RAMAN SPECTROSCOPY FOR THE FORENSIC EXAMINATION OF REACTIVELY DYED COTTON

by

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Certificate of Authorship and Originality

I certify that the work in this thesis has not been previously submitted for a degree nor has been submitted as part of the requirements for a degree except as fully acknowledged in the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all the information sources and literature used are indicated in the thesis.

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31st October 2005

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List of Abbreviations

AFP	Australian Federal Police
CI	Colour Index
CCD	Charged Coupled Device
CCTV	Closed Circuit Television
DP	Discriminatory Power
DNA	Deoxyribose Nucleic Acid
EFG	European Fibres Group
ENFSI	European Network of Forensic Science Institutes
ESI-MS	Electrospray Ionisation – Mass Spectrometry
ESI-MS/MS	Electrospray Ionisation – tandem Mass Spectrometry
FBI	Federal Bureau of Investigation
FPF	Fibre Plastic Fusion
FSS	Forensic Science Service
FTIR	Fourier Transform Infrared
GSR	Gun Shot Residue
IR	Infrared
MPFSL	Metropolitan Police Forensic Service Laboratory
MSP	Microspectrophotometry
mW	milli-watt
NIR	Near Infrared
nm	nano-meter
PCA	Principal Components Analysis
PCM	Plastic Coating Mark
Py-GC	Pyrolysis Gas Chromatography
SERRS	Surface Enhanced Resonance Raman Spectroscopy
SERS	Surface Enhanced Raman Spectroscopy
SWGMAT	Scientific Working Group for Materials
TLC	Thin Layer Chromatography
TWGFIBE	Technical Working Group for Fiber Examinations
UV	Ultraviolet
Vis-MSP	Visible Microspectrophotometry
XAM	Neutral medium improved white Gurr (Xylene based mountant)
ΔE	energy change
μm	micro-meter

Abstract

In the 21st Century, fibre examiners are faced with the constraints of time and money. Rapid advances in DNA technology has seen fibre evidence undervalued due to it being time consuming, costly and (when competing with DNA) perceived to be less discriminatory. However, DNA is not present in all cases and when present may require other evidence to interpret the circumstance/s. The only way forward for fibre examinations is through research that will address issues of time and money whilst increasing their evidentiary value by demonstrating reliability through improved statistical information.

Raman spectroscopy was investigated as it is a technique that is growing in popularity for the analysis of materials. It is quick, discriminatory, non-destructive and requires minimal sample preparation. The ability of Raman spectroscopy to discriminate black/grey and blue cotton fibres was investigated to determine how effective the technique would be for analysing this often undervalued fibre evidence.

Various parameters of Raman spectroscopy were investigated to determine the optimal conditions for the analysis of the samples selected. The major variable investigated was laser wavelength with the 785nm and 632.8nm lasers providing the best results. Results indicated that, at least, the major dye component could be identified using Raman spectroscopy.

The discriminatory power of the technique was then investigated for a sample set of 11 black/grey and blue cotton samples as well as a set of 91 black and blue cotton fibres of unknown dye types. This was then compared to the discriminatory power of microspectrophotometry (MSP) for the same sample sets as well as the discriminatory power of the combined techniques.

In analysis of both sample sets, Raman spectroscopy showed a lower discriminatory power than microspectrophotometry. However, combining the two techniques significantly increased the discriminatory power. Using

chemometrics increased the discrimination provided by Raman spectroscopy indicating that chemometrics may be used as an aid for interpreting data.

Whilst the advantages of Raman spectroscopy were evident during these studies one major limitation of the technique was also highlighted. The problem of fluorescence has long been regarded as the major drawback to using Raman spectroscopy for fibre examinations. The problem was overcome in some instances by changing the laser wavelength but not all.

Even with the occurrence of fluorescence, it was shown that, for the sample sets investigated, Raman spectroscopy (when combined with microspectrophotometry) provided a level of discrimination not able to be achieved with microspectrophotometry as a single technique. Therefore, Raman spectroscopy should be considered when undertaking analysis of these sample types.

Keywords: Fibre evidence, Reactive Dyes, Raman Spectroscopy, Microspectrophotometry, Chemometrics

Chapter 1: Introduction

1.0 Summary

The research carried out in this thesis utilises three general areas of knowledge - textile science, forensic fibre examinations and Raman spectroscopy. Specifically, the document focuses on the use of Raman spectroscopy for the forensic examination of black/grey and blue reactively dyed cotton fibres.

Chapter one introduces the first two key areas, textile science and forensic fibre examinations. The first section of this chapter deals with the general nature of textiles and covers such things as history, classification of fibre types and production statistics. This section also introduces dyes and outlines the importance of dyes in the textile industry.

The second section of this chapter is an assessment of current fibre examination methodologies and a brief history of fibre examinations. This section introduces the problems that are currently facing fibre examiners and the research that is being conducted to address these problems.

1.1 Textiles

It is necessary for forensic fibre examiners to fully exploit fibre and textile evidence during analysis. This requires not only specialised forensic knowledge but also knowledge of textiles, their structure and manufacturing processes. To assess the value of fibre evidence it is often necessary to also know production figures, fashion changes, sudden arrival of new material/s, dye variability, and other factors. What follows is a brief summary of textiles as they relate to this

research. Information can also be obtained from catalogues [1], journals [2], websites [3-7] and texts [8-10]. Every three years a comprehensive summary of journal articles on fibres and textiles is condensed and presented at INTERPOL Forensic Science Symposia [11-13]. This provides an excellent resource not only for forensic based journal articles but also for general textile references. The book "Forensic Examination of Fibres" edited by Robertson and Grieve is an excellent reference for fibre examination and general textile information [14].

1.1.1 History and Classification

Textiles have been used by man since ancient times for protection against the elements. Progressively, textiles were employed for an increasingly diverse range of uses, such as furnishing, bedding and sailing. Today textiles pervade all aspects of our lives.

The basic component of a textile is a fibre. At the basic level all fibres fall into one of two categories: **natural fibres** and **man-made fibres**. Natural fibres are fibres that occur naturally such as wool and cotton. Man-made fibres are fibres that have been manufactured from either naturally occurring fibre-forming polymers (e.g. viscose) or synthetic fibre-forming polymers (e.g. acrylics) as well as those made from inorganic materials (e.g. carbon) [9]. The classification of fibres is summarised in Figure 1-1 [15].

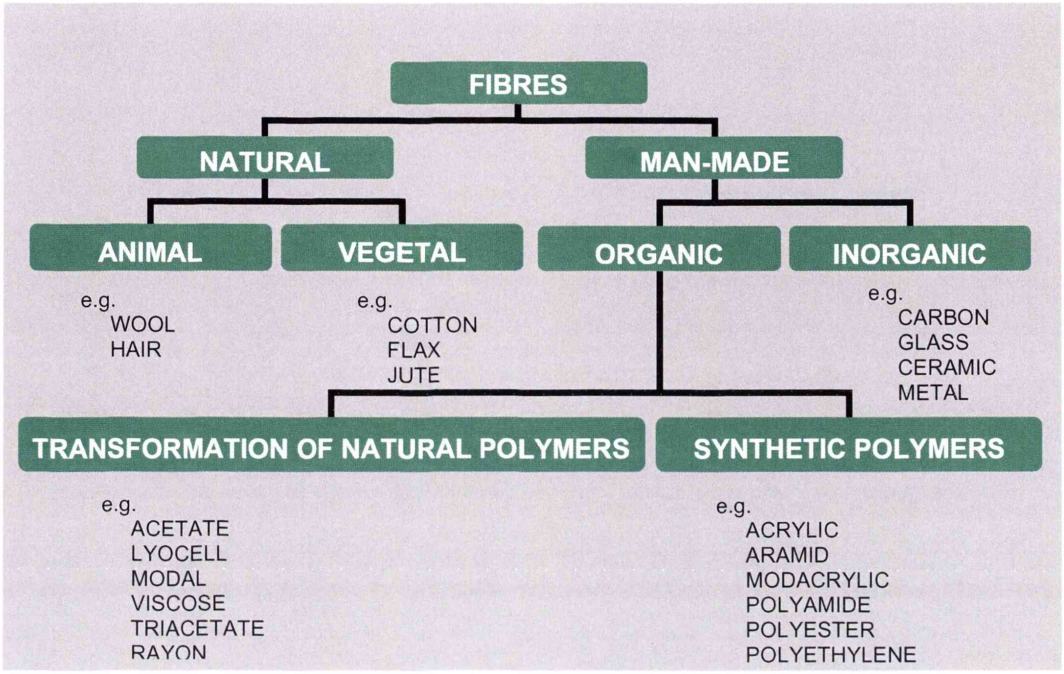


Figure 1-1 Classification and sub-classification of fibres [15]

Originally all textiles were composed of natural fibres, such as cotton, flax and jute. With the increasing demand for fibres that could perform a variety of functions, the invention and production of man-made fibres emerged. The first man-made fibre was rayon (a transformation of the natural polymer cellulose) introduced in 1911 [16]. The discovery of synthetic polymers in 1930 made possible the production of organic synthetic polymer fibres. The first man-made polymer fibre was nylon in 1939 [16]. The development of new fibres continues even today [17, 18].

Fibres themselves are only the starting point of the finished "textile". A fibre is the smallest unit of a textile material that has a length many times greater than its diameter. A fibre can be spun with other fibres to form a yarn that can be woven or knitted to form a fabric. The fabric can then be cut and sewn to form a garment (Figure 1-2 is a simplified diagram of fibres to finished textile). The manufacturing processes used to turn fibres into finished products will alter the original fibre by means of dyeing and/or finishing.

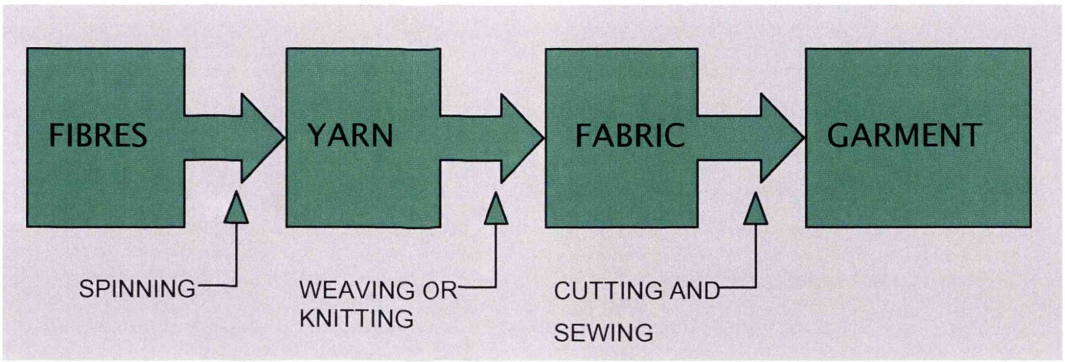


Figure 1-2 Conversion of fibres to finished product (garment)

A way of classifying textiles, suggested by Adolf [8], is by their end use. This rough outline of textile end-uses is helpful to the forensic examiner for classification purposes. There are three generic categories of fibre based products:

1. clothing for men, women and children – *apparel*
2. textiles for use in the home – *household textiles, furnishings, upholstery, floor coverings*, and
3. technical textiles – *e.g. medical and industrial textiles.*

The first two categories are generally composed of natural and/or synthetic fibres. The third category, technical textiles, is predominantly composed of man-made fibres. It is this third category that has driven the manufacture of new and improved fibres types (such as the high-performance aramids, fluorofibres and carbon fibres) [8].

1.1.2 World Fibre Production

As demand has shaped the quality of fibres manufactured so does demand influence the quantity of fibres produced each year. In previous years cotton has been the most common fibre produced (representing 58% of total world fibre production in 1970) [19]. Today, its share of production has decreased to be only

slightly higher than that of polyester (38% of world fibre productions in 2002- refer to Figure 1-3) [19].

World-wide production percentages of fibres, for the year 2002, are shown in Figure 1-3 and a comparison of the production values are shown in Table 1-1 for the years 1980 and 2002 [19]. What is interesting to note is that the two fibre types that have driven the increase in production quantities (fibre production has approximately doubled in this period) are cotton and polyester. Cotton production has increased ~150% and polyester production has increased ~400%. These values indicate that there is a growing trend towards man-made fibres, though cotton still remains an important fibre type.

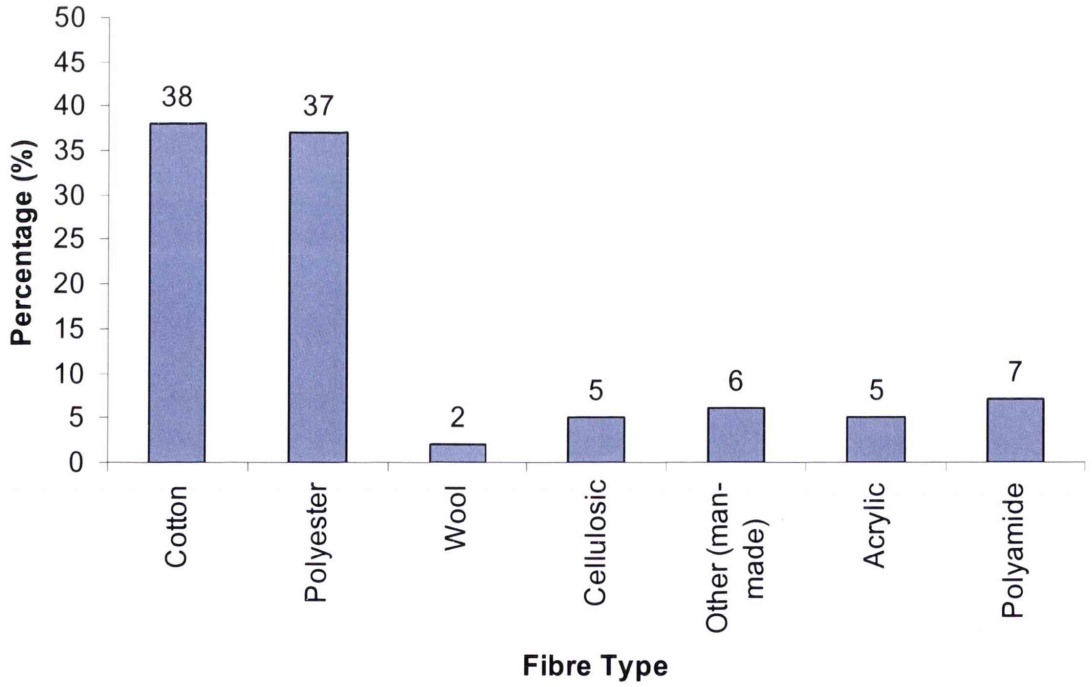


Figure 1-3 World production of fibres in 2002 [19]

Table 1-1 World production figures for fibres (1980 and 2002) [19]

Fibre Type	1980 (1,000 tonnes)	2002 (1,000 tonnes)
cotton	13,840	21,100
wool	1,600	1,300
polyester	5,130	21,000
polyamide	3,150	4,000
acrylic	2,060	2,700
other (man-made)	290	3,300
cellulosic	3,560	2,700
sum	**29,630**	**56,100**
relative share (%) for cotton	**46.7**	**37.6**

1.1.3 Cotton Fibres and Forensic Science

The importance of cotton in forensic investigations is highlighted by the large number of garments submitted to forensic laboratories that are made wholly or partially of cotton [20]. In 1992, 23% of all fibre comparisons submitted to the Metropolitan Police Forensic Service Laboratory (MPFSL) involved cotton [21].

Cotton is an important fibre type in garments and forms a large majority of fibres found in textiles from studies of random fibre populations [22-25]. The population of cotton fibres in these studies represent a larger percentage of fibres types than what is indicated in production figures (as seen above).

The presence of cotton has been shown to be more prevalent in Australia than in European countries possibly due to the climate [25]. This indicates that the importance of cotton fibres in Australian forensic investigations arises from the predominance of this fibre type in clothing.

1.2 Dyes

Fibre type and quantities produced are not the only things that a fibre examiner should be aware of. A third aspect of textiles which is extremely important is colour. Colour is a prime feature used to discriminate fibres because of its high level of variability. It is particularly important in the examination of natural fibres due to the low variability in the fibre substrate itself.

Colour is defined as the net response of an observer to visual physical phenomena involving visible radiant energy of varying intensities over the wavelength range ~350 to 700 nanometres (nm) [26]. The electromagnetic spectrum, with the visible section expanded, is shown in Figure 1-4.

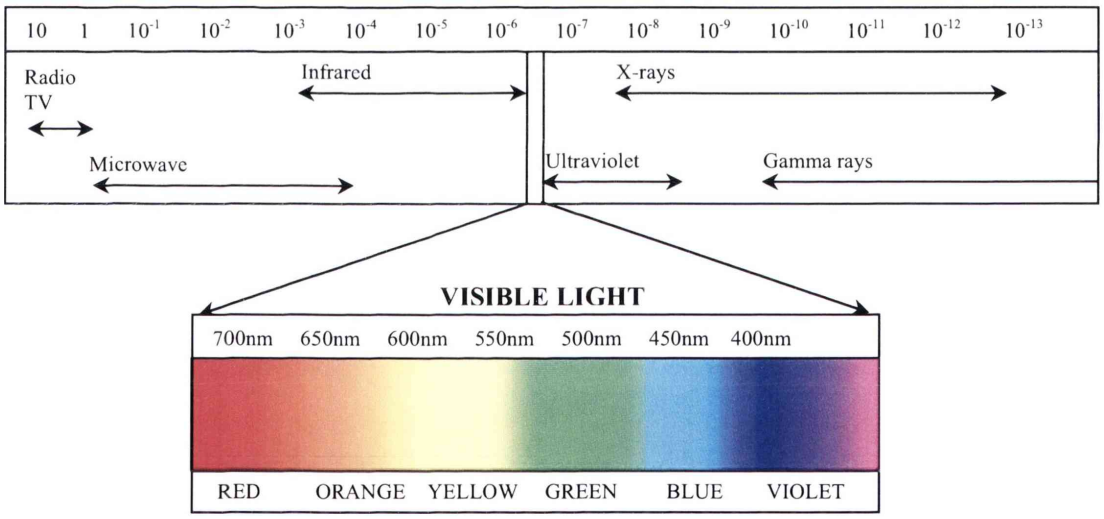


Figure 1-4 Electromagnetic spectrum with visible light detail [26]

Colour is imparted to textiles through the application of dyes. Dyes are aromatic compounds that absorb electromagnetic energy with wavelengths in the visible range (~350-700nm). When light from a source strikes a dyed textile surface different portions of the light wavelengths are absorbed by the dye depending on the structure and light absorption characteristics of the dye. Light that is not absorbed by the dye is reflected from the surface as diffuse light and this is the

colour the observer sees. Table 1-2 is an example of absorbed and observed light waves.

Table 1-2 Colour after absorption/reflectance (adapted from) [26]

Wavelength (nm)	Light absorbed	Colour observed
435-480	Blue	Yellow
490-500	Blue-green	Red
500-560	Green	Purple
580-595	Yellow	Blue
605-700	Red	Blue-green

1.2.1 History of Dyes

Colorants have been used by man for painting and the dyeing of skin and clothes for thousands of years. Until the middle of the 19th century, all colorants used were of natural origin. Natural inorganic pigments include: soot, ochre, hematite and manganese oxide. Natural organic colourants such as the aromatic compounds from plants, insects, fungi and lichens, were employed for dyeing textiles.

Synthetic dye manufacturing started in 1856 when the English chemist W.H. Perkin, in an attempt to synthesise quinine, obtained instead a bluish substance with excellent dyeing properties. This substance became known as Tyrian purple (also called aniline purple or mauveine) (refer to Figure 1-5). New dyes soon began to appear on the market and by the early 1900's synthetic dyestuffs had almost completely supplanted natural dyes [27].

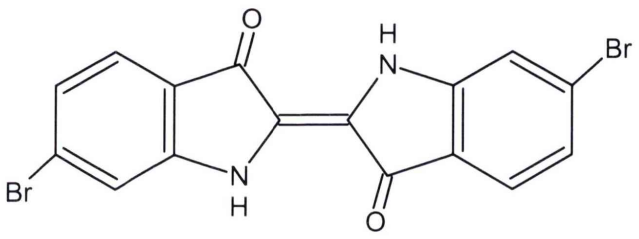


Figure 1-5 Structure of Tyrian purple

To be effectively applied, dyes must have an affinity for the fibre substrate. The dyes on fibres are physically bound by one or more physical forces including hydrogen bonding, Van der Waals or ionic forces. In certain cases they are chemically bound by covalent bonds. Fibres have differing dyeing characteristics and affinities dependent on the chemical and morphological structure of the fibre [26]. Table 1-3 shows the major dye classes and the fibre types that each class has an affinity for.

Table 1-3 Dye class and related fibre affinity [26]

		Fibre Type										
		Cotton	Viscose	Wool	Silk	Modacrylic	Acrylic	Polyester	Polypropylene	Acetate	Nylon	Protein
Dye Class	acid			✓	✓		✓		✓		✓	✓
	basic					✓	✓	✓			✓	
	direct	✓	✓									
	disperse						✓	✓	✓	✓	✓	
	reactive	✓		✓							✓	
	sulphur	✓										
	vat	✓										
	metallised	✓		✓					✓		✓	
	azoic	✓	✓									
	ingrain	✓										

1.2.2 Classification of Dyes

Dyes may be classified two ways: by chemical structure or by application method. Classification by chemical structure is done by chemical classes, the

major classes are summarised in Table 1-4. An example of an azo dye is given in Figure 1-6. Included is its classification by application (i.e. Acid).

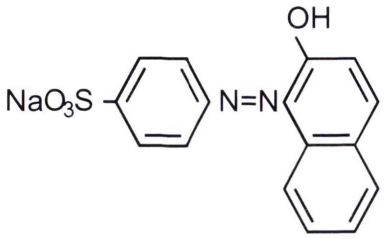


Figure 1-6 An azo dye - CI Acid Orange 7

Table 1-4 Dyes classes based on chemical structure [28]

Chemical Class	Distinctive structural feature	General characteristics	Main application class(es)
Azo	-N=N-	All hues, but yellow to red most important	Dominate most, but not vat dyes
Carbonyl	C=O	All, but blue most important	Important in most applications
Pthalocyanine	16-membered heterocyclic ring, metal complex	Blue and green only	Most important in pigments
Triarylcarbonium ion	Positively charged carbon	All hues, but reds and blues most importantly	Cationic dyes and pigments
Sulphur dyes	Complex polymeric S-containing species	Most dull colours, such as blacks and browns	Often considered as an application class itself
Methine	-C=	All hues, but yellow most important	Disperse, cationic
Nitro	$-NO_2$	Many yellows	Disperse, hair dyes
Inorganic	Range of inorganic types	All hues, white and metals	Exclusively pigments

The most common method for classifying dyes is by colour, structure and application method as is seen in the Colour Index (CI). The CI was created in 1924 (and revised every three months) by the Society of Dyers and Colourists and the American Association of Textile Chemists and Colorists. The Colour Index (3rd Edition, issue 2) lists about 28,000 commercial dye names, representing ~10,500 different dyes, of which ~4,500 are currently produced. Each different dye is given a CI generic name determined by its application characteristics and its colour (e.g. CI Reactive Black 5 is a reactive black dye). The Colour Index classifies dyes into 15 different categories. Of these 15 classes five are associated with cotton dyeing (reactive, sulphur, vat, azoic and ingrain). These are discussed below, also included is a discussion on metallised dyes (which are not listed in the Colour Index as a separate class).

1.2.2.1 Metal complex dyes

Among acid and reactive dyes, many metal complex dyes can be found. These are strong complexes of one metal atom (usually chromium, copper, cobalt or nickel) and one or two dye molecules.

1.2.2.2 Direct dyes

Direct dyes are relatively large molecules with high affinity for cellulose fibres. Van der Waals forces make them bind to the fibre. In the Colour Index, the direct dyes form the second largest dye class with respect to the amount of different dyes: about 1600 direct dyes are listed with only ~30% of them in current production.

1.2.2.3 Vat dyes

Vat dyes are water-insoluble dyes that are widely used for dyeing cellulose fibres. The dyeing method is based on the solubility of vat dyes in their reduced (leuco) form. Reduced with sodium dithionite, the soluble leuco vat dyes impregnate the fabric. Next, oxidation is applied to bring back the dye in its insoluble form.

1.2.2.4 Anionic dyes and ingrain dyes

Azoic dyes and Ingrain dyes (naphthol dyes) are the insoluble products of a reaction between a coupling component (usually naphthols, phenols or acetoacetylamides) and a diazotised aromatic amine. This reaction is carried out on the fibre.

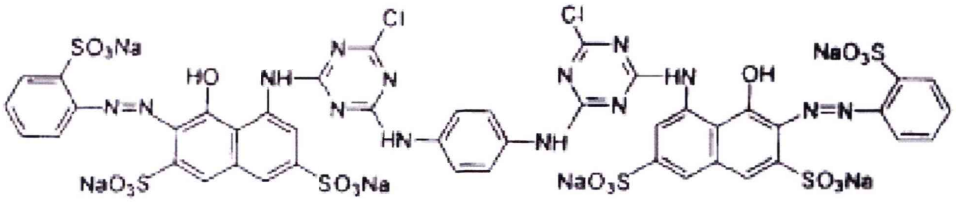
1.2.2.5 Sulphur dyes

Sulphur dyes are complex polymeric aromatics with heterocyclic S-containing rings and represent with 15% of the global dye production. Dyeing with sulphur dyes involves reduction and oxidation, similar to vat dyeing. These dyes are mainly used for dyeing cellulose fibres.

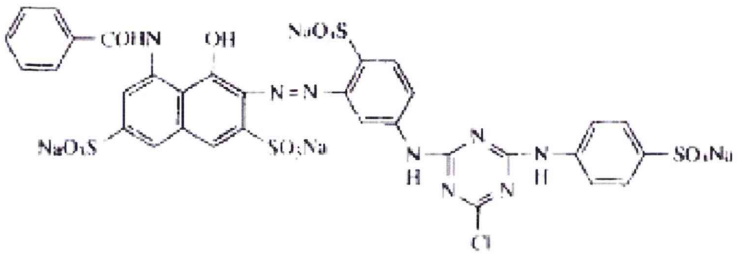
1.2.2.6 Reactive dyes

By far the most important dye class for cellulosic fibres is the reactive dye group. Reactive dyes are dyes with reactive groups that form covalent bonds with OH-, NH-, or SH-groups in fibres (cotton, wool, silk, nylon). The reactive group is often a heterocyclic aromatic ring substituted with chloride or fluoride. Another common reactive group is vinyl sulphone. The use of reactive dyes has increased ever since their introduction in 1956, especially in industrialised countries.

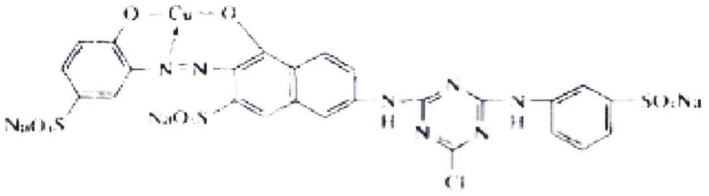
In the Colour Index, the reactive dyes form the second-largest dye class (with acid dyes forming the largest) with respect to the amount of active entries. Approximately 600 of the ~1050 different reactive dyes listed are in current production. Some examples of red reactive dyes are shown below in Figure 1-7.



CI Reactive Red 120



CI Reactive Red 4



CI Reactive Red 7

Figure 1-7 Examples of red reactive dyes [29, 30]

Before the introduction of reactive dyes, cotton was often dyed with direct, vat, sulphur and azoic dyes. The characteristic structural features of a typical reactive dye molecule are:

- the reactive system enabling the dyes to form covalent bonds between the dye and the substrate
- the chromophoric grouping contributing to the colour for cellulose
- a bridging group linking the reactive system to the chromophore and
- solubilising groups.

The most important reactive dye is CI Reactive Black 5.

CI Reactive Black 5

The top 5 selling textile dyestuffs are [28]:

1. Indigo
2. CI Disperse Blue 79
3. CI Sulphur Black 1
4. **CI Reactive Black 5**
5. CI Acid Black 194

CI Reactive Black 5 is the most popular reactive dye in the world. Large volumes (over 40,000 tonnes used annually) are produced by many manufacturers. The popularity of this dye is predominantly due to its cost efficiency.

C I reactive Black 5 in its pure form is not a black but rather a navy blue. It is usually used in its shaded form where a red component is added to give a full black.

The dye is made in two stages:

1. First the mono azo is synthesized from equimolar amounts of intermediates under appropriate conditions. This is a red dye (see Figure 1-8).

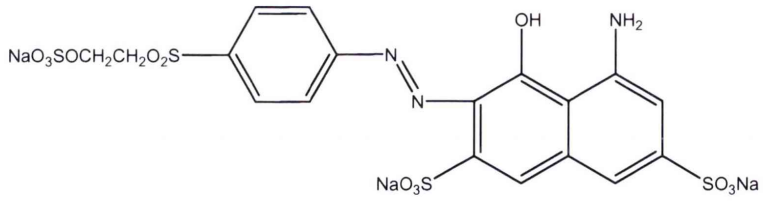


Figure 1-8 Red dye created during first step of Reactive Black 5 manufacture

2. Next the second azo group is introduced by changing the conditions however the amount added is not enough to allow for 100% conversion to the Bisazo form. This means that NOT ALL THE RED DYE is converted into the bisazo navy blue form. This residual red acts as a built

in shading component to give a full black. This is a complex process to control.

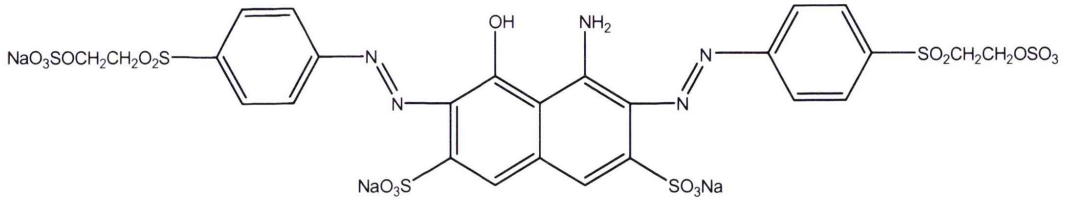


Figure 1-9 Bisazo CI Reactive Black 5 in its pure form

Different manufacturer's black dyes, based on C I Reactive Black 5 can be:

- different shades
- contain different proportions of mono and bis azo
- contain different other shading components.

1.2.3 Production Statistics

Recent statistics on the global production and use of dyes and on the relative distribution between the different dye classes are not readily available. The most recent readily available data is from 1991 [31]. It is reasonable to assume that the total sale and consumption approximately equals the production.

Table 1-5 Total sale of dyes (with the exclusion of solvent and pigment dyes) in 1991 [31]

Dye Class	World Production (1,000 tonnes)
acid	100
basic	44
direct	64
disperse	157
reactive	114
sulphur	101
vat	40
azoic	48
sum	**668**
relative share (%) for reactive dyes	**17**

1.3 Textiles as Forensic Evidence

The large volume of textiles surrounding us means that they will be directly and/or indirectly involved during the commission of crime. It is therefore necessary to understand the mechanisms of textile transfer/damage and the role that textile trace evidence has in a forensic investigation.

'Textile' is a broad definition that encompasses everything from the fibre to an end product (such as garment or bedding). Textile evidence can vary enormously and be present in a wide range of crimes. It may be present as a fibre (microscopic), yarn, tuft or garment (macroscopic). Macroscopic textile evidence is visible to the naked eye, with or without light enhancement. This type of evidence would be examined macroscopically and may also be examined microscopically.

Evidence that exhibits **individual characteristics** is able to be associated with a common/single source with a high degree of probability [16]. In textile examinations this type of individualisation generally only occurs with macroscopic textiles showing tear matches. However, textile evidence is overwhelmingly discovered as fibres, tufts and yarns that cannot be individualised. This type of evidence shows **class characteristics** that can only be associated with a group and not with a single source [16].

The purpose of a fibre examiner is to examine all aspects of textile evidence present at a crime scene and related areas for comparison and intelligence purposes. Some examples of textile evidence and their use in forensic investigations are:

Substrate: Textiles can act as substrates for other forensic evidence such as: glass, gun shot residue (GSR), paint, soil, DNA, blood and other body fluids.

Damage to textiles: Forensic examinations of textile damage, generally deal with mechanical effects on textiles such as; damage by normal wear and tear,

tear, cut, puncture, abrasive damage, tensile failure, animal damage, heat and microbial damage. This type of evidence may be useful in cases of homicide and suicide and also in cases of sexual assault, rape and armed robbery [32]. Figure 1-10 shows an example of damage to a textile due to normal wear and tear (reprinted from [33]).

Figure 1-10 T-Shirt showing variations due to normal wear and tear (UV light damage indicated by areas circled) [33]

Texture transfer: The weave pattern (or logo) of a textile may be transferred to another surface during contact with blood, paint or even during a high impact collision (such as a hit and run) [34].

Ropes and cordage: The examination of ropes and cordages generally involves the determination of whether two pieces have come from the same source [35]. Examination investigates construction as well as materials used.

Pattern matching (video): Patterns of garments (e.g. floral or stripes) may be matched with video footage in cases where the pattern is unique through wear and tear or where the garment patterns are random. This type of textile evidence is useful in cases of armed robbery and terrorist acts, where there is footage from closed circuit television (CCTV) or other sources [36].

Fibre Plastic Fusions / Plastic Coating Marks: These are contact traces that occur almost exclusively in car accidents and can assist the examiner in determining the seating arrangements of the occupants at the moment of impact [37]. The traces occur when friction heat (from the sudden increase in kinetic energy) causes the plastic interior surfaces of the vehicle to soften momentarily and then harden trapping fibres (Figure 1-11 left image). These traces are known as "fibre plastic fusions" (FPF). The transfer can occur both ways as plastic residues may be left on clothing, these traces are known as "plastic coating marks" (PCM) Figure 1-11 right image.

Figure 1-11 Examples of (left) Fibre Plastic Fusion, and (right) Plastic Coating Mark (reprinted from [34])

Fibre transfer: This can be a one-way or two-way transfer of fibre material from clothing and furnishings. The ability and opportunity of fibres to be transferred during a crime are great and the potential for the use of this type of class evidence should be apparent at nearly all major crime scenes [38].

The examination of trace evidence can provide large quantities of information regarding crime/s. Fibre evidence is one of the most common trace evidence

found at a crime scene [39] and whilst fibre transfer[1] evidence is only class evidence it can still be used to support (and sometimes refute) association between people and places.

1.3.1 How Fibres Come to be Evidence

During the commission of a crime a perpetrator may come into contact with the victim and his/her environment and as a result leave and/or remove fibres. Fibres may also be left on a vehicle during a hit and run case or even become caught in glass during a break and enter. As with other trace evidence (such as soil) fibre transfer follows Locard's principle [40].

Locard's Principle

In the 1920's, Edmond Locard's study of dusts from numerous sources [40] laid the foundation for what became known as "Locard's Exchange Principle" [41]. The principle is summarised as follows:

> *"Whenever two objects come into contact, there is always a transfer of material. The methods of detection may not be sensitive enough to demonstrate this or the decay rate may be so rapid that all evidence of transfer has vanished after a given time. Nonetheless, the transfer has taken place."* [41]

[1] From this point on "fibre evidence" will refer to *fibre transfers* associated with a crime or criminal activity.

In essence, Locard's principle is that every contact leaves a trace. If every trace could be detected and identified, it would be possible to deduce the participants of the contact [38]. With fibre evidence being the most common physical evidence found at a crime scene [39] it then follows that a major key to establishing association between the perpetrator and the crime/victim lies with the successful detection, collection, identification and interpretation of fibre evidence.

The value of fibre evidence lies in its potential to link aspects of a crime (e.g. a person and a crime scene or two people in the instances of assault) [39]. Fibre evidence and examinations can:

1. establish a sequence of events
2. link a weapon with a victim or suspect
3. help to corroborate a victim's account of circumstances surrounding an assault
4. provide leads to investigators about a victim's surroundings at the time of an incident
5. link together a number of different (sometimes apparently unrelated) victims or criminal activities
6. establish a high probability that contact or some other association has taken place between people and/or objects and
7. eliminate a suspect from an investigation.

Despite the obvious forensic significance of fibre evidence it was a long time between the formulation of Locard's principle and the general acceptance of the evidentiary value of fibres [11].

1.3.2 Prevalence and Importance of Fibres

In the United States a catalyst for fibre examinations was provided by evidence presented at the Wayne Williams case in 1983 [42, 43]. Whilst fibre evidence had often been an important part of criminal cases before, the Williams trial differed from previous cases in that the case involved:

1. a large number of victims, 30 children and men
2. fibre evidence presented was not used to support other testimony and validate other evidence, rather
3. fibre evidence was the primary evidence and all other testimony and evidence was used to support the fibre evidence.

This high-profile case provided an impetus for fibre examinations. Many questions concerning the significance of fibre evidence and examination procedures were brought to the attention of the public. In 1984, a year after the Wayne Williams case, separate sessions were devoted to hair and fibre evidence at the 10th International Association of Forensic Sciences (Meeting in Oxford, England) for the first time [39]. This trend was maintained in Europe, where the European Fibres Group (EFG) was formed in 1993 [44], and in the United States, with the formation of the Technical Working Group for Fiber Examinations (TWGFIBE) in 1994 [45], which later became part of the Scientific Working Group for Materials (SWGMAT). Currently in the EFG there are 33 laboratories that are full members and 21 associate members from across Europe. There are also six world members [46].

These groups were formed to investigate and evaluate forensic fibre methods as well as to disseminate information relating to forensic examination of hair and fibres [44, 47]. To achieve this purpose, the EFG meets regularly and the proceedings of the meetings are published [48-50]. The formation of these

groups has resulted in a large quantity of information on fibre examinations from both research and case work in the last ten-to-fifteen years.

1.3.3 History of Fibre Examinations

Forensic fibre analysis has been carried out on a routine basis in laboratories around the world for many years [39] and their usefulness in providing information in criminal cases is limited only by the skill and knowledge of the investigator (subject to certain practical considerations) [39].

In the 1960's, forensic examination of fibres was relatively simple. Loose fibres were removed from the crime scene or from textiles examined in the laboratory using strips of adhesive tape as first proposed by Max Frei-Sulzer, in 1951, in Switzerland [51]. This method of retrieval is essentially unchanged today. Fibres were mounted as a bundle on a glass slide and flooded with benzene. Most of the examination procedure was based on the microscopic properties of the fibres [52].

The 1970's saw the introduction of major analytical improvements. Smalldon (1973) utilised advances in infrared (IR) spectroscopy for the classification of polymer fibres, and Feeman [53] (1970) used Thin Layer Chromatography (TLC) for the identification and comparison of dyes, thereby creating schemes for fibre dye analysis. Pounds and Smalldon [54, 55] carried out research on the mechanisms of fibre transfer, persistence and retention.

In the 1980's, fibre colour dominated the development of analytical methods. TLC eluent systems for major classes of fibres were developed and Microspectrophotometry (MSP) was introduced into fibre comparison sequences in England. Foundation work for MSP was carried out in Switzerland between 1959 and 1976.

In 1999, the SWGMAT Fiber sub-group published its Forensic Fiber Examination Guidelines [56] and in 2001 The EFG published its Manual of Best

Practice [46]. The Manual of Best Practice was published with a view to universally raising the standards of forensic fibre examination and to address the EFG's major aim:

> *"To ensure that work carried out on a daily basis by its members is of the highest quality and is fit for purpose. The techniques used should be performed to a standard that enables the result from case and research work in different countries to be comparable".* [46]

The large quantity of research carried out by individuals and by organisations (such as the EFG and SWGMAT) has greatly impacted the **collection**, **analysis** and **interpretation** of fibre evidence. This has resulted in new and more efficient/discriminatory techniques being developed as well as large volumes of data being collated to aid in interpretation.

1.4 Collection of Fibres

The search for fibres at the crime scene and in the laboratory is based on the belief that there has been a transfer (either one-way or two-way) of fibres between the victim, perpetrator and surroundings. The Australian Federal Police (AFP) procedures state that "as a general rule where fibres may be important, recovery of these should be considered first" [57]. This is because fibres are not normally visible to the naked eye, are often readily lost and the techniques for the recovery of other trace evidence such as glass and paint will generally result in their loss.

At the crime scene any items suspected of having fibres or other trace material are collected and packaged individually. These items may include the victim as well as surfaces from the victim's environment. For exhibits that cannot be packaged and sent to the laboratory, tape lifts are done at the crime scene.

Fibre collection techniques can vary from country to country and laboratory to laboratory. Fibre recovery may be by tweezers, tape lifting, scraping and vacuuming. The SWGMAT Fiber Guidelines support taping, vacuuming (with a specialised filter system) and scraping [58]. However, it has been found that there is an increased risk of contamination with the scraping technique [59]. The EFG does not recommend vacuuming and scraping for the recovery of fibres. It does, however, endorse taping, individual fibre removal with forceps, combing (especially in the case of fibres in head hair) and brushing (when there is a large amount of debris on the substrate) [46].

Fibre taping can be additive, where multiple applications of a single tape can be used to cover a large area, or taping can be 1:1, where one area of tape equals the same area of taped surface [60]. The latter method has advantages over additive taping for crime reconstruction but can be prohibitive due to large number of tapes that are generated [60].

1.5 Analysis of Fibres

The method of analysis will vary depending on the fibre evidence recovered as well as the information available pertaining to the case. When a source is **known** (e.g. suspect's clothing and/or environment) comparison with the fibres collected from the victim and/or environment (known as **questioned** fibres) can be conducted. When a source is unknown and only the fibres from the victim and their surroundings are available, identification of the evidence can provide useful 'intelligence' information.

The following flow-chart (Figure 1-12) is a summary of the techniques used by the AFP (and described in Robertson and Grieve [61]) in the identification and analysis of fibres for comparison purposes.

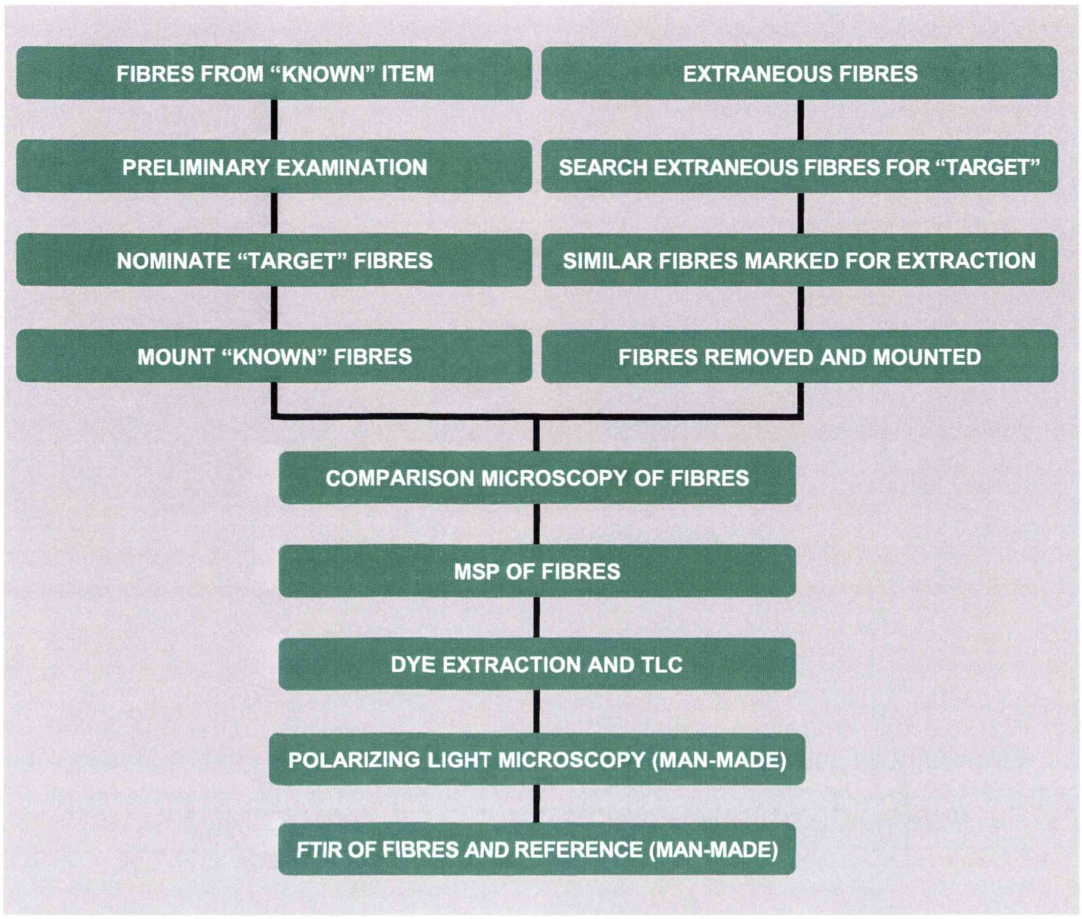


Figure 1-12 Procedure for the collection and examination of hairs and fibres [61]

1.5.1 Identification

The identification of fibre type/s from a target[2] garment and the comparison of these with any fibres from a suspect and his/her environment have the potential to provide valuable evidence of association. If fibres have been transferred during the commission of a crime, but a suspect has not been apprehended, the

[2] A target garment (or recipient garment) is defined as one that has received transferred fibres. A target fibre is a fibre type selected after evidential assessment to use for active searching in trace materials 46. Group, E.F., *Manual of Best Practice*. 2001.

identification of fibre/s can provide probative information that may help with investigative leads.

Saferstein [16] defines identification as "the process of determining a substance's physical or chemical identity". Figure 1-13 shows what procedures can be followed for identification of fibres [62].

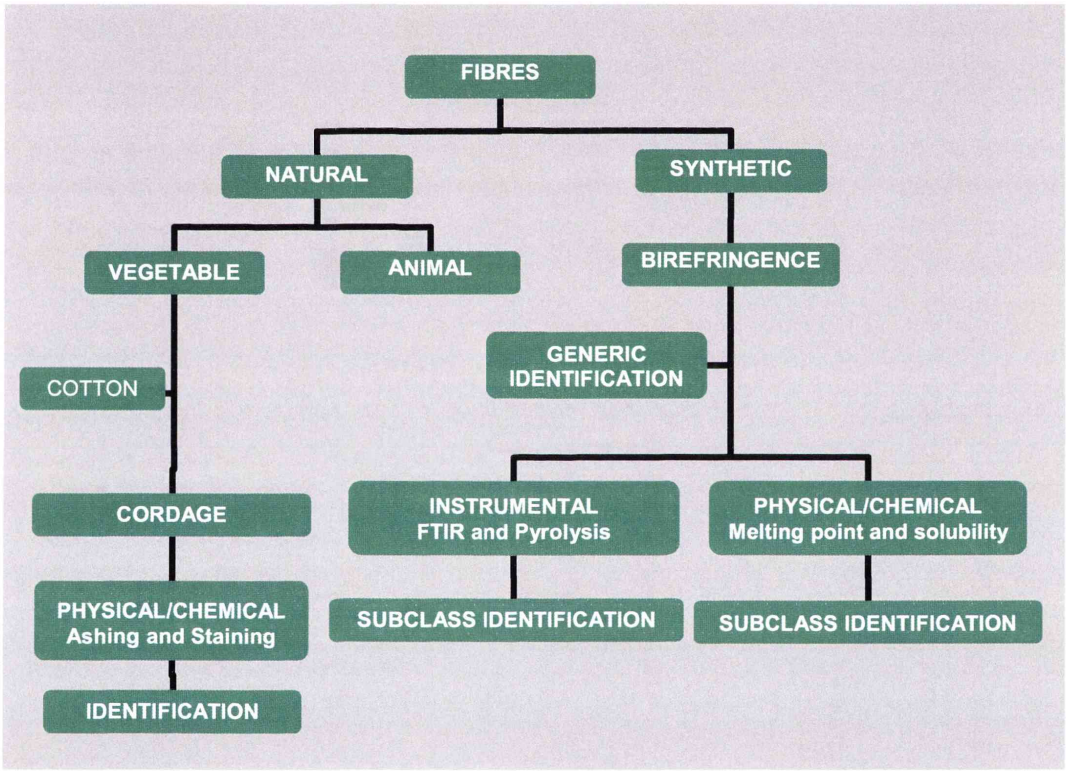


Figure 1-13 Flow chart showing scheme of fibre identification [62]

At the identification stage of an examination the sample/s will be classified into either naturally occurring or man-made fibres. The generic type of man-made fibres is established through the use of polarized light microscopy and further sub-typing can be achieved through the use of Infrared Spectroscopy. The identification of vegetable fibres is based on characterisation of colour, the lumen and its relative width, cross markings, type and distribution of pits and crystals. Identification for both man-made and natural fibres is achieved predominantly through microscopy.

1.5.1.1 Microscopy

Microscopy is the essential technique for fibre examinations [41, 63]. It provides the quickest and least destructive means of determining morphological characteristics [46]. Several types of microscopy can be used at the identification stage of analysis, the two most important are:

1 White light/transmitted light (bright-field microscopy): This is the simplest method of fibre identification. The sample is placed under white light (at high magnification) and morphological features are identified which are useful in identifying fibre type. This technique is particularly useful for cotton and wool and to some extent other vegetable fibres. Synthetic fibres are more difficult to identify using this technique alone as they can be relatively featureless and differ only in chemical makeup. For this reason, the application of birefringence is useful.

2 Birefringence: This microscopic technique exploits the anisotropic and psuedocrystalline properties of polymeric synthetic fibres. Anisotropic fibres possess different physical properties (refractive indices) in different directions of the structure.

Not all polymeric samples can be identified using microscopy therefore further techniques are required for identifying synthetic fibres.

1.5.1.2 Infrared spectroscopy

This is an invaluable spectroscopic technique that is able to identify the constituent polymers present in a fibre (synthetic man-made fibres only) [64, 65]. This means the generic type of fibre can be readily identified and in the cases where the fibre contains co-polymers, Infrared (IR) spectroscopy allows for sub-classification within a generic type. The usefulness and sensitivity of this technique has been shown through many studies, especially that by Grieve et al. in the sub classification of acrylic fibres [66].

1.5.1.3 Pyrolysis

Pyrolysis is the decomposition of a chemical structure at high temperatures resulting in molecular fragments. The fragments are analysed and identified by coupling the pyrolysis with GC/MS to characterise the original sample.

In all cases of identification, comparison with authenticated reference samples[3] is imperative and identification should be as specific as possible [46]. This technique's high discrimination is offset by its destructive nature and requirement for a large sample size. Some difficulties exist with this technique, predominantly with respect to repeatability, data sharing and databasing. As a result this technique is not routinely used for casework.

1.5.2 Comparison

Saferstein [16] defines comparison as "the process of ascertaining whether two or more objects have a common origin". When fibres are available from both the victim and the suspect the objective of analysis is to determine if the samples exhibit no difference at each comparison stage. Comparison of man-made fibres takes into account features such as: generic sub-type, colour, cross sectional shape, size and distribution of delustrant, surface features and inclusions. Comparison of vegetable fibres requires the use of features of identification listed in the section above for both the known and questioned fibres. The techniques used for comparison are outlined in Figure 1-14 [62].

[3] A textile reference sample is one that has the same classification and sub-classification as the questioned/known fibre identified during the examination process. This sample comes from an authenticated source and is generally colourless.

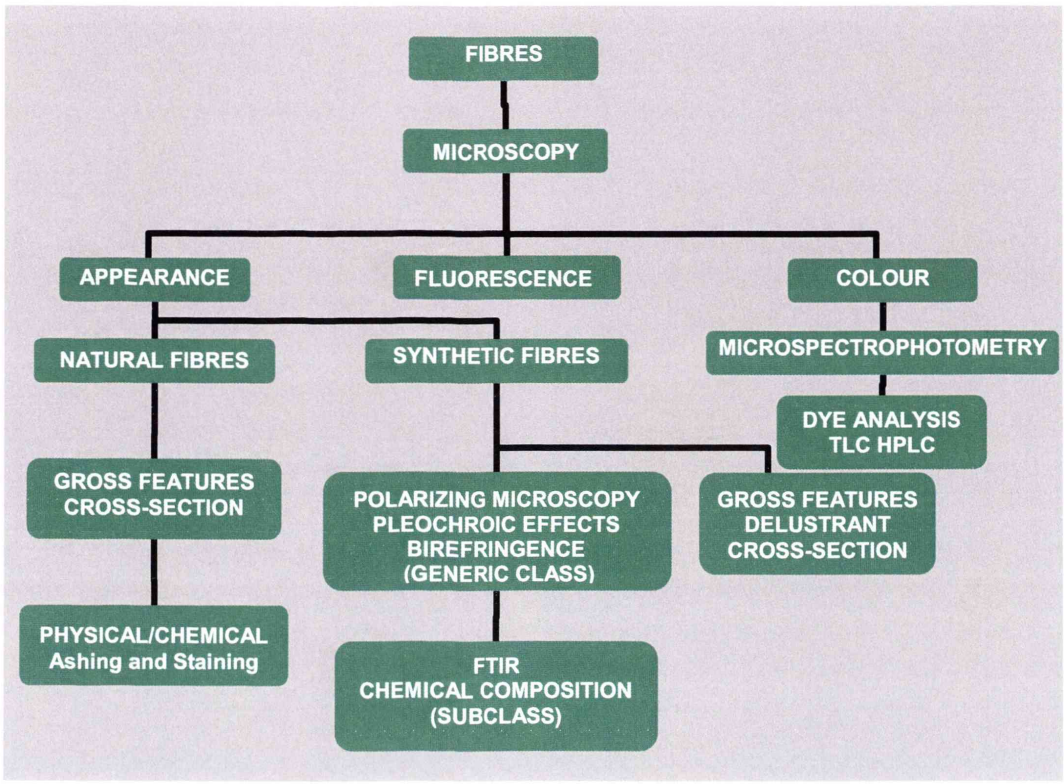


Figure 1-14 Flow chart showing scheme of fibre analysis/comparison [62]

The comparison of suspect fibres with fibres from the target item begins with the searching of the tape lifts using low-power stereomicroscopy. At this stage, only general colour and morphology is considered.

1.5.2.1 Morphological comparison

Morphological comparison is achieved with a comparison microscope which consists of two identical microscopes linked via an optical bridge. This set-up allows for the simultaneous viewing of two samples. This simple yet powerfully discriminating technique is able to detect the presence (or absence) of delustrant and is able to observe shape and morphological features as well as colour. With the addition of polarising filters birefringence of the samples can also be compared. Other light and filtering systems can be added to the instrument which allow for the comparison of fluorescent characteristics under different wavelengths. Care must be exercised when using this technique as small

variations in colour may occur between fibres from the same source (particularly with natural fibres).

1.5.2.2 Colour comparison

The colour of a sample is one of the most important factors for discriminating samples. The human eye (aided by a stereomicroscope) is capable of discerning subtle differences in colour. However, the colour of two fibres can appear identical yet have measurable differences. Some techniques for discriminating between similarly coloured fibres are:

Microspectrophotometry (MSP)

Microspectrophotometry (MSP) belongs to the wide range of spectroscopic methods. MSP is a special method within UV-vis spectroscopy. It allows the measurement of the absorption of electromagnetic radiation of many kinds of material in the visible region and (depending on the instrumental equipment) the ultraviolet region (UV) of the electromagnetic radiation spectrum.

Microspectrophotometry (MSP) has long been established as the method for objective fibre colour comparison and is used world-wide. Research into this technique started in the 1950's [51] and still continues today [20, 67] with a particular focus on the extra discrimination provided by the utilisation of the UV region.

Due to its popularity as an efficient and effective test, it is an integral part in the fibre examination sequence [57]. However, MSP is unable to provide any information pertaining to individual dyes used in the colouration of a fibre.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) [68, 69] is a rapid and inexpensive technique that is useful in discriminating between two fibres that have similar MSP spectra but contain dyes of different chemical structure. However, this technique is

partially destructive and suffers from a lack of sensitivity and difficulties with reproducibility.

1.5.2.3 Chemical composition comparison

Comparison of the chemical composition of the fibre substrate is especially important when analysing man-made fibres as surface morphology discrimination is limited. The principle method of comparison is IR analysis.

Fourier Transform Infrared Analysis (FTIR)

FTIR for comparisons is used in the same way as FTIR for identification (in the previous section). A small amount of the fibre is prepared on the slide. For comparison purposes the fibres must be aligned and mounted in the same manner to remove any instrument bias. This micro-technique provides class and sub-class information for man-made fibres. Analysis of plant fibres is limited as the base structure for all sub-classes is cellulose.

Chemical physical tests

Sometimes it is necessary to use destructive tests in order to provide more information on the fibres, for instance melting point analysis and solubility testing. When doing a comparison the reference sample is always analysed first and as little as possible of the question material is used. This technique is relatively unused today but can provide a quick discrimination between classes and some sub-classes.

1.6 Interpretation of Fibre Evidence

The fact that fibre evidence is class evidence poses a significant problem for fibre examiners. The interpretation of fibre evidence seeks to overcome this problem by showing that whilst fibres are not unique they still have evidentiary

value. Interpretation places the evidence in context and adds significance to the findings from the analysis stage.

The evidential value of fibres is dependant on many factors surrounding the crime. The factors considered in determining the evidential value of fibres are shown in Figure 1-15 [16].

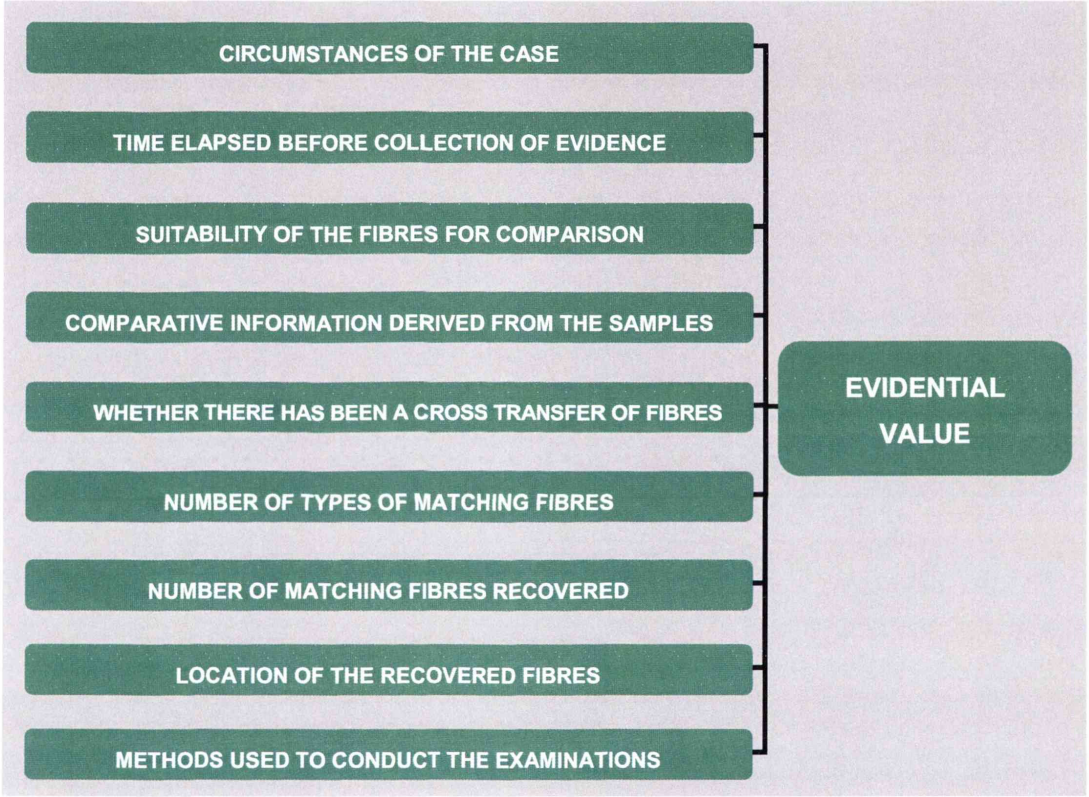


Figure 1-15 Factors affecting the evidential value of fibres [16]

The nine factors that affect the evidential value of the fibres shown above rely on case specific information (and whilst this information provides important information it is not always possible to evaluate all factors). Further information can be gathered about the fibre that can inform the investigator of the general evidential value of the fibre/s in question. This information is based on occurrence of the fibre/s in the general population. This information on fibre trends is vital when interpreting fibre evidence.

1.6.1 Fibre Frequency and Trends

Information gathered from population, transfer and persistence studies can be used to place fibre evidence in context. Fibres that are commonly occurring and have high persistence and transfer characteristics will have the lowest probity value as evidence and vice versa for fibres that are less common with low persistence and transfer characteristics. In order to determine the evidential value of the fibre evidence, it is important to understand the case circumstances (as summarised in Figure 1-15) as well as the frequency information pertaining to the fibre/s in question. The frequency information required has been summarised by Grieve as shown in Figure 1-16 [52].

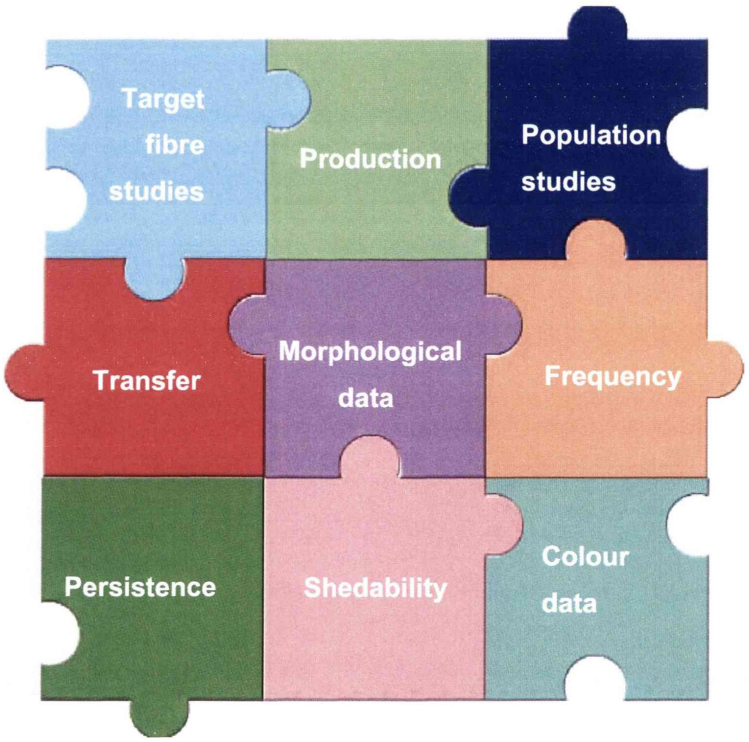


Figure 1-16 Data considered for interpretation of fibre evidence [52]

Data obtained from frequency information can help determine the probability of a random non-discrimination (chance of finding two non-related fibres with same morphological, colour and chemical characteristics).

Different studies have been developed to help measure evidentiary value; the major areas of transfer, persistence, and frequency are explored below.

1.6.1.1 Transfer and persistence

Transfer and persistence studies seek to determine the ability of a fibre to transfer and persist on a substrate/s. The ability of a fibre to be transferred from a textile in contact with another textile was first established by Pounds and Smalldon in 1975 [54], who then went on to study the persistence of these fibres after the initial contact [55]. Other studies have examined the transfer of fibres to material substrates such as cinema seats [70], the transfer and the persistence of fibres on head hair [23, 71, and 72], transfer of fibres to car seats [73-76], and persistence of fibres under open air conditions [77]. Transfer and persistence studies have consistently shown that natural fibres shed significantly more readily and persist on substrates longer than man-made fibres. Grieve [22] found that of 1760 fibres found on outdoor surfaces 1328 of them were cotton (~ 75 %).

Transfer studies do not only deal with one-way transfer but can also investigate two-way transfer. One recent study conducted by Merciani et al. [78] looked at the cross transfer of fibres with a focus on the dependencies that could exist between the number of fibres transferred in one direction and the number of fibres transferred in the other direction.

1.6.1.2

1.6.1.3 Frequency

Frequency studies seek to determine how common a fibre is. The assessment of this can be through the use of databases, population studies, target fibres studies or a combination of all three.

- **Databases**: usually consist of fibres used in clothing garments. Table 1-6 is a comparison of the findings from three textile databases. Home and Dudley [79] developed a database of garments in England compiled from items submitted to the Metropolitan Police Forensic Science Laboratory for two

seasons - November/December and June/July. Biermann and Grieve [80] developed a database of garments from mail order catalogues in Germany for a Spring/Summer season and an Autumn/Winter season. Langdon [25] developed a database based on garments submitted for analysis at Australian forensic laboratories as well as from a two week survey of clothing worn by students (from September and March). The databases are very similar for the most common fibre types (cotton and polyester) and colours (white, black and blue). The greatest variation is seen in the less common fibre types and colours.

Table 1-6 Comparison of findings from three textile databases

	Home and Dudley [79]	**Biermann and Grieve [81]**	**Langdon [25]**
Fibre Type (in order of frequency)	Cotton Polyester Polyamide Viscose Acrylic Wool	Cotton Polyester Polyamide Viscose Acrylic Wool	Cotton Polyester Rayon/viscose Wool Elastane Acrylic
Fibre Colour (in order of frequency)	White Black Red	Black/grey White Blue	Blue Black White Green Red Purple

- **Population studies**: these studies look at the fibre type and/or colour population on a specified surface. In a population study different surfaces are samples and the fibres collected are classified in to generic type and colour. Table 1-7 is a comparison of the findings from three population studies. Roux and Margot [24] conducted a population study in Switzerland during February and March. This study looked at car seats as the surface. Grieve and Biermann [22] looked at the population of coloured fibres on outdoor surfaces in Germany. Cantrell et al. [70] looked at the fibre population on cinema seats in Sydney, Australia. These studies found cotton to be the most common fibre type, as was found in the three databases outlined above. The significant difference between

the population studies and the databases is the population of man-made fibres. Man-made fibres were much less frequent in the population studies. This has been attributed to the fact that man-made fibres shed less readily than natural fibres.

Table 1-7 Comparison of the findings from three different population studies

	Roux and Margot [24]	Grieve and Biermann [22]	Cantrell et al. [70]
Fibre Type (in order of frequency)	Cotton Wool Animal Viscose Polyacrylonitrile Polyester	Cotton Polyester Polyamide Viscose Acrylic Wool	Cotton Polyester Rayon/viscose Wool Elastane Acrylic
Fibre Colour (in order of frequency)	Black/grey Blue Colourless Red Purple Brown	Colourless Blue (denim) Black/grey Red	Black/grey Blue

Target fibre studies: as the name suggests these studies assess the population of a specific fibre type (and colour) in the general population [82, 83]. This is achieved by selecting a target fibre and then sampling different surfaces to search for matches that occur by chance. Target fibre studies have found that it is rare to find two unrelated items that have foreign fibres that are analytically indistinguishable.

1.6.2 Bayesian Analysis/Inference

The interpretation of evidence approach outlined above provides the same weighting to the fibre evidence if fibres of the same type and colour are found in separate cases. The Bayesian approach utilises the same background information (fibre frequency, transfer and persistence etc.) whilst also incorporating probabilities of guilt or innocence by utilising information pertaining to the case [84].

Bayesian inference is a statistical inference in which probabilities are interpreted as degrees of belief. The methods of Bayesian inference are a formalisation of the scientific method involving collecting evidence that points towards or away from a given hypothesis. There can never be certainty (i.e probality of 1), but as evidence accumulates, the degree of belief in a hypothesis changes; with enough evidence *it* will often become very high (almost 1) or very low (near 0).

Bayesian inference is used as a logical basis for discriminating between conflicting hypotheses (generally the prosecutions hypothesis of guilt, H_0, and the defence hypothesis of not guilt, *not*H_0) by producing a "posterior odds". The posterior odds are calculated by multiplying the estimate of the degree of belief in a hypothesis **before** knowing the evidence (known as the "prior probability") with an adjustment of degree of belief (known as the "likelihood ratio") **after** knowing/incorporating the evidence. The calculation is shown in Equation 1.

Posterior odds = Likelihood ratio x Prior odds

$$\frac{\Pr(H_0 \mid E)}{\Pr(notH_0 \mid E)} = \frac{\Pr(E \mid H_0)}{\Pr(E \mid notH_0)} \times \frac{\Pr(H_0)}{\Pr(notH_0)}$$

Equation 1 Bayesian posterior odds equation

Posterior odds = odds on the hypothesis after the evidence, *E*.

Prior odds = probability of H_0 divided by the probablilty of *not*H_0 before evidence *E*.

Likelihood ratio = a number which is the ratio of two probabilities for the same assertion (e.g evidence, *E*) assuming alternate conditions, H_0 and *not*H_0.

In many cases, the impact of observations (e.g. evidence) is summarised as the likelihood ratio. It can then be presented in court as a statement of likelihood with wording such as "The evidence is LR (value determined from likelihood ratio, refer to equation 2) times more likely if H_0 is true than if *not*H_0 is true".

$$\mathrm{LR} = \frac{\Pr(E \mid H_0)}{\Pr(E \mid notH_0)}$$

Equation 2 Bayesian likelihood ratio

Pr(*E* | *H*$_0$) = probability that H_0 (usually the prosecution's hypothesis) is true given the evidence, *E*.

Pr(*E* | *not*H$_0$) = probability that *not*H_0 (usually the defence hypothesis) is true given the same evidence, *E*.

The Bayesian framework has been applied to different case scenarios involving fibre evidence [86, 87]. Some examiners argue that a Bayesian framework will remain theoretical as numerical data (from surveys etc) for various parameters is absent. Aitken and Taroni [88] argue that the Bayesian approach does not require the evidence to involve data as this approach considers "probabilities as measures of belief". In this way the provision of estimates is through objective probabilities (data) and subjective probabilities (based on examiners knowledge and experience).

Recently a large volume of publications have been devoted to the Bayesian approach [20, 84, 86, 87, 89-91]. However, due to its complexity, the Bayesian

approach has not been adopted by the majority of fibre experts [92, 93]. It is believed that Bayesian theory is helpful as an aid for systematically assessing evidence, even if numeric data for some parameters is absent [94].

1.7 Research and Fibre Examinations

In the 21st Century, the modern fibre examiner is faced with several problems - the greatest being the pressure of time and money. The rapid advance in DNA technology has seen fibre evidence undervalued due to the fact that it is time consuming, costly and (when competing with DNA) perceived to be less discriminatory [95-97]. However, DNA is not present in all cases, and when present, may require other evidence for the interpretation of the circumstances [98]. In order to realise the potential of fibre evidence is through research that will decrease the problem of time and money and increase the evidentiary value of fibres.

The preeminent objective of forensic work is to demonstrate high evidentiary value in as a highly objective manner as possible. DNA research has substantiated a high degree of evidential value because of the high statistical probability that when found at a crime scene, the DNA is from a specific person, i.e., individualistic. This is ideal in demonstrating that the person (subject) on trial is guilty or innocent beyond reasonable doubt. Improved evidentiary value can be demonstrated through an analytical method that is strong in discriminating material from a high number of other materials, such as, fibers containing dyes with the same color. Should the dye be one of many used, the higher the statistical (evidentiary) value is.

One way of increasing the evidentiary value of fibres is to determine how common the fibre is (as outlined above) [92]. Another way is through the research of new (or improved) analytical techniques that may provide greater discrimination of fibres [92]. New and/or improved techniques have the added

advantage of reducing analysis time (generally). Some techniques that are being researched are:

Electrospray Ionisation: Tuinman et al. [99] analysed acidic dyes used in Nylon fibres by applying electrospray ionisation mass spectrometry (ESI-MS) and tandem mass spectrometry (ESI-MS/MS). The combination of these two techniques was shown to provide both qualitative and quantitative information about the dyes present. Huang et al. [100] also used electrospray ionisation mass spectrometry to analyse dyes from a number of chemical classes including acid, direct and disperse dyes.

Pyrolysis Gas Chromatography (Py-GC): Flax fibre qualities were compared by Morrison and Archibald [101] and it was found that this technique had potential for identifying fibre additives.

Chemical Imaging: Chemical imaging is not an advance in technique, but rather a series of advances in instrumentation that allows the combination of several techniques into one. It combines optical microscopy, digital imaging and spectroscopy (visible, fluorescence, IR and Raman) for the morphological and spectral characteristics of samples [102]. Recently, Payne et al. [103] used chemical imaging for colourmetric and fluorescence microscopic analysis (400-720nm) of a range of fibres. Comparison with some conventional microspectrophotometers showed that, in some instances, chemical imaging performed better. Chemical imaging has distinct advantages over multi instrumental analysis in that there is limited (or no) sample preparation, is non-destructive, allows for visual presentation of samples, and samples can be analysed *in-situ*.

1.7.1 Research into *in-situ* Dye Analysis

In the past three years there has been a considerable focus on the analysis of dyes *in-situ*. This is not only seen as a way of decreasing analysis time, but, also

removes the problems associated with dye extraction and thin layer chromatographic analysis.

UV-Visible Microspectrophotometry: Advances in instrumental technology has meant that analysis has been possible in the UV region (240-400nm) for microspectrophotometry. Grieve et al. [20] analysed 225 samples of black cotton by UV-visible microspectrophotometry. The authors were generally able to group spectra based on dye type. The discriminating power of the technique varied from 0.13 for sulphur dyes to 0.93 for reactive dyes. This study highlighted the fact that spectral information below 400nm (UV) is important and increases the discriminatory power of MSP. The importance of the UV region was again highlighted by the same authors in their study of orange and green cotton fibres [104]. Suzuki et al. [105] also used the UV region to successfully discriminate structurally similar indigo derivatives.

Raman Spectroscopy: Research investigating the use of Raman spectroscopy for fibre examinations has shown that this technique has potential for fibre examinations [106-111]. This technique and the research currently being conducted is investigated in the next chapter (Chapter 2: Raman spectroscopy).

Chapter 2: Raman Spectroscopy

2.0 Summary

In 1928, Sir CV Raman discovered that when radiation was passed through a transparent medium, a small fraction of the scattered radiation differed in wavelength from that of the incident beam [112]. Furthermore, the shifts in wavelength were dependent on the chemical structure of the molecules [113]. This became known as Raman scattering and was due to the incident photons of light interacting with the molecule and changing the distance between the nuclei. Raman spectroscopy is the technique based on the detection of these vibrational and rotational changes of the molecule.

Raman spectroscopy of today is vastly different than that of its infancy, mostly due to technological advances that have made analysis quick and discriminatory. This chapter looks at the theory of Raman spectroscopy and the developments that have occurred since its characterization in 1928. There is also an in-depth look at the research that has been carried out in the field of fibres and dyes/pigments. This research demonstrates the potential of this technique for the forensic examination of fibres.

2.1 Theory

Raman spectra are obtained by using a powerful laser source (UV, visible or infrared monochromatic radiation) to irradiate a sample (refer to Figure 2-1). Once the monochromatic light impinges on the sample it interacts either elastically or in-elastically and the resulting light is scattered. The spectrum of

the scattered radiation is measured using a suitable spectrometer. The scattered radiation is of three types, **Stokes**, **anti-Stokes**, and **Rayleigh**.

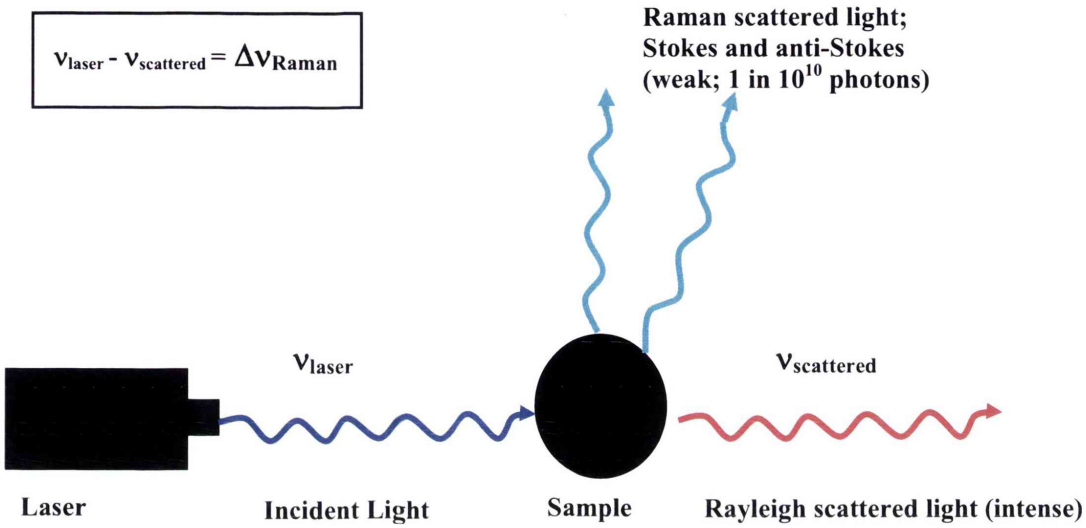


Figure 2-1 Schematic of the Raman Effect showing Raman and Rayleigh scattering (adapted from [114])

The quantum theory of Raman scattering treats radiation (of frequency ν) as a stream of photons (particles) that have energy $h\nu$ (h is Planck's constant). If the photons collide with molecules, and the collision is perfectly elastic, they will be unchanged in the deflection. Placing a detector at right angles to the incident beam will record photons of energy $h\nu$, known as **Rayleigh scattering** (which is equal to radiation of frequency ν) [115].

$$E = h\nu$$

Equation 3 Rayleigh scattering

An inelastic collision between the photons and molecules, results in the molecules either gaining or losing amounts of energy. This change in energy is

in accordance with the quantal laws, where the energy change (ΔE) is the difference between two of its allowed energy states. If the molecule gains energy, ΔE, the photon is scattered with energy $h\nu - \Delta E$ and the radiation will have frequency $\nu - \Delta E/h$ (known as **Stokes radiation**). If the molecule actually loses energy, the scattered frequency will be $\nu + \Delta E/h$ (known as **anti-Stokes radiation**) [115].

$$E = h\nu - \Delta E$$

Equation 4 Stokes radiation

$$E = h\nu + \Delta E$$

Equation 5 Anti-Stokes radiation

The energy changes that produce Stokes and anti-Stokes emission are depicted on the right of Figure 2-2. These emissions differ from the Rayleigh radiation by frequencies that correspond to $\pm \Delta E$ ($+\Delta E$ for anti-Stokes and $-\Delta E$ for Stokes). If the bond were infrared active it would have energy of ΔE also. Therefore, the infrared peak frequency and the Raman frequency shift are identical [113].

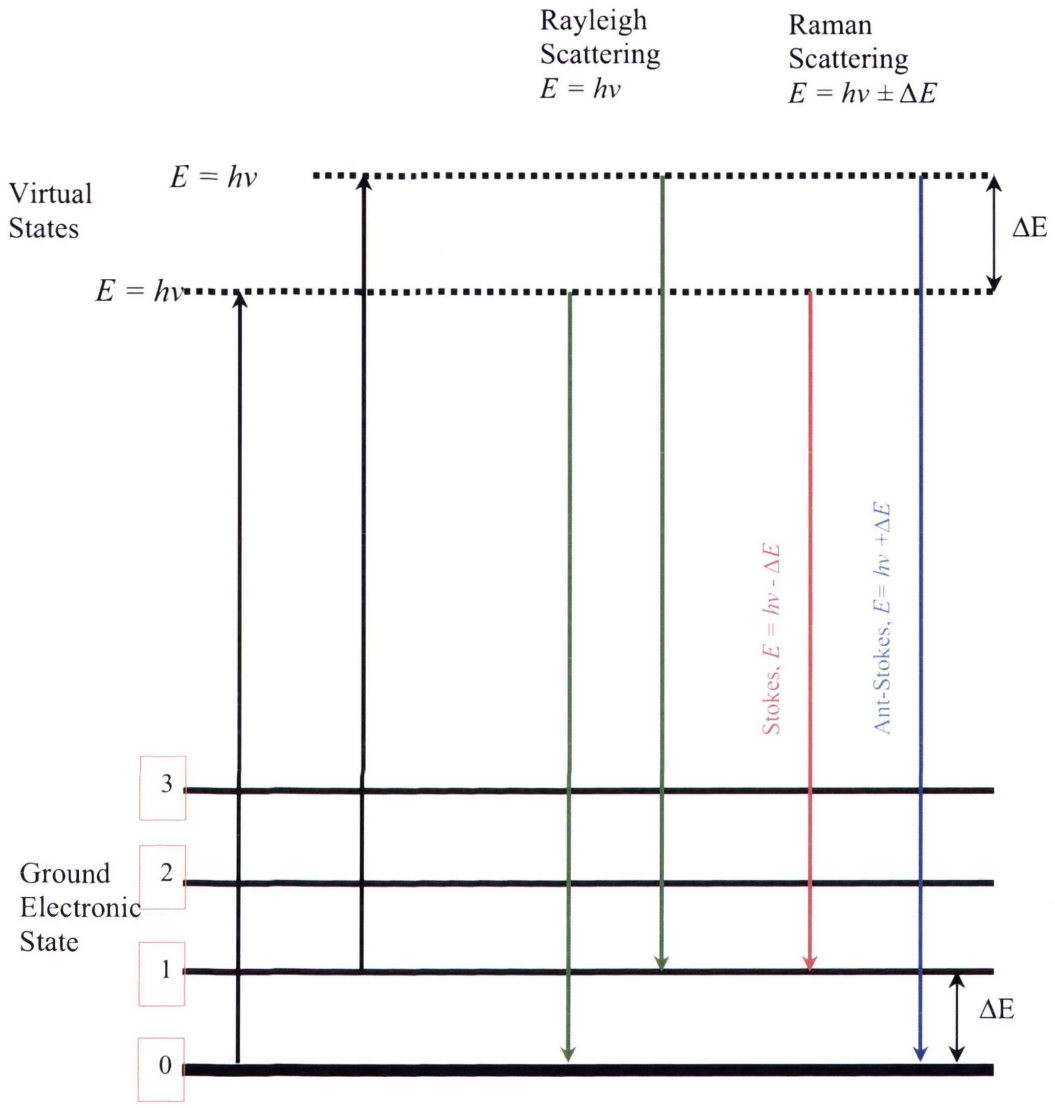


Figure 2-2 Origin of Rayleigh and Raman scattering, adapted from [113]

Figure 2-3 is an extract of a Raman spectrum [113] it shows that the Rayleigh scattering is at the same wavelength of the excitation source (argon ion laser, 480.0nm). Generally, anti-Stokes lines are less intense than the corresponding Stokes lines. For this reason the Stokes region of the spectrum is used (the negative sign is reversed and the Raman shift is expressed in cm^{-1}).

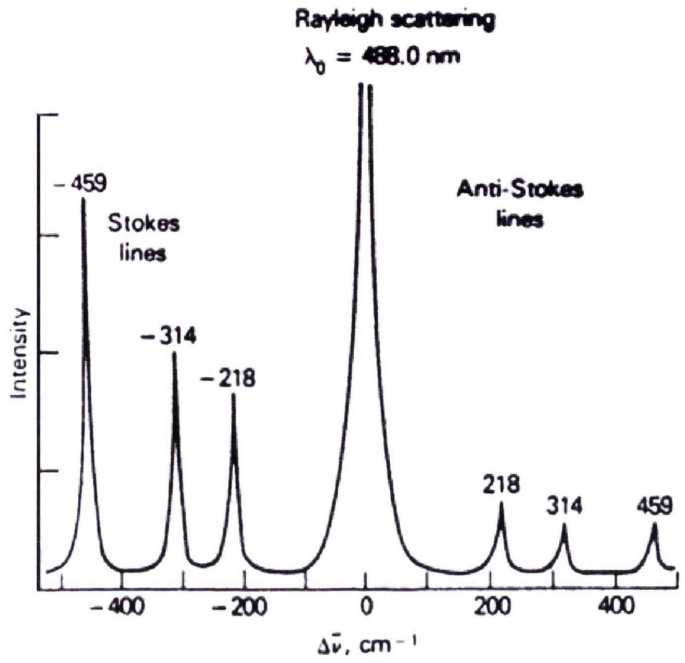


Figure 2-3 Raman spectrum for CCl_4, from [113]

Raman intensities are usually directly proportional to the concentration of the species and whilst Stokes' radiation is generally more intense than anti-Stokes' radiation, the total radiation scattered is extremely small at any other than the incident frequency. Thus, sensitive apparatus is needed for its analysis. The search for such instrumentation has underpinned a large proportion of the research undertaken in Raman spectroscopy.

2.2 History and Developments

The scattering of monochromatic radiation with change of frequency was predicted theoretically in 1923 by the Austrian quantum physicist A Smekal [116]. Inelastic scattering was first reported in Calcutta by Raman and co-worker Krishnan [112], and almost simultaneously by Landsberg and Mandelstam [117] in Moscow, both in 1928.

Some historical details of the developments in Raman spectroscopy both from the theoretical and instrumental point of view are summarized in Figure 2-4. The development of Raman spectroscopy depended largely on the availability of suitable tools, and significant advances have followed the invention of new or improved technology (technological advances are depicted on the left of the timeline and Raman spectroscopy developments are depicted on the right).

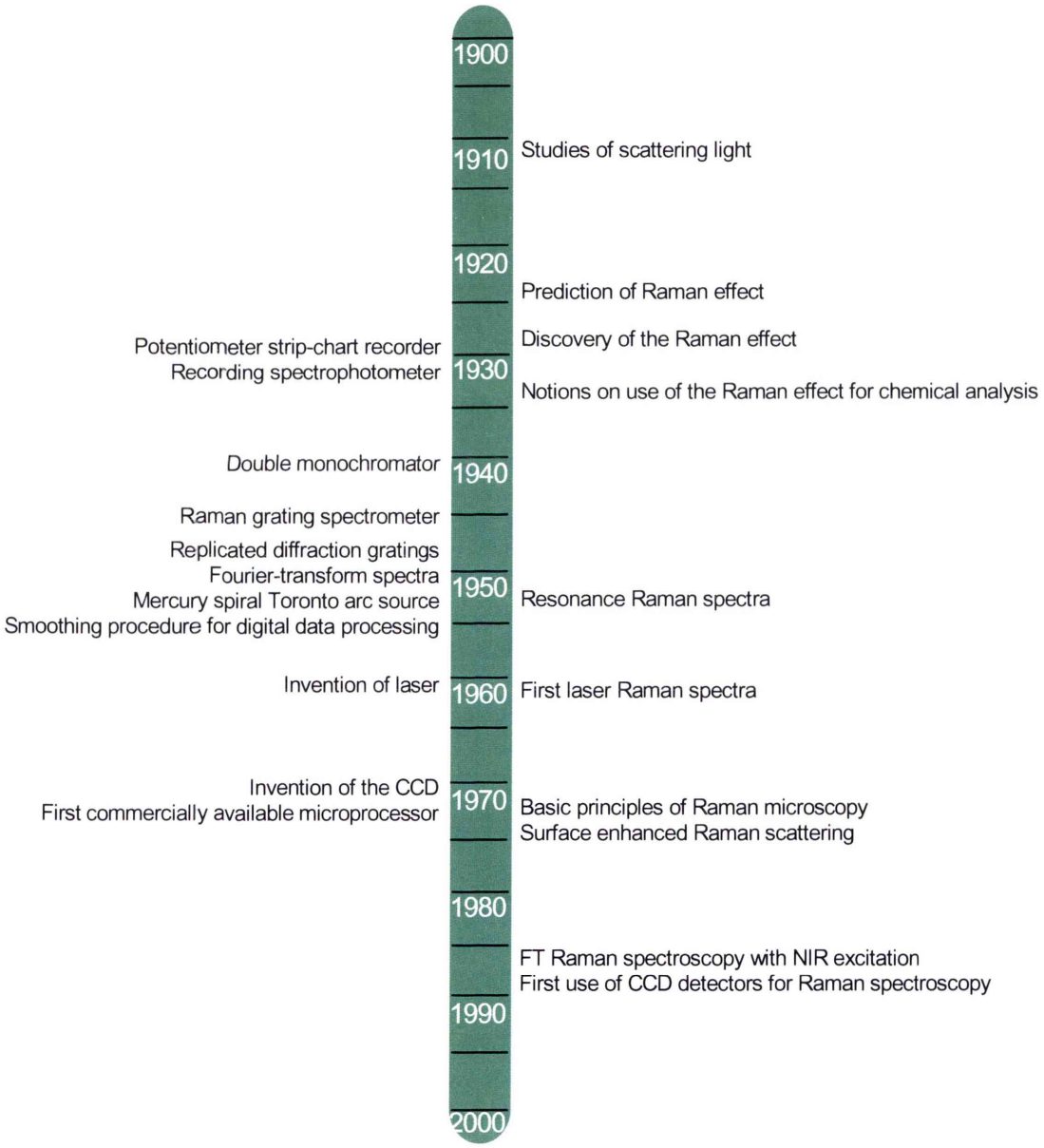


Figure 2-4 Timeline of advances in Raman instrumentation and theory [118, 119]

In the early days of Raman spectroscopy spectra could be obtained with simpler apparatus than those required for infrared measurements. As a result, at the end of the 1930s scientists working on infrared analysis often had to resort to Raman data collections for reference material. This was due to the fact that Raman spectra were more extensive and better catalogued than the corresponding infrared data [119].

Early experimental work looked at improving the radiation sources used for analysis. The mercury lamp, filtered to give essentially monochromatic radiation from one of the prominent mercury lines, became the standard source during the 1930s. This was superseded by the mercury Toronto arc lamp after its introduction in 1952 [120]. However, the biggest advance in the quality of Raman spectra came with invention of the laser in 1960 [121], and was soon after applied as a monochromatic source for Raman analysis. In 1962 Porto and Wood [122] reported the use of a pulsed ruby laser for exciting Raman spectra. The advantages of the laser included its capabilities for focusing onto a very small sample, thus enabling excellent spectra to be obtained routinely from materials in short supply. Also, the laser overcame some problems with stray light, thereby improving the detection power and allowing the study of low frequency vibrations. The capabilities of the laser permitted, in certain circumstances, the avoidance of the problems of fluorescence and absorption encountered with many samples. Following the demonstration of FT Raman spectroscopy in 1986 [123], the use of Nd:YAG lasers operating at 1064nm were used to decrease the fluorescence level.

Initially analysis by Raman spectroscopy encountered problems with high levels of elastic scattering (i.e. Rayleigh scattering). This was overcome by fitting the monochromator with two or three dispersion stages. These systems were able to reduce the level of Rayleigh scatter by 10 or more orders of magnitude at Raman shifts of only a few cm-1. However, as a result of this advance there was an increase in the size and price of the instrument and a decrease in the throughput of the optical system. The search for alternatives resulted in the development of

high efficiency holographic notch filters for rejection of Rayleigh light, which made successive dispersion stages unnecessary. These filters were efficient in the elimination of Rayleigh scattering (Figure 2-3), which greatly reduced the expense of the system and increased its efficiency.

The resurgence of Raman spectroscopy in its modern form has primarily resulted from the development of Fourier Transform Raman spectroscopy. This multiplex technique enabled analysis in the far infrared (thereby removing fluorescence interference), whilst maintaining recordable Raman intensities. The first practical demonstration of FT Raman was by Chantry and Gebbie in 1964 [124, 125]. Their experiment was only of academic interest since little of the technology needed to produce a viable commercial instrument did not exist at that time.

The 1960's and early 1970's saw a rapid and significant increase in the performance of almost every component of conventional Raman spectrometers. In 1986, near infrared (NIR) excitation and a commercial interferometer-based FT-IR spectrometer were combined to record a Raman spectrum.

Another major advance has been the use of charge coupled device (CCD) as a detector. The CCD was invented in 1970 by Boyle and Smith [126] however, its use as a detector for Raman spectroscopy was first reported in 1987 [127]. The charge-coupled device detector (CCD) enhanced performance capability and was able to be used as a two-dimensional array providing both spectral and spatial information simultaneously. This type of detection was a vast improvement over the photomultiplier and intensified photodiode-array technology which had been used previously [128].

The basic principles of Raman micro-spectrometry were developed in the early 1970's [129]. Coupling a Raman spectrometer with an optical microscope greatly increased the types of samples that could be analysed [129]. The advantage of Raman micro-spectrometry was that it allowed for spatial resolution of ~1μm. As a consequence, any component larger than 1μm in a sample could

be illuminated by the micro-Raman set-up and its characteristic Raman spectrum can be recorded free of interference from the surrounding media. This greatly enhanced the capabilities of Raman spectroscopy especially for small samples and samples containing micro-inclusions.

Modern instruments are capable of being bench mounted. An external view of the Renishaw 2000 model is shown in Figure 2-5 and a schematic of the model and its various components is given in Figure 2-5.

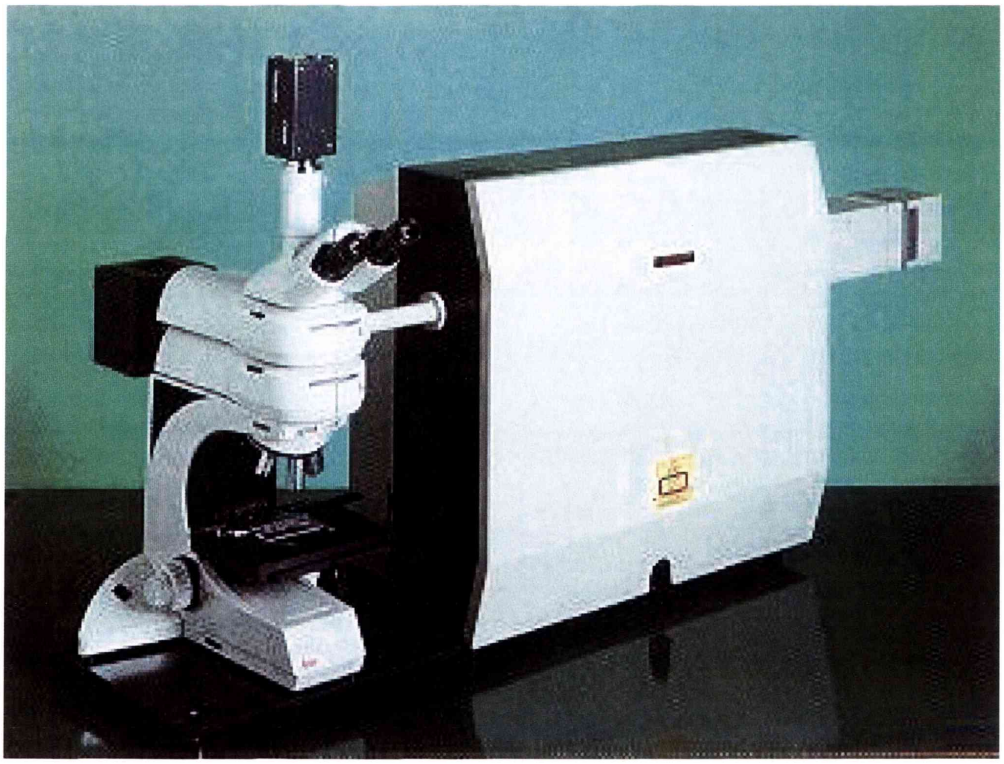
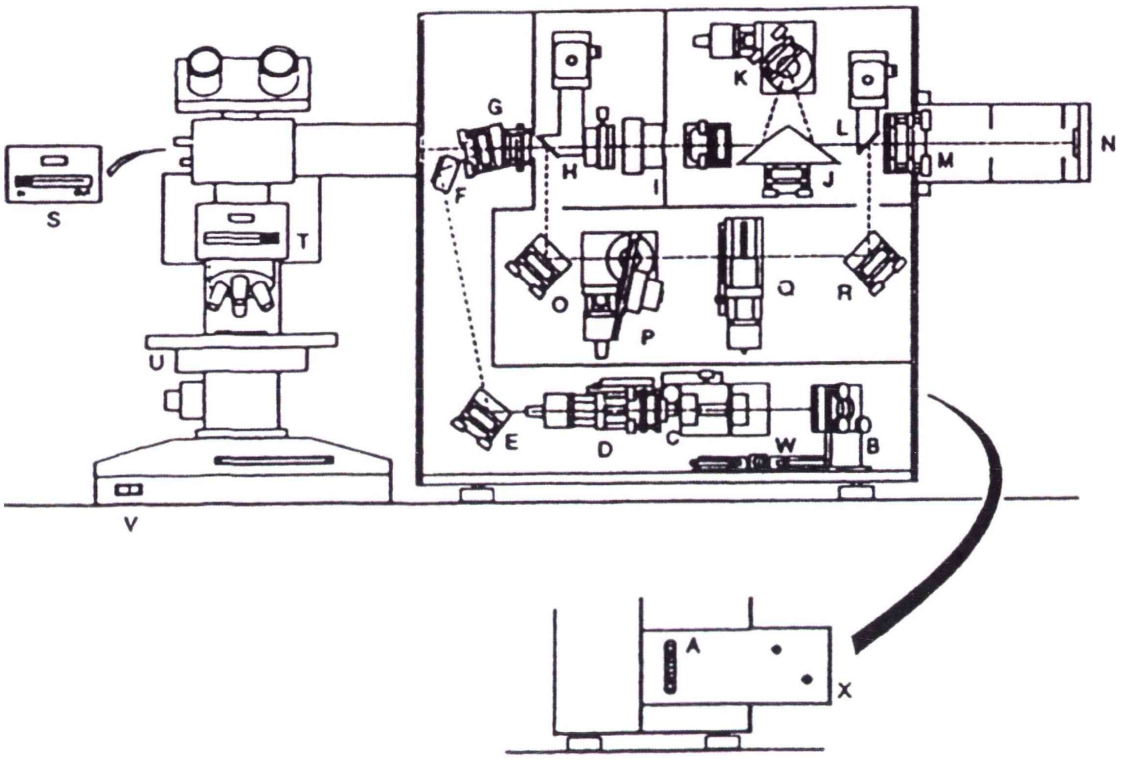


Figure 2-5 The 'Renishaw' Raman Spectroscopy System 2000, external view [130]



KEY

A Laser attenuation filter wheel
B Laser alignment mirror
C Objective lens and 10μm pinhole
D Spot focus adjustment lens (x 4 objectives)
E Adjustable mirror
F Fixed mirror
G Dichromic beam splitter (holographic notch filter) and polariser
H Wedge mirror
I Spatial Mirror
J Isosceles triangle mirror
K Grating
L Wedge mirror
M Focusing lens
N CCD Detector
O Adjustable mirror
P Filter disc and drive motors
Q Fabry-Perot etalon filter
R Adjustable mirror
S Laser light mirror control
T White light 50:50 beam splitter control
U Mechanical stage
V On/off switch for white light
W Interlock switches and defeat key-switch
X Delivery optics tube containing alignment mirror

Figure 2-6 Schematic of Renishaw 2000 System [128]

2.3 Raman Signal Enhancement

Several sub-types of Raman spectroscopy have been developed to enhance the weak Raman signal. These include: Resonance Raman Scattering (RR), Surface Enhanced Raman Scattering (SERS), and Surface Enhanced Resonance Raman Scattering (SERRS).

2.3.1 Resonance Raman Scattering (RR)

Resonance Raman scattering (RR) occurs when the photon of energy (of the exciting laser beam) matches that of an electronic transition of a chromophoric group within the system under study. Under these conditions bands belonging to the chromophore are *selectively* enhanced by factors of 10^3 to 10^5. The first resonance Raman spectra was reported in 1951 by Khalilov et al. [131] in their study of the variation of the intensity of Raman lines with the frequency of excitation.

2.3.2 Surface Enhanced Raman Scattering (SERS)

This development arose from a phenomenon first reported by Fleishman [132] who was using Raman spectroscopy to investigate the adsorption of pyridine on silver electrode surfaces. It was established that a roughened silver surface gave a Raman signal enhancement of about 10^6 [133].

2.3.3 Surface Enhance Resonance Raman Scattering (SERRS)

The SERRS effect is considered to be a combination of the RR and SERS effects and was first reported in 1983 [134]. When a laser excitation wavelength

matches the absorbance maxima of a chromophore (as is possible with many dyes) and if the dyes remain in close proximity to a metal surface, the intensities of the signals generated can be up to ten orders of magnitude greater than that of conventional Raman spectroscopy. Since the Raman bands of a chromophore are selectively enhance when in resonance, SERRS gives less complex spectra than SERS, but they are still highly characteristic of a particular molecule.

2.4 Raman Spectroscopy Research

Raman spectroscopy has been an integral analytical tool in many industrial laboratories [128]. Whilst Raman is becoming more popular in industry, it does not have the well established reputation as that of Fourier-transform infrared spectrometry (FTIR) or microspectrophotometry (MSP) in the field of forensic science. However, several forensic specialties have utilised Raman spectroscopy for research and novel purposes.

2.4.1 Raman and Forensic Samples

Applications of Raman spectroscopy in forensic analysis [135] have included drug identification [136], paint sample analysis [137], identification of hazardous materials [138], analysis of lipsticks [139] and pigments in paintings and illuminations [129].

Archaeometry and conservation researchers have found Raman spectroscopy to be the best technique for analysing pigments and paints in samples. The technique is non-destructive and analysis can be *in-situ*. This has proved to be particularly advantageous in the analysis of historic and fragile works.

The research that has been carried out on the Raman analysis of dyes and pigments provides critical background information for the experiments carried

out in this study. For this reason the key papers are reviewed and presented in the next section.

2.4.2 Raman and Dyes/Pigments

Raman microscopy is now recognized to be the analytical procedure which is the most specific and sensitive for *in situ* analysis of pigments on historical artefacts [140]. The technique has been applied to the field of archaeometric analysis principally by Clark et al. [141-151] and also by Coupry et al. [135, 152, 153], and Gardiner et al. [154, 155]

Clark & Gibbs [148, 150] demonstrated that Raman spectroscopy analysis may be applied to historical artefacts suffering conservation problems. Andreev et al. [156] analysed plants, historic textiles and the lakes used for dyeing, with FT-Raman (Nd^{3+}: YAG laser excitation at 1064nm). The technique was found to be discriminatory and non-destructive. Analysis of the textiles and lakes with FT-Raman showed that it was possible to distinguish the main dye component by using Raman bands.

A Raman spectroscopic library of natural and synthetic pigments was created by Bell et al. [157] as there was an increasing amount of Raman studies into archaeometry. Sixty pigments, known to be used before ~1850 AD, were analysed by Raman spectroscopy. Fifty-six of the pigments yielded high quality spectra using 514.5nm and 632.8nm lasers. This library was expanded on by Burgio et al. [158] who included binders and minerals and also increased the range of laser wavelengths used for analysis (780.0nm, 647.1nm, 632.8nm and/or 514.5nm). Burgio et al. [159] also utilised Raman spectroscopy to identify the pigments used in 3 separate manuscripts. Sampling of the pigments from manuscripts is generally forbidden. Therefore, the non-destructive aspect of Raman spectroscopy was a definite advantage.

Coupry et al. [153] used laser excitation at 514.5nm to analyse natural and synthetic indigo samples by Raman microspectroscopy. The authors found that analysis of dyes fixed on threads was simple and required no chemical pre-treatment. Barnes et al. [160] also used Raman spectroscopy for the analysis of dyes. The authors looked at two closely related dyes, Orange II and Para Red, and were able to elucidate the species present in basic and neutral solutions.

2.4.3 Raman and Fibres

Edwards et al. [161] utilised FT-Raman for the analysis of untreated natural fibres (flax, ramie, jute, cotton, sisal and coconut). Edwards and Falk [162] looked at the yellow-brown coloration formed in the degradation process of linen fabrics by Raman spectroscopy.

Jahn et al. [163] studied flax fibres grown under well managed conditions and then submitted to NaOH chemical treatments, so called mercerization. The extent of the polymorphic transformation of cellulose I into cellulose II taking place within the crystalline domains of the fibre cellulose was dependent on the alkali concentration. FT Raman spectroscopy turned out to represent an ideal tool for detecting the polymorphic transformation of the cellulosic fine structure of the flax fibres in vivo.

Carter et al. [164] stated that Raman studies had not been previously undertaken as wool fluoresces strongly in the UV/Vis regions. The FT-Raman spectrum of wool was obtained using near-IR excitation (1064nm). No significant fluorescence was observed and the spectra could be obtained routinely. No sample damage was observed for laser powers up to 400mW.

Hendra et al. [165] and Maddams et al [166] produced high quality Fourier transform Raman spectra, free from fluorescence, for a series of commercial polyamides of the single number nylon type from nylon 3 to nylon 12. With this newly developed technique, it was possible to record spectra routinely and to

characterize each nylon spectrum thus identifying them for analytical purposes. The authors also found that sampling by Raman was easier than that for IR.

Kazarian et al. [167] found that FT-Raman spectroscopy provided insight into the subtle aspects of the crystalline structures in PET (poly ethylene tereph-2-thalate), and was also very useful for the detection of azo dyes impregnated into PET from supercritical fluid solution.

Bourgeois and Church [168] found that Raman spectroscopy overcame the problem of determining a small amount of additive in a vast excess of host material. They observed that FT-Raman was a powerful tool for direct analysis of low levels of dyestuffs in acrylic fibres.

Studies have been conducted into Raman spectroscopy for the analysis of dyed and colourless fibres (industrial and forensic context) [108, 110, 161, 165, 166, 168-171] and have shown that Raman spectroscopy has real potential as an analytical tool for fibre examinations. These studies form a significant part of the background information for the ensuing research and as such are discussed in detail in the following chapters

2.4.4 Raman Spectroscopy and the European Fibres Group (EFG)

The potential of Raman spectroscopy for forensic fibre examinations was recognised by the European Fibres Group in 2002 when it formed the Raman sub-group [49]. The group was tasked with investigating aspects of Raman spectroscopy as they related to fibre examinations.

Since its inception the Raman sub-group (headed up by Professor Massonnet of the University of Lausanne, Switzerland) have undertaken several studies including: a survey of laboratories and the use of Raman spectroscopy [50], a study on red wool and acrylic fibres [106], a study on green cotton [107] and

most recently a study on the detection limit of Raman spectroscopy for green cotton samples (as well as respective dye components) [172].

This research has significantly contributed to the changing perception of Raman spectroscopy and its incorporation into the fibre examination sequence. By far the most comprehensive studies carried out on the use of Raman spectroscopy for fibre analysis have been by the Raman sub-group ([106, 107, and 172]). Raman instruments from six different manufacturers were tested as well as nine different laser wavelengths ranging from blue (λ=458nm) to near infrared-NIR (λ=1064nm). These represent the largest comparison studies of Raman analytical parameters carried out on identical fibre samples.

The studies on Raman spectroscopy, detailed above, prove its usefulness as an analytical technique for fibre examinations (in both an industrial and forensic context). With its potential for fibre examinations clearly demonstrated this research seeks to explore Raman spectroscopy as another application for the forensic analysis of black/grey and blue reactively dyed cotton as well a clearly identifying the broad potential of Raman spectroscopy in fibre examinations. The direction and aims of this research are detailed in the next chapter.

Chapter 3: Direction and Aims

3.0 Summary

In the examination of fibre evidence from a crime scene and related areas and people, the aim is to use a sequence of examination techniques that provides the most discrimination. This is in order to state that the either (a) these fibres do not have a common origin, or (b) these fibres show no differences and could have a common origin. This can be used in substantiating (or discounting) prosecution and defence hypotheses about the events surrounding the crime.

The statement of likelihood provided by the expert witness is dependent on the weight of the fibre evidence. As seen in Chapter 1 factors affecting the weight of the evidence vary from information provided about the incident to how common the fibre is in random fibre populations. The discrimination provided by the examination sequence is another important factor (refer to Figure 1-15); as a sequence that provides greater discrimination will add more weight than one that provides less discrimination.

The aim of this research is to determine the discrimination of Raman spectroscopy when examining fibre evidence. The perception of Raman spectroscopy, in the forensic science field is changing. When this research was initially undertaken the perception was, that Raman spectroscopy would be a technique that would not add anything to that provided by techniques already in the fibre examination sequence. Laboratories were hesitant to invest in an expensive tool without verification of its capabilities. Over the past three years, research carried out primarily by the European Fibres Group – Raman sub-group has shown the potential of this technique for fibre examinations to be greater than that first believed.

This research seeks to further add information about the discrimination provided by Raman spectroscopy. Careful selection of samples (both substrate and colour) was required to address current areas of need for forensic examination of fibres. Hence, this research focused on a sample set comprised of black/grey and blue reactively dyed cotton fibres. Sample selection is discussed in section 3.1 below.

The advantages of Raman spectroscopy have already been demonstrated through previous studies. It is a non-destructive analytical technique requiring minimal sample preparation, allowing for *in-situ* analysis and providing molecular information about the sample. This research seeks to capitalise on these advantages whilst also investigating any limitations of the technique when determining the discrimination provided for the sample set.

The primary aim of this research was to investigate the suitability of Raman spectroscopy for the analysis of black/grey and blue reactively dyed cotton fibres. In achieving the primary aim, the research investigated four secondary objectives:

1. determine suitable conditions for analysing fibre and dye samples
2. compare the discriminatory power of Raman spectroscopy with that of Microspectrophotometry
3. investigate the use of chemometrics for the Raman spectral data collected from the sample set, and
4. construct a database of common reactive dyestuffs.

Each of these secondary aims as well as the premise behind sample selection is discussed further in the following sections of this chapter.

3.1 Sample Selection

The objective here was to select a sample set where Raman's ability to allow for structural elucidation might prove more discriminatory than current techniques. In considering this, the fibre substrate selected was cotton, the colours selected were black/grey and blue, and the dye type selected was reactive. The rationale behind these selections is explained below.

3.1.1 Fibre Type and Colour

Cotton fibres have been chosen for this study because they are widely available (accounting for 38% of world fibre production in 2002 [4]) and the extraction of certain dyes from single cotton fibres is problematic in forensic fibre comparison. In addition there has been an increased world-wide popularity of cotton textiles over synthetic materials for garment manufacture.

Blue and black/grey cotton are two of the most commonly encountered colour/class combinations in Australian [70] and overseas fibre population studies [24, 80]. In databases that have been compiled on garments (in Australia [25] and Overseas [24, 80]) cotton is the most common fibre type. In the database recently compiled by Langdon [25] black/grey and blue accounted for 62% of colour variations and cotton fibres accounted for 52.6% of samples (this database considered both pure and blended fibre types). Top five fibre/colour combinations in the Australian database are listed in Table 3-1.

Table 3-1 Top five fibre/colour combinations in the Australian Database [25]

Fibre type/colour	Number of instances
Cotton/Blue	134
Cotton/Black	103
Cotton/White	82
Polyester/Black	71
Polyester/Blue	53

Analysis of cotton fibres is based predominantly on their colour (comparison microscopy) as the cotton fibre itself is generic cellulose and cannot be further sub-classed using FTIR as is the case with man-made fibres. Black/grey and blue cotton are traditionally regarded as coloured samples that provide the least discrimination with the techniques available to the fibre examiner due to the commonality of these fibre types. In many cases MSP can discriminate the fibres based on minor variations in colour. The possibility of further discrimination through Raman spectroscopy based on the molecular structure of the individual dyes used would further dispel the contention that these fibres are of little to no evidential value.

3.1.2 Dye Type

Reactive dyes have been chosen for this study because they have traditionally been difficult to extract from cotton fibres requiring complex extractions or destruction of the sample [110] for TLC analysis. As stated above, cotton fibres are discriminated primarily on colour, either through MSP (spectrophotometric detection of variation in colour) or through TLC (extraction and separation of dye components). The latter is particularly problematic for reactively dyed fibres due to the destructive nature.

Furthermore reactive dyes are popular with manufacturers due to their excellent colourfastness and consequently represent a high proportion of dyed cotton fibres in the market. In 2004 reactive dye consumption accounted for 49% of dye usage for cellulosic fibres (refer to Table 3-2). This has risen from 37.5% in 1992 highlighting the growing trend of reactively dyed cotton.

Table 3-2 Estimated annual consumption of dyes for cellulosic fibres [173]

Dye Class	World Usage (1,000 tonnes)		
	1988[a]	1992	2004
reactive	60	109	178
direct	74	60	68
sulphur	90	70	70
vat	36	21	22
indigo	12	12	12
azoic	28	18	13
sum	**300**	**290**	**363**
relative share (%) for reactive dyes	**20**	**37.5**	**49**

[a]does not include China, India and Eastern Europe

The colourfastness of reactive dyes is attributed to the bonding between the fibre and dye. Reactive dyes can be mono- or bi-functional with each of the functional sites binding to the cotton fibre. Functional groups are often a heterocyclic aromatic ring substituted with chloride or fluoride. Another common reactive group is vinyl sulphone. Bifunctional dyes can be either homogenous (the two reactive sites are the same) or heterogeneous (the two reactive sites are different) with respect to the functional group.

Reactive dyes with more than one reactive group in the dye molecule have greater fixation properties. Reactive systems are often classified according to the number of reactive groups contained in the dye molecule, as either mono-, bi- or poly-functional reactive systems.

Figure 3-1 is a schematic of a heterogeneous, bi-functional dye with reactive (bonding) groups of sulphone and monofluorotriazine. This bonding makes reactive dyes difficult to extract for analysis by Thin Layer Chromatography

(TLC) and consequently results in partial or complete destruction of the fibre [110].

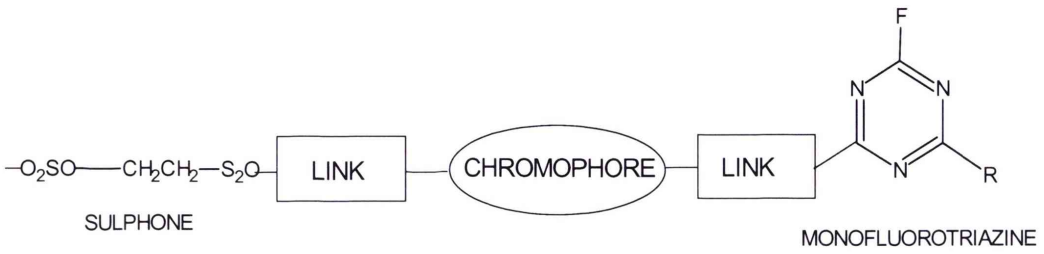


Figure 3-1 Schematic representation of the components of a typical bi-functional dye with reactive groups

Figure 3-2 shows the principle behind the binding of cotton to a triazyl reactive dye by a substitution reaction at the chlorine sites of the heterocyclic aromatic ring (vinyl sulphone reaction sites undergo addition reactions to bind with cotton).

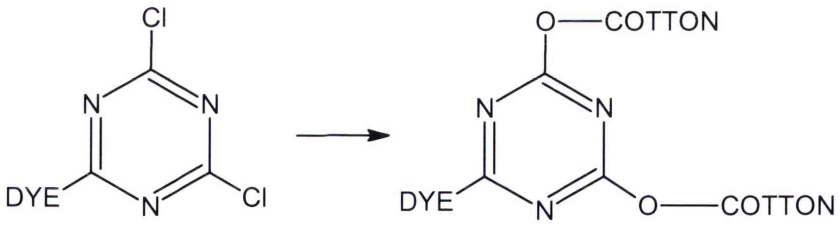


Figure 3-2 Principle of cotton dying with triazyl reactive dye

Once the samples were selected it was necessary to explore the secondary aims of this document.

3.2 Optimisation of Methodology

This section explores the variable parameters in Raman spectroscopy. The parameter that has the greatest impact on results is the laser wavelength used for excitation of the sample. Laser wavelength can vary from the UV (approximately 320nm) to the near-infrared (1064nm). Previous studies have shown that varying the laser wavelength can provide different information about the sample set.

So far, no single laser wavelength has proved to be optimal for every sample colour and substrate. It is this limitation which has had the greatest negative effect on the implementation of Raman spectroscopy in the forensic science field. Unlike Infrared spectroscopy, there are a large number of parameters that can vary. This has meant there has been limited usefulness of Raman spectroscopy for comparisons within and between laboratories. In order to overcome this limitation, a range of colours and substrates need to be investigated to determine optimal parameters for the specific fibre/colour combination. Therefore, it is necessary for this study to determine the optimal laser excitation/s for the black/grey blue reactively dyed cotton sample set.

Other variables to be investigated in this chapter include:

- laser strength
- mounting variations
- sample analysis time, and
- spectrometer resolution.

The aim of this chapter is to determine the optimal analysis conditions for the sample set. Once determined these conditions will be used in subsequent analyses.

3.3 Comparison of Raman Spectroscopy and Microspectrophotometry

The current fibre examination technique utilises MSP and TLC for discriminating between cotton fibre samples. As stated above TLC is problematic for the sample set chosen and as such is not used for comparison with Raman spectroscopy.

Microspectrophotometry (MSP) is the most utilised technique in fibre examinations. MSP is a quick technique that discriminates fibres based on the spectrophotometric colour of the sample. It is a well established technique in the fibre examination process. However, as MSP's discrimination is confined to the colour of a sample, theoretically MSP may show no discrimination between fibres dyed to the same shade even though the colour may have been achieved using dyes of different molecular structure. The fibre examination sequence is already a long and involved process, but if Raman can be shown to provide greater discrimination (on its own or within a sequence of techniques) then the value of its introduction to that sequence will be established.

This chapter aims to compare the discrimination of the sample set by Raman spectroscopy to that provided by microspectrophotometry. In addition the discrimination of the combined techniques will also be investigated.

3.4 Use of Chemometrics for Raman Spectral Data

Raman analysis produces a spectrum of the sample being investigated. The spectra can be discriminated visually where the differences are obvious (and consistent within the sample) and where the sample set is of a sufficiently small size (to allow for direct comparison of all spectra). In instances where the

spectra are not so easily discriminated or the sample set is of a large size (thereby introducing problems of comparison) visual comparison becomes difficult.

It is under such circumstances that chemometrics can be used as a valuable aid in discriminating the sample set. Chemometrics interprets data based on mathematical modelling which detects spectral variations between samples.

This chapter aims to use chemometrics for discriminating samples within the sample set. It will focus on samples that result in visually similar Raman spectra but which are known to have different dye components. If chemometrics is able to differentiate visually similar Raman spectra than this will further increase the discrimination provided by Raman spectroscopy for the sample set.

3.5 Library of Common Reactive Dyes

Libraries are commonly used as an aid for interpretation by fibre examiners. They allow fibre examiners to compare fibre evidence with known samples for identification purposes. If Raman spectroscopy is to be utilised by fibre examiners, a library of known Raman dye spectra will provide invaluable information to examiners for interpreting results of unknown sample.

Since Raman is an emission process, the appearance of a Raman spectrum is highly dependent on individual instrument characteristics such as line shape, excitation source, and detector response. Such dependencies have hindered the development of standard Raman spectral libraries. The lack of such spectral data bases, which have become so important for other spectroscopic techniques, has been problematic in validating Raman-based analytical methods.

The aim of this chapter is to construct a database of the dyes used in the course of this research and include some other common reactive dyestuffs. This library is shown at Appendix 1.

Chapter 4: Optimisation of Methodology

4.0 Summary

Raman spectroscopy was investigated to determine the optimal conditions for the analysis of the commonly encountered black/grey and blue cotton fibres dyed with reactive dyes. The major variable investigated was laser wavelength.

In this chapter a single blue cotton fibre, its three dye components and an undyed cotton fibre were analysed with 5 different laser wavelengths from two different Raman microprobe spectrometers. The quality of the spectra, fibre degradation and speed of acquisition were used to determine that, under the conditions used, the 785nm and 830nm lasers gave superior results. The 632.8nm laser wavelengths provided good results with little acquisition time and no spectral degradation. Results indicate that at least the major dye component could be identified using Raman spectroscopy.

4.1 Introduction

This chapter deals with the determination of optimal system parameters for mounting, resonance, minimisation of fluorescence and minimisation of spectral and sample degradation. It is important to determine these parameters for both the specific fibre substrate of cotton, and the black/grey and blue colours.

As discussed in Chapter 1, colour is one of the most distinguishing features of fibres and textiles due to its high variability. It is especially important in the analysis of natural fibres, such as cotton, as there is little to no discrimination within the generic classes based on morphology or chemistry. Colour is imparted to textiles (or fibres) through dyes and pigments which are, generally,

non-polar compounds. Raman spectroscopy is being investigated as it provides molecular information of non-polar compounds and therefore shows potential as an *in-situ* dye analysis technique that may provide greater discrimination of coloured natural fibres.

Raman has advantages over other analytical techniques, such as MSP and TLC, due to ease of sample preparation and analysis. It does however have some inherent disadvantages, the two greatest being:

- fluorescence, and
- degradation of the sample through thermal factors and light exposure (pyrolysis and photochemical reactions) [115].

For Raman spectroscopy to be an effective technique it is necessary to investigate methods of minimising the effects of fluorescence and degradation. Of these two problems fluorescence is the one that proves to be the most common [106].

4.1.1 Fluorescence

Impinging a sample with photons (from a laser) will cause excitation of some molecules from ground state (S_0) to an excited state (S'_1). The Raman effect results in the molecule either gaining energy or losing energy to the vibration and rotation of the molecule (ΔE_{Raman}) refer to Figure 4-1. Fluorescence, however, is a radiative decay process (from S'_1 to S_1) that occurs after excitation of the electronic molecular states [174] and also produces an energy change – thereby a change in emitted wavelength ($\Delta E_{Fluorescence}$). Figure 4-2 shows the decay process that result in fluorescence.

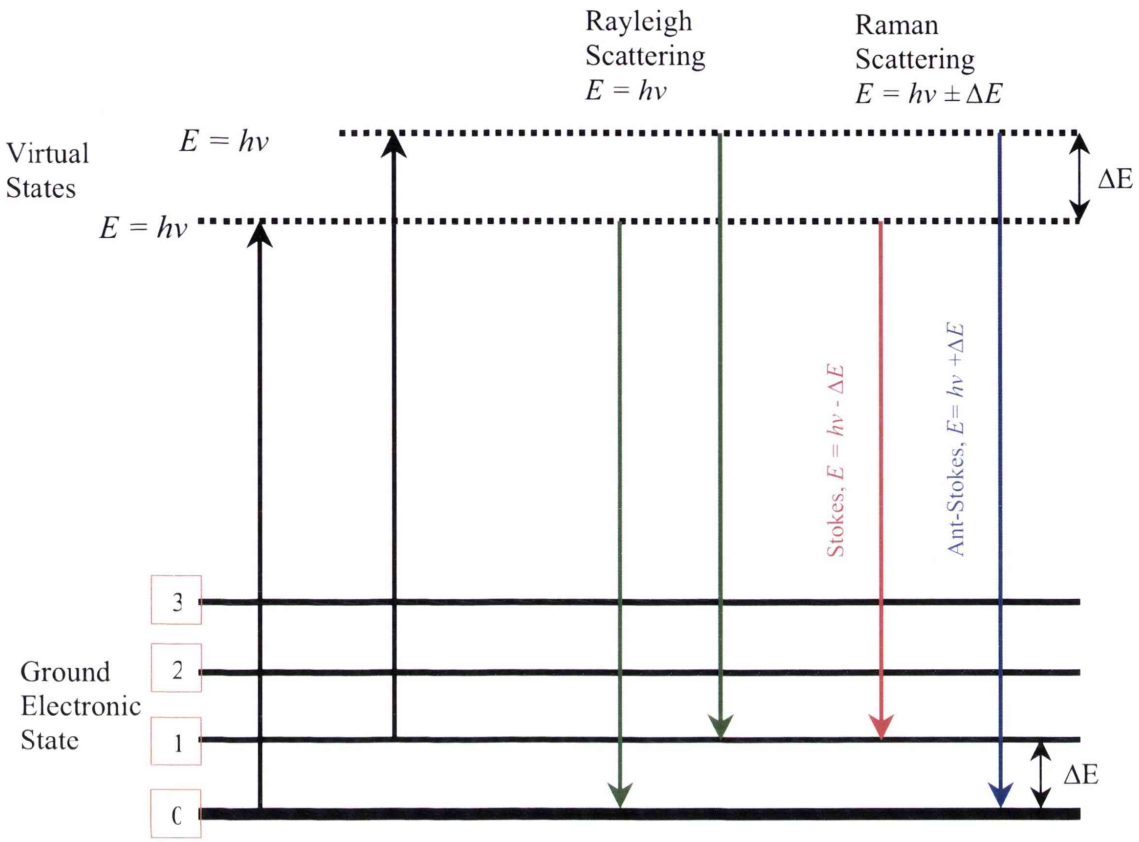


Figure 4-1 Origin of Rayleigh and Raman scattering, adapted from [113]

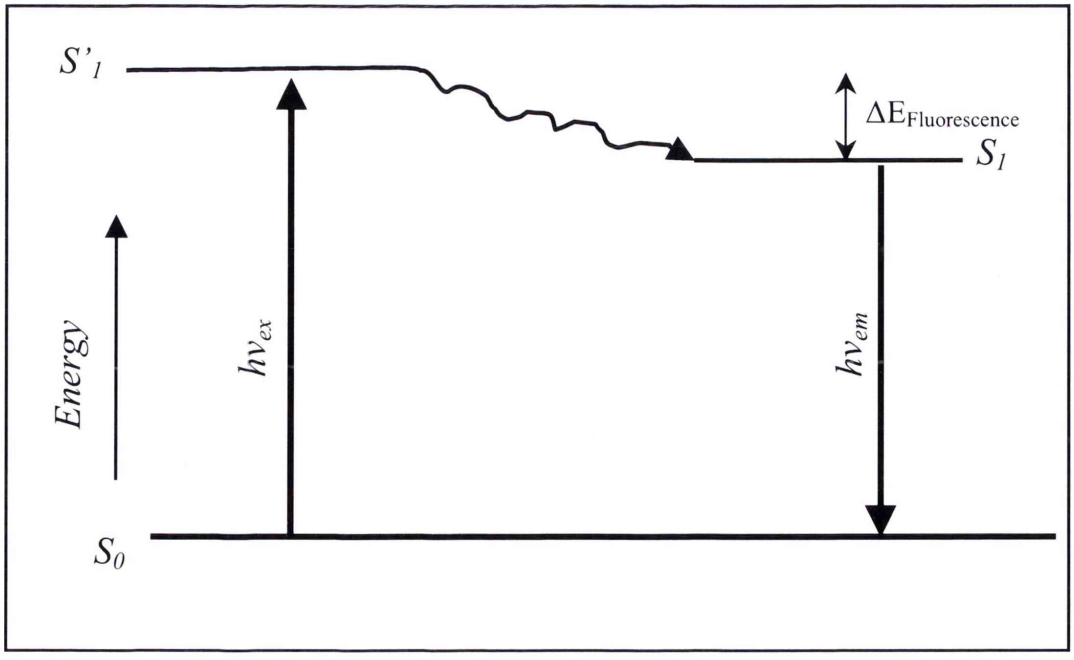


Figure 4-2 Simplified *Jablonski Diagram* showing fluorescence

In Raman spectroscopy the problem arises due to the fact that the spectrometer measures changes in photon energy (i.e. wavelength) after molecular excitation. If fluorescence occurs, the resultant energy change will be recorded along with the energy change as a result from the Raman Effect.

The energy change from the Raman Effect occurs in a small number of instances (1 in 10^6). If energy changes due to fluorescence outnumber those produced by the Raman Effect, in frequency and/or intensity, fluorescence will swamp any Raman signal. Fluorescence is an unpredictable phenomenon that does not occur in every instance and appears as a large featureless background that swamps Raman bands from the sample being analysed [175]. It can in some instances be reduced or removed altogether by;

- changing the laser wavelength [106],
- photobleaching [176], or
- the application of metal colloids e.g. Surface Enhanced Resonance Raman Spectroscopy - SERRS [170] and Surface Enhanced Raman Spectroscopy - SERS [175].

Photobleaching is a light-induced change in a chromophore, resulting in the loss of its absorption of light of a particular wavelength. [176] Application of metal colloids can enhance the Raman spectrum of the sample by sometimes as much as a factor of ten, thereby nullifying the effect of fluorescence [170, 175].

Changing the laser wavelength requires no sample preparation (unlike SERS or SERRS) and will not change the sample (unlike photobleaching). Changing the laser wavelength is the simplest and least destructive alternative for reducing fluorescence and for this reason this approach was investigated in this chapter.

4.1.2 Changing Laser Wavelength to Overcome Fluorescence

Previous studies have looked at a range of laser wavelengths for specific fibre/dye samples. Table 4-1 is a summary of the fibre types, colour and the wavelengths used in each study. Each study is then discussed with respect to fluorescence and how it was overcome.

Table 4-1 Fibre type, colour and wavelengths that have been investigated

Reference	Fibre Type	Colour	Wavelength/s (nm)
Lang et al. [108]	polyester polyacrylonitrile	colourless	514.5 and 476.5
Bouffard et al. [177]	polypropylene	various pigments	632.8
Bourgeois and Church [168]	acrylic	red and blue	1064
Keen et al. [169]	rayon wool polypropylene polyester nylon	colourless colourless colourless colourless, red colourless, red	632.8 632.8 632.8 632.8 and 780 632.8 and 780
Kokot et al. [110]	cotton	red	1064
Miller et al. [111]	various man-made	colourless	785
Jochem et al. [171]	polyacrylonitrile viscose	various various	514 and 633 514 and 633
Massonnet et al. [106]	acrylic wool	red red	458, 488, 514, 532, 633, 685, 785, 830,1064 for all
Massonnet et al. [107]	cotton	green	458, 488, 514, 532, 633, 685, 785, 830,1064

It is important to note that studies have predominantly used Raman spectroscopy for the analysis of man-made fibres. The research in this document is focussed on natural fibres therefore the results of the above studies, whilst providing important background information, cannot be directly correlated to the expected results of this research.

4.1.2.1 Man-made fibre studies

Lang et al. [108] studied polyester and polyacrylonitrile fibres using laser wavelengths of 514.5 and 476.5nm. The authors found that analysis using these laser wavelengths was limited by fluorescence which they were not able to overcome.

Bouffard et al. [177] also encountered fluorescence when analysing pigmented polypropylene (various colours) with a laser wavelength of 632.5nm as well as problems associated with sample heating. Keen et al. [169] and Miller et al. [111] overcame fluorescence problems by analysing colourless man-made samples with NIR lasers (780nm for Keen et al. [169] and 785nm for Miller et al. [111]). These three studies show that, for man-made fibres, changing laser wavelengths from visible light to NIR can overcome fluorescence interference.

Interestingly, Jochem et al. [171] found that using visible lasers (514 and 633nm) produced spectra wholly attributed to the pigments used in the colouration of the man-made fibre sample set with no contribution from the fibre substrate. In this study fluorescence was not a significant problem. Conversely, Bourgeois and Church [168] and Keen et al. [169] found that analysing coloured man-made fibres (with NIR lasers) produced peaks that could be attributed to the substrate (acrylic for Bourgeois and Church [168] and polyester and nylon for Keen et al. [169]) and to the dye. Both sets of authors used spectral subtraction techniques to isolate the Raman spectrum associated with the dye. However, Bourgeois and Church [168] found that it was possible to generate artefacts in the subtraction spectrum through small shifts in the position of the laser line passing through the interferometer. The implication of the above studies for this research is that it is

important to investigate a variety of laser wavelengths, in the visible and in the NIR, not only for the minimisation of fluorescence but also in the possibility of deriving different information about the dye and the fibre substrate.

4.1.2.2 Natural fibre studies

There have been several studies into the use of Raman spectroscopy for natural fibres. Keen et al. [169] were able to analyse an uncoloured wool fibre using a laser excitation of 632.8nm with no fluorescence interference. Kokot et al. [110] analysed the different methods of dye fixation on a red coloured cotton fibre using a 1064nm laser and encountered no fluorescence problems. The authors were able to successfully discriminate (with the aid of chemometrics) the differently fixed samples from their Raman spectra.

By far the most comprehensive studies carried out on the use of Raman spectroscopy for fibre analysis have been by the members of the European Fibres Group. Massonnet et al. [106, 107] used Raman instruments from six different manufacturers and utilised a sum of nine different laser wavelengths ranging from blue (λ=458nm) to near infrared-NIR (λ=1064nm). These represent the largest comparison studies of Raman analytical parameters carried out on identical fibre samples. The first study analysed two red acrylic fibres and one red wool fibre. For the chosen fibre and dye samples, red lasers (λ=633 and 685nm) gave the poorest spectral quality whereas blue (458nm), green (514nm) and near infrared lasers (785, 830 and 1064nm) provided average results. Blue (488nm) and green lasers (532nm) globally gave the best quality spectra, indicating that dye chemistry and colour is a determining factor in deciding laser wavelengths for natural fibres when trying to reduce fluorescence. The second study analysed two sets of green cotton. Within each set the two samples had the same dyes but in different ratios. The study found that dye identification was easier with Raman spectroscopy than with Microspectrophotometry and that using a range of laser wavelengths provides the most information.

All of the above man-made and natural fibre studies highlight the necessity to investigate different laser wavelengths. This is not only to counteract the occurrence of any fluorescence interference but also to determine the optimal laser wavelength for the **specific** sample set (colour and fibre substrate) to be investigated.

4.1.3 Effect of Mounting Conditions

Mounting samples under different conditions can change the resultant Raman spectrum as the covering and mountant can contribute to the spectrum. Several studies have investigated the effect of sample mounting when analysing with Raman spectroscopy. Table 4-2 is a summary of various mounting methods that have been investigated by different authors.

Table 4-2 Mounting methods used in various studies

Reference	Mounting (covering)	Mounting (base or mountant)
Bourgeois and Church [168]	glass	specially constructed cell
Keen et al. [169]	no cover (fibres taped at each end)	glass slide metal plate
Miller et al. [111]	no cover glass coverslip	glass slide aluminium foil permount (mountant)
Jochem et al. [171]	no covering	specially constructed aluminium cell

Bourgeois and Church [168] designed a cell specifically to optimize the Raman signal in their fibre experiments (refer to Figure 4-3). The fibres were compressed between a glass window and an adjustable concentric metal rod which was located with a screw fitting. This arrangement ensured that unwanted

voids were not present in the sampling area and that a high density of sample was impinged by the laser beam.

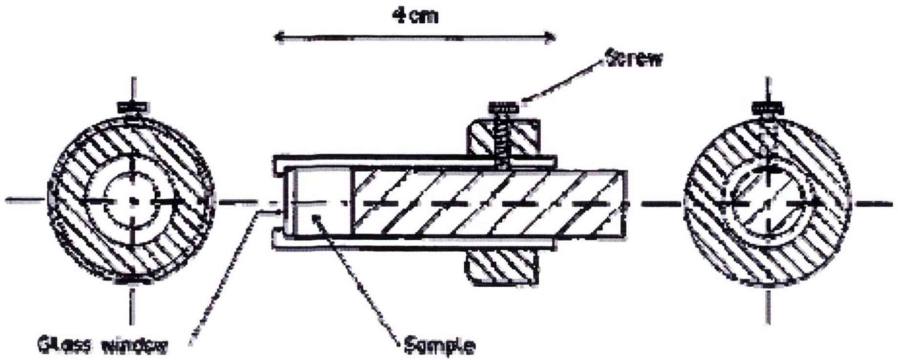


Figure 4-3 Specially constructed cell used by Bourgeois and Church [168]

Jochem et al. [171] also constructed a cell for the analysis of their fibre samples. This was simply an aluminium slide with a small hole that the fibre was placed over. In this way the authors avoided the influence of a specimen holder.

Keen et al. [169] analysed their samples on either glass slides or metal plates and encountered no problems with this form of sample mounting.

Miller et al. [111] also examined samples with no covering on glass slides and metal plates. Initially the authors taped the end of their fibre samples on to glass slides and then analysed the exposed fibres. They found that this method produced an underlying fluorescence spectrum from the glass. The authors found that changing the glass to aluminium foil removed the underlying spectrum and produced clear spectra for the samples.

Miller et al. [111] went on to analyse samples under glass coverslip and mounted in Permount. This was to simulate analysing the sample after it has undergone MSP analyses without de-mounting the sample. The authors found that occasionally there was still the underlying spectrum from glass and in addition there was an underlying spectrum associated with the Permount. The authors employed spectral subtraction to obtain the fibre spectrum.

The above studies highlight the different ways in which samples can be mounted for analysis. Typically the best results are produced by direct laser impingement on the samples. However, as highlighted by the Miller et al. [111] study, sample information can be obtained when fibres are mounted in less than ideal situations (i.e. Permount). If samples can be analysed directly after mounting then this could potentially reduce time spent in changing the mounting conditions after microscopy or MSP.

4.2 Aims

The purpose of this chapter is to investigate various aspects of Raman spectroscopy for the analysis of black/grey and blue reactively dyed cotton, namely:

- different mounting techniques for the dye standards and the fibre sample, and
- the effect of varying the laser wavelength on the dye and dyed fibre spectra.

Once these parameters have been analysed the optimal conditions for analysis of further samples will be determined.

4.3 Materials and Methods

4.3.1 General

Four samples (dyed fibre and three individual dye components) were analysed using two Raman spectrometers and a combined total of five different laser wavelengths. A fifth sample (undyed cotton) was analysed with 785 nm wavelength laser. Sample mounting, fluorescence, sample degradation and the

effect on the sample spectra of varying the laser wavelengths were all investigated. Samples were also analysed by visible microspectrophotometry in order to assess which excitation wavelengths might lead to resonance effects in the corresponding Raman spectra.

4.3.2 Samples

The sample set consisted of five components; an undyed cotton fibre, a dyed fibre and the three individual dye components used in the colouration of the fibre. The dyed fibre was Australian cotton with shade name *Moroccan Blue* (assigned by manufacturer: Rocklea Spinning Mills Pty Ltd, Melbourne Australia). This fibre was a dark shade of blue achieved through the combination of three reactive dyes, Synazol Gold-Yellow, Synazol Red and Sumifix Navy Blue. The dyes themselves were fine particles of intense colour and varying shape. The concentrations of the dyes used in the colouration of the fibre are unknown.

The fibre was initially analysed under two mounting conditions to determine best mounting conditions. In all subsequent examinations the fibre sample was examined in its original state and was mounted on double sided tape with direct contact with the laser (i.e. no covering). The dye samples were analysed predominantly by diluting the samples and allowing them to dry to a thin film on aluminium slides and occasionally by analysing the dye particles themselves.

4.3.3 Raman Microprobe Conditions

Two instruments were used in this study:

1. **Foster and Freeman FORAM 685/2 spectrometer** with Charged Coupled Device (CCD) array detector (thermoelectrically cooled) with Ramskan 2 version 1.1.0.2 software (Foster and Freeman) and a 50x objective. Sample

excitation was through a 685nm diode laser. The operating laser power was 4.5 mW at 100% laser power (laser power could be adjusted to 50% and 25% power) and in general a spectrum was accumulated for ten scans. The CCD array detector was operated at room temperature, and all the spectra were measured over 2100-400 cm^{-1} at 8 cm^{-1}.

2. **Renishaw System RM1000** (Renishaw Plc, Wotton-under-Edge, UK) equipped with a Leica microscope and GRAMS software (Galactic Industries Corporation). A 50x objective (numerical aperture of 0.75 and a working distance of 0.37 mm) was used for measurements. The microspectrometer was operated non-confocally at excitation wavelengths of 514.5nm, 632.8nm, 785nm and 830nm, with laser output powers of 20 mW, 14-17 mW, 300mW and 300mW respectively. Analysis was performed using either 100% laser power or 50% laser power depending on intensity of spectra and also any evidence of spectral degradation. The accumulated exposure time of the CCD detector was between 10 seconds and 120 seconds and the instrumental spectral resolution ranged from 1.5 to 2.5 cm^{-1}. The spectral range investigated was from 200 cm^{-1} to 1800 cm^{-1}.

4.3.4 Microspectrophotometer Conditions

The microspectrometer was a SEE2100 equipped with GRAMS/32 software (Galactic Industries Corporation) and using a 50x objective for measurements. The microspectrophotometer was operated, in transmission mode, across the wavelength range of 400nm to 850nm with a spectral resolution of 5nm.

4.3.5 Experiments

4.3.5.1 Mounting variations

In order to determine the effectiveness of analysing a sample after it had been mounted it was necessary to look at all components that may contribute to the final spectrum. Two techniques were investigated to determine the best method for analysis of the coloured cotton fibres: (i) the fibre was placed on double sided tape and analysed with no covering (refer to Figure 4-4) and (ii) the fibre was mounted in XAM beneath a glass coverslip (refer to Figure 4-5). For method (ii) XAM and glass (separately and combined) were analysed to determine their spectral contributions. The 785nm laser source was used for analysis.

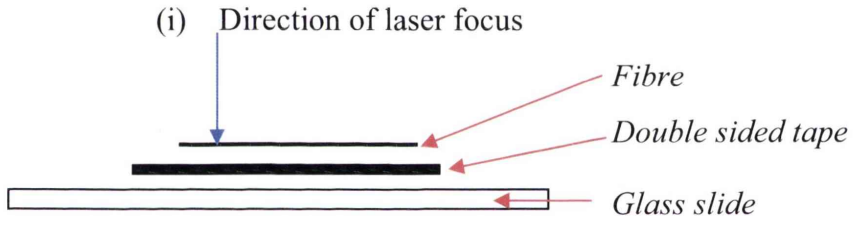


Figure 4-4 Fibre mounted on double sided tape

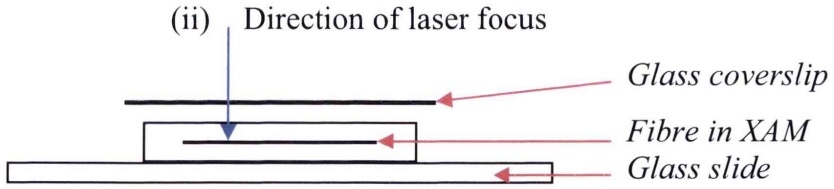


Figure 4-5 Fibre mounted in XAM beneath a glass coverslip

4.3.5.2 Dye analysis

Two techniques were investigated to determine the best method for analysis of the individual dye components: (a) the dye particles were placed on a slide and

the 632.8nm laser focussed directly on them, and (b) the dye was diluted in distilled water and allowed to dry to a thin film on an aluminium slide and then analysed using the 632.8nm laser.

4.3.5.3 Sample degradation

Sample degradation is a very real possibility when analysing samples with high intensity lasers, so it was therefore necessary to determine if any degradation of the sample (and the subsequent spectra) would occur during long analysis times. To achieve this, the four samples (dyed fibre and three dye components) were imaged at time = zero and the spectra collected at four time intervals (1, 5, 10 and 25 accumulations of 20 seconds) for the laser strengths 50% and 100%. The samples were visually checked before and after every analysis and the spectra were investigated for variations.

4.3.5.4 Microspectrophotometry

Each of the four samples was analysed using microspectrophotometry to investigate the potential for resonance enhancement of the Raman spectra at different laser wavelengths. The cotton sample was mounted on a glass slide with a glass coverslip and Hystomount as the mountant. The dye particles were ground up and mounted on a glass slide under a glass coverslip. The samples were analysed in the visible region in transmission mode.

4.3.5.5 Varying the laser wavelength

Three aspects of the samples were analysed by varying the laser wavelengths. The first was how the spectra of each sample may vary (e.g., relative peak intensities, resonance enhancement effects) with differing laser wavelengths. This was investigated by analysing each of the four samples (dyed fibre and three dye components) with the five different lasers and visually comparing the resultant spectra.

The second aspect was the variation in observed fluorescence as the laser wavelengths are changed. This was investigated by analysing the spectra of any

sample that exhibited fluorescence at any wavelength and visually comparing it with the spectra of the same sample from other wavelengths.

The third aspect was the quality (intensity and fluorescence) of the spectra for all samples as the wavelengths varied, this was investigated by visually comparing the spectra for samples at each wavelength and determining which wavelength gave the best overall result. The results were compared using a 1-4 rating system (see data treatment section below).

4.3.6 Data Treatment

For ease of comparison most spectra were baseline corrected using the software specific to each instrument. For the Renishaw instrument piecewise polynomial, 2^{nd} order polynomial and 4^{th} order polynomial functions were utilised, depending on the general shape of the spectra. For the FORAM instrument piecewise polynomial, 2^{nd} order polynomial and 4^{th} order polynomial functions were also utilised, once again depending on the general shape of the resultant spectra.

Analysis of Raman results was carried out through visual comparison of the raw and baseline corrected spectra, consideration of spectral acquisition times and the observation of any fibre degradation. Spectra were rated between 1 and 4:

- 1 - no spectra due to fluorescence (refer to Figure 4-6)
- 2- some spectral details with large amounts of fluorescence (refer to Figure 4-7)
- 3- good spectral details with some fluorescence (refer to Figure 4-8) and
- 4- excellent spectral details with little to no fluorescence (refer to Figure 4-9).

Analysis of microspectrophotometer results was carried out using the GRAMS/32 software to determine absorption maxima and minima as well as the presence of any shoulders.

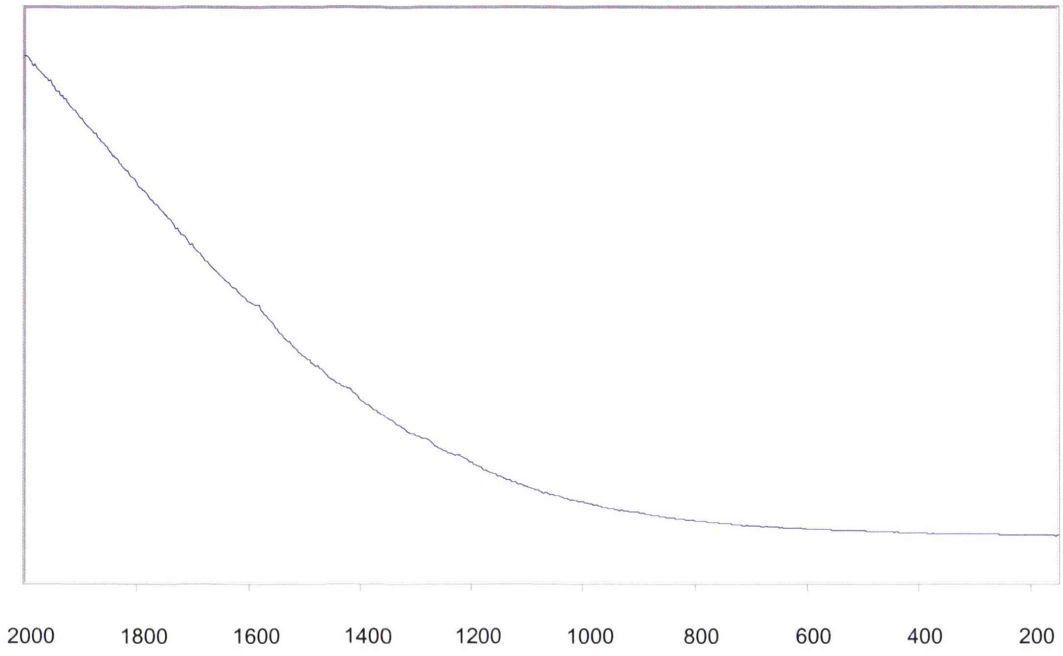


Figure 4-6 Example of: 1-no spectra due to fluorescence

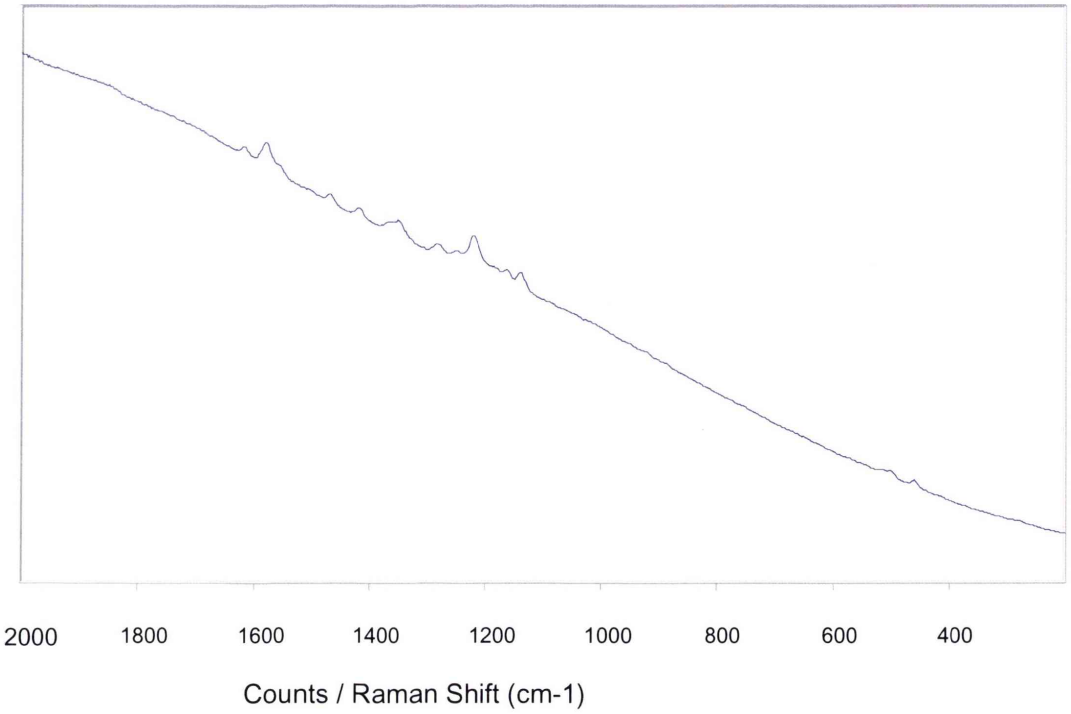


Figure 4-7 Example of: 2- some spectral details with large amounts of fluorescence

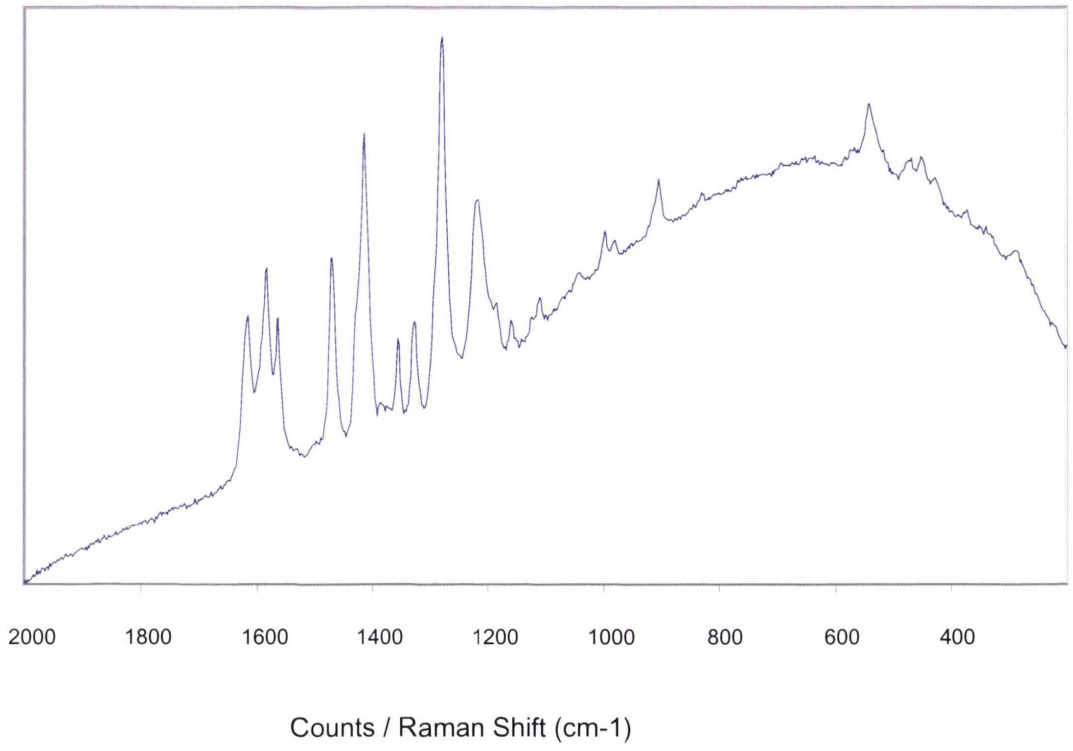


Figure 4-8 Example of: 3- good spectral details with some fluorescence

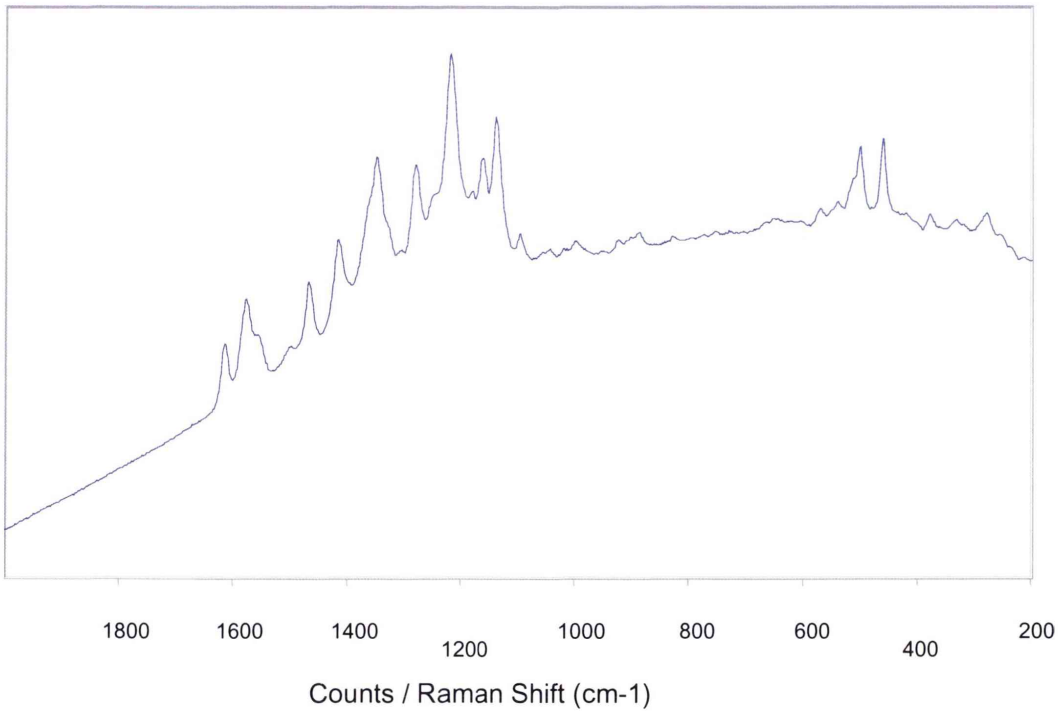


Figure 4-9 Example of: 4- excellent spectral details with little to no fluorescence

4.4 Results and Discussion

4.4.1 Mounting Variations

Initially the sample was analysed with no covering (mounting technique (i) from method and materials section pg 79). This produced a true spectrum of the sample as there was direct laser impingement (refer to red spectrum in Figure 4-10). The spectrum is characterised by a strong peak in the ~500cm^{-1} region and medium peaks in the 1100-1600cm^{-1} range.

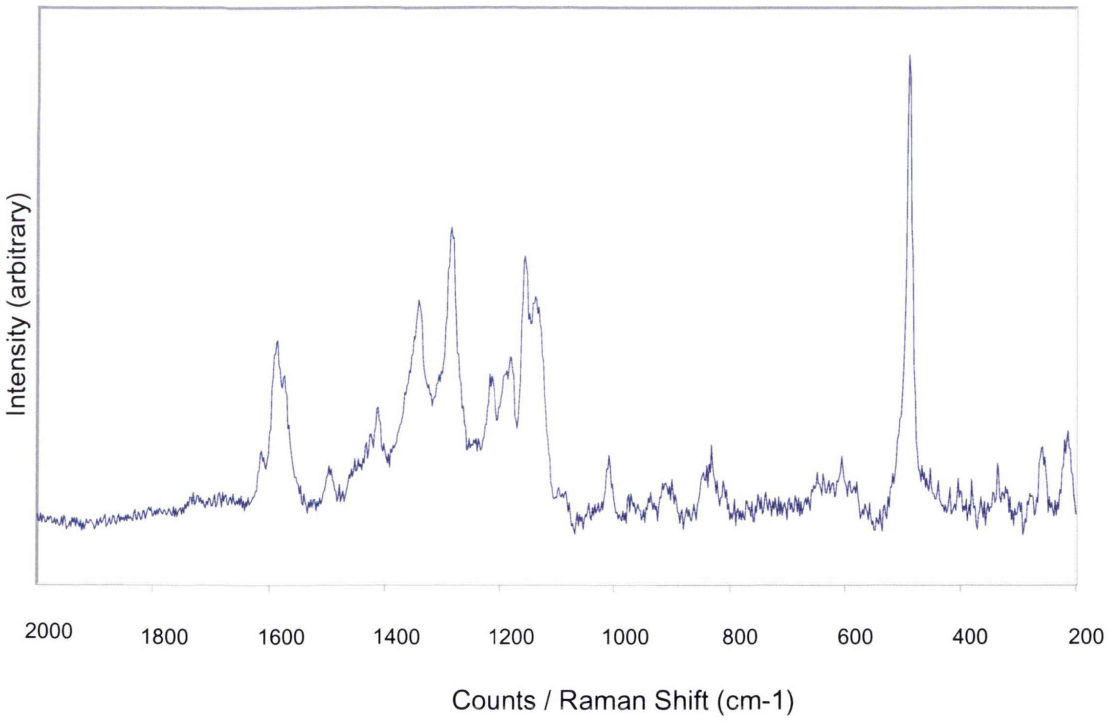


Figure 4-10 Raman spectrum of dyed fibre with no covering (method (i))

For the second mounting condition the individual components of glass and XAM, as well as the combination of glass and XAM were analysed. The top spectrum in Figure 4-11 shows the combination of glass and XAM. This

spectrum is dominated by a broad peak in the ~1400cm^{-1} region from the glass (bottom spectrum in Figure 4-11). The smaller peaks in the 400-1200cm^{-1} range are due to the XAM (middle spectrum in Figure 4-11).

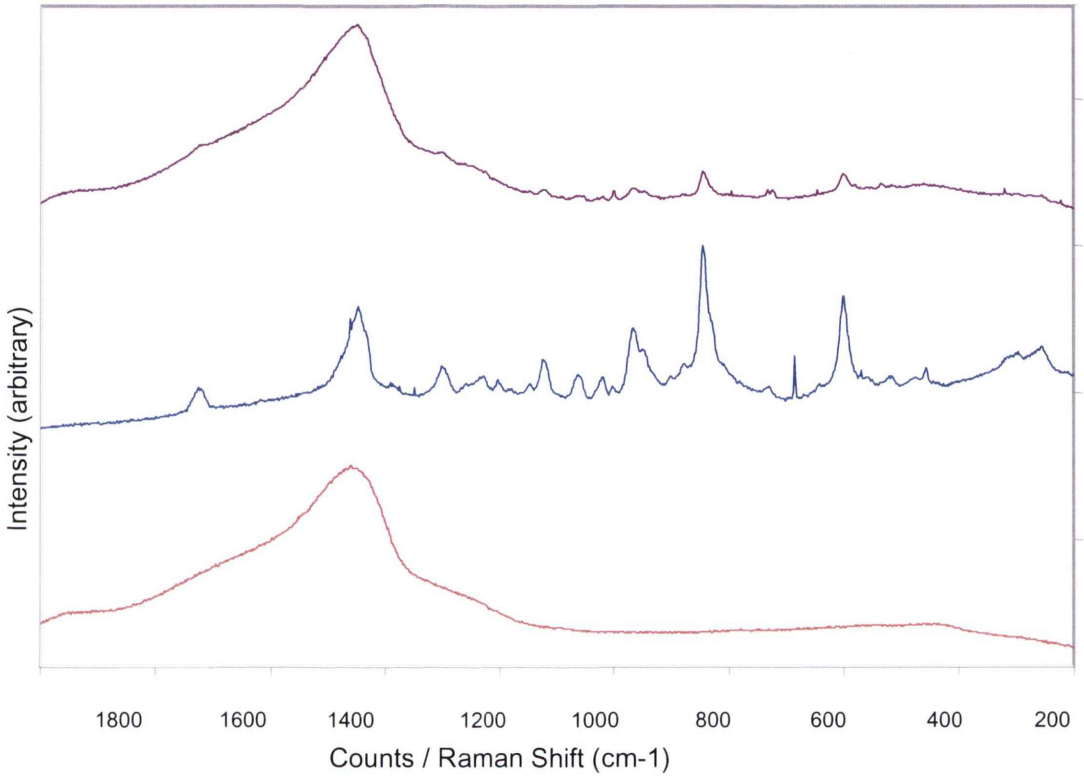


Figure 4-11 Raman spectra of: (top) XAM through glass coverslip, (middle) XAM, and (bottom) glass coverslip

The fibre was then analysed in the mounted conditions (refer to blue spectrum in Figure 4-12). This produced a significantly different spectrum to that of the uncovered fibre. This spectrum was dominated by a broad, intense peak at ~1450 cm^{-1} which can be attributed to glass. Some of the smaller peaks observed in the mounted spectrum may be attributed to the fibre sample as they have a similar Raman shift (refer to asterisk in Figure 4-12). The other smaller peaks can be attributed to the XAM contribution.

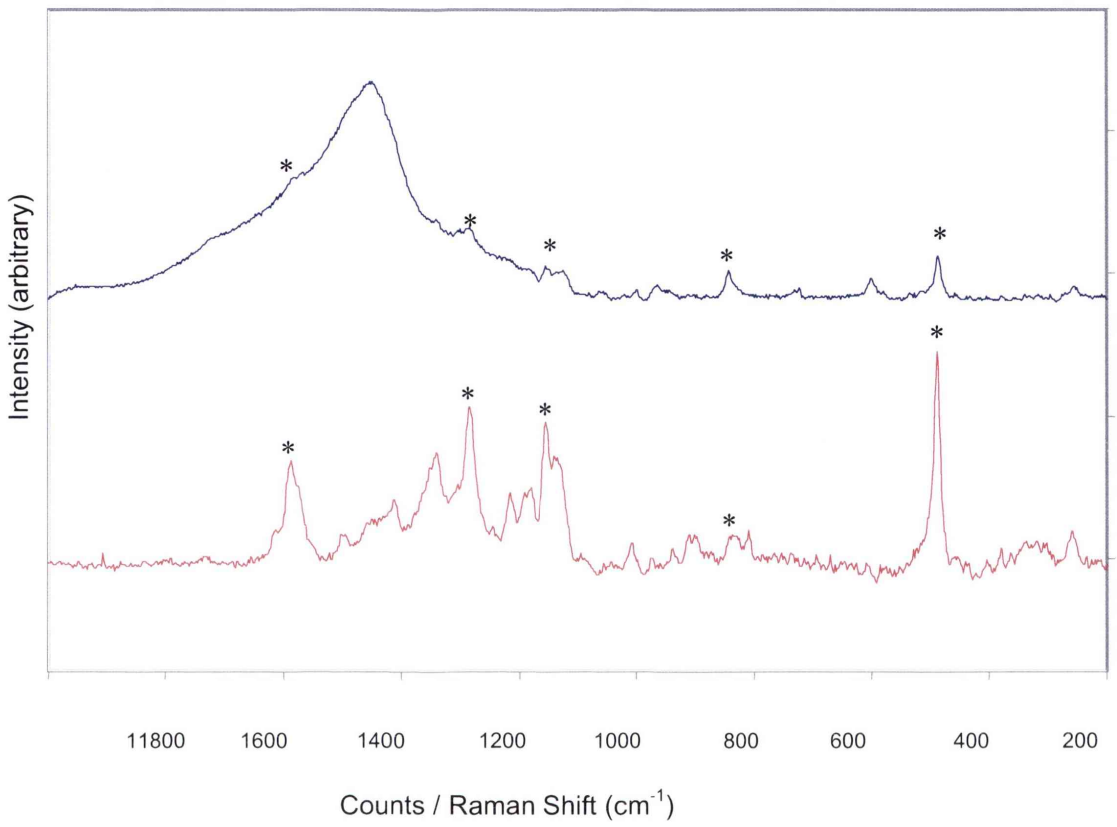


Figure 4-12 Raman spectra of: (top) Black cotton through glass coverslip (mounted in XAM), and (bottom) black cotton sample (no covering).

Spectral subtraction was used to determine if the spectrum of the dyed fibre could be extracted from that of the fibre in mountant. Figure 4-13 shows the spectrum of the result of subtracting the glass and mountant spectra from the spectrum of the fibre in mountant (blue spectrum). This is compared with the spectrum of the dyed fibre from direct laser impingement (red spectrum). Peak positions and relative intensities from the subtracted spectrum are comparable to that of the dyed fibre. There are some small variations in peak position and a slight increase in noise in the subtraction spectrum.

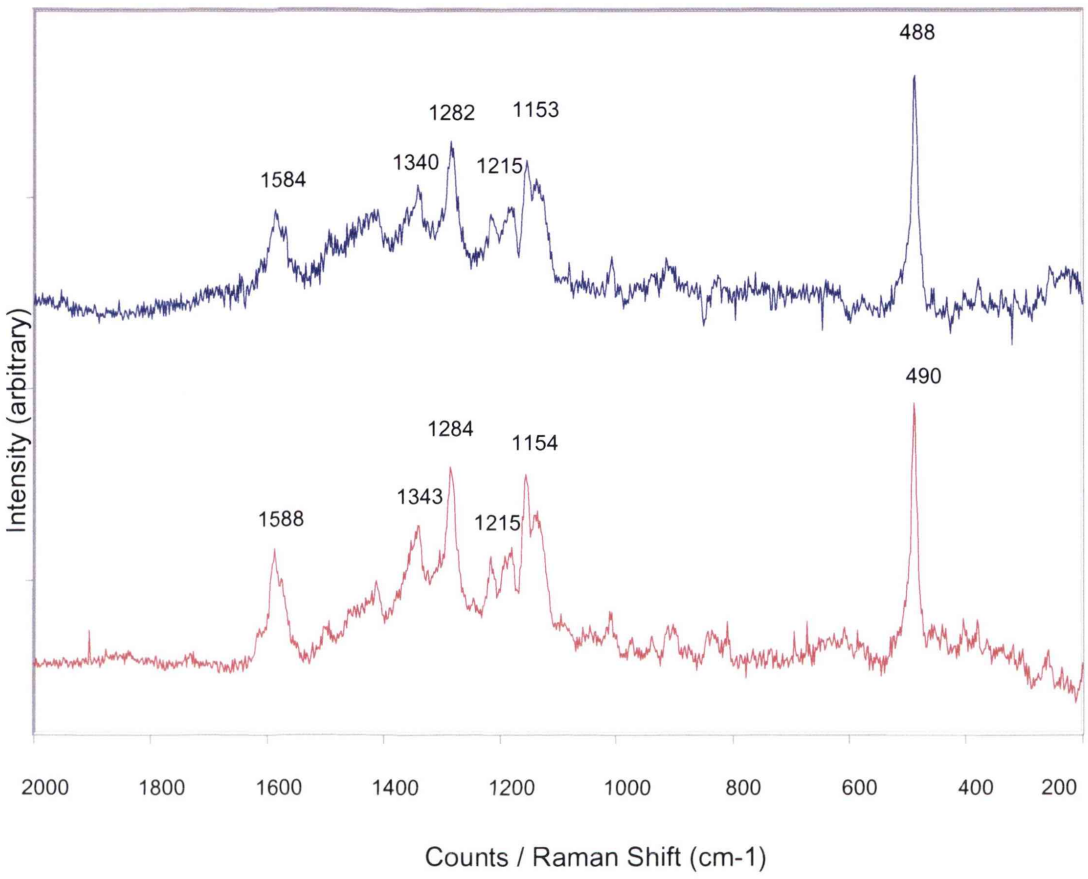


Figure 4-13 Raman spectra of (top) Black cotton mounted (glass and mountant subtraction), and (bottom) Black cotton no covering or mountant

The above result indicates that spectral subtraction can be used to determine the Raman signal produced by the dyed fibre when mounted. The use of spectral subtraction could greatly assist fibre examiners by removing the need to demount fibres prior to Raman analysis. This would decrease analysis time as well as the possibility of losing fibres. However, more analysis is required (i.e. analysis of components of mounting) and spectral subtraction is only useful if peaks from the mountant as well as the fibre can be seen in the resultant spectra. Some care is also needed in interpretation as spectral subtraction can result in peak shifts and some instances have been reported where artefact peaks have been created. This is seen in the study conducted by Miller and Bartick [111].

Miller and Bartick [111] found that when they subtracted contributions from the mountant and glass artefact peaks were produced (refer to Figure 4-14 - Raman spectra of a nylon 6 Fibre mounted on a glass slide in Permount with a coverslip. (A) nylon 6 mounted in Permount (arrows show major Permount peaks); (B) Permount on aluminium foil; (C) circled in red - nylon 6 mounted in Permount after subtraction of Permount and glass slide and baseline – arrows indicate artefact peaks); (D) nylon 6 spectrum obtained on aluminium foil-covered slide).

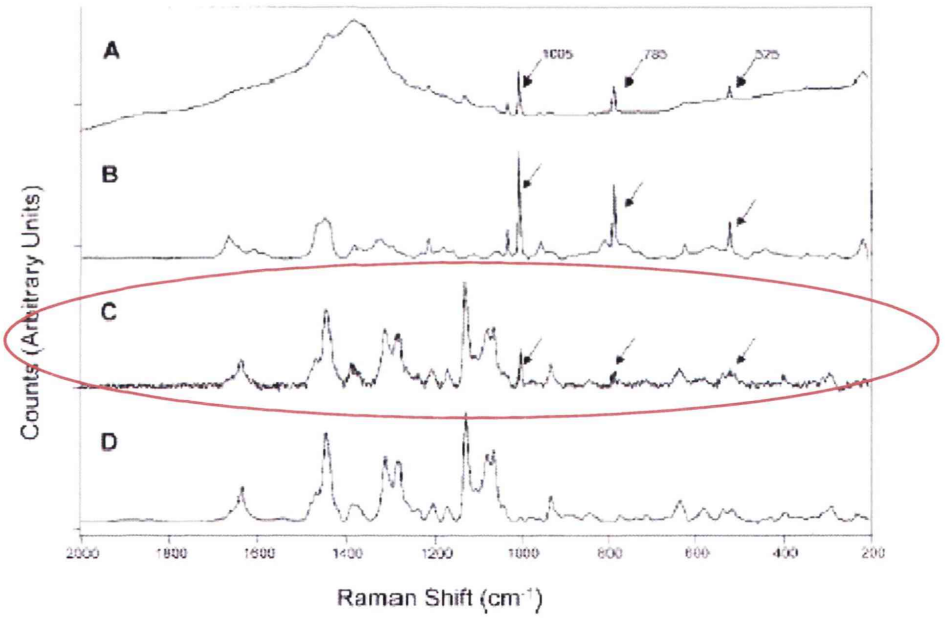


Figure 4-14 Spectral subtraction from Miller and Bartick [111] - arrows in spectrum C (circled in red) indicate artefact peaks

4.4.2 Dye Analysis

Analysis of a single dye particle proved problematic as the particle was an irregular shape. This made focussing the laser for maximum signal difficult and produced spectra that had low intensity and hence a poor signal to noise ratio, as seen in the bottom spectrum of Figure 4-15. By diluting the dye in deionised water and allowing it to dry on an aluminium slide the size of the particles was

significantly reduced and focussing the laser beam was easier on the flatter surface. The spectrum of the diluted and dried sample had a significantly higher intensity and less noise as seen in the top spectrum of Figure 4-15. This analysis technique also provided a good quality spectrum in a shorter analysis time.

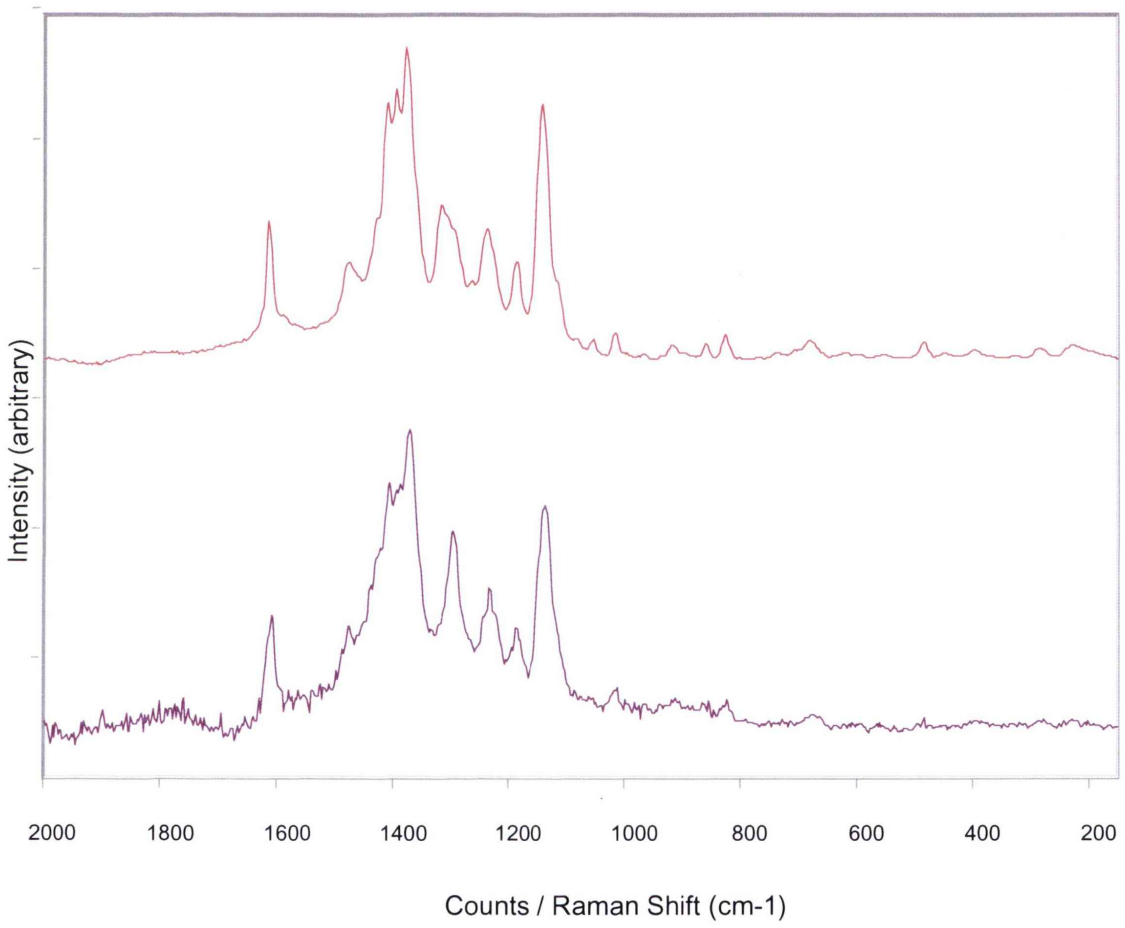


Figure 4-15 Sample mounting variations (632.8nm laser). Top spectrum: Synazol Gold - Yellow diluted and dried on aluminium slide. Bottom spectrum: Synazol Gold - Yellow dye particle

The analysis of the dye sample by the two different methods has shown that sample preparation significantly affects the resultant Raman spectra. The observed differences were attributed to two factors; the flatness of the surface presented to the laser, and the size of the particles. Having a flat surface for laser contact means that more of the Raman scattered light will be directed back along

the path of the laser to be collected by the CCD detector, while an irregular surface will mean that there will be a reduction in the collected Raman signal.

Pellow-Jarman et al. [178] studied the effect of particle size and their experimental data indicated that particle size does play a role in the intensity of Raman scattering. By analysing varying sizes of particles it was shown that the smaller the particle, the greater the intensity of the resultant Raman spectrum. The observation of our study concur with published results: a significant increase in intensity is observed when dye samples were diluted and dried.

4.4.3 Sample Degradation

Pyrolysis appears as a dark spot on the sample (due to burning) and the presence of a broad double peak at approximately 1330 and 1580 cm^{-1} from the carbon residue. Figure 4-16 is a Raman spectrum of carbon (from carbon nanotubes) showing the characteristic broad double peak **[179]**.

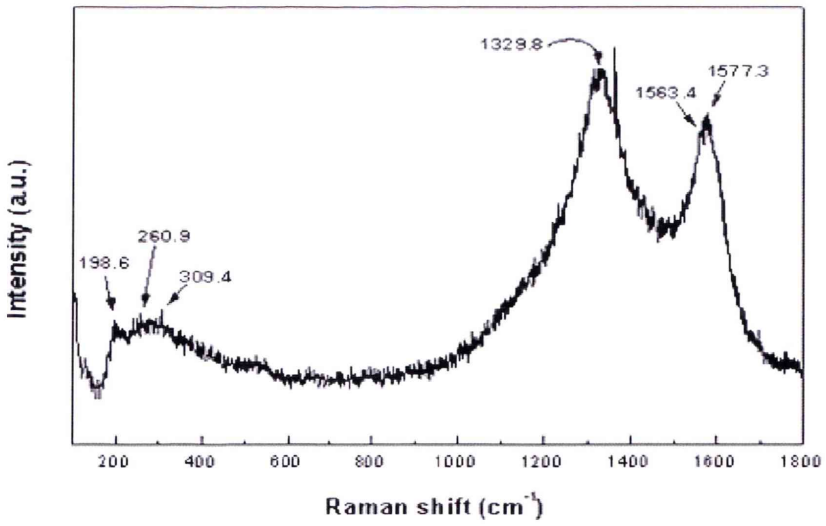


Figure 4-16 Raman spectrum of carbon (from carbon nanotubes) [179]

Analysis of the surface of sample A1, as well as the resultant spectra showed no degradation from pyrolysis or photochemical reactions after 400 seconds. In Figure 4-17 red circles indicate approximate point of laser contact.

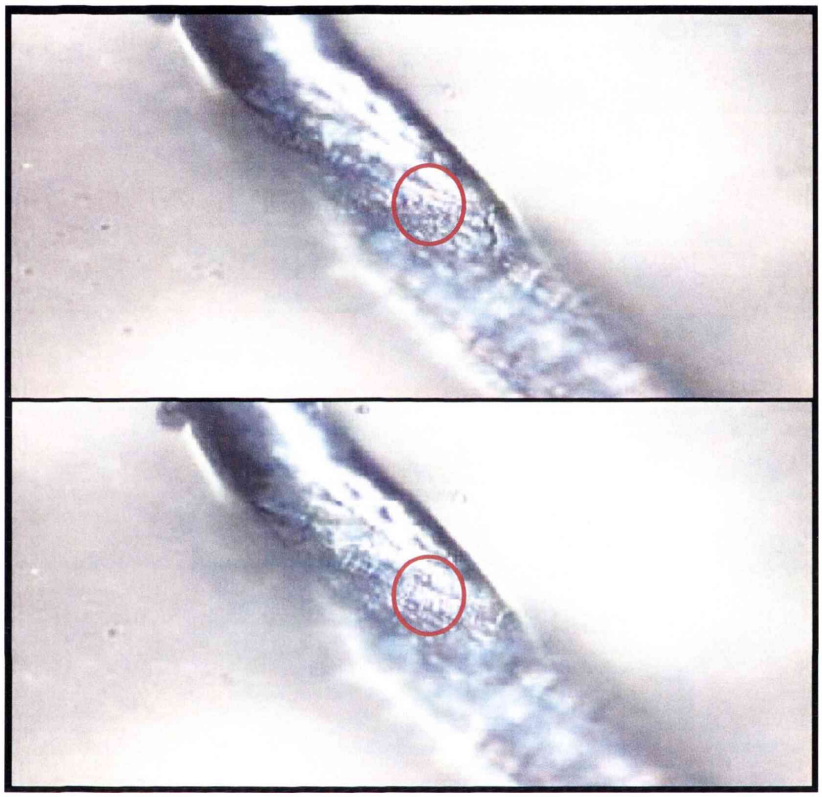


Figure 4-17 Images (using Renishaw Instrument imaging) of sample A1 at (top) time = 0 and (bottom) laser =100%, 20 x accumulations of 20 seconds

Photochemical changes appear as peak shifts or changes in relative peak intensities of the Raman spectra of the sample. Visual examination of the spectra also shows that there is no significant degradation of the sample (refer to Figure 4-18). The amount of noise in the top spectra of Figure 4-18 is due to the fact that there are fewer accumulations (i.e. less analysis time). Peak positions and relative peak intensities are the same for the two spectra.

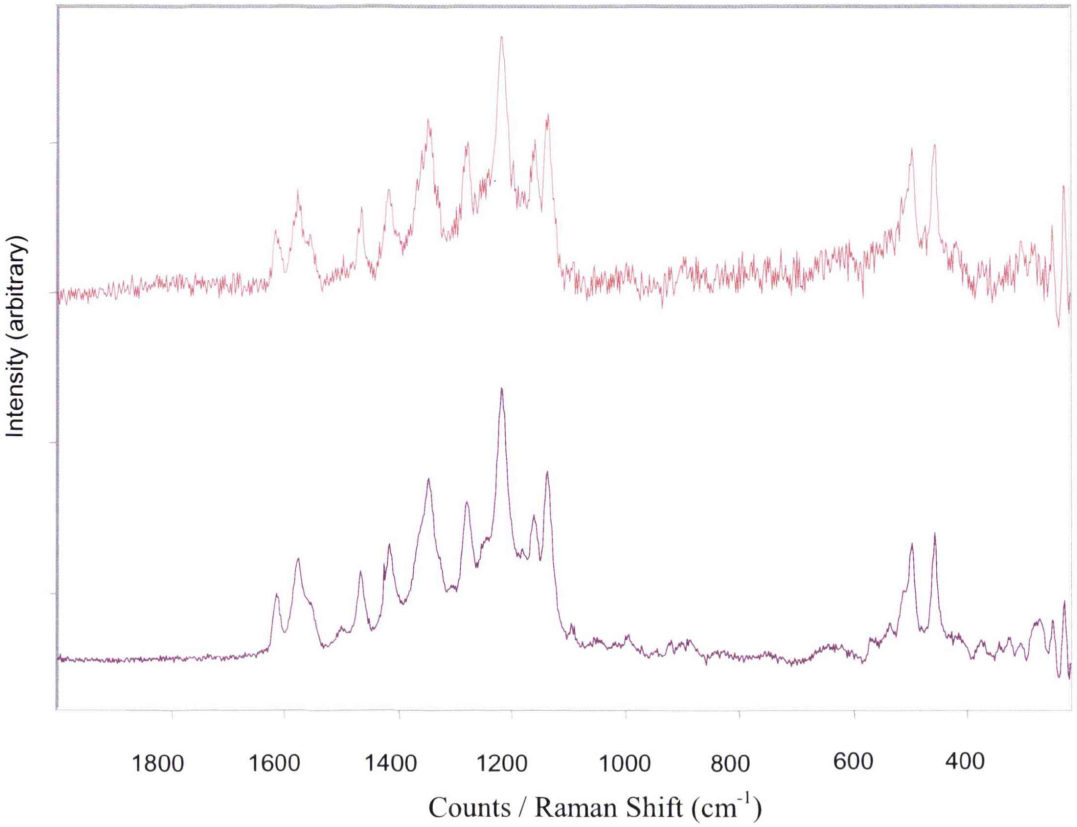


Figure 4-18 Raman Spectra of (top) A1 sample 100% laser and 1 accumulation of 20 seconds (bottom) A1 sample 100% laser and 25 accumulation of 20 seconds

The above results show that the analysis of samples by Raman spectroscopy truly is non-destructive in this case. However, care must be taken when analysing samples that the conditions do not contribute to degradation of the sample as well as the resultant spectra. Laser power varies from instrument to instrument and must be considered when preparing samples for analysis.

4.4.4 Fluorescence

Fluorescence is seen in Raman spectra as a large featureless background as shown in the spectrum of undyed cotton using the argon laser at 514.5nm (Figure 4-19), where the fluorescence completely masks any vibrational (Raman) information from the cotton fibre.

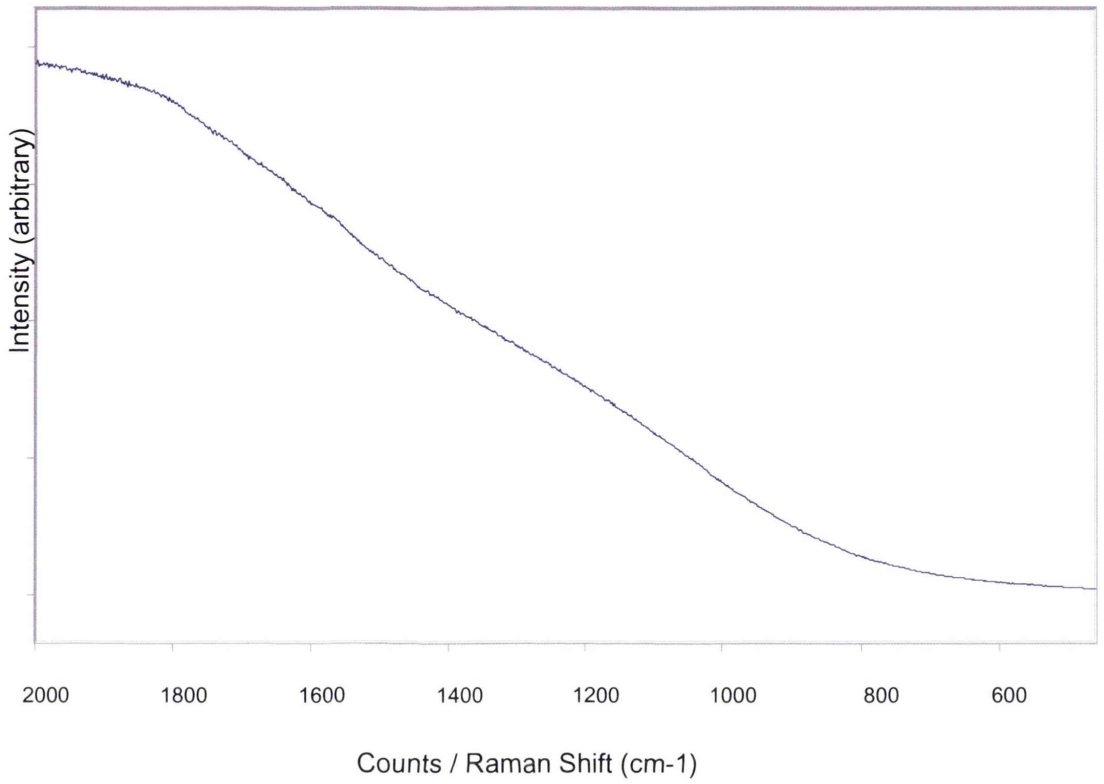


Figure 4-19 Raman spectrum of fibre - undyed cotton at 514.5nm showing signal swamping due to fluorescence

In some instances small Raman peaks can be observed above the fluorescence. This can be seen in the 1700-1000 cm^{-1} region of the *Moroccan Blue* dyed fibre sample analysed at 514.5nm (Figure 4-20). When this type of highly fluorescent spectrum is baseline corrected for comparison, the result is a spectrum that has a very poor signal to noise ratio (Figure 4-21). In the case of the baseline corrected *Moroccan Blue* dyed fibre spectrum at 514.5nm, the Raman signal is almost completely swamped by the fluorescence and rendered useless. The amount of background fluorescence is therefore a factor in determining the overall quality of the spectra.

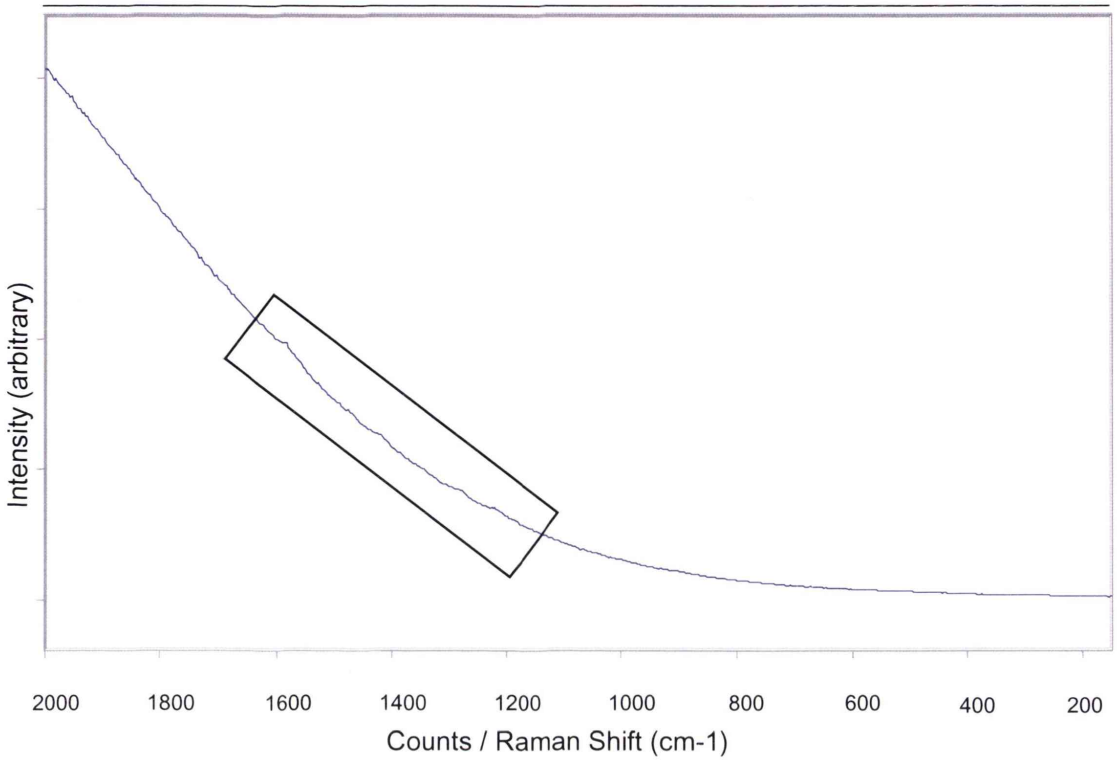


Figure 4-20 Raman spectrum at 514.5nm of Moroccan Blue fibre. Some sample signal above the fluorescence (boxed area)

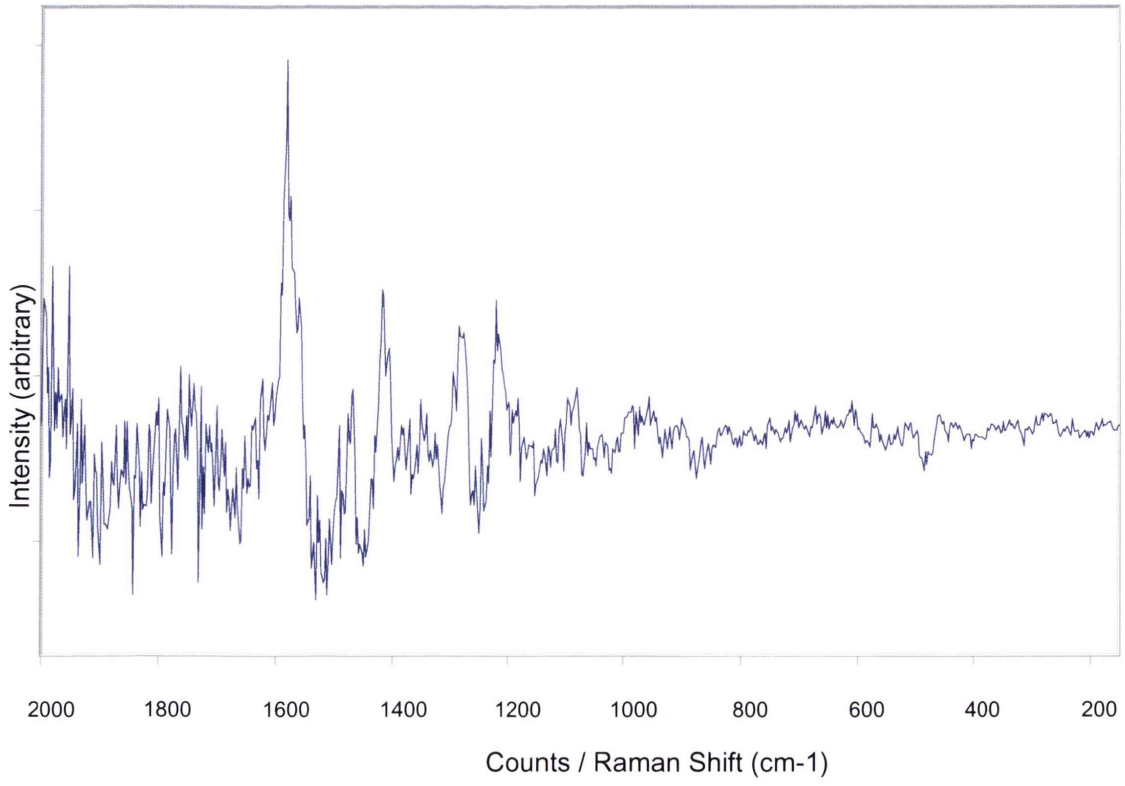


Figure 4-21 Raman spectrum of fibre Moroccan Blue at 514.5nm, baseline corrected

Fluorescence is one of the major problems associated with Raman spectroscopy and in this study the observed fluorescence of the dyed fibre (when using Argon and Helium-Neon visible lasers) appeared to be related to the cotton substrate itself. This problem was overcome, through no manipulation or destruction of the sample, by analysing using NIR lasers (785nm and 830nm).

These results highlight the need for an instrument that provides the flexibility of multiple laser wavelengths in order to achieve the optimal results for the samples being investigated.

4.4.5 Microspectrophotometry

The visible microspectrophotometry (MSP) results for all four samples are shown in Figure 4-22 and summarised in Table 4-3. The spectrum of the dyed fibre indicates that the yellow dye and the red dye contribute to the overall colour, though less significantly than the blue dye.

MSP in the visible region was investigated for the purpose of predicting resonance enhancement in the Raman spectra of the highly coloured samples. Resonance enhancement of Raman bands can occur when the wavelength of the laser used to excite the sample coincides with some part of a UV-visible absorption band. Not all Raman bands will necessarily be enhanced; only those that represent vibrations of the part of the molecule associated with the UV-visible absorption band.

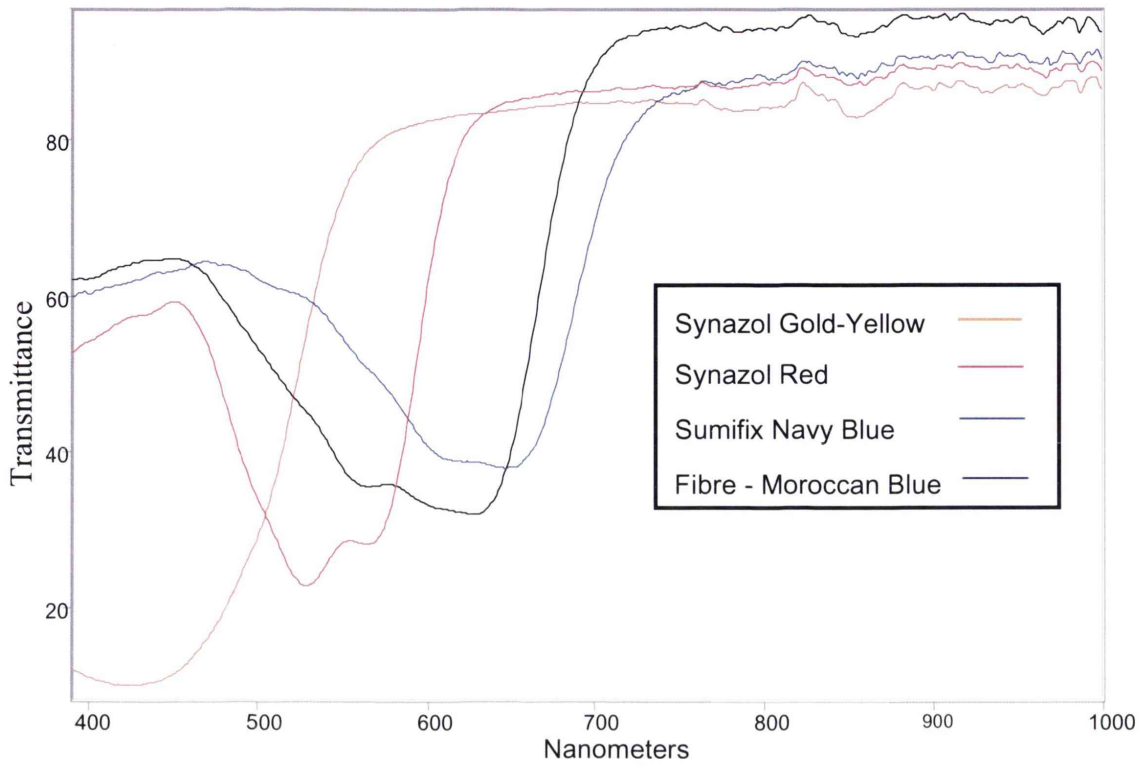


Figure 4-22 Vis-Microspectrophotometry (SEE 2100) results for (from left) Synazol Gold-Yellow, Synazol Red, Sumifix Navy and dyed fibre Moroccan Blue

Table 4-3 Visible-Microspectrophotometry peak maxima, minima and shoulder

Sample	Peak 1	Peak 2	Shoulder	Minima 1	Minima 2
Synazol Gold –Yellow	422nm	-	-	-	-
Synazol Red	529nm	567nm	-	451nm	553nm
Sumifix Navy	652nm	600nm	512nm	475nm	-
Fibre –Moroccan Blue	629nm	564nm	600nm	457nm	579nm

4.4.6 Varying the Laser Wavelength

Because of resonance enhancement effects, fluorescence and the ν^4 dependence of scattering intensities on laser frequency, Raman spectra of the same substance, at different laser wavelengths, can exhibit different relative peak intensities. Therefore, caution has to be exercised when measuring and interpreting Raman spectra, as data from different laser wavelengths cannot always be compared directly. A good example of this effect can be seen in the spectra of the dye component Sumifix Navy Blue analysed at four laser wavelengths (Figure 4-23).

Relative peak intensities vary over all four laser wavelengths (though the differences between the spectra at 785 and 830nm are slight). Particularly notable is the observation that the 514.5nm spectrum is utterly different from the spectra at the other wavelengths.

The observed differences can only be due to either: (i) resonance enhancement at 514.5nm which is not occurring at the other three wavelengths, or (ii) vice versa. At first, option (i) seems unlikely since the MSP intensity of the blue dye at 514.5nm is low, but option (ii) is impossible since the blue dye has no intensity at 830nm in the MSP spectrum. In other words, if resonance enhancement was following the shape of the main peak in the MSP plot, the spectra at 633 and 785nm would differ greatly from that at 830nm - but this is not observed; all three are very similar. The visible MSP band which is linked to the resonance effect may not have its maximum at the maximum in the overall curve, it may instead be an underlying band which is obscured partially or completely by another. A small shoulder is observed in the visible microspectrophotometry spectrum of the blue dye in the region of 514.5nm (refer to Figure 4-22).

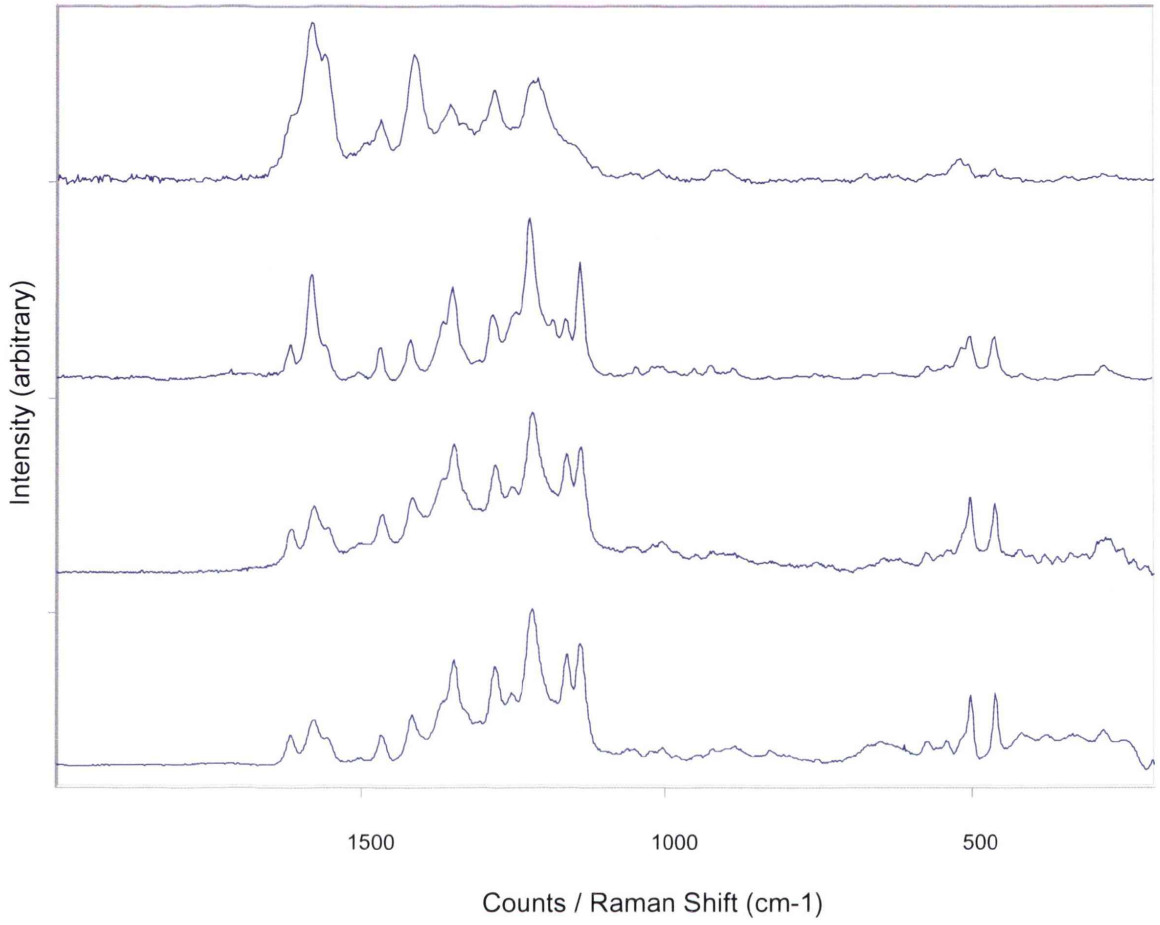


Figure 4-23 Analysis of Sumifix Navy dye with (from top) 514.5nm, 632.8nm, 785nm and 830nm laser sources

The spectrum of the yellow dye using the lasers at visible laser wavelengths (514.5nm and 632.8nm) shows distinctly different peak positions when compared with spectra collected using the NIR lasers (785 and 830nm) (refer to Figure 4-24), most notably with the disappearance of a peak at 1400 cm^{-1} (as you move from visible to NIR) and the change in peak shape at 1300 cm^{-1}. The range of laser wavelengths investigated in this study meant that a range of variations in the spectra of the one sample could be observed.

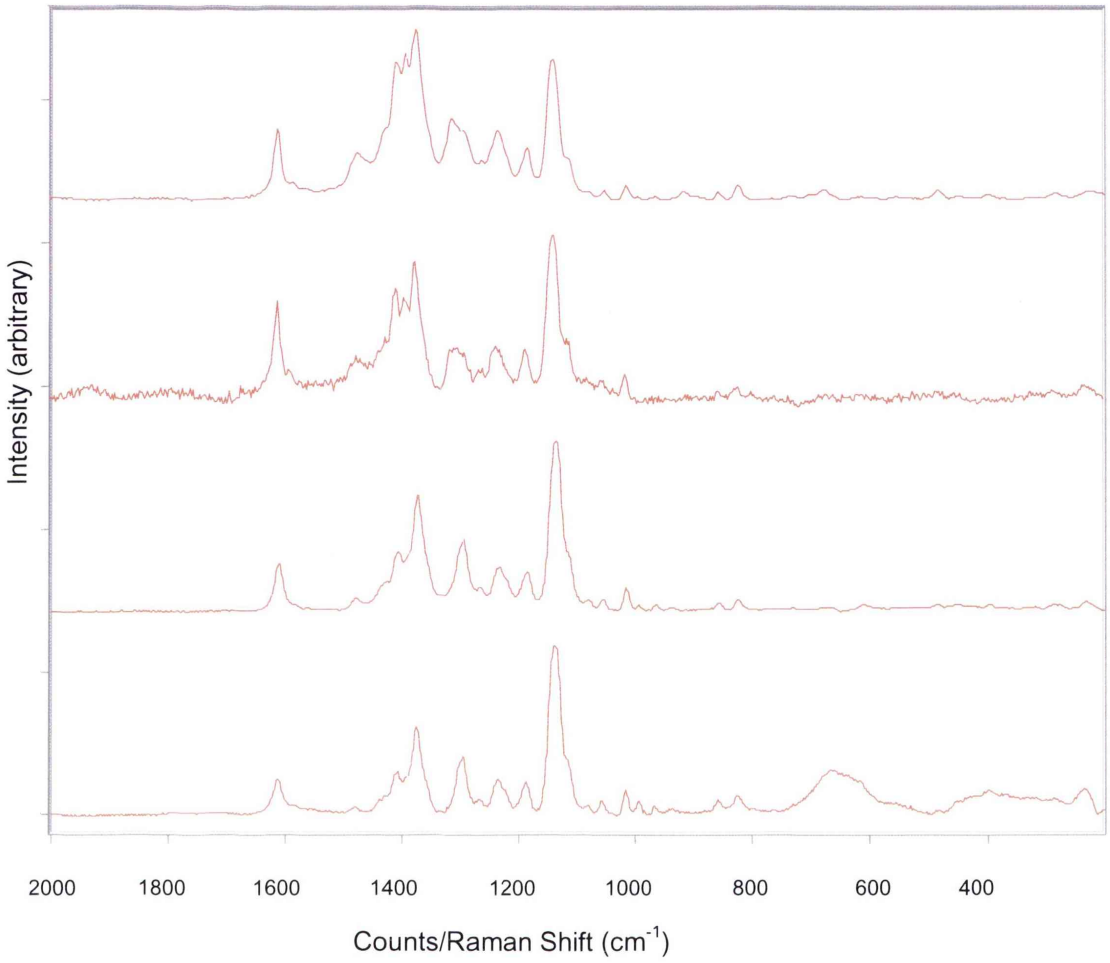


Figure 4-24 Analysis of Synazol Gold-Yellow dye with (from top) 514.5nm, 632.8nm, 785nm and 830nm laser sources

4.4.6.1 Optimising the laser wavelength, λ

The argon laser wavelength at 514.5nm was not a practical wavelength for analysis (refer to Figure 4-25) as the spectrum of the dyed fibre (top) was difficult to obtain due to fluorescence. Large amounts of fluorescence meant that very little of the fibre's resultant spectrum was visible, resulting in a poor signal to noise ratio for the baseline corrected spectra. The spectra of the dyes, however, were easy to achieve. This indicates that the fluorescence comes from the cotton fibre itself.

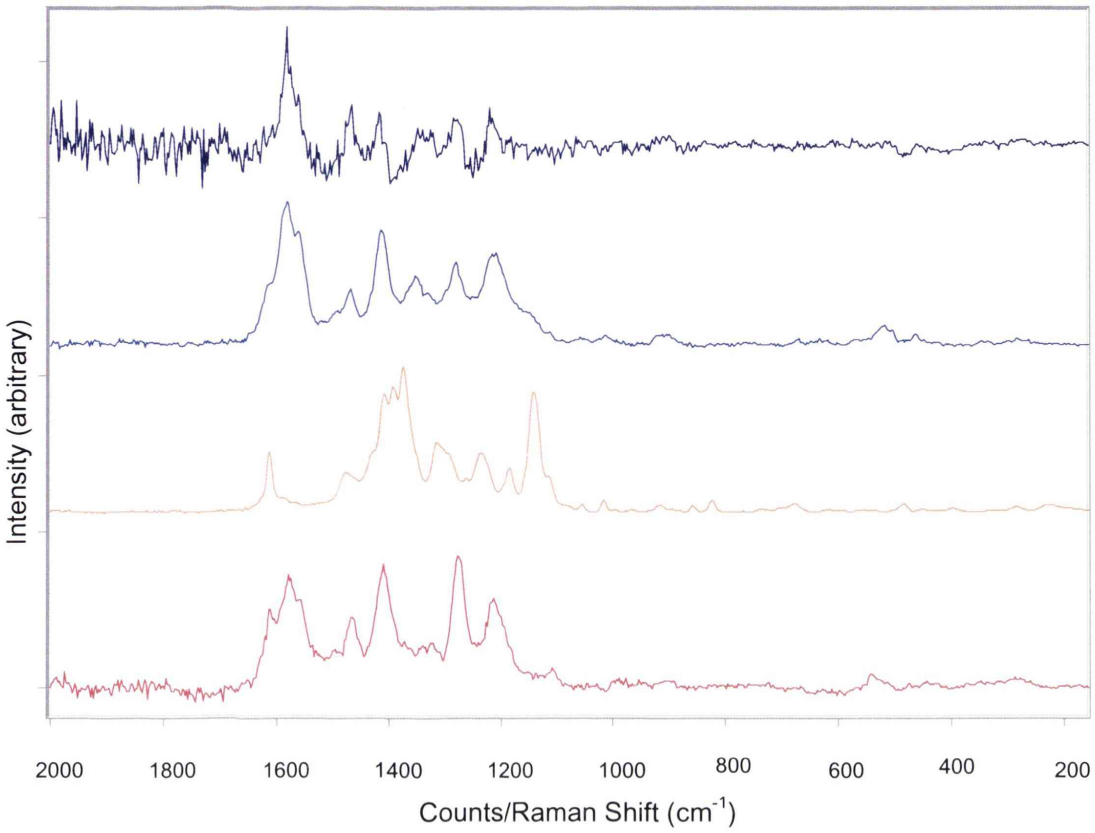


Figure 4-25 Raman spectra at 514.5nm analysis of (from top) dyed fibre - Moroccan Blue, Sumifix Navy dye, Synazol Gold-Yellow dye and Synazol Red dye

Using a 514.5nm laser source, resonance enhancement would be expected of the red component (Synazol Red) of the dyed fibre (refer to Figure 4-22), but as the Raman spectrum of the dyed fibre is poor it was impossible to determine if this was the case. Instead the spectrum of the dyed fibre most closely resembles that of the navy blue dye and has some peak positions similar to the red dye.

Using helium-neon laser source at 632.8nm gave significantly better results for the dyed fibre (Figure 4-26); though the yellow dye by itself (third spectrum) did exhibit strong fluorescence. It was observed that the spectrum of the fibre (top spectrum) is almost wholly due to the contribution of the navy blue dye (second spectrum) - no yellow, no red and no cotton contributions can be discerned. This phenomenon may be due to resonance enhancement of the blue dye (Sumifix Navy) component (refer to Figure 4-22), or, to the fact that the blue dye had a significantly greater concentration than the other two dye components. As the

concentrations of the dyes are unknown it is difficult to verify the latter. Another possibility is simply that the Raman spectrum of the blue dye is intrinsically more intense that that of the other dyes and cotton.

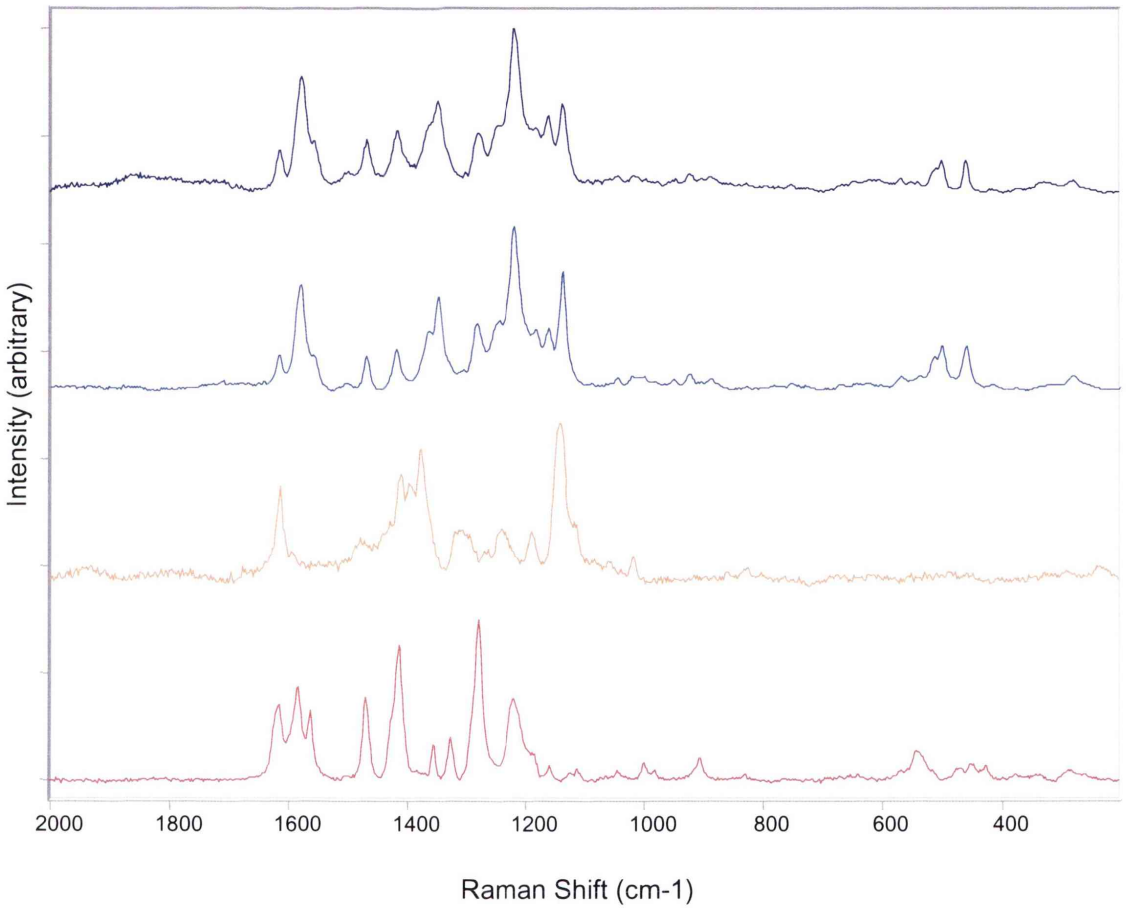


Figure 4-26 Raman spectra at 632.8nm analysis of (from top) dyed fibre - Moroccan Blue, Sumifix Navy dye, Synazol Gold-Yellow dye and Synazol Red dye

Whilst there is a significant decrease in resolution with the FORAM 685/2 system (refer to Figure 4-27) differences between the three dye components can still be observed. The spectrum of the navy blue dye (bottom spectrum) is the sole contributor to the observed spectrum of the dyed sample (top spectrum) as was seen with the analysis using the 632.8nm laser.

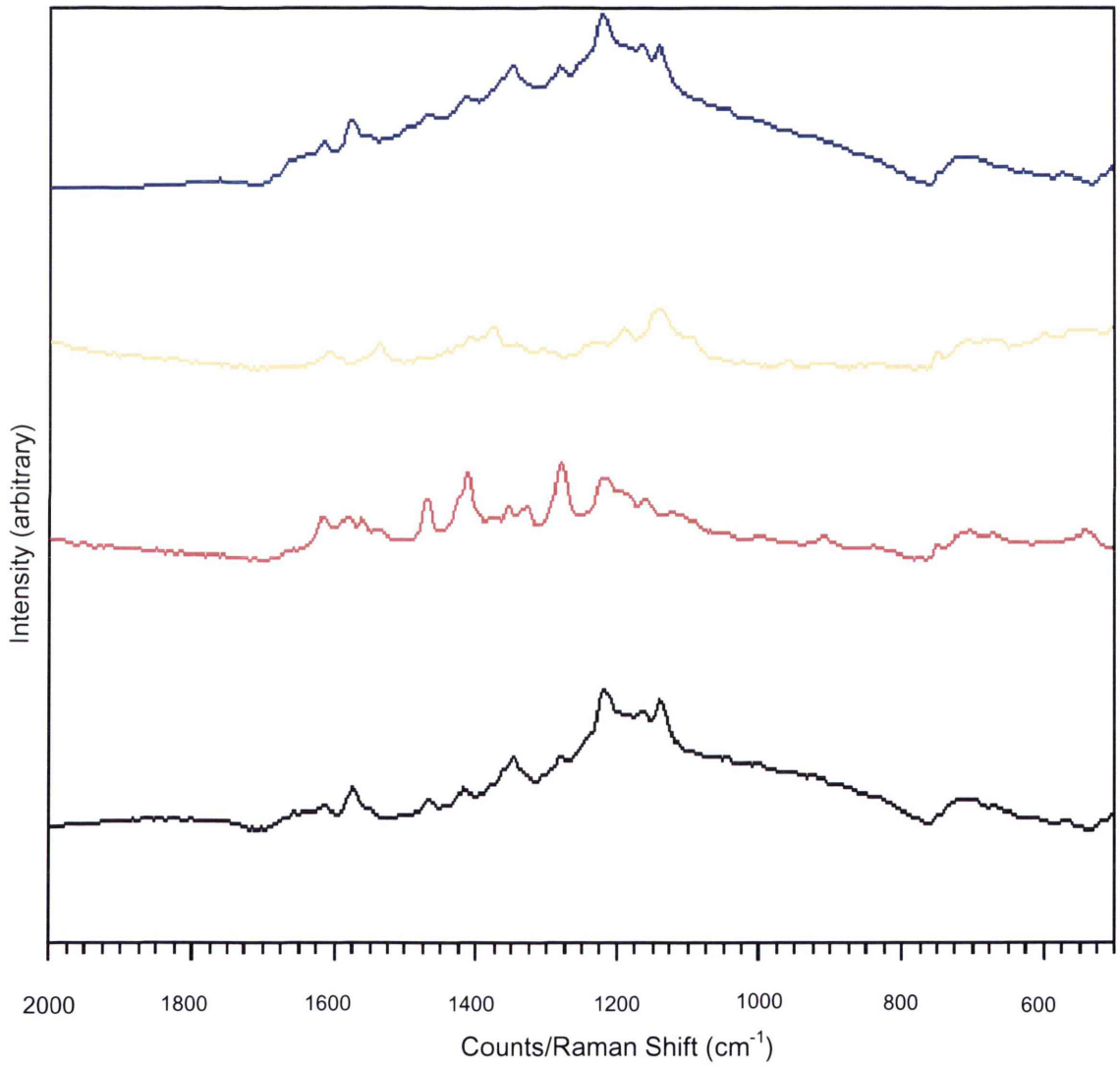


Figure 4-27 Raman spectra 685nm analysis of (from top) dyed fibre - Moroccan Blue, Synazol Gold-Yellow dye, Synazol Red dye and Sumifix Navy dye

The high powered NIR laser at 785nm gave excellent spectra for the dyes and dyed fibre (refer to Figure 4-28) as did the NIR laser at 830nm (refer to Figure 4-29). It is interesting to note that using the NIR lasers (ie 785nm and 830nm) there is a possible contribution of the yellow dye (in the 1150 cm-1 region) to the dyed fibre spectrum (see the red boxed sections on Figure 4-28 and Figure 4-29). There was a marked increase in the relative intensity of the spectra as the laser wavelength moved from the visible (514.5, 632.8 and 685 nm) to the NIR (785nm and 830nm) and a marked decrease in fluorescence. The spectrum of the navy blue dye is the major contributor to the dyed fibre spectrum at all laser wavelengths and the cotton substrate does not interfere as can be seen from the 785nm analysis of the dyed fibre, the blue dye and the raw cotton (Figure 4-30).

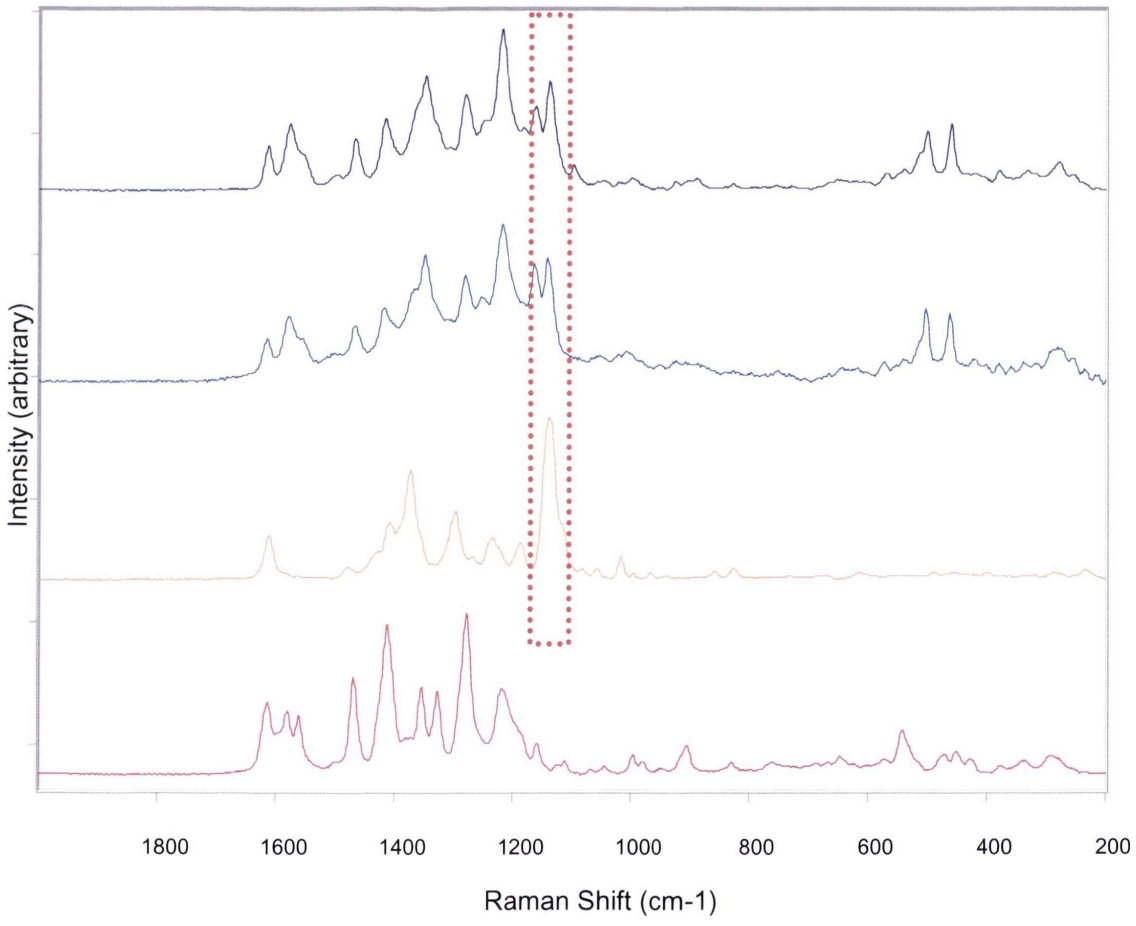


Figure 4-28 Raman spectra from 785nm analysis of (from top) dyed fibre - Moroccan Blue, Sumifix Navy dye, Synazol Gold-Yellow dye and Synazol Red dye

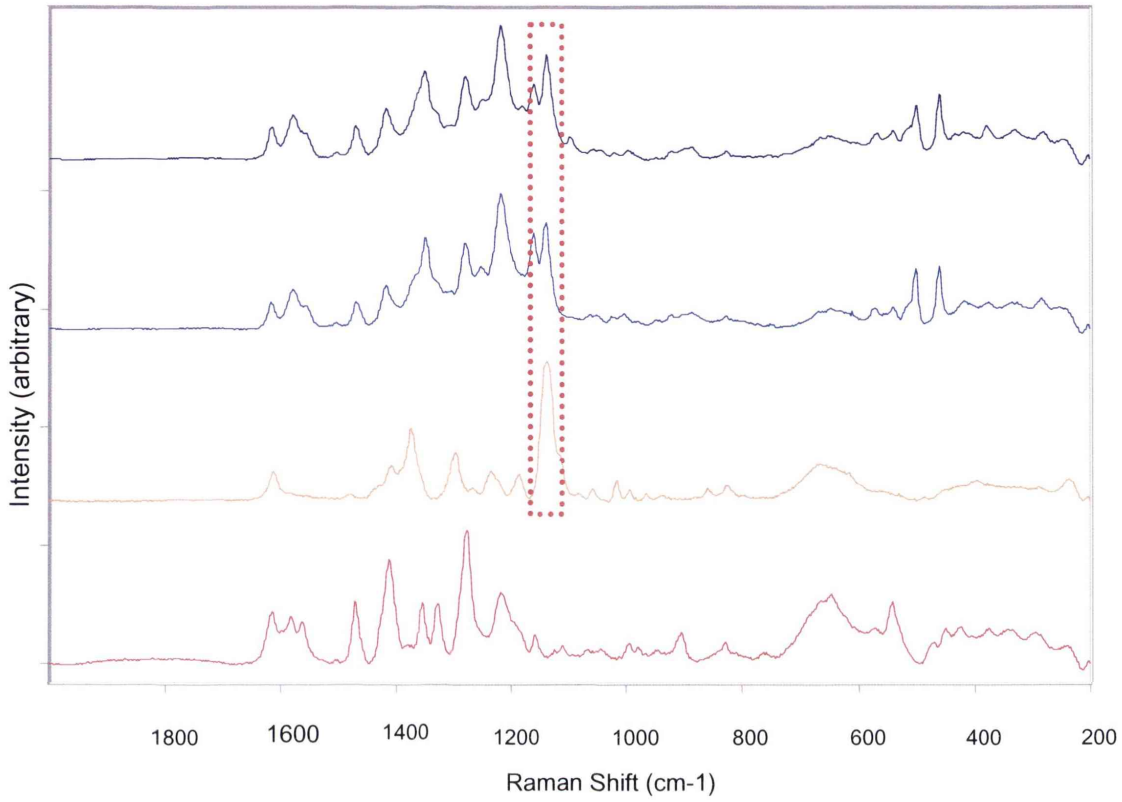


Figure 4-29 Raman spectra from 830nm analysis of (from top) dyed fibre - Moroccan Blue, Sumifix Navy dye, Synazol Gold-Yellow dye and Synazol Red dye

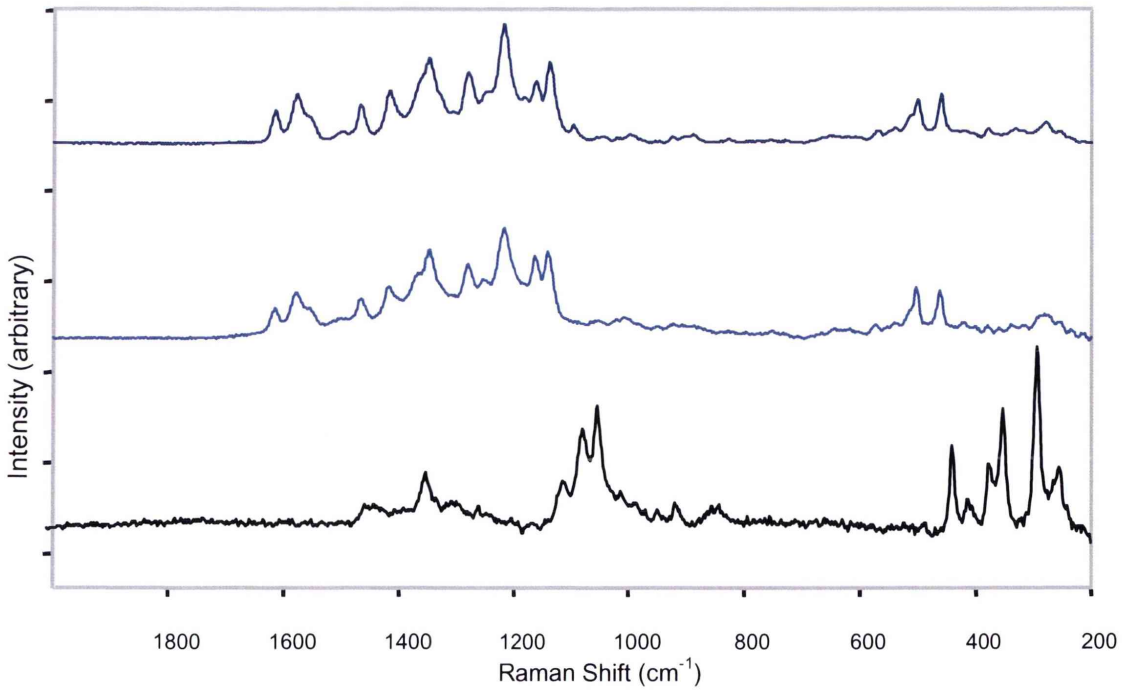


Figure 4-30 Raman spectra from 785nm analysis of (from top) dyed fibre – Moroccan Blue, blue dye - Sumifix Navy Blue and cotton

The dominance of the blue dye spectra observed at all laser wavelengths was surprising. It was expected that at least some spectral details from the cotton substrate would be present in the spectra of the dyed fibre as was seen by Kokot et al. in their study of red reactively dyed cotton fibres [110]. Jochem and Lehnert, however, found that strongly pigmented synthetic fibres resulted in a spectrum wholly attributed to the pigment [171] with no interference from the matrix when analysing with visible lasers. However, the opposite held true for their analysis with NIR laser which was found to be well suited for characterising the polymer but failed to provide useful data on the pigments. Jochem and Lehnert [171] also observed that for specific samples (not all) the dyed fibre spectrum was associated with only one pigment when two or three had been used and considered that it may have been due to selective resonance enhancement of the particular pigment.

The fact that the two visible laser wavelengths (632.8nm and 685nm) and the NIR lasers (785nm and 830nm) all show the domination of the blue dye in the spectrum of the dyed fibre would indicate that this is due, in the most part, to the concentration or native Raman intensity of the blue dye and not to any resonance enhancement of that dye.

Resonance enhancement occurs when the laser excitation wavelength is at or near to the wavelength of the coloured chromophore in the sample being analysed. This results in the selective enhancement of certain peaks in the Raman spectra of the sample. Dyes contain chromophores, delocalised electron systems with conjugated double bonds, and auxochromes, electron-withdrawing or electron-donating substituents that cause or intensify the colour of the chromophore by altering the overall energy of the electron system.

In this study, the spectrum of the navy blue dye component did vary significantly with laser wavelength because of resonance enhancement effects (different Raman bands are enhanced at different laser wavelengths). No resonance enhancement effects of the other two dye components were observed in the fibre spectrum, however, because the spectrum of the blue dye dominated the

spectrum of the dyed fibre at all excitation wavelengths. Thus the Raman spectrum of the fibre varied with laser wavelength, but in this particular case only because of variations in the spectrum of one dye component. It can be envisaged that in other cases, variations in the Raman spectrum of a dyed fibre could arise from simultaneous variations in more than one dye component as the laser wavelength is changed. In a simple example, the spectrum of a fibre could resemble that of one dye component at one excitation wavelength, but then appear more similar to another dye component at another laser wavelength.

Visual comparison of the spectra resulted in the following grading table (Table 4-4) which shows that laser wavelengths 785nm and 830nm provided the best spectra with the least interference by fluorescence with very similar spectral results.

Table 4-4 Summary of results from the various Raman laser wavelengths

Sample Name	514.5nm	632.8nm	685nm	785nm	830nm
Dyed Fibre	1	2	3	4	4
Synazol Gold-Yellow	4	2	3	4	4
Synazol Red	2	3	3	4	4
Sumifix Navy	3	4	3	4	4

1- no spectra due to fluorescence, 2- some spectral details with large amounts of fluorescence, 3- good spectral details with some fluorescence, 4- excellent spectral details with little to no fluorescence

4.5 Conclusions

Raman Spectroscopy is a non-destructive technique requiring only a small amount of sample and with very little sample preparation involved. These are highly desirable aspects in forensic science, and in particular fibre examinations. With respect to forensic fibre examinations this technique is still in its

preliminary stages of development, and for this reason it is important to explore various techniques and applications of Raman spectroscopy.

This situation poses a challenge to the forensic discrimination of dyed fibres using Raman spectroscopy, as in many cases there will be no single Raman spectrum for a given fibre or dye component. Therefore in situations where ease of analysis and comparison is required, the consistent use of a single NIR laser wavelength is strongly recommended, as this will reduce the likelihood of both fluorescence and resonance effects. In better equipped laboratories, the ability to collect several Raman spectra (at different excitation wavelengths) of a given sample might increase the potential for discrimination, particularly between very similar samples.

Various aspects of Raman spectroscopic analysis were investigated using a dyed fibre and its three dye components. The study observed the effects of the method of mounting the dye particles, spectral degradation, fluorescence, resonance enhancement and the general effects of changing the laser wavelengths for two Raman spectrometers (FORAM 685/2, and Renishaw RM1000 System).

It was determined that the best method for analysing dyes required the dilution of the dye in distilled water followed by drying on an aluminium slide to produce a smaller grain size. Spectral degradation was found to not be a concern for these samples for any laser wavelength or analysis time studied. The laser wavelength-dependent resonance enhancement of one dye component was observed, and due to the high concentration of this component in the dyed fibre, the spectrum of the fibre varied with laser wavelength accordingly. Fluorescence was minimised by changing the laser wavelengths, with NIR wavelengths minimising fluorescence as might be expected. Through visual comparison of the spectra it was determined that the NIR diode laser source at 785nm and the NIR laser source at 830nm provided superior results for the sample set investigated. Two laser wavelengths will be used in the next study one visible (632.8nm) and one NIR (785nm) to provide maximum information about the dye s used in colouration.

This study has shown that Raman spectroscopy can be used, in this case, to analyse reactively dyed cotton fibres without interference from the cotton substrate, thereby providing molecular information about the main dye used. However, caution must be taken in the choice of laser wavelengths, because of both fluorescence and the changes in sample spectra that can occur with laser wavelength. For these reasons, NIR laser excitation is recommended.

Chapter 5: Comparison of Raman Spectroscopy and Microspectrophotometry

5.0 Summary

Raman spectroscopy was investigated to determine the discriminatory power of the technique for a sample set of eleven black/grey and blue cotton samples as well as a set of 91 black and blue cotton fibres (of unknown dye types). This was then compared to the discriminatory power of Microspectrophotometry (MSP) for the same sample sets as well as the discriminatory power of the combined techniques. For the known sample set Raman spectroscopy provided a discrimination power of 0.873, MSP provided a discrimination power of 0.927 and the combined techniques provided a discrimination power of **0.982**. For the unknown sample set Raman spectroscopy provided a discrimination power of 0.799, MSP provided a discrimination power of 0.832 and the combined techniques provided a discrimination power of **0.962**.

The results from this study indicate that, as a single technique, MSP provides greater discrimination than Raman spectroscopy. However, because the samples were discriminated based on different aspects of the fibre (i.e. MSP-colour, Raman-molecular structure of the major dye) the combination of the two techniques provided the greatest discrimination. These results indicate that whilst Raman spectroscopy will not supercede MSP the introduction of the technique into the fibre examination sequence would be valid when analysing black/grey and blue cotton samples.

5.1 Introduction

Comparing the discrimination provided by Raman spectroscopy with that provided by Microspectrophotometry will assist in determining the usefulness of Raman spectroscopy as a technique for fibre examinations. The fibre examination process outlined in Chapter 1 is a relatively long procedure and for a new technique to be added to this process it must provide a degree of discrimination that warrants the additional time and expense. Within the fibre examination process the current techniques used for analysing colour are Microspectrophotometry and Thin Layer Chromatography (TLC)

Microspectrophotometry (as outlined in Chapter 1) provides a non-destructive and objective measurement of colour [180]. Studies have been conducted into the ability of microspectrophotometry to discriminate between similarly coloured fibres [181, 182] and these studies have clearly shown the effectiveness of this technique for discrimination. However, MSP provides little molecular information about the specific dye used [67] and, theoretically, the problem may arise where two fibres are dyed to the exact same shade using dyes of different molecular structure (i.e. chemically different).

Thin Layer Chromatography (TLC) is another method employed for analysing colour and is able to discriminate between chemically different dyes. However, it is semi/completely destructive and in the case of reactive dyes is an involved process [68]. TLC is particularly problematic when trying to extract reactive dyes from cellulosic fibres due to the covalent bonding between the dye and the cotton fibre substrate.

A study by Wiggins et al. [183] studied the discrimination provided by comparison microscopy, visible microspectrophotometry, UV microspectrophotometry and thin layer chromatography for a set of 29 black and 47 blue reactively dyed cotton fibres. Whilst a large proportion of all samples could be discriminated not all could be differentiated using the four techniques.

In the study, there were 406 pair-wise comparisons for the reactively dyed black cotton. After comparison microscopy 10 pairs matched. Visible microspectrophotometry reduced this to **8** matching sets, but UV microspectrophotometry and TLC could not reduce the matches further.

For the reactively dyed blue cotton samples there were 1081 pair-wise comparisons. After comparison microscopy 49 pairs matched. Visible microspectrophotometry reduced this to 19 with UV microspectrophotometry further reducing it to 16 matching pairs. TLC also reduced the 19 pairs matching after visible microspectrophotometry to the same **16** matching pairs identified after UV microspectrophotometry. The results from this study indicate that TLC does increase discrimination and that there is potential for further discrimination in these sample sets

Raman spectroscopy is able to provide molecular information of the dyes used non-destructively. This advantage means that Raman spectroscopy has great potential for the forensic examination of fibres. Several studies have already investigated the discrimination of Raman spectroscopy compared with other techniques. A summary of the samples and techniques used is provided in Table 5-1.

Table 5-1 References with fibre type and colour as well as the techniques used

Reference	Fibre Type	Colour	Comparison
Bourgeois & Church [168]	acrylic	red and blue	FT Raman and IR
Best et al. [140]	none	various pigments	Multiple and Raman
Miller et al. [111]	various	colourless	Raman and IR
Jochem et al. [171]	polyacrylonitrile viscose	various various	Raman and IR
Massonnet et al. [107]	cotton	green	Raman and UV/Vis MSP

5.1.1 Raman Spectroscopy for Pigments

Best et al. [140] produced a detailed analysis of the strengths and weaknesses of a variety of different possible techniques for pigment analysis. These techniques included; scanning electron microscopy (SEM), X-ray fluorescence (XRF), X-ray diffraction (XRD), PIXE (particle-induced X-ray emission), PIGE (particle-induced gamma-ray emission), infrared spectroscopy, ultraviolet/visible spectroscopy, optical microscopy and Raman spectroscopy. The results of the analysis are summarised in Table 5-2. The authors concluded that Raman microspectroscopy is the best single technique for the analysis of pigments. The advantages of Raman microspectroscopy are that it: is highly specific; is sensitive; has high spatial resolution (0.5-1.0 μm); is generally immune to interference and can be operated *in-situ*.

Table 5-2 Comparison of Raman spectroscopy with other techniques for the analysis of pigments [140]

Technique	*In situ*	Specificity	Sensitivity	Spatial Resolution	Immunity to Interference
SEM	no	bad	good	excellent	good
XRF	no	good	good	good	good
XRD	no	good	fair	poor	poor
PIXE/PIGE	yes	good	good	poor	good
Raman	yes	excellent	excellent	excellent	good
IR	yes	good	good	fair	bad
UV/VIS	yes	poor	good	fair	fair
Optical Microscopy	yes	bad	good	good	good

5.1.2 Raman Spectroscopy for Undyed Fibres

Miller and Bartick [111] analysed 70 fibres with Infra-red spectroscopy (IR) and Raman spectroscopy. Table 5-3shows some of the generic classes and subclasses of fibres analysed. This study shows that Raman spectroscopy is useful for the analysis of fibre substrates, and in the example of acetate analysed provides more discrimination than IR spectroscopy.

Table 5-3 Ability to use IR vs. Raman analysis in fibre class and subclass identification of some non-dyed fibres [111]

Fiber class	IR		Raman	
	Class	Subclass	Class	Subclass
Acetate	good	poor/difficult	good	good
Acrylic	good	good	good	poor
Modacrylic	good	good	good	good
Natural	good	good	good	good
Nylon	good	good	good	good
Polyester	good	good	good	good
Rayon	good	No subclass	good	No subclass
Spandex	good	good	good	good

5.1.3 Raman Spectroscopy and Coloured Man-made Fibres

Bourgeois and Church [168]found that the infrared spectra, of acrylic fibres, were dominated by intense polymer absorptions. By comparison the dye bands were very weak with insufficient detail to provide much useful information on the nature of the dye. The FT Raman spectra (1064nm laser wavelength) were much more revealing as the methyl acrylate residue of the polymer was relatively featureless in the FT Raman spectrum whilst spectral features attributable to the dye were quite prominent and readily distinguishable from the polymer spectrum.

Jochem and Lehnert [171] found that in the IR spectra only few and relatively weak pigment bands could be found. This is seen in their results for the deep blue fibre *Dolan 29* in Figure 5-1. The pigment bands are super-imposed and largely obscured, predominantly by the usually strong absorption of the fibre polymer matrix in the range from 1000 to 1800 cm^{-1}. In contrast, the corresponding Raman spectrum of the same fibre is dominated by signals of the pigments (Raman spectrum is inverted in Figure 5-1). This is due to high Raman scattering cross-section of typical dye molecules compared with typical polymers.

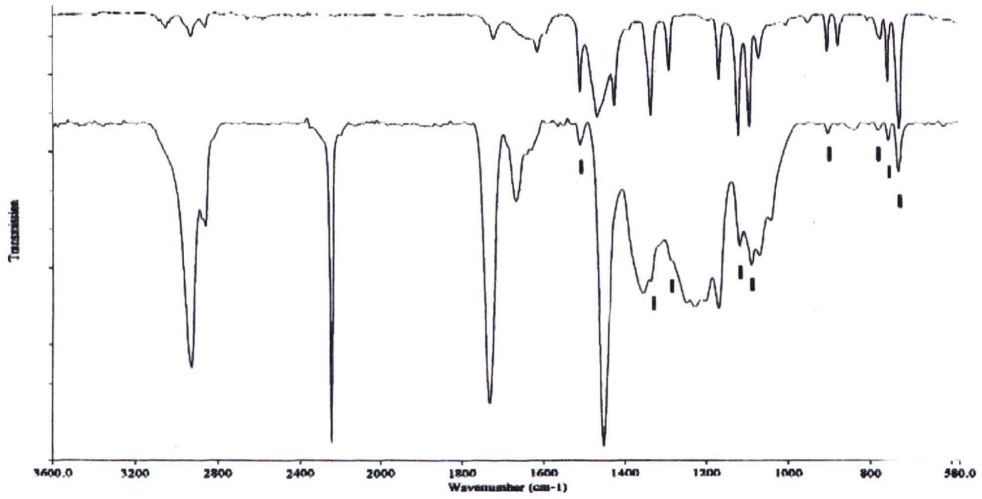


Figure 5-1 Comparison of a pigmented fibre by (top) Raman spectroscopy - spectra inverted and (bottom) IR spectroscopy [171]

5.1.4 Raman Spectroscopy and Dyed Natural Fibres

The most relevant study carried out, to date, was that of Massonnet et al. [107]. The authors analysed four green cotton fibres dyed with reactive dyes. Four dyes were used. Samples 1 and 2 were dyed with Dyes (i) and (ii) though with different ratios. Samples 3 and 4 were dyed with Dyes (iii) and (iv) also in different ratios. The authors then compared the discrimination provided by Raman spectroscopy (from nine different laser wavelengths) to that provided by Visible-Microspectrophotometry.

Discrimination of the two sample sets (samples 1 and 2 discriminated from samples 3 and 4) was easily achieved with both techniques. Within the same colour group (S1/S2)/(S3/S4), Raman spectroscopy had difficulties in differentiating samples based on intensity variations only; however cotton bands, when present, helped to discriminate these pairs of samples. Microspectrophotometry analysis found more intensity variations observed between S3/S4 than S1/S2; however, inter-laboratory variations observed (spectral intensity) for both techniques. It was observed that dye identification was easier with Raman spectroscopy than with microspectrophotometry.

The above studies demonstrate the necessity of comparing Raman spectroscopy with other techniques for the particular sample set to be investigated. With respect to microspectrophotometry the study by Massonnet et al. [107] clearly shows that discrimination by this technique is through differences in colour and that Raman spectroscopy discriminates based on the molecular structure of the dye (and the cotton substrate). Comparing these techniques for the sample set in this study is necessary to determine if Raman spectroscopy will provide a higher level of discrimination

5.2 Aims

This chapter aims to examine a known sample set of cotton fibres and their respective dye components as well as an unknown sample set of cotton fibres to determine the discriminatory power of Raman spectroscopy compared to the discriminatory power of MSP. TLC was not attempted on the sample set due to the inherent problems and the destructive nature of extracting reactive dyes from cotton fibres.

The known sample set comprised of cotton fibres dyed with known combination of dyes (though concentration of dyes used was not known). This sample set was

used as to not only determine the discrimination provided by both techniques but as a further investigation into the effect of laser wavelength (from Chapter 1).

The unknown sample set comprised of dark coloured cotton samples collected randomly. This sample set was used to not only determine discrimination provided by the techniques but to also investigate the types of Raman spectra observed in the general population of dark coloured cottons.

5.3 Materials and Methods

5.3.1 General

Eleven (11) black/grey and blue cotton samples were analysed using Raman spectroscopy and Visible-Microspectrophotometry. The resultant spectra for each technique were grouped based on similarities (and absence of differences) to determine the discrimination of each technique for this sample set. The two techniques were combined (in a sequential manner) and the discrimination of the combined techniques was also determined.

Analysis was also conducted on random black and blue cotton samples collected by Langdon [25]. These samples had unknown dye classes and dye combinations. Raman analysis of this sample set was compared to the UV-Vis MSP results achieved by Langdon [25].

5.3.2 Samples

5.3.2.1 Known sample set

The sample set consisted of eleven (11) dyed black/grey and blue cotton fibres of different (reactive) dye combinations from Rocklea Spinning Mills Pty Ltd, Melbourne (Australia). The dyed fibres were Australian cotton with shade

names and colours attributed by the manufacturer. The sample numbers and shades are shown in Table 5-4.

Table 5-4 Sample numbers and shade (assigned by manufacturer)

Sample Number	Shade (attributed by manufacturer)
1	blue
14	blue
18	blue
20	blue
25	black
31	black
32	grey
35	grey
38	black
39	black
40	black

Samples were not separated based on manufactures shade name as some black samples appeared blue at a microscopic level. This is not uncommon for black fibres as they may actually be dyed a shade of blue that appears black to the naked eye, this is especially so for reactively dyed black fabrics [20].

The dye components for each shade are listed in Table 5-5. The relative concentrations of the dyes were unknown.

Table 5-5 Sample numbers and their dye components

Sample No.	Dye 1 (Yellow)	Dye 2 (Red)	Dye 3 (Blue/Black)	Dye 4 (Blue/Black)
1	Synazol Gold-Yellow HF 2GR	Synazol Red HF 6BN	Sumifix Navy Blue BF	-
14	Synazol Gold-Yellow HF 2GR	Synazol Red HF 6BN	-	Synazol Black B
18	-	Sumifix Scarlet 2GF	Everzol BR Blue R	Sumifix Supra Blue BRF
20	-	Synazol Red HF 6BN	Everzol BR Blue R	-
25	-	-	-	Synazol Black B
31	Synazol Gold-Yellow HF 2GR	Synazol Red HF 6BN	-	Synazol Black B
32	Cibacron Orange LSBR	Cibacron Red LSB	Cibacron Blue LSBR	-
35	Cibacron Orange LSBR	Cibacron Red LSB	Cibacron Blue LSBR	-
38	Lanasol Yellow CE	-	Lanasol Navy CE	Cibacron Navy LSN
39	Cibacron Orange LSBR	Cibacron Red LSB	-	Cibacron Black LSN
40	Synazol Gold-Yellow HF 2GR	Synazol Red HF 6BN	Synazol Black HF GRP	Synazol Black B

In all examinations the fibre samples were examined in their original state. The samples were mounted on double sided tape with direct contact with the laser (i.e. no covering) for Raman spectroscopic analysis. The dye samples were analysed by diluting the samples and allowing them to dry to a thin film on an

aluminium slide. The samples were mounted on a glass slide with glass coverslip and Hystomount for Visible-MSP analysis.

5.3.2.2 Unknown sample set

This sample set consisted of 100 samples collected from cinema seats by Langdon [25]. Langdon analysed these fibres by UV-Vis Microspectrophotometry and the fibres and the UV-Vis MSP results are used in this study for comparison purposes. The following information is a summary of the samples from Langdon [25]:

> "Extraneous fibres were collected from ten cinema seats in Cinema 3 at Pacific Cinemas Tuggeranong (Canberra, ACT). In this cinema the seats were arranged either side of a single centre aisle. Seats were selected from each side of the centre from five rows in the middle of the cinema. Each of these seats was two in from the centre aisle. The cinema seats were divided into eight sections, the seat into quarters and the seat base into quarters. Each section was then tape lifted with a piece of Scapa crystal 1250 tape, which was then placed onto a clear overhead transparency. The eight tapes from the one seat were placed onto the one overhead.
>
> Each tape was then divided into a 1cm grid and each square individually numbered. A set of random numbers was generated using the webpage http//www.randomizer.com and these were used to select a random order of the 1 cm squares on the tape. Each selected square was then searched in order for both black and blue cotton fibres. Both colours were selected since black cotton, when present as single fibres, can appear to be dark blue in colour and therefore difficult to objectively separate the two colours during selection. If no blue or black cotton fibres were present in a selected square the next square was searched. This procedure was repeated until 100 blue or black cotton fibres were collected."

After MSP analysis these samples were stored in XAM on glass slides (under glass coverslips). The fibre samples were removed for Raman analysis using

xylene. The samples were cleaned in xylene, dried, and then mounted on double sided tape. Nine of the one hundred samples had undergone some form of degradation during storage and were not used for the subsequent Raman analysis. The results achieved by Langdon [25] were all reclassified before comparison with the Raman spectra.

5.3.3 Raman Microprobe Conditions

The Raman instrument used in this study was the Renishaw Raman spectrometer System RM1000 (Renishaw Plc, Wotton-under-Edge, UK). An x50 objective (numerical aperture of 0.75 and a working distance of 0.37 mm) was used for measurements. The microspectrometer was operated non-confocally at an excitation of wavelength of 632.8 and 785nm (300 mW). Laser power varied between 50% and 100% depending on intensity of spectra and also any evidence of spectral degradation. The accumulated exposure time of the CCD detector was between 10 and 120 seconds and the instrumental spectral resolution ranged from 1.5 to 2.5 cm^{-1}.

5.3.4 Microspectrophotometer Conditions

5.3.4.1 Known sample set

The microspectrophotometer was a SEE2100 equipped with GRAMS/32 software (Galactic Industries Corporation). A dry 50x objective was used for measurements. The microspectrophotometer was operated, in transmission mode, across the wavelength range of 400nm to 850nm with a spectral resolution of 5nm.

5.3.4.2 Unknown sample set

The following conditions were used by Langdon [25] for the UV-Vis MSP analysis of the samples collected from the cinema seats:

"The selected fibres were removed from the tapes with fine tweezers and mounted separately on quartz slides using a mix of glycerine and water. These fibres were then analysed using the SEE 2100 Microspectrophotometer (x15 objective, 12μm aperture, range 200nm to 2100nm). The percentage of light transmitted by each fibre was measured ten times at ten different locations along the fibre. This was done for both the visible and the UV regions and the ten measurements in each region were then averaged. Adding the average of the visible region to the average of the UV region produced the final spectrum."

5.3.5 Experiments

5.3.5.1 Known sample set

Raman spectroscopy analysis of fibres and dye components

During the analysis of samples to determine optimal wavelength (Chapter 4) it was observed that the Raman analysis of the dyed fibre resulted in a spectrum wholly attributed to the navy blue dye component. Through investigation of resonance effects it was determined that this was due not to any resonance that may have occurred but rather was due to the concentration of the blue dye. This experiment sought to analyse the eleven samples and their respective dye components to see if this phenomena was observed over the whole sample set. Of particular interest are the lightly coloured (and hence lower concentration of dye) grey fibres.

The fibre samples were mounted on double-sided tape allowing for direct laser contact with the 785nm and 632.8nm lasers. The resultant spectra were then used in determining the discrimination provided by Raman spectroscopy for the sample set.

Microspectrophotometry analysis

The dyed fibres were mounted on glass slides with Hystomount. Analysis was in transmission mode over the visible range. Spectra were examined visually to determine similarity (and differences) in order to investigate the discrimination provided by microspectrophotometry for the sample set (refer to Equation 6).

Comparison of MSP and Raman spectroscopy results

The resultant spectra were visually examined and grouped based on similarities (and absence of differences) for each technique (Raman and Vis-microspectrophotometry) and for the combined techniques. The discriminatory power was then calculated (refer to Equation 6).

5.3.5.2 Unknown sample set

Raman spectroscopy analysis of fibres

The samples provided by Langdon [25] were demounted using xylene and remounted on double-sided tape allowing for direct laser contact with the 785nm laser. The resultant spectra were then compared visually and the discrimination of Raman spectroscopy was determined for the sample set (refer to Equation 6).

Microspectrophotometry analysis

Microspectrophotometry in the UV and Visible range was carried out by Langdon [25]. All spectra were re-examined by the author and grouped based on visible comparison to determine discrimination provided by UV/Vis MSP for the sample set(refer to Equation 6).

Comparison of MSP and Raman spectroscopy results

After classification by microspectrophotometry the samples were further classified by Raman spectroscopy and the discrimination of the combined techniques determined (refer to Equation 6).

5.3.6 Data Treatment

5.3.6.1 Known and unknown sample sets

Raman Spectra

Analysis of Raman results was carried out through visual examination of the spectra. For ease of comparison, all spectra were baseline corrected using the software specific to the instrument (GRAMS/32): piecewise polynomial; 2nd order polynomial and 4th order polynomial functions were utilised, depending on general shape of spectra.

MSP Spectra

Analysis of MSP results was carried out through a visual examination of the spectra (aided by the GRAMS/32 software) and took into account; [57]

- general shape
- peak positions
- presence or absence and shape of shoulders
- troughs and plateaus

Discrimination Power

The discriminatory power (DP) of each technique (and of the combined techniques) was determined by applying the following calculation (Equation 6):

$$DP = \frac{\text{(number of discriminated pairs)}}{\text{(number of possible pairs)}}$$

Equation 6 Calculation for Discrimination Power

5.4 Results and Discussion

5.4.1 Known Sample Set

5.4.1.1 Raman spectroscopy analysis of fibres and dye components

Raman analysis of the samples and their respective dye components indicated that either the blue or the black dye was the only contributor to the spectrum of the dyed fibre for both laser wavelengths. There were two exceptions to this observed trend being the grey samples 32 and 35 at 785nm laser wavelength.

An example of dominance by a black dye (Synazol Black B) is given below in (Figure 5-2) for the 785nm analysis and in Figure 5-3 for the 632.8nm analysis

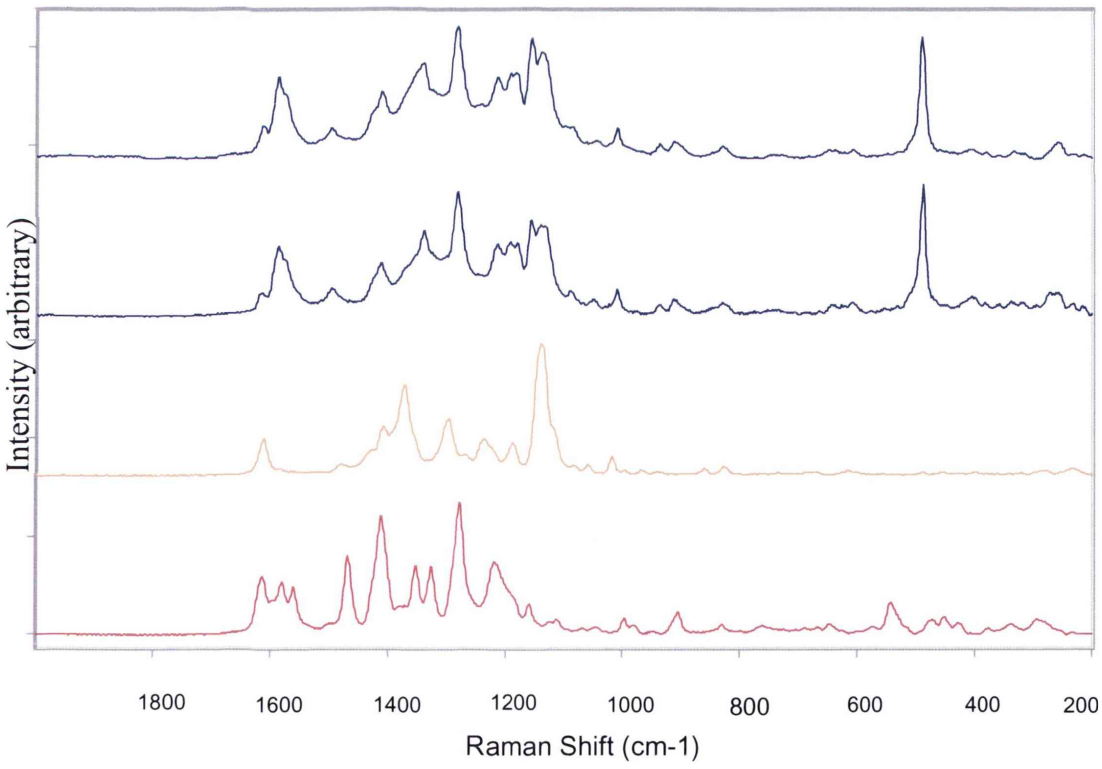


Figure 5-2 Raman spectra (785nm laser) of (top) sample 31 and its three component dyes Synazol Black B (2nd), Synazol Gold-Yellow HF 2GR (3rd), and Synazol Red HF 6BN (bottom)

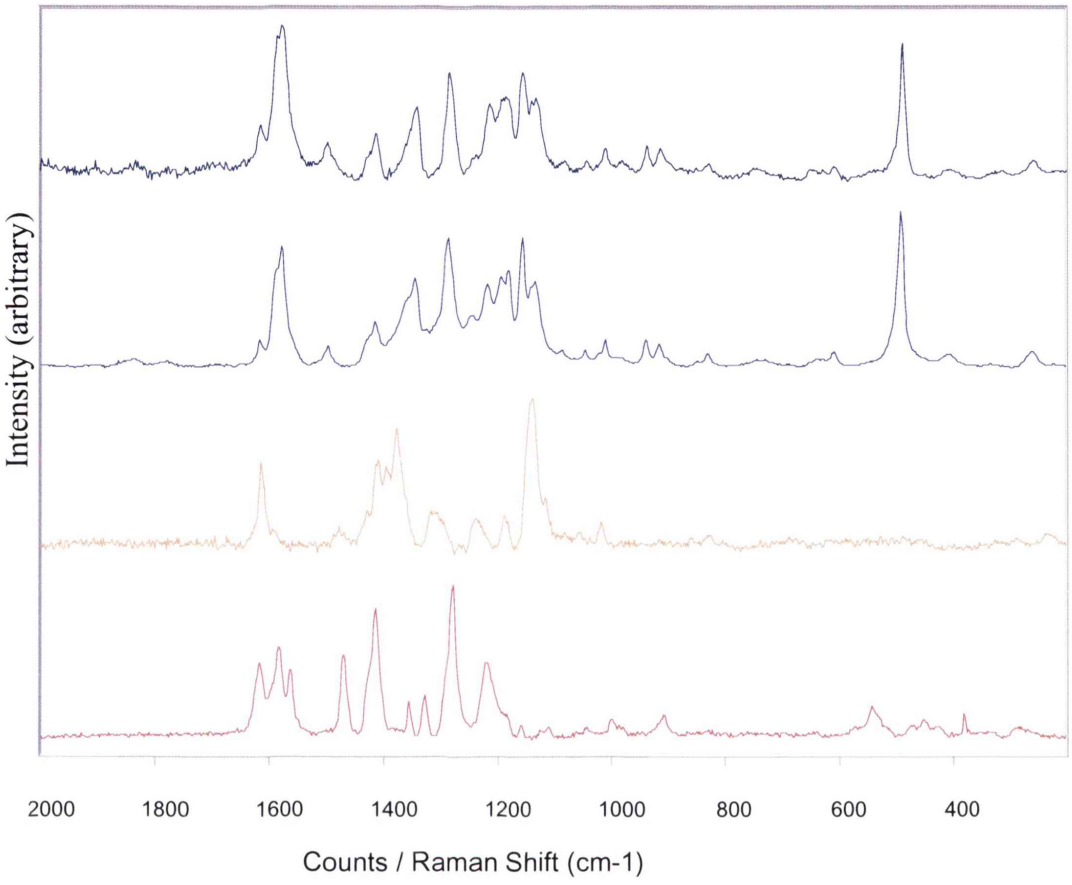


Figure 5-3 Raman spectra (632.8nm laser) of sample 31 (top) and its three component dyes Synazol Black B (2nd), Synazol Gold-Yellow HF 2GR (3rd), and Synazol Red HF 6BN (bottom)

Samples that were dyed with the same black dye exhibited similar spectra. Figure 5-4 shows the spectra of three dyed fibres (sample 14, sample 25 and sample 40) for 785nm and Figure 5-5 for the 632.8nm laser. Each of these fibres had different dye combinations (see Table 5-5 Sample numbers and their respective dyes). Common to all samples was Synazol Black B which can be seen to be the dominant contributor to all three sample spectra. These samples could not be discriminated visually from each other. This was a similar result to that in Chapter 4 with the dominance observed of the blue dye. In the above samples the reasoning for the observed dominance is the same as that reached in Chapter 4: that the concentration of the black dye resulted in the spectrum of the black dye swamping any contribution of the minor dye inclusions.

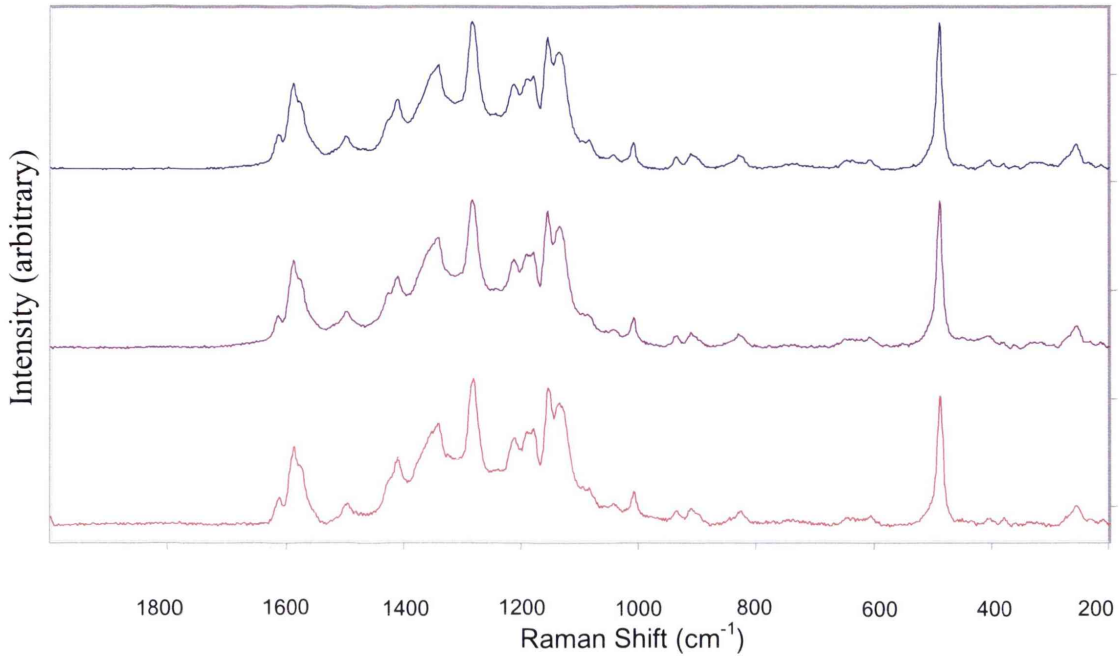


Figure 5-4 Raman spectra (785nm laser) of (top) sample 14, (middle) sample 25 and (bottom) sample 40 - each containing dye Synazol Black B

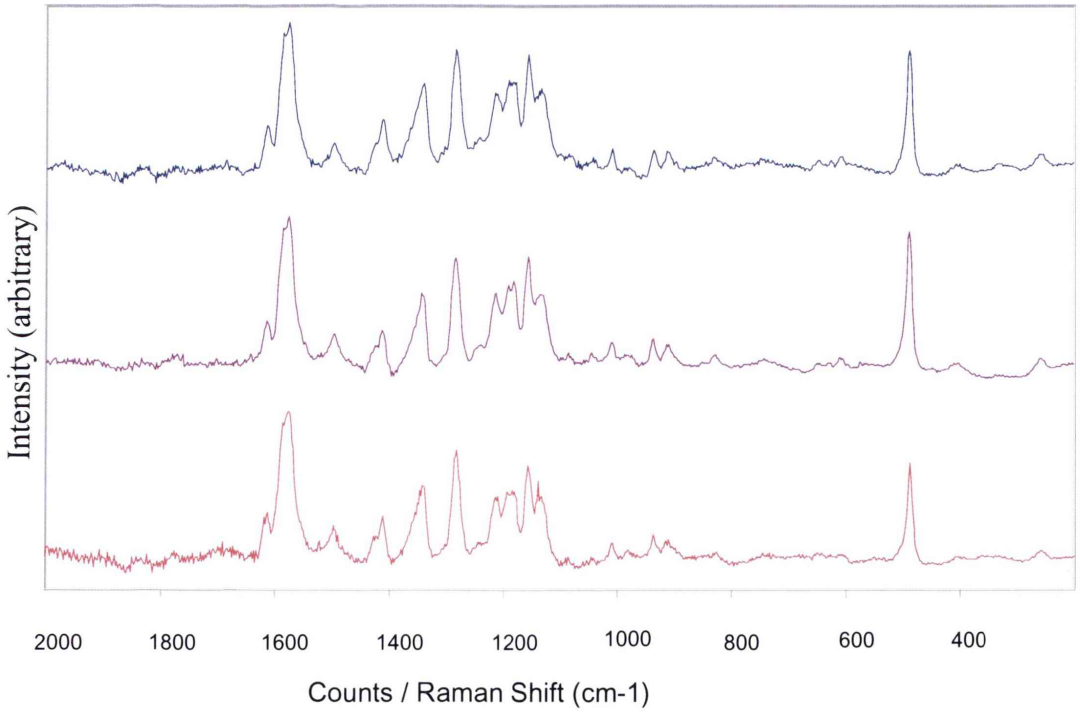


Figure 5-5 Raman spectra (632.8nm laser) of (top) sample 14, (middle) sample 25 and (bottom) sample 40 - each containing dye: Synazol Black B

Samples 38 and 39 could not be discriminated even though dye combinations were different for the two samples. It was observed that the major dye components, whilst named differently, (Cibacron Navy LSN and Cibacron Black LSN), exhibited Raman spectra that were visually similiar. The Raman spectra for the two dyes are shown in Figure 5-6 for the 785nm laser analysis and for the 632.8nm laser analysis.

This highlights the fact that dyes can have similar spectra that result from dominating Raman scattering vibrations of a major dye component of that fibre, but, the dyes themselves can have different colours. This indicates that molecular structures of dyes need not be significantly different to be named differently by a single manufacturer.

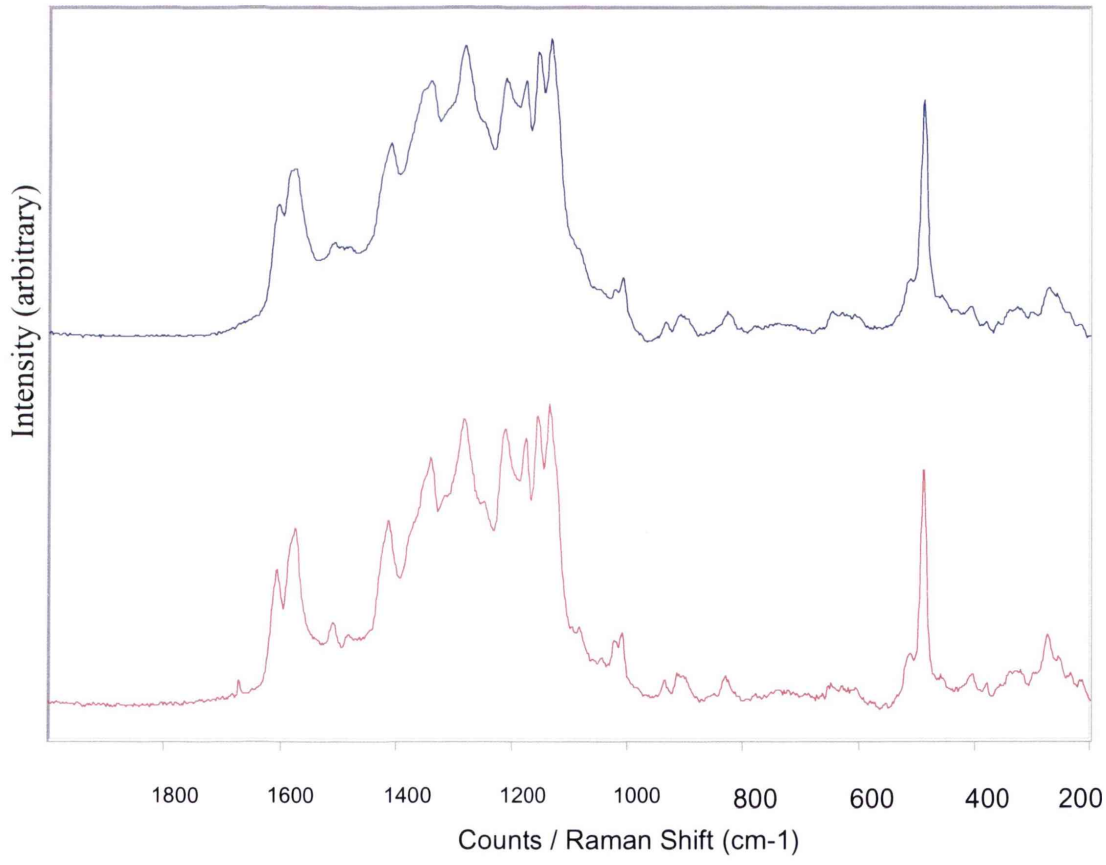


Figure 5-6 Raman spectra (785nm laser) of Cibacron Navy LSN (top), and Cibacron Black LSN (bottom)

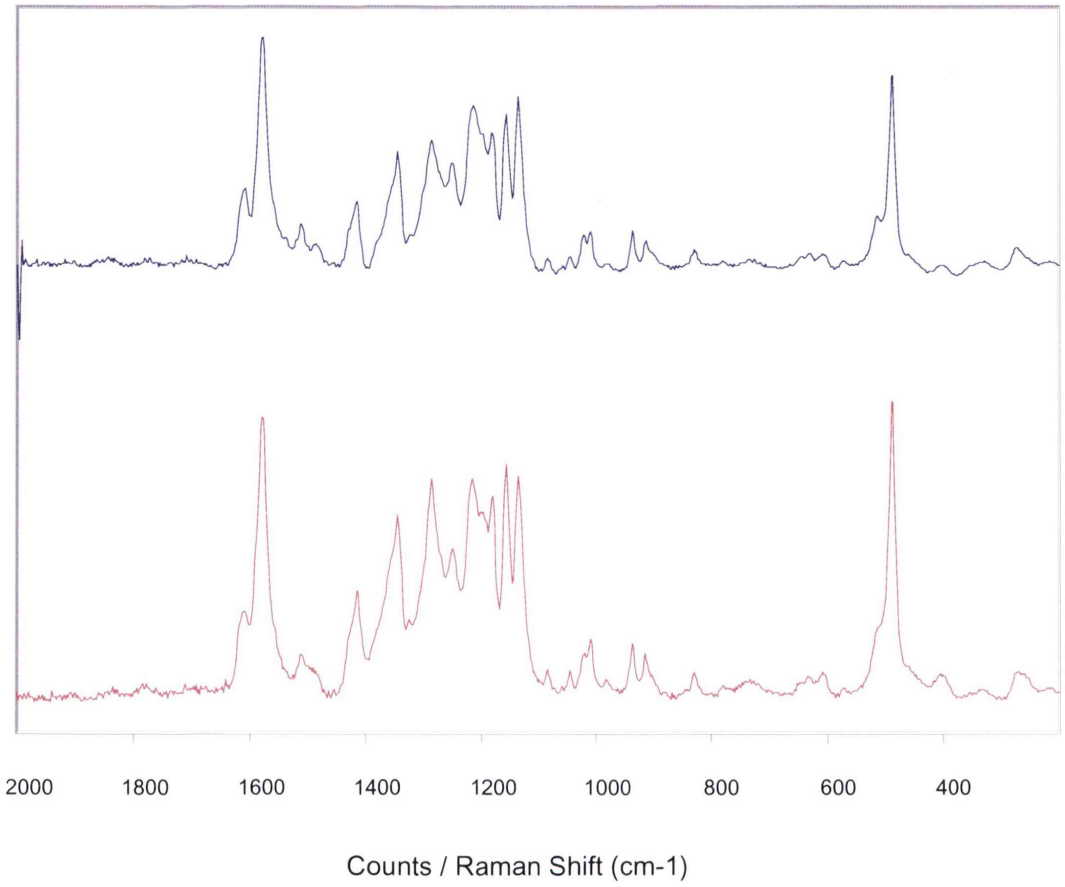


Figure 5-7 Raman spectra (632.8nm laser) of Cibacron Navy LSN (top), and Cibacron Black LSN (bottom)

Grey samples 32 and 35

The two grey samples (32 and 35) analysed had the same dye components and it was expected that they would not be differentiated visually from each other. All other samples in the sample set showed spectral contributions only from the major black or blue dye. However, the first observation for samples 32 and 35 was that the two samples had spectral contribution also from the orange dye (Cibacron Orange LSBR). This contribution of the orange dye in the grey samples was attributed to the lower concentration of blue used to achieve the lighter grey shade. Not all peaks from the orange dye were evident in the dyed

fibre spectrum and the blue dye was the most significant contributor. Figure 5-8 shows the results for sample 32 with its dye components. The boxed area shows sections of spectral contribution from the orange dye to the dyed fibre spectrum of sample 32.

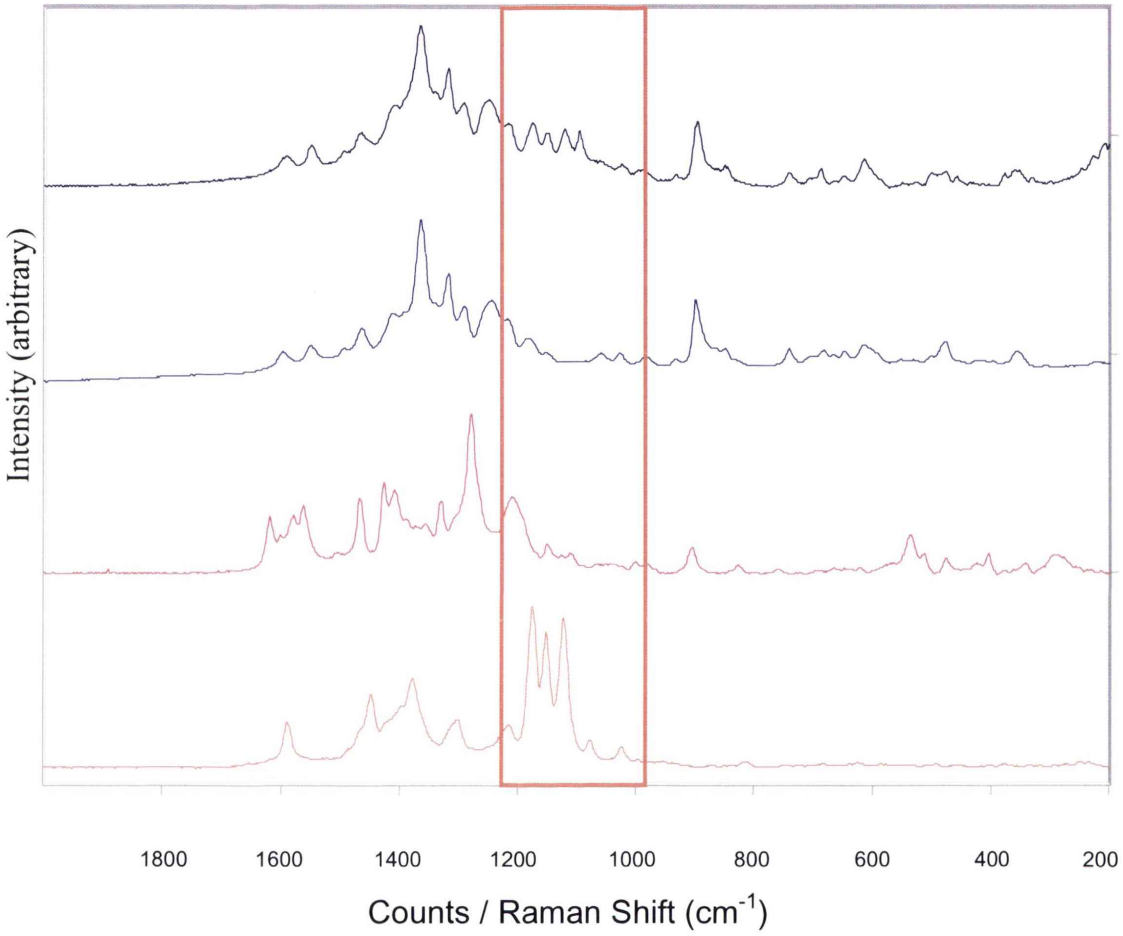


Figure 5-8 Raman spectra (785nm laser) of (top) sample 32, (2nd) Cibacron blue LSBR, (3rd) Cibacron Red LSB, and (bottom) Cibacron Orange LSBR

Figure 5-9 shows the results for sample 35 with its dye components. The boxed area shows sections of spectral contribution from the orange dye to the dyed fibre spectrum of sample 35. Sample 35 shows evidence of another spectral contributor as peaks in the 300-500 cm^{-1} region are not from any of the dyes.

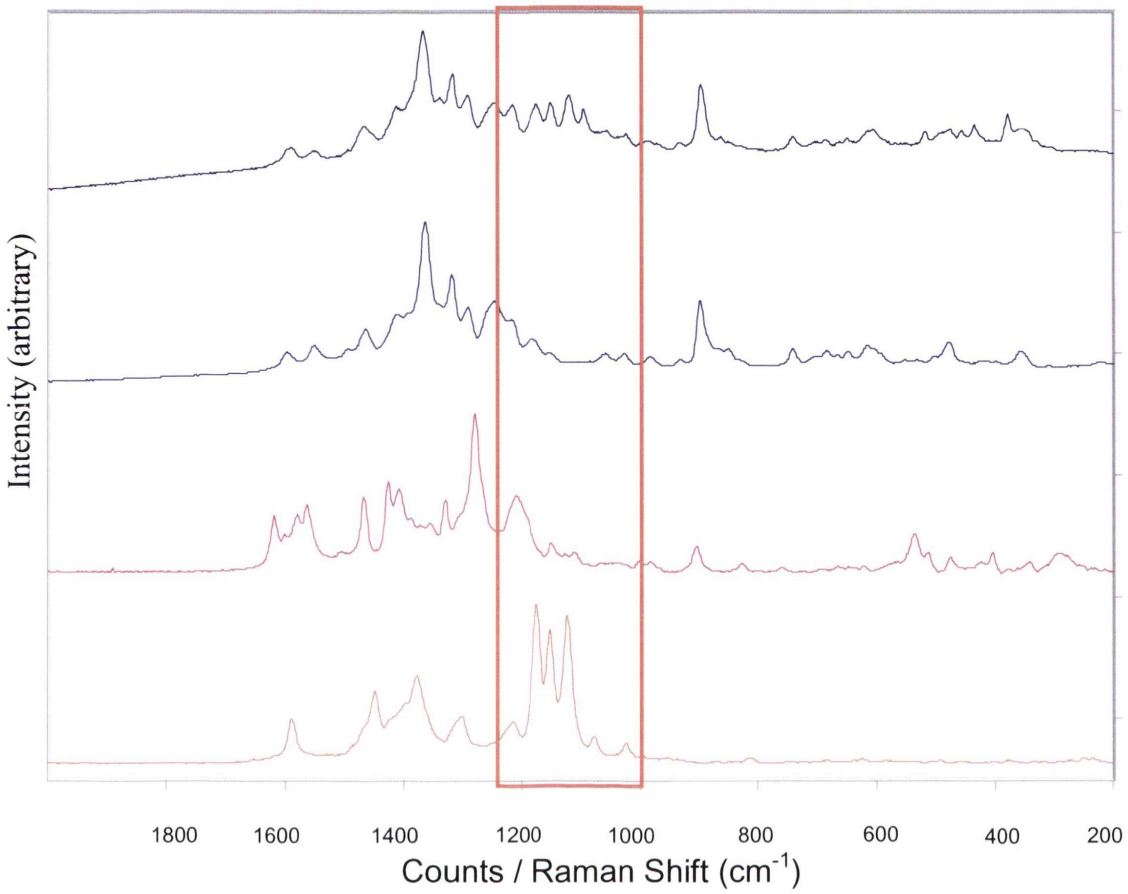


Figure 5-9 Raman spectra (785nm laser) of (top) sample 35 (2nd) Cibacron blue LSBR (3rd) Cibacron Red LSB, and (bottom) Cibacron Orange LSBR

Comparing sample 32, sample 35 and cotton shows that the extra peak contribution in sample 35 (Figure 5-10) are from the cotton substrate. The presence of these cotton peaks meant that sample 32 and sample 35 could be discriminated even though they had the same dye components. These results are corroborated by the study conducted by Massonnet et al. [107] where authors were able to discriminate between samples of same dye combinations but different ratios by the presence (and absence) of cotton peaks. The implications of these results and those of Massonnet et al. [107] is that lighter coloured fibres may show even further discrimination with Raman spectroscopy as variations due to other dyes present may be observed.

These results also corroborate the theory that the dyed fibre samples that exhibited spectra that could only be attributed to the one major dye was due to the concentration of the major black or blue dye.

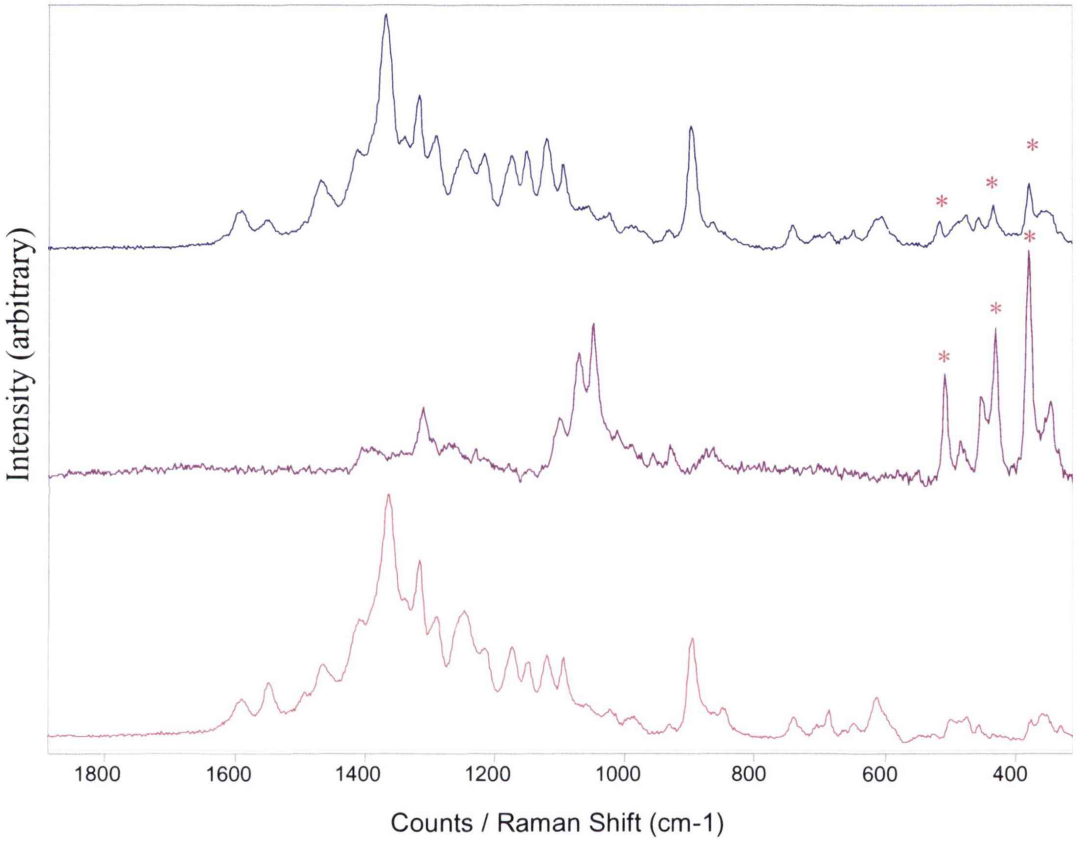


Figure 5-10 Raman spectra (785nm laser) of (top) sample 35, (middle) cotton, and (bottom) sample 32

The contribution of the orange dye observed in the spectrum of samples 32 and 35 was not observed when analysing with the 632.8nm laser (refer to Figure 5-11 and Figure 5-12) nor were the cotton peaks observed in sample 35 when analysing with the same laser wavelength. This means that the visible laser wavelength of 632.8nm laser was unable to discriminate between these two samples. This further emphasises the need to explore multiple wavelengths for the sample set being examined.

Discrimination of the entire sample set at the two laser wavelengths is investigated in the following sections.

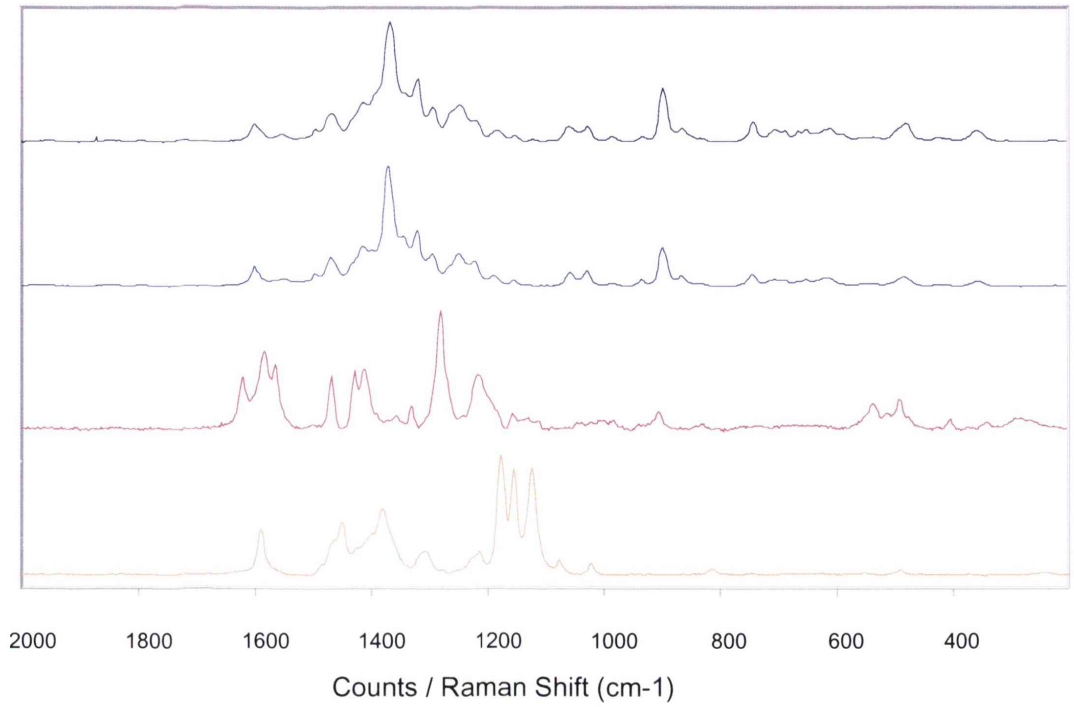


Figure 5-11 Raman spectra (632.8nm laser) of (top) sample 32, (2nd) Cibacron blue LSBR, (3rd) Cibacron Red LSB, and (bottom) Cibacron Orange LSBR

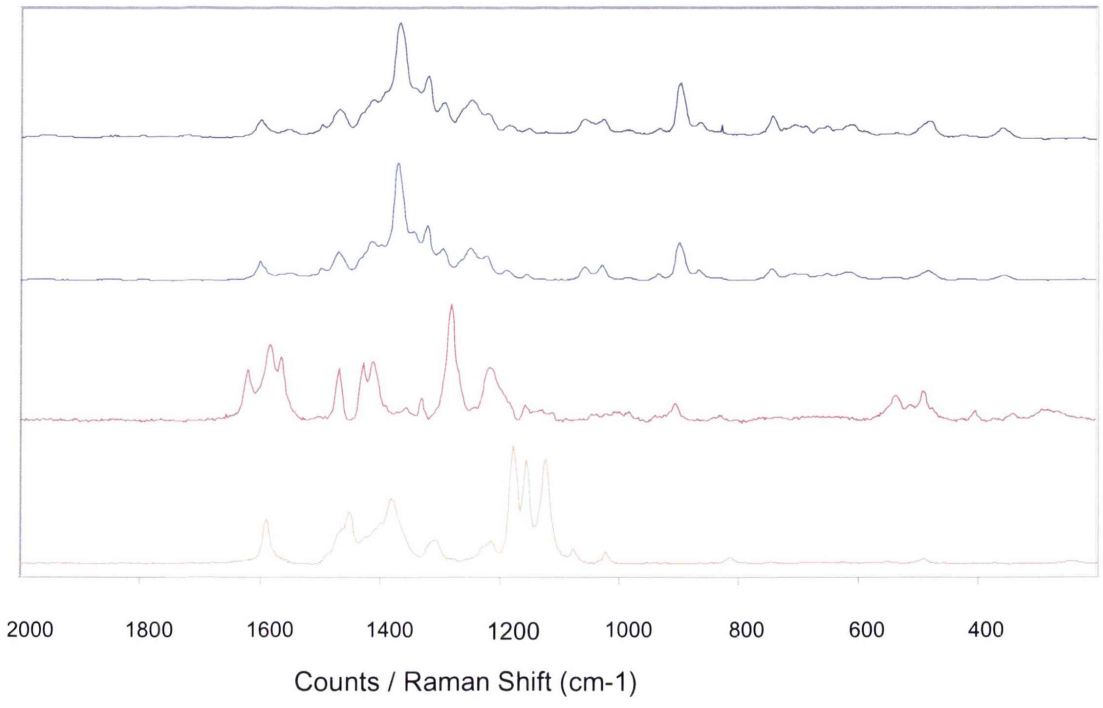


Figure 5-12 Raman spectra (632.8nm laser) of (top) sample 35, (2nd) Cibacron blue LSBR, (3rd) Cibacron Red LSB, and (bottom) Cibacron Orange LSBR

Discrimination of sample set by Raman spectroscopy - 785nm laser

Raman spectroscopy analysis using the 785nm laser allowed for the samples to be classified into six groups (refer to Figure 5-13 to Figure 5-17). Five groups showed distinct spectra associated with the dyes used and the sixth group was composed of two samples that fluoresced. The two samples that fluoresced (samples 18 and 20) had the same major dye component that also fluoresced using this laser wavelength. Analysis of all of the component dyes for the samples was carried out and the results are at Appendix1 – Library of Reactive Dyes along with other dyes analysed.

The 11 samples produce 55 pair-wise comparisons and after 785nm Raman spectroscopy analysis there were 7 pairs left not discriminated. The discrimination power (DP) of 785nm Raman spectroscopy analysis for this sample set is 0.873.

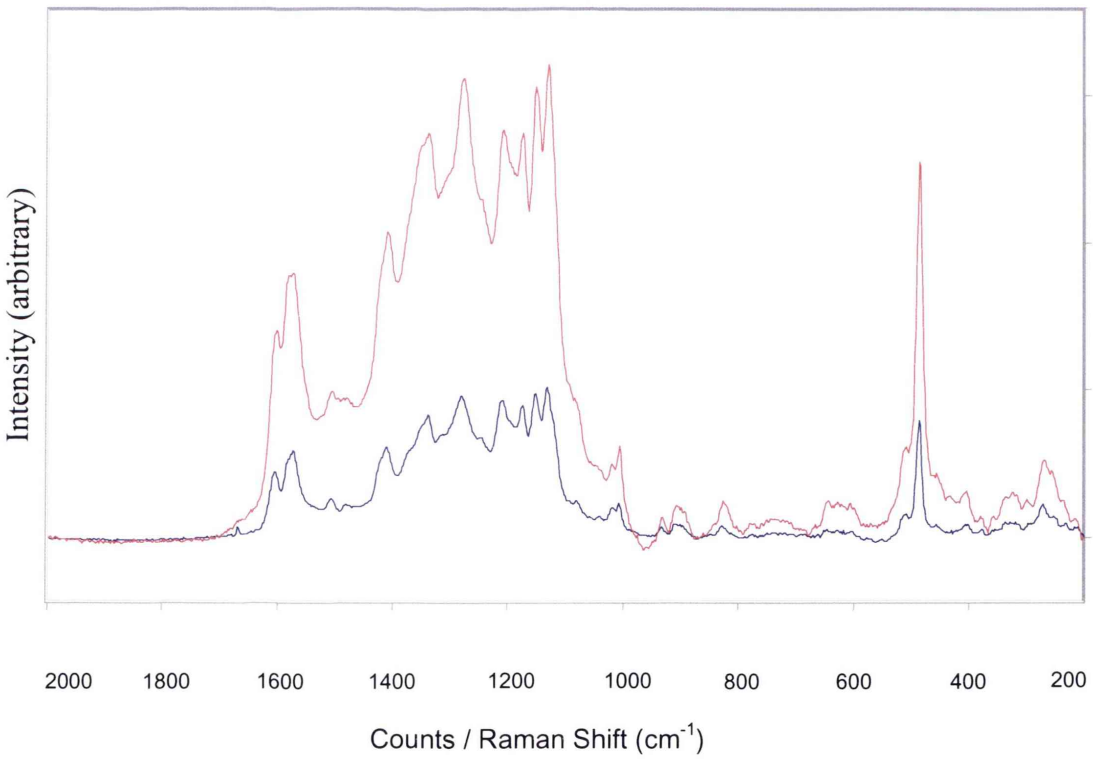


Figure 5-13 Raman spectra (785nm laser) of (red) sample 39, and (blue) sample 38.

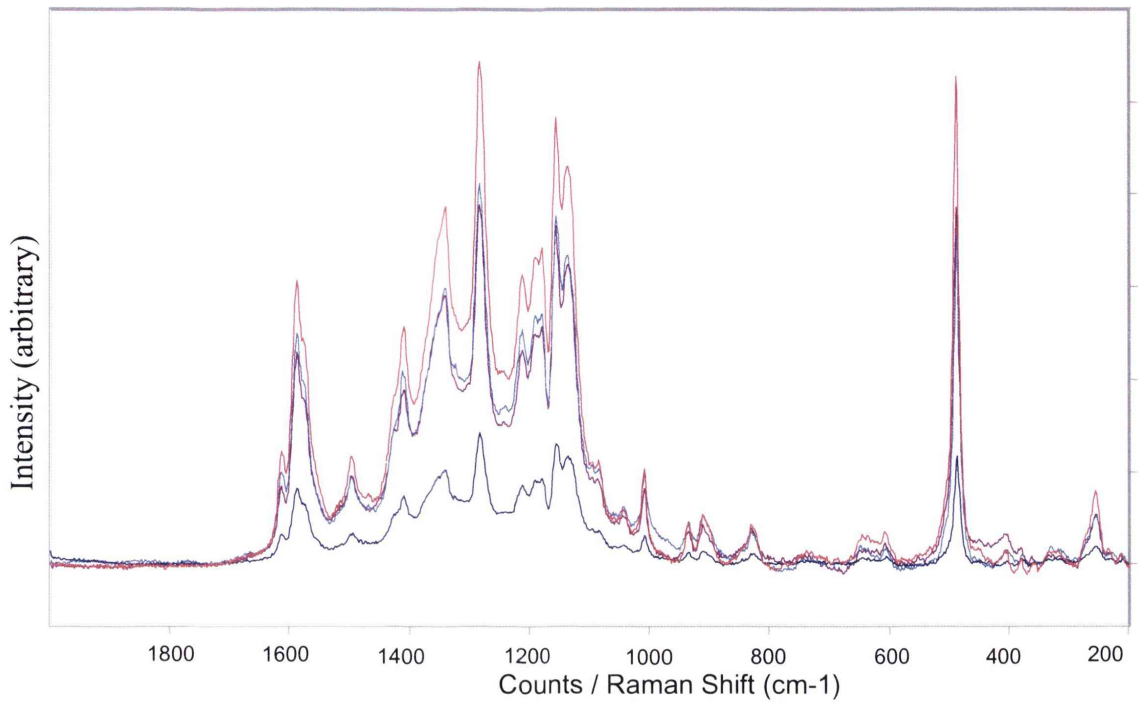


Figure 5-14 Raman spectra (red) sample 14 (light blue) sample 31 (purple) sample 25, and (blue) sample 40 - 785nm

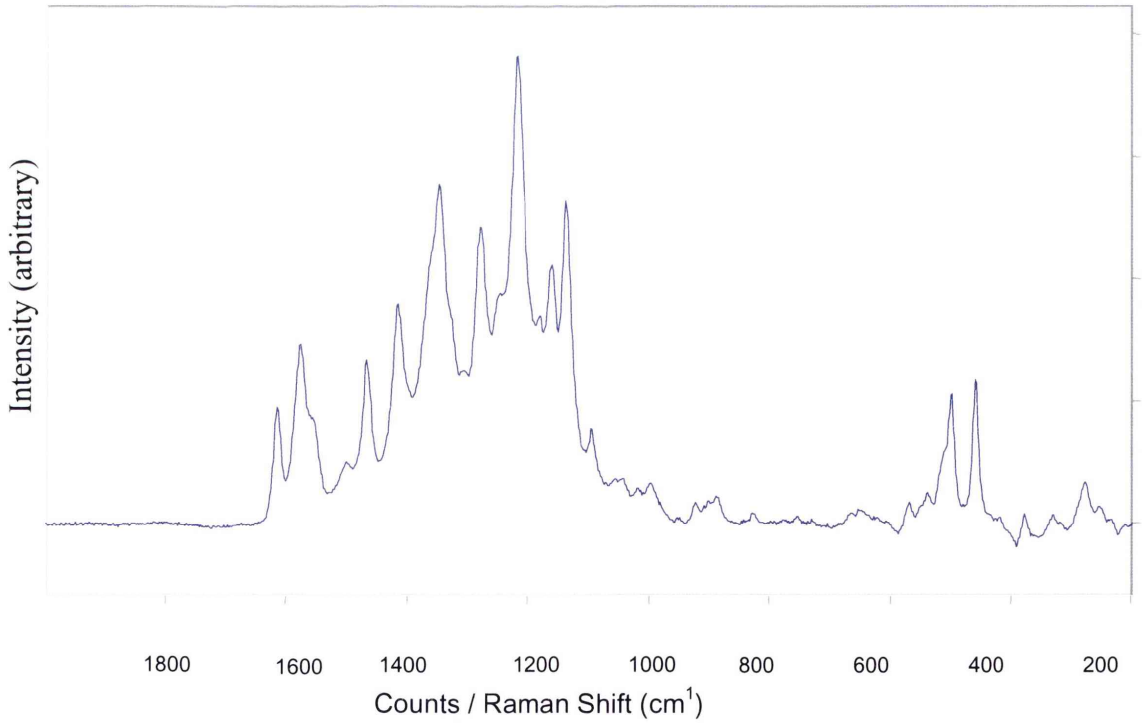


Figure 5-15 Raman spectrum of sample 1 - 785nm

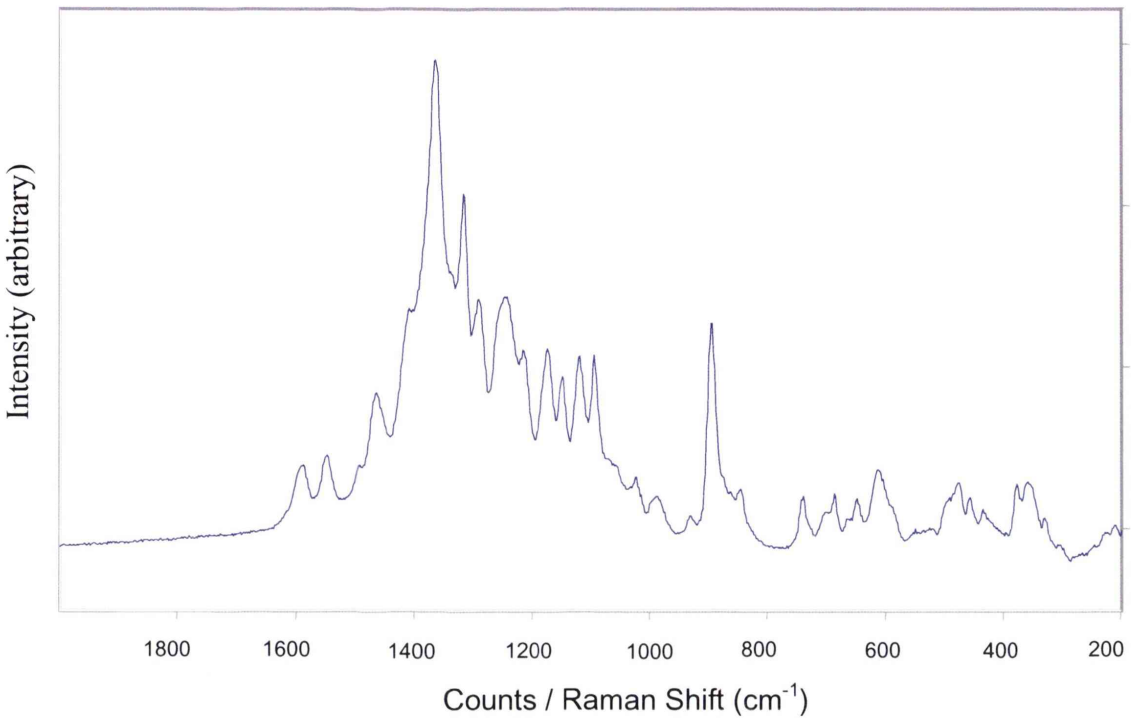


Figure 5-16 Raman spectrum of sample 35 - 785nm

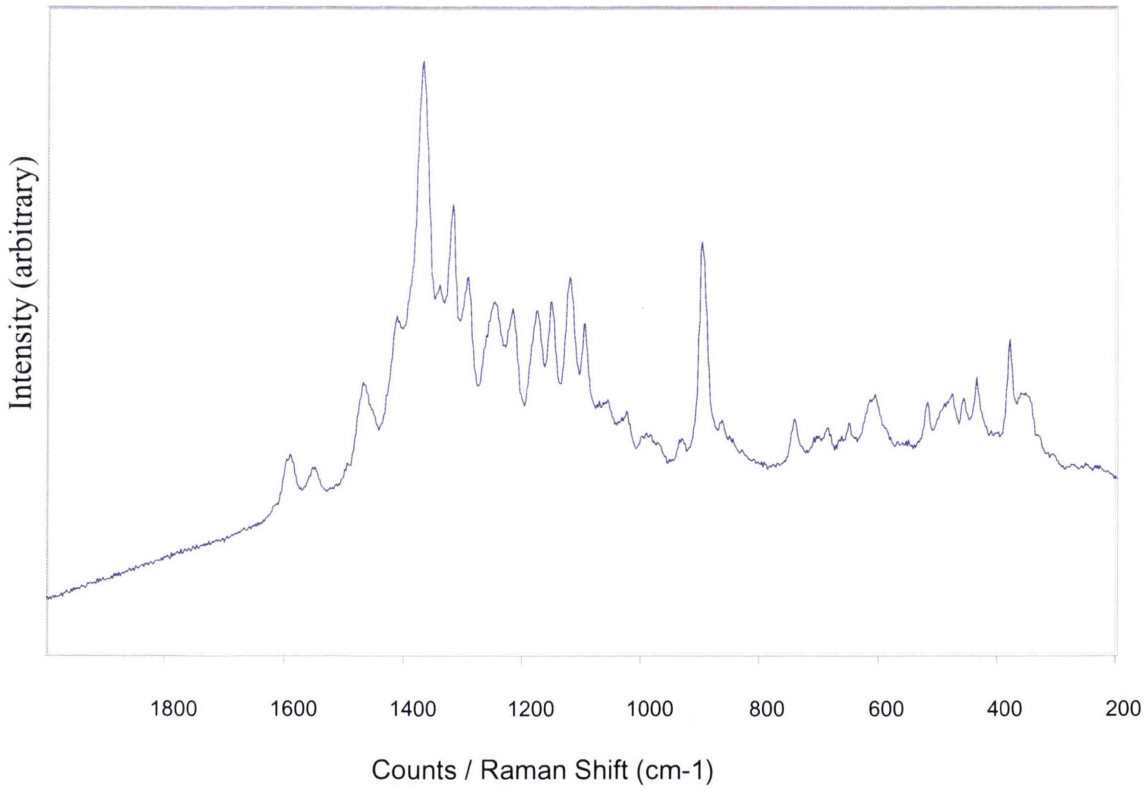


Figure 5-17 Raman spectrum of sample 32 - 785nm

Discrimination by Raman spectroscopy - 632.8nm laser

Raman spectroscopy analysis using the 632.8nm laser allowed for the samples to be classified into five groups (refer to Figure 5-18 to Figure 5-21). Four groups showed distinct spectra associated with the dye and the fifth group was composed of two samples that fluoresced. Like the 785nm analysis samples 18 and 20 fluoresced and once again the dye component was responsible.

The 11 samples produce 55 pair-wise comparisons and after 632.8nm Raman spectroscopy analysis there were 8 pairs left not discriminated. The discrimination power (DP) of 632.8nm Raman spectroscopy analysis for this sample set is 0.855. The discrimination of the sample set by this laser wavelength is less than that provided by the 785nm laser therefore the 785nm laser is used in subsequent comparisons to the discrimination provided by microspectrophotometry.

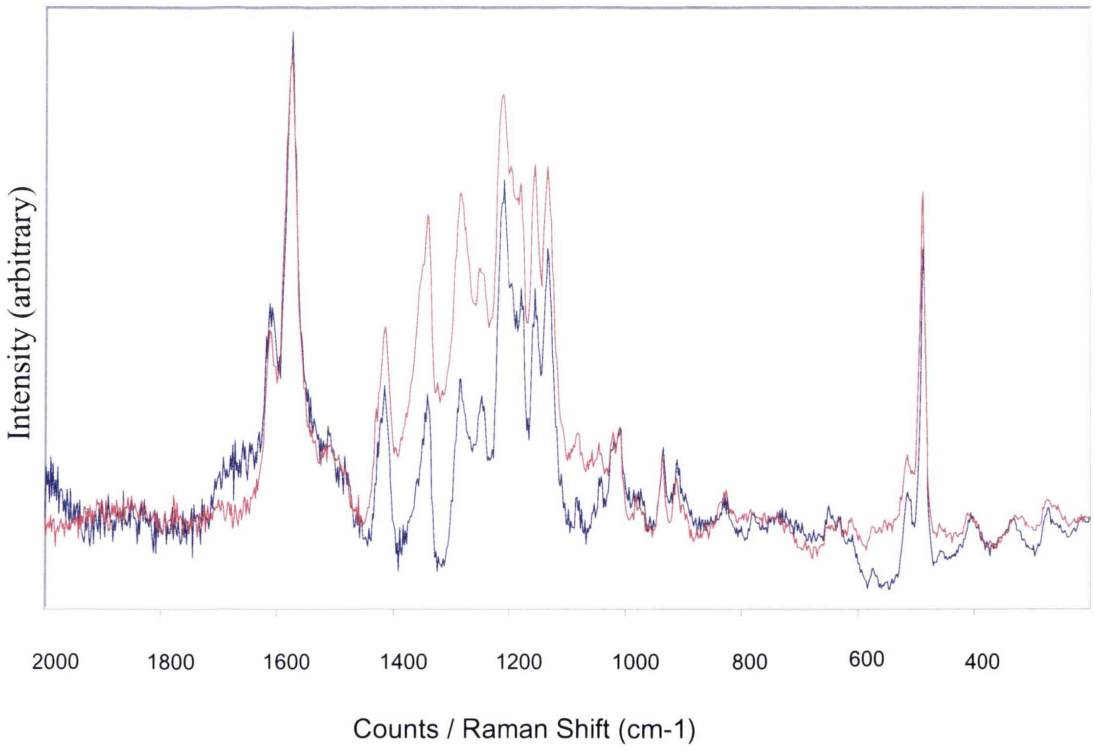


Figure 5-18 Raman spectra of (red) sample 39, and (blue) sample 38 - 632.8nm

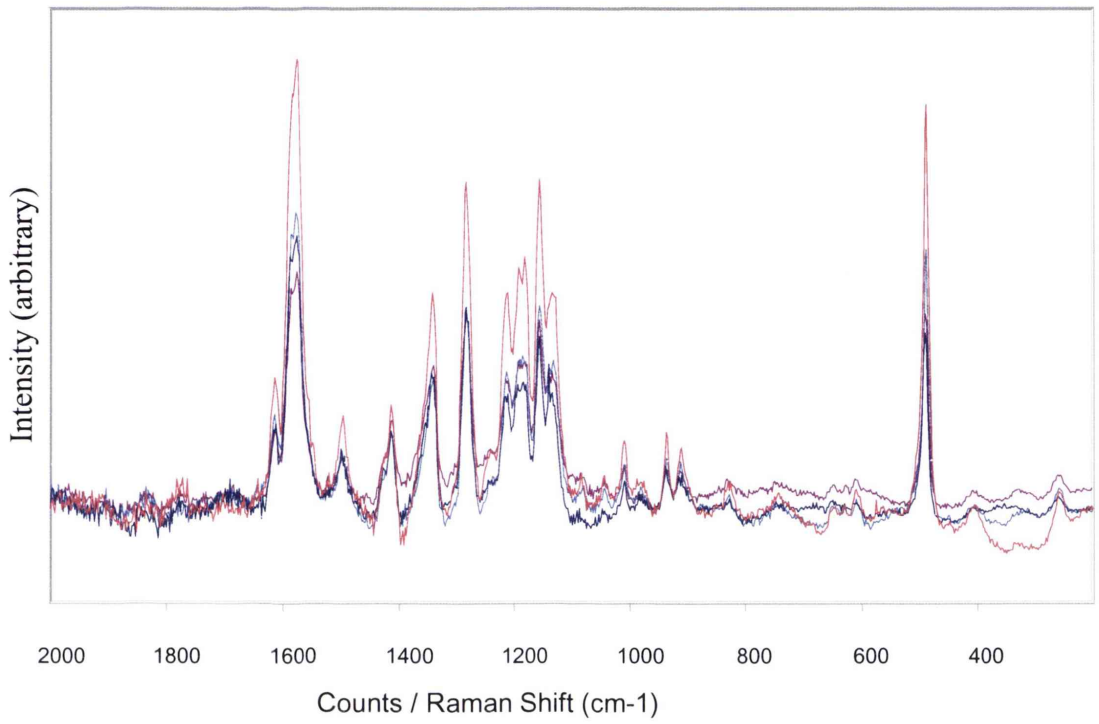


Figure 5-19 Raman spectra (red) sample 14 (light blue) sample 31 (purple) sample 25, and (blue) sample 40 - 632.8nm

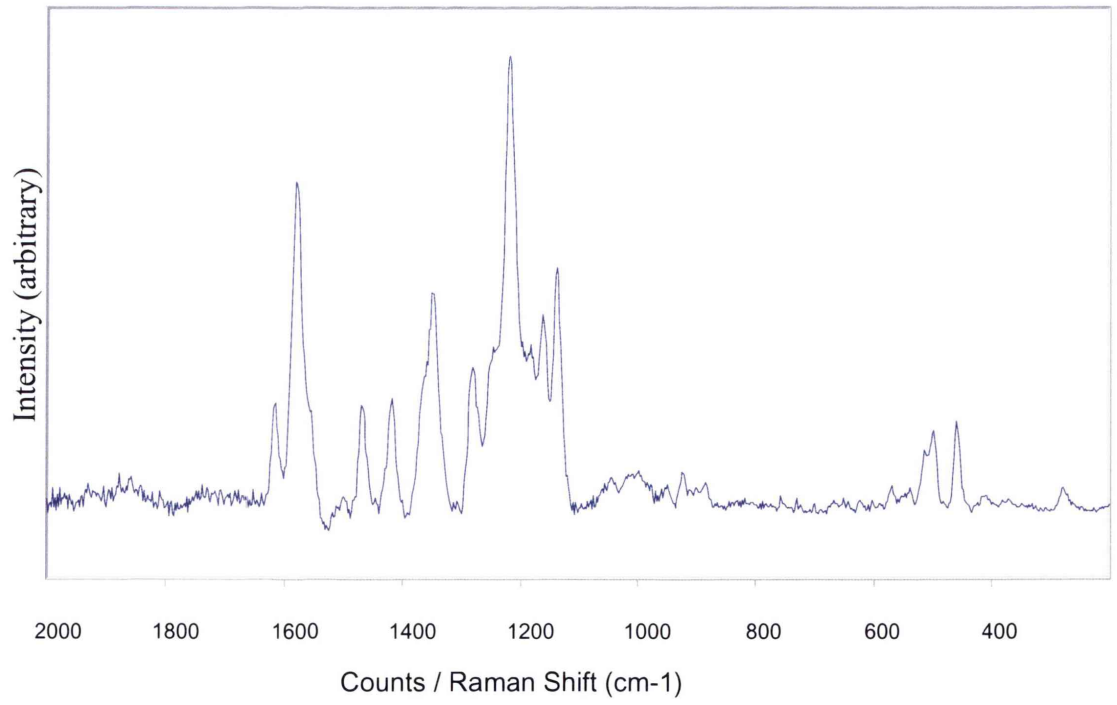


Figure 5-20 Raman spectrum of sample 1 - 632.8nm

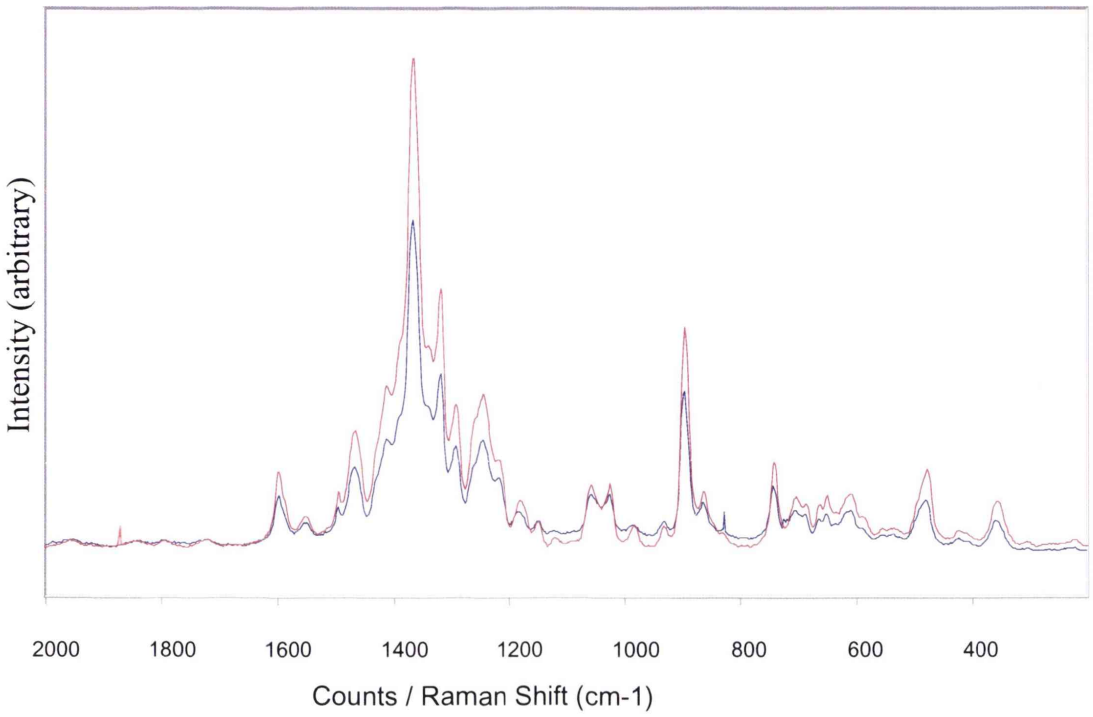


Figure 5-21 Raman spectra of sample 32 (green) and sample 35 (red) - 632.8nm

5.4.1.2 Discrimination by Visible-Microspectrophotometry

Visible-MSP analysis allowed for the samples to be classified into eight groups (refer to Figure 5-22 to Figure 5-28). Visible-MSP provided more discrimination than Raman spectroscopy (8 groupings for MSP compared to 6 groupings for Raman spectroscopy). The Vis-MSP analysis highlights the fundamental problem with Raman spectroscopy – fluorescence – in both the 632.8nm and 785nm Raman spectroscopy analysis samples 18 and 20 fluoresced. Using Vis-MSP, not only were the samples able to produce spectra but they were also able to be discriminated at this step.

The 11 samples produced 55 pair-wise comparisons and after Vis-MSP there were only 4 pairs left not discriminated. The discrimination power (DP) of Vis-MSP for this sample set is 0.927. Vis-MSP provided the best discrimination for analysis with a single technique.

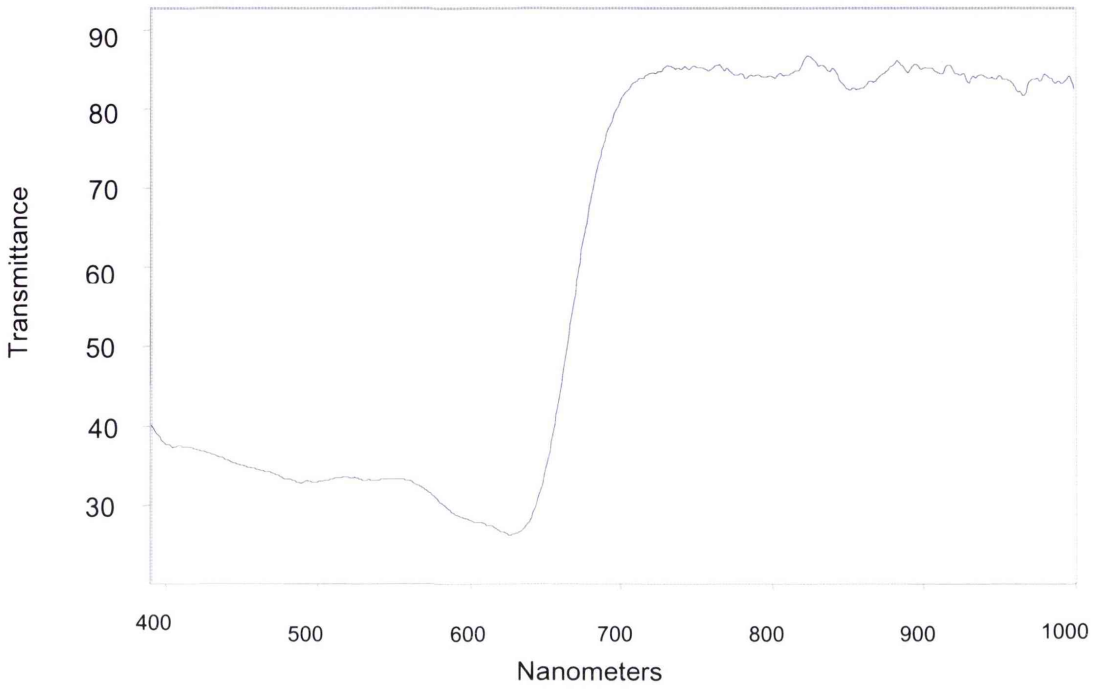


Figure 5-22 Vis-MSP spectrum of sample 39

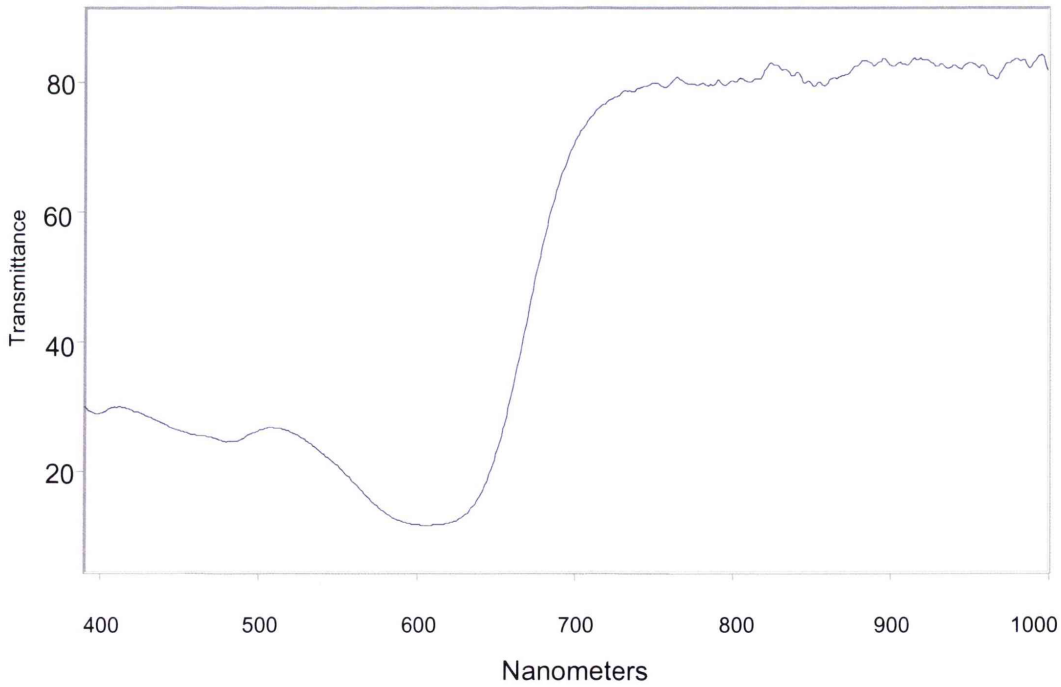


Figure 5-23 Vis-MSP spectrum of sample 25

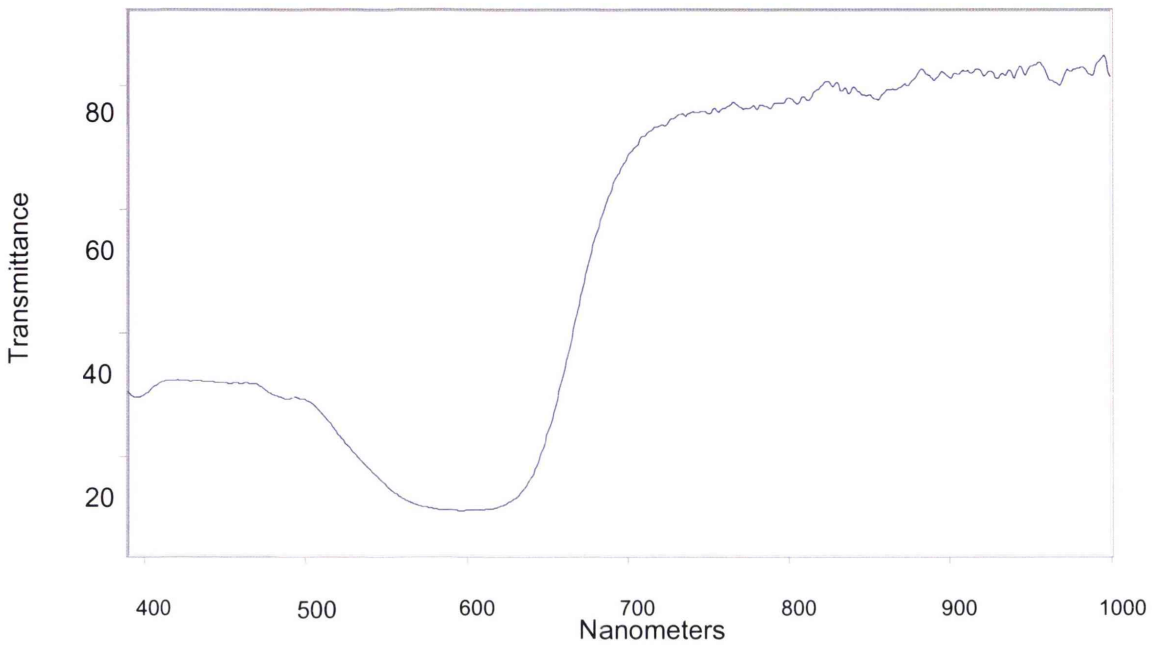


Figure 5-24 Vis-MSP spectrum of sample 14

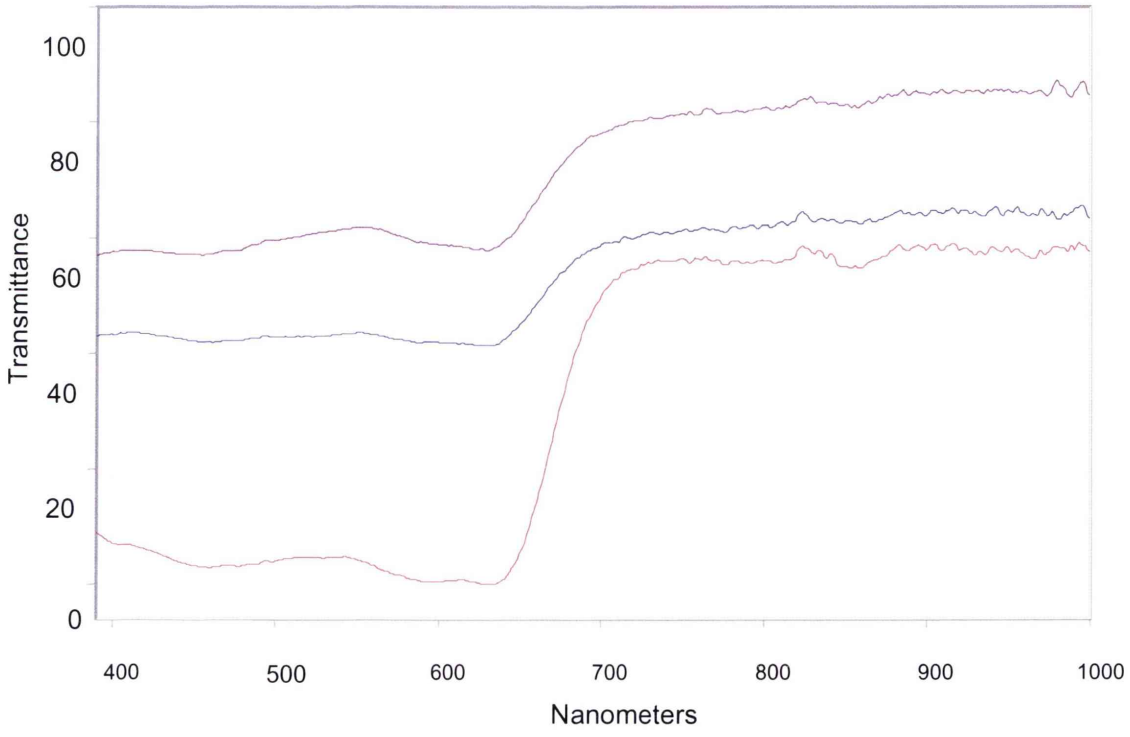


Figure 5-25 Vis-MSP spectra of samples 32, 35 and 38

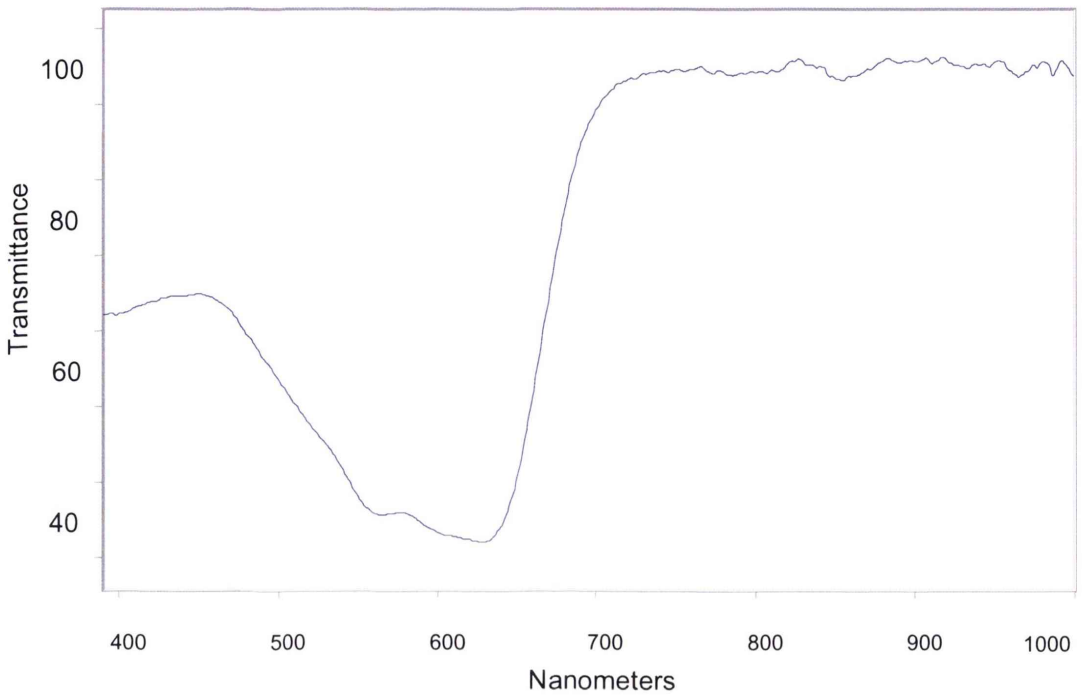


Figure 5-26 Vis-MSP spectrum of sample 1

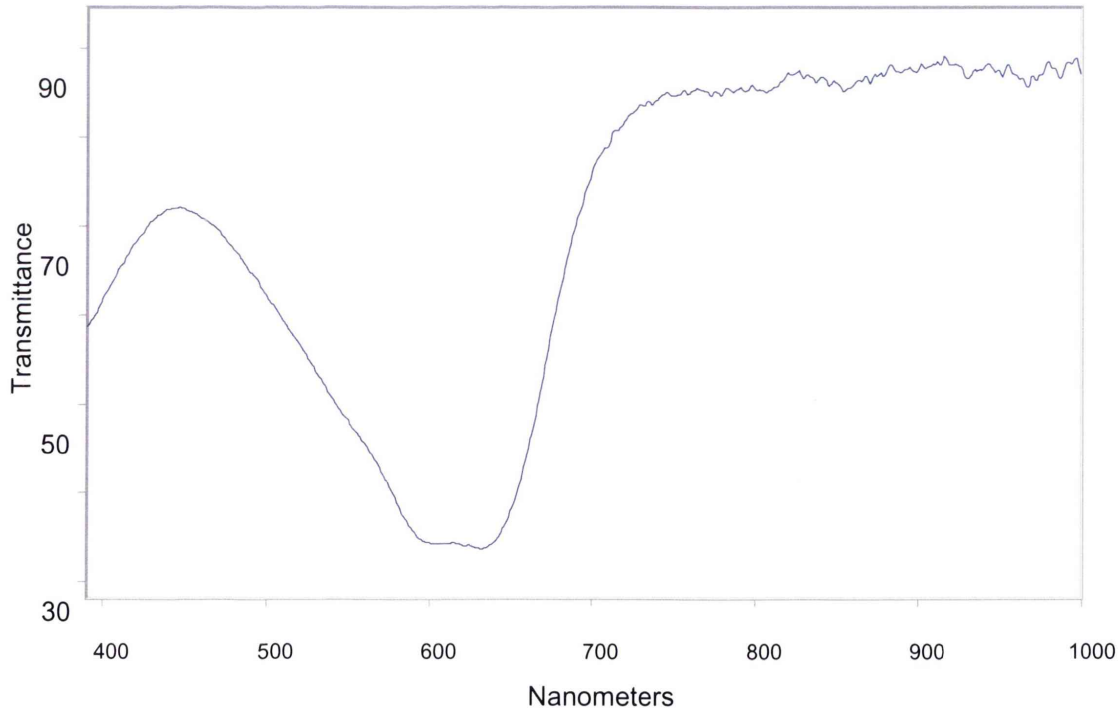


Figure 5-27 Vis-MSP spectrum of sample 18

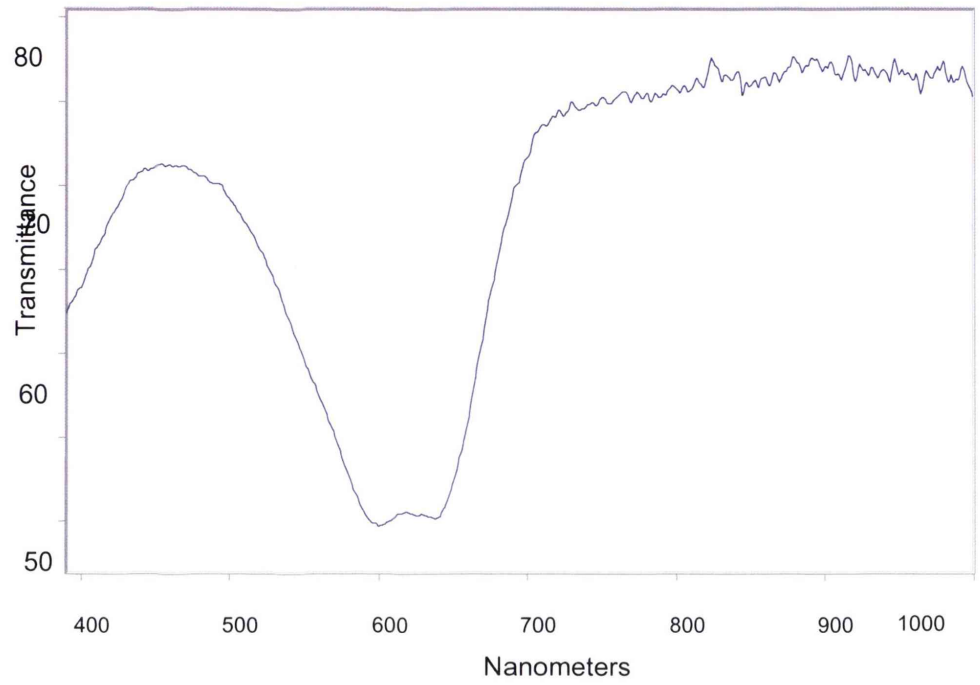


Figure 5-28 Vis-MSP spectrum of sample 20

5.4.1.3 Discrimination by combined techniques

By combining the two techniques the discrimination of the sample set could be increased. The eleven samples were able to be separated into ten groups. It was important at this stage to recognise that using Raman spectroscopy (785nm laser) followed by Vis-MSP (Figure 5-29) requires more analysis than if using Raman spectroscopy after Vis-MSP (refer to Figure 5-30). This was due to the lower discrimination of Raman spectroscopy compared to microspectrophotometry for the sample set.

Using an analysis sequence of Visible-MSP followed by Raman spectroscopy provided more discrimination than using a single technique with the 55 pair-wise comparisons are reduced to 1 pair not discriminated. By combining the techniques the discriminatory power (DP) rose from 0.873 for 785nm Raman spectroscopy and 0.927 for Vis-MSP to **0.982**.

Visible-MSP discriminated based on colour and could detect additions of minor dye colours, whereas Raman spectroscopy discriminated based on the molecular structure of the major dye component.

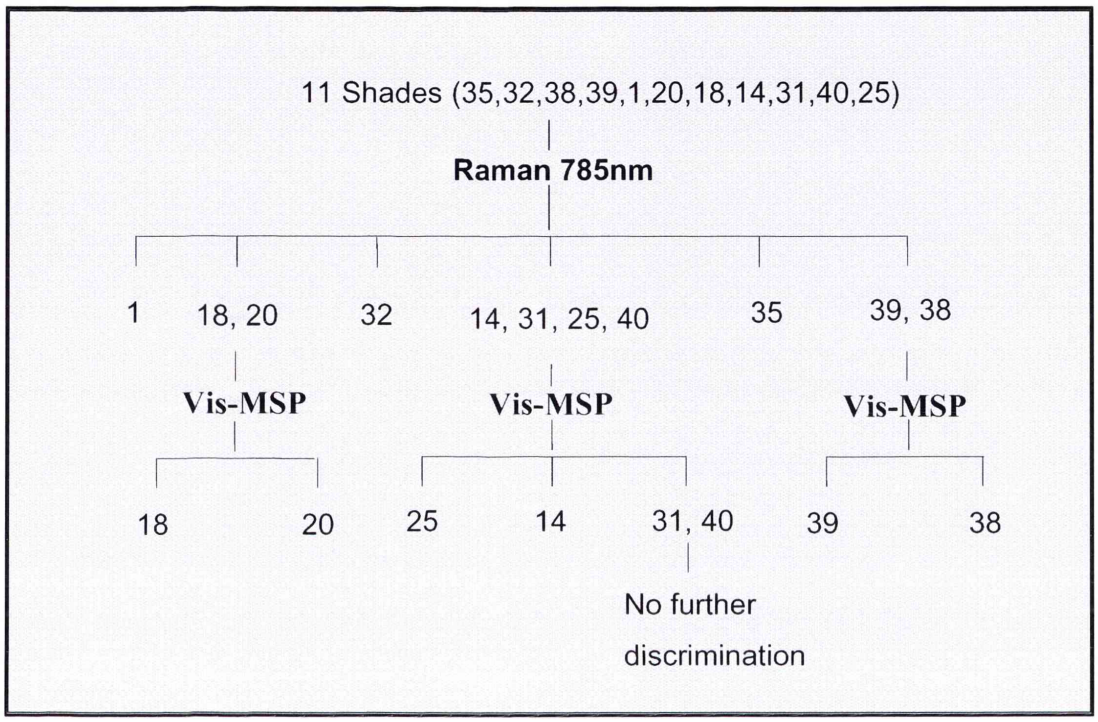


Figure 5-29 Flowchart of sample discrimination using Raman spectroscopy then Vis-MSP

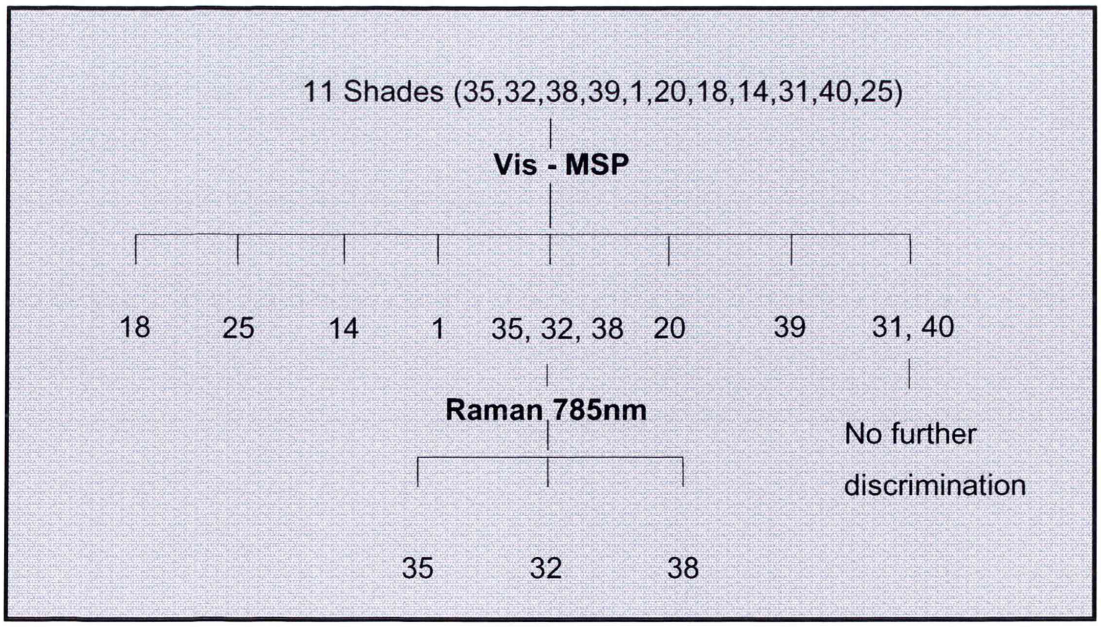


Figure 5-30 Flowchart of sample discrimination using Vis-MSP then Raman spectroscopy

5.4.2 Unknown Sample Set

5.4.2.1 Raman spectroscopy analysis

Eighty-four of the ninety-one random samples collected by Langdon [25] could be classified into six general types. The remaining seven spectra were distinctive from all the others and were grouped together as a seventh category. Figure 5-31 shows the relative frequency of the seven groups. A representative of group I-VI follows. Over half of the fibres could be placed in the two most common groups with the rest forming much smaller groups.

The 91 samples result in 4095 pair-wise comparisons. After analysis with Raman spectroscopy (785nm laser) 824 pairs could not be discriminated. The discriminatory power (DP) for Raman analysis of this sample set was 0.799.

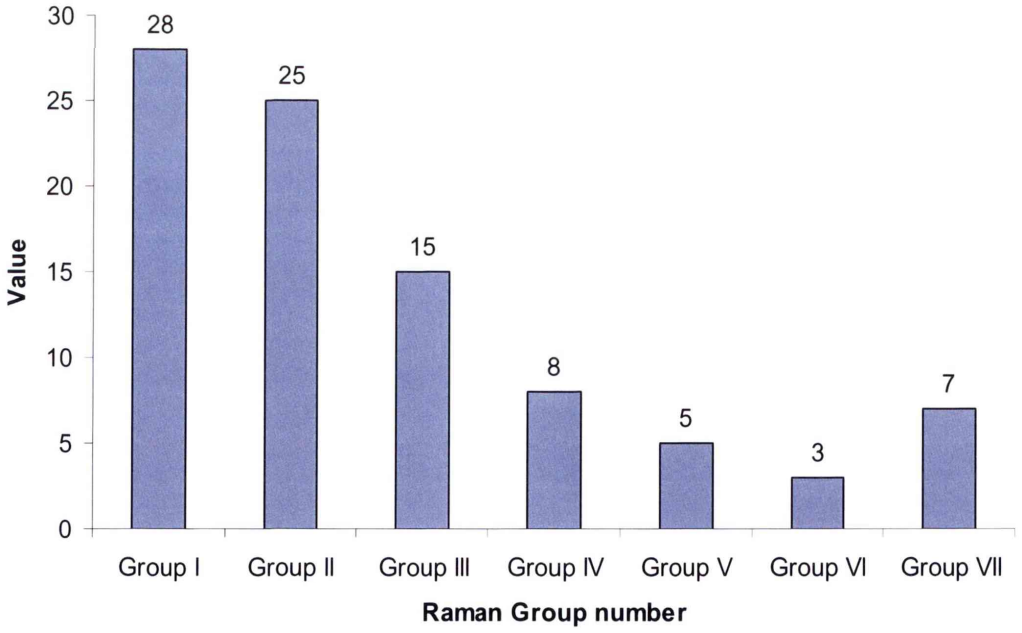


Figure 5-31 Frequencies of the seven different spectral categories (Raman)

The largest group (Group I) accounted for 28 of the 91 samples and was characterised by the peak positions and relative intensities shown in Table 5-6. An example spectrum for this group is shown in Figure 5-32.

The spectra of this Raman group are very similar to the Raman spectrum of Reactive Black 5 (refer to Appendix 1 – Library of Reactive Dyes). Industry inquiries have revealed that Reactive Black 5 is the most common dye used by manufacturers in the colouration of black and dark-blue fibres.

Table 5-6 Peak positions and relative intensities for Raman spectral group I

Peak position (cm^{-1})	Relative intensity
260	very weak
489	strong
1008	very weak
1137	medium
1154	strong
1181	medium
1214	medium
1283	strong
1343	medium
1411	weak
1586	medium

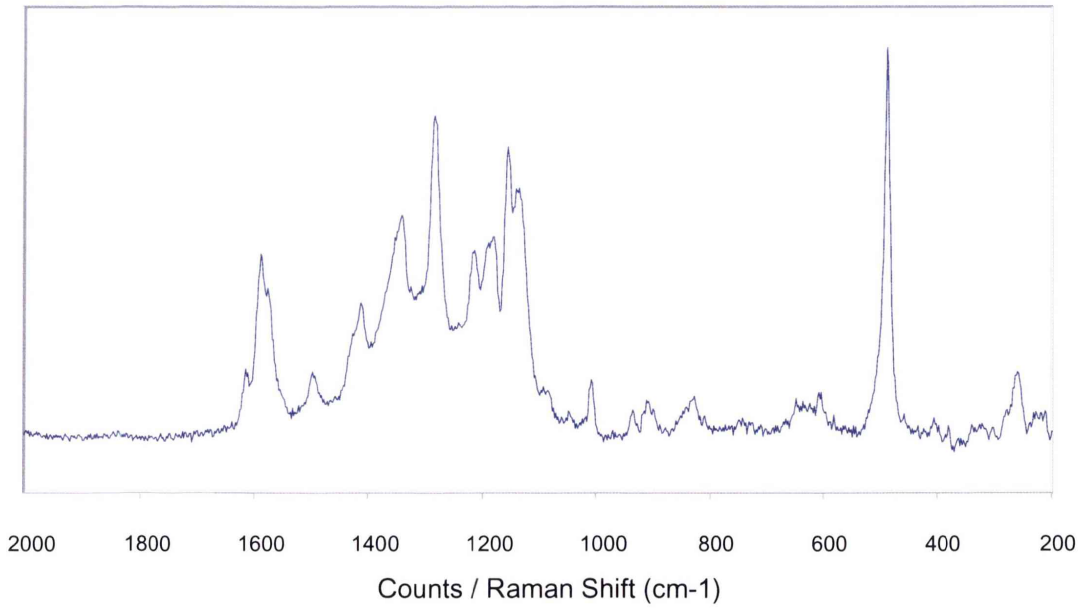


Figure 5-32 Example Raman spectrum from Group I

The second largest group (Group II) accounted for 25 of the 91 samples and was characterised by the peak positions and relative intensities shown in Table 5-7. An example spectrum for this group is shown in Figure 5-33.

Table 5-7 Peak positions and relative intensities for Raman spectral group II

Peak position (cm^{-1})	Relative intensity
380	weak shoulder
441	weak shoulder
525	medium
948	weak
1069	weak
1380	strong (very broad)

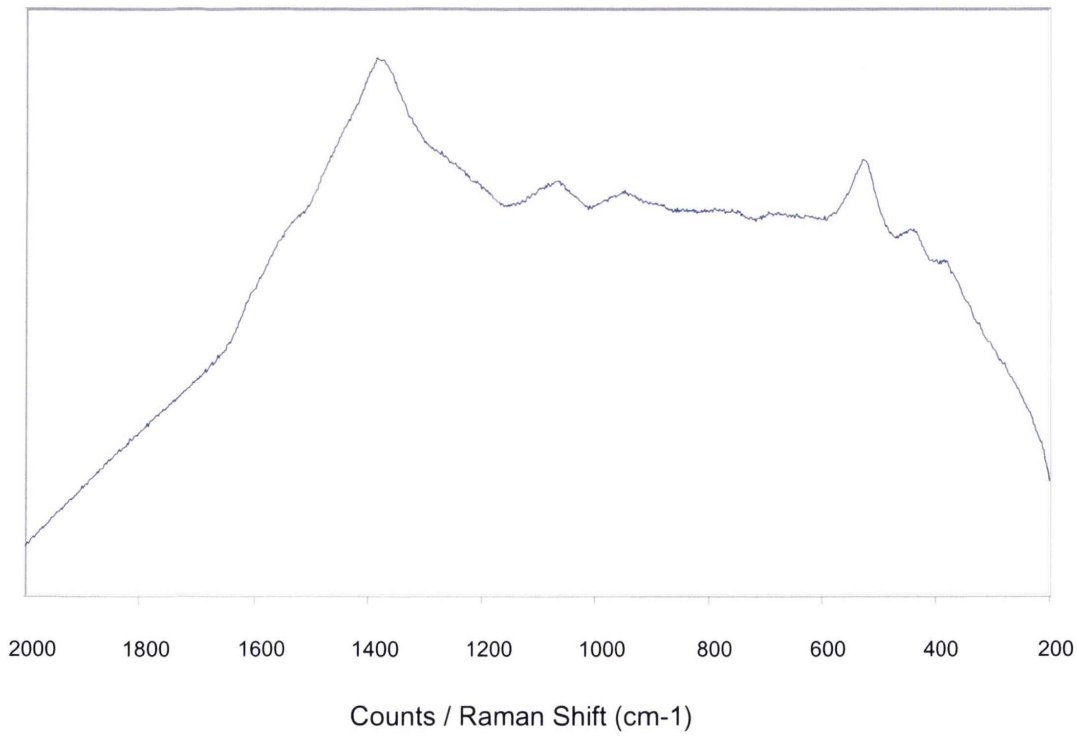


Figure 5-33 Example Raman spectrum from Group II

The third largest group (Group III) accounted for 15 of the 91 samples and was characterised by samples that fluoresced. This highlights the inherent problems faced by Raman spectroscopy (weak signal and high occurrence of fluorescence).

It is these problems that indicate that Raman would not be feasible as a stand-alone technique for forensic fibre examinations.

Group IV accounted for 8 of the 91 samples and was characterised by the peak positions and relative intensities shown in Table 5-8. An example spectrum for this group is shown in Figure 5-34.

Table 5-8 Peak positions and relative intensities for Raman spectral group IV

Peak position (cm^{-1})	Relative intensity
252	medium
266	medium/weak
277	medium/weak
545	medium
598	weak
1311	very weak
1461	very weak
1573	strong

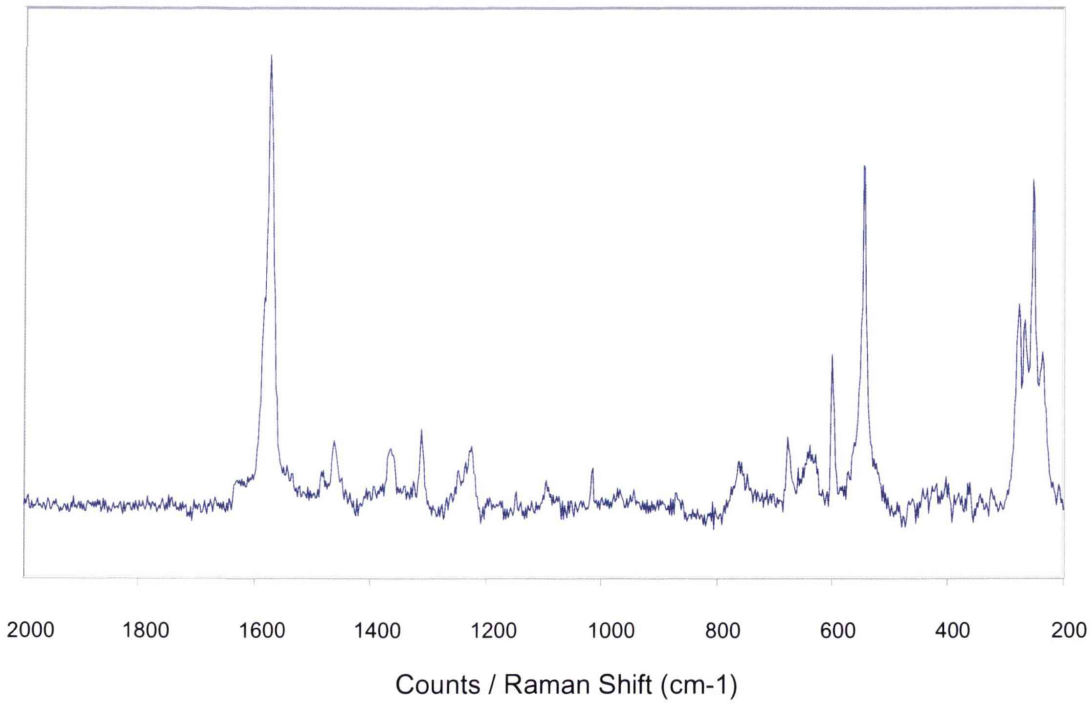


Figure 5-34 Example Raman spectrum from Group IV

Group V accounted for five of the 91 samples and was characterised by the peak positions and relative intensities shown in Table 5-9. An example spectrum for this group is shown in Figure 5-35.

Table 5-9 Peak positions and relative intensities for Raman spectral group V

Peak position (cm^{-1})	Relative intensity
279	weak
416	medium
739	medium
856	weak
1074	medium
1151	strong
1169	strong
1208	medium
1327	strong
1579	weak
1612	medium
1725	weak

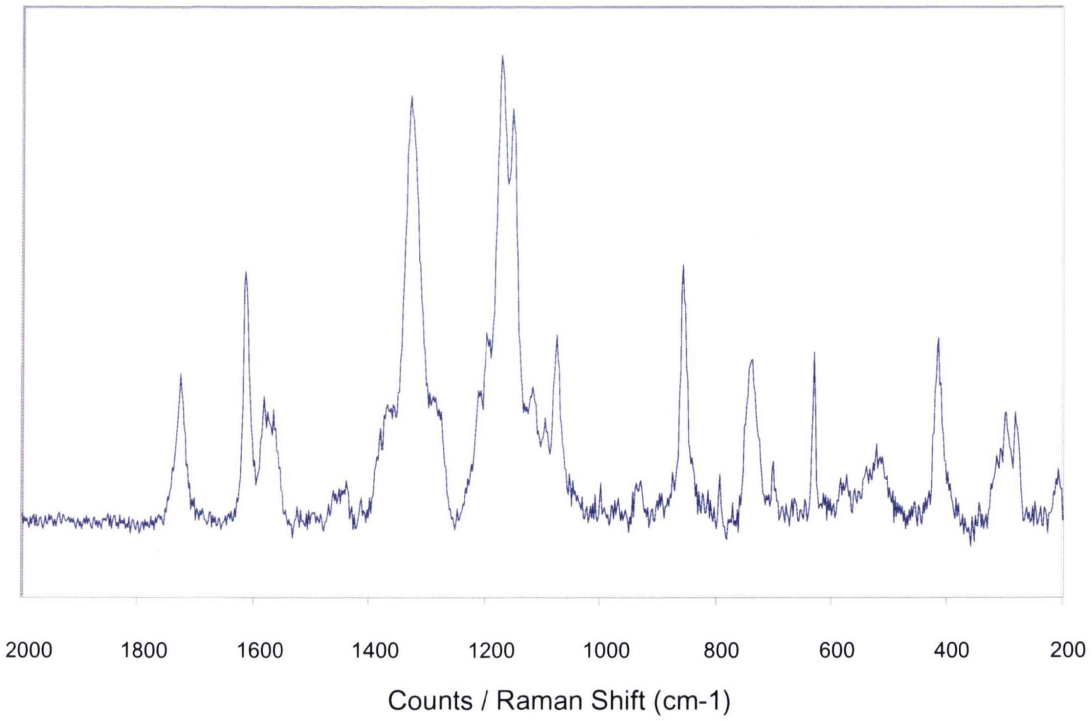


Figure 5-35 Example Raman spectrum from Group V

Group VI accounted for three of the 91 samples and was characterised by the peak positions and relative intensities shown in Table 5-10. An example spectrum for this group is shown in Figure 5-36.

Table 5-10 Peak positions and relative intensities for Raman spectral group VI

Peak position (cm^{-1})	Relative intensity
281	weak
304	weak
423	medium
631	medium
734	weak
751	weak
857	medium
1076	medium
1124	medium
1161	strong
1203	medium
1331	strong
1581	medium
1613	strong
1726	weak

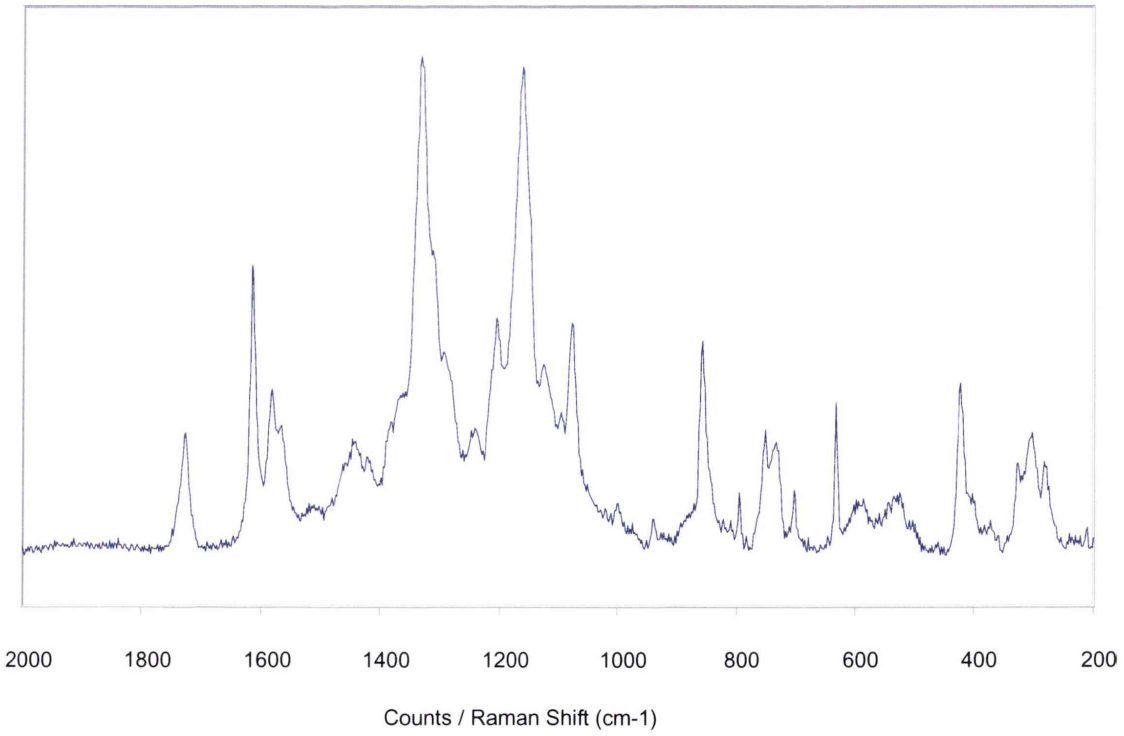


Figure 5-36 Example Raman spectrum from Group VI

5.4.2.2 Microspectrophotometry analysis

Eighty-five of the 91 random samples could be classified into seven general types. The remaining six spectra were distinctive from all the others and were grouped together as an eighth category. Figure 5-37 shows the relative frequency of the eight groups. A representative of groups A-G follows. Over half of the fibres could be placed in the two most common groups with the rest forming much smaller groups.

The 91 samples result in 4095 pair-wise comparisons. After analysis with UV-Vis microspectrophotometry 687 pairs could not be discriminated. The discriminatory power (DP) for microspectrophotometry analysis of this sample set was 0.832.

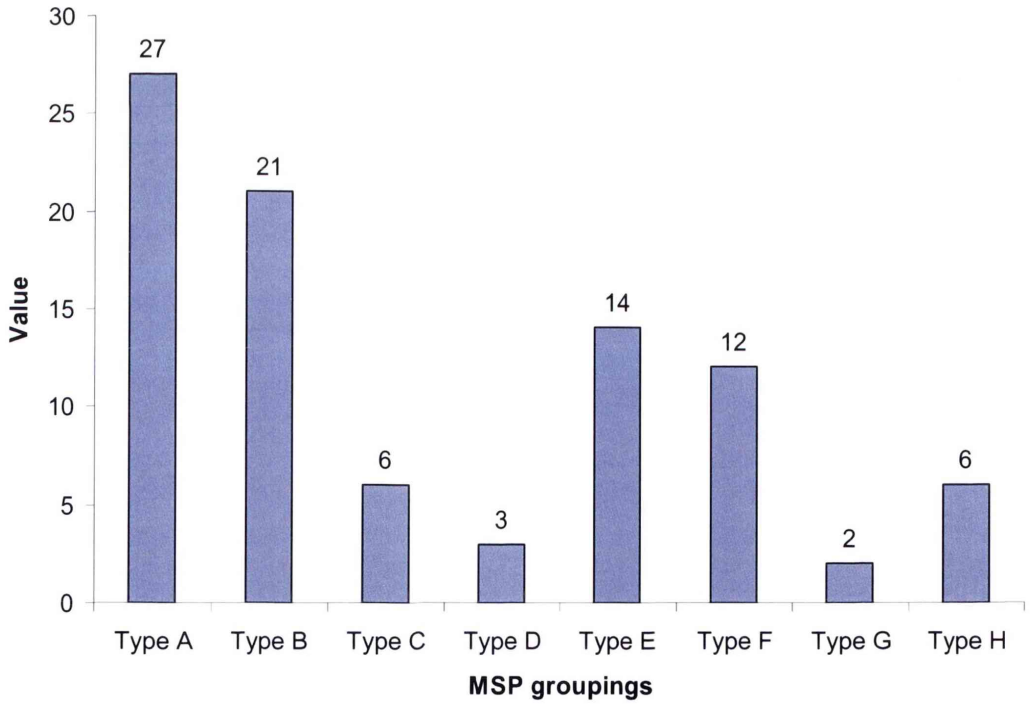


Figure 5-37 Frequencies of the eight different spectral categories (MSP)

The largest group (Group A) accounted for 27 of the 91 samples and was characterised by a spectra with two relatively sharper peak, one at approximately 365nm and another, less sharp, at 500nm with a broad peak between these two at about 430nm. An example spectrum of this group is shown in Figure 5-38

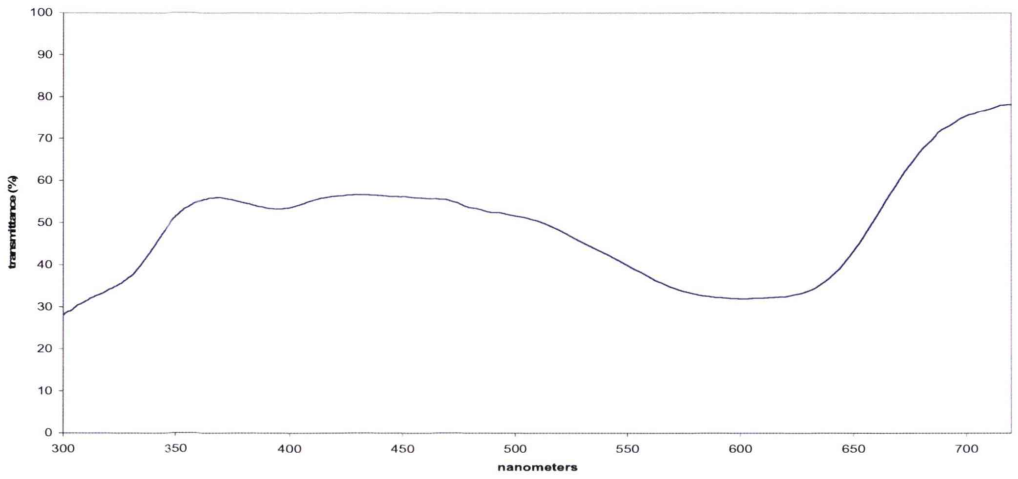


Figure 5-38 Example UV/Vis-MSP spectrum from Group A

The second largest group (Group B) accounted for 21 of the 91 samples and was characterised by a spectra that had a single distinctive peak at 425nm and also had a broadening of the peak at ~520nm. An example spectrum from this group is shown in Figure 5-39

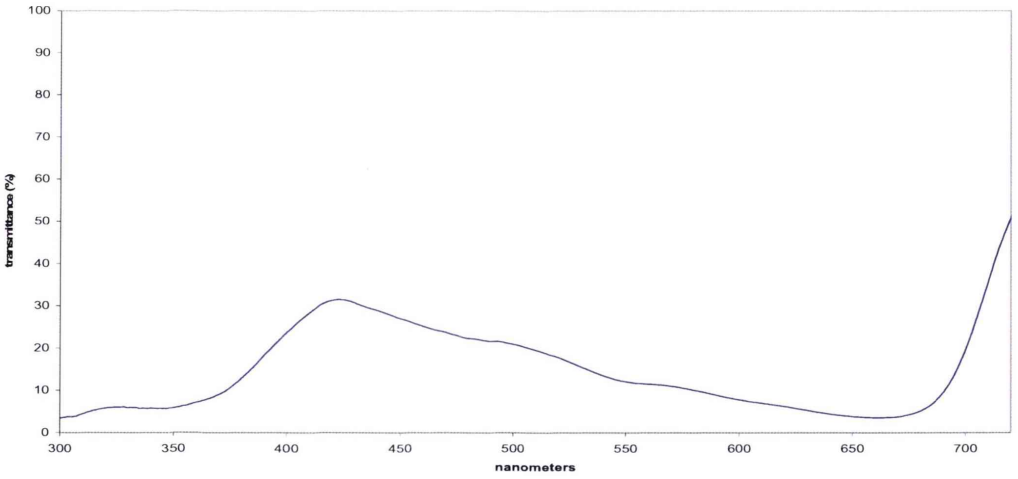


Figure 5-39 Example UV/Vis-MSP spectrum from Group B

Group C accounted for 6 of the 91 samples and was characterised by a broad peak from ~510nm to 550nm in addition to peaks at 365nm and 430nm. An example spectrum from this group is shown in Figure 5-40

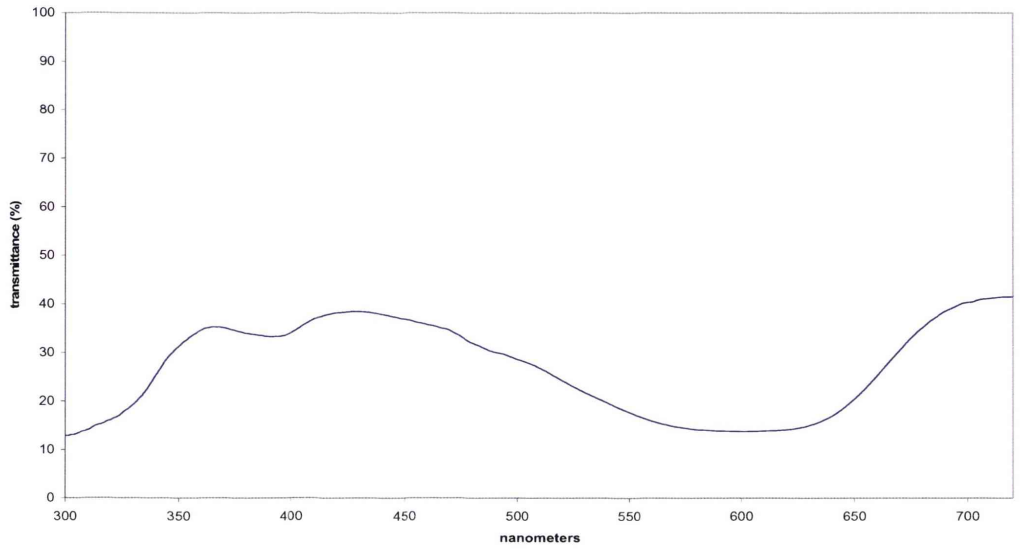


Figure 5-40 Example UV/Vis-MSP spectrum from Group C

Group D accounted for 3 of the 91 samples and was characterised by a very featureless spectra with only a broad peak at ~500nm. An example spectrum from this group is shown in Figure 5-41

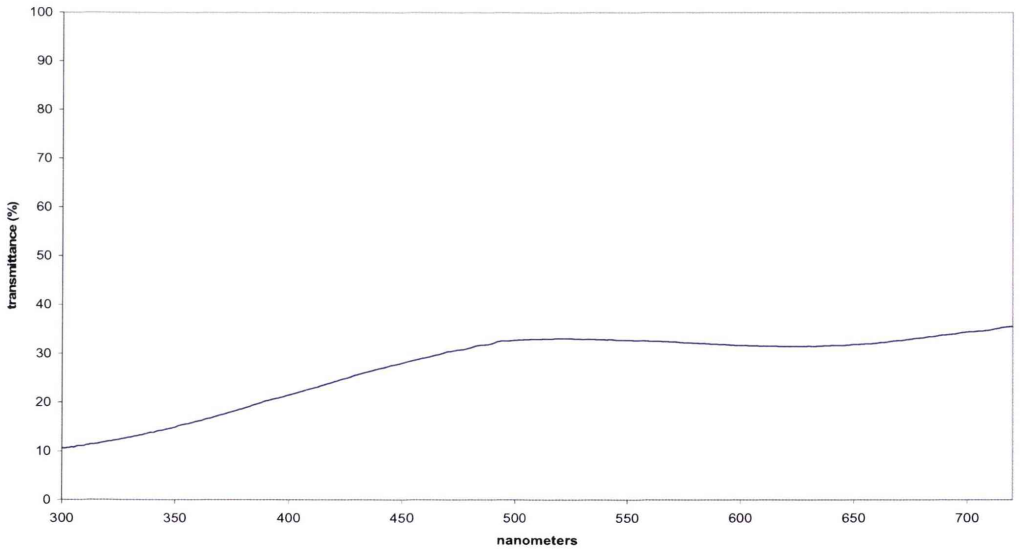


Figure 5-41 Example UV/Vis-MSP spectrum from Group D

Group E accounted for 14 of the 91 samples and was characterised by a very featureless spectra with only a broad peak at ~450nm. An example spectrum from this group is shown in Figure 5-42

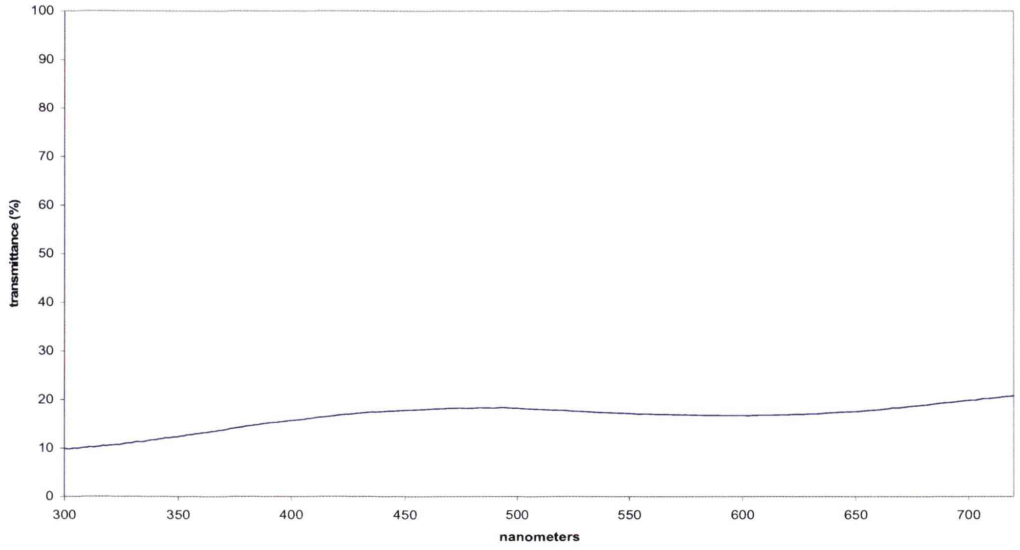


Figure 5-42 Example UV/Vis-MSP spectrum from Group E

Group F accounted for 12 of the 91 samples and was characterised by peaks at 365nm, 430nm and 540nm. An example spectrum from this group is shown in Figure 5-43.

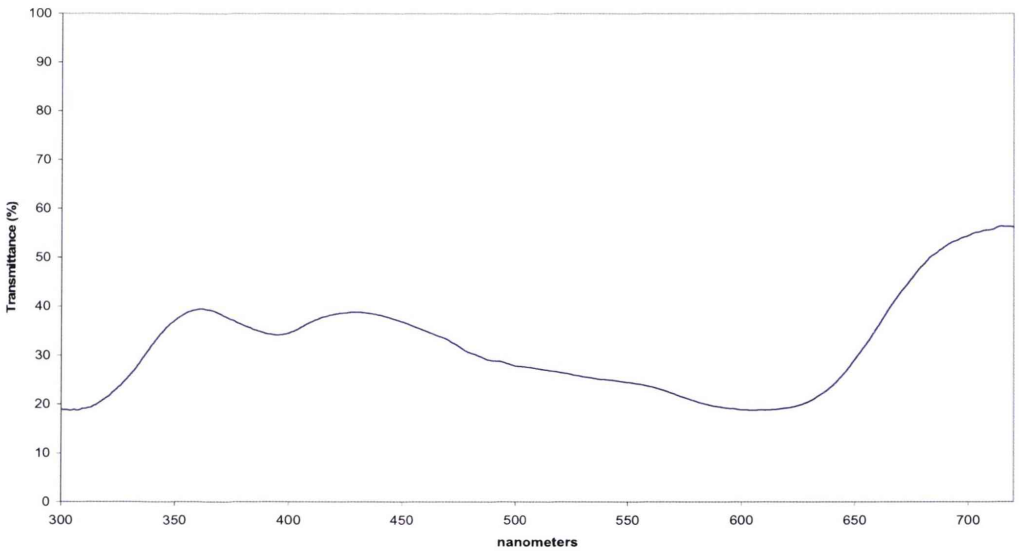


Figure 5-43 Example UV/Vis-MSP spectrum from Group F

Group G accounted for 2 of the 91 samples and was characterised by peaks at 365nm and 560nm. An example spectrum from this group is shown in Figure 5-44.

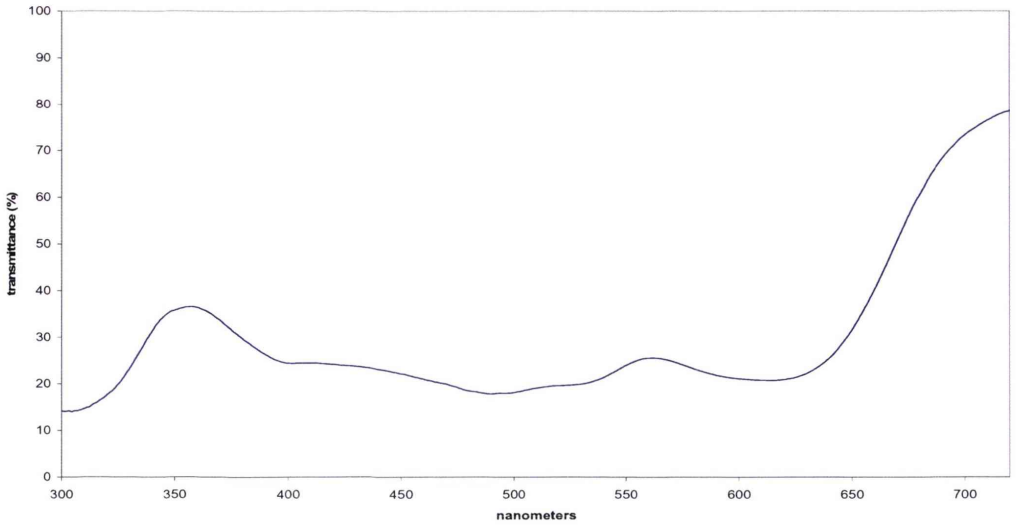


Figure 5-44 Example UV/Vis-MSP spectrum from Group G

5.4.2.3 Combining the two techniques

By combining the two techniques the discrimination of the unknown sample set could be increased significantly. The 91 samples were able to be separated into 38 groups. Using an analysis sequence of UV-Vis MSP followed by Raman spectroscopy (785nm) provided more discrimination than using a single technique with the 4095 pair-wise comparisons reduced to 157 pairs not discriminated. By combining the techniques the discriminatory power (DP) rose from 0.799 for 785nm Raman spectroscopy and 0.832 for UV-Vis MSP to **0.962**.

The groupings from combining the two techniques are shown in Table 5-11 and are discussed in the following pages.

Table 5-11 Groupings created when analysing with the two techniques: Raman spectroscopy and UV-Vis MSP

MSP / Raman	Group A	Group B	Group C	Group D	Group E	Group F	Group G	Group H
Group I	9	4	1	2	3	6	1	2
Group II	8	8	1	0	6	1	0	1
Group III	5	3	0	1	2	3	0	1
Group IV	0	3	1	0	3	0	0	1
Group V	2	0	1	0	0	1	1	0
Group VI	0	2	1	0	0	0	0	0
Group VII	3	1	1	0	0	1	0	1

Analysis by UV-Vis MSP allowed for organisation of 27 of the 91 samples into Group A-type spectra. Subsequent analysis by Raman spectroscopy was able to further sub-categorise these samples into seven groups (refer to Figure 5-45).

The samples comprising MSP Group A showed the highest percentage of fluorescence with 5 of the 27 samples fluorescing.

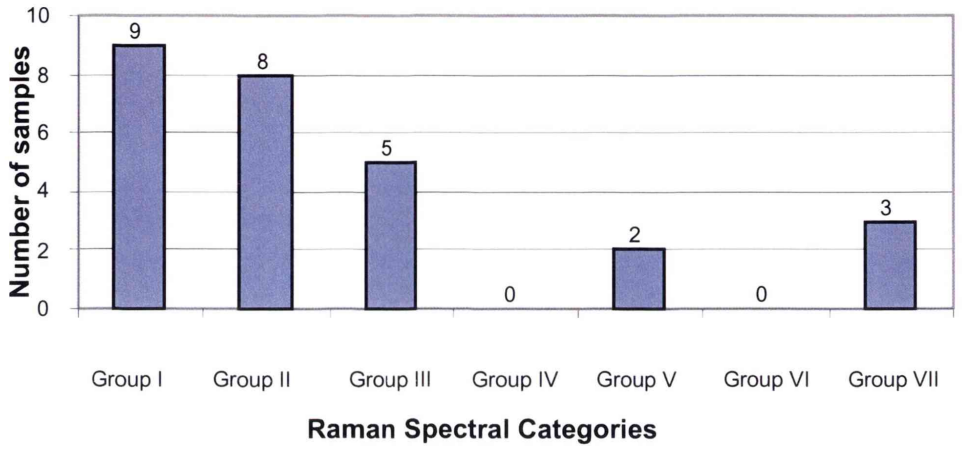


Figure 5-45 Raman spectral sub-classes in MSP Group A

Analysis by UV-Vis MSP allowed for organisation of 21 of the 91 samples into Group B-type spectra. Subsequent analysis by Raman spectroscopy was able to further sub-categorise these samples into six groups (refer to Figure 5-46).

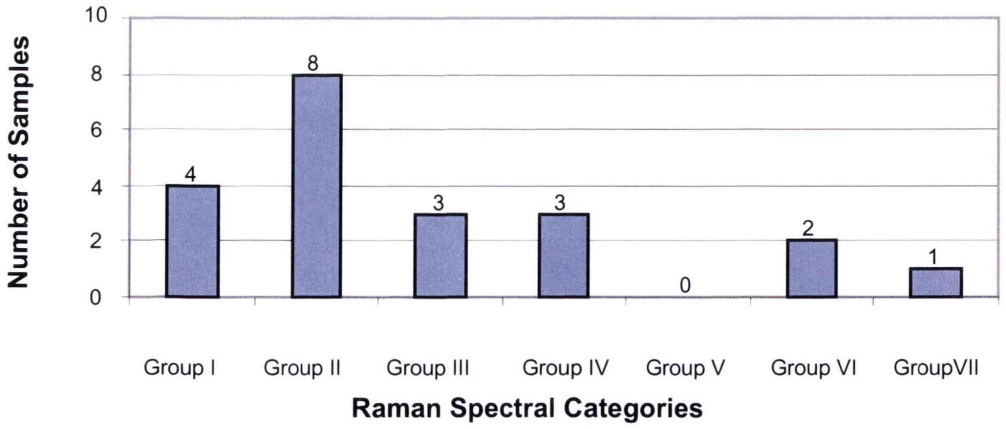


Figure 5-46 Raman spectral sub-classes in MSP Group B

Analysis by UV-Vis MSP allowed for organisation of six of the 91 samples into Group C-type spectra. Subsequent analysis by Raman spectroscopy was able to further sub-categorise these samples into six groups (refer to Figure 5-47). The samples comprising MSP Group C showed the highest discrimination by Raman spectroscopy and no samples in this group fluoresced.

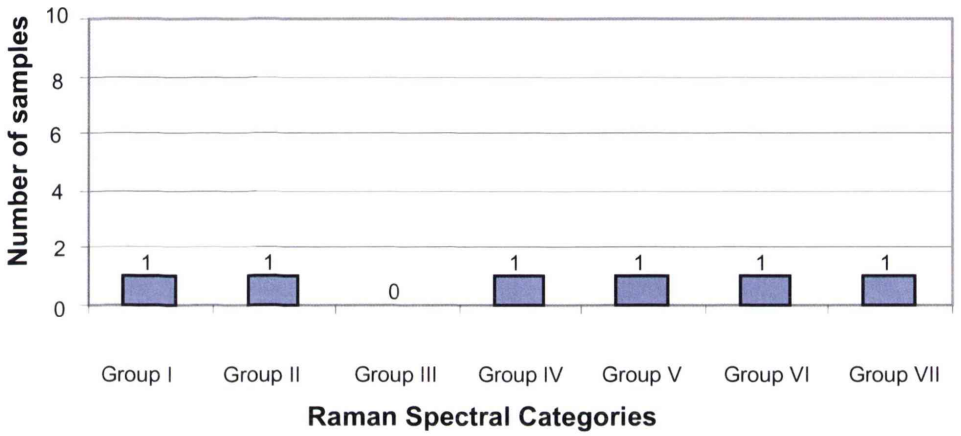


Figure 5-47 Raman spectral sub-classes in MSP Group C

Analysis by UV-Vis MSP allowed for organisation of 27 of the 91 samples into Group D-type spectra. Subsequent analysis by Raman spectroscopy was able to further sub-categorise these samples into two groups (refer to Figure 5-48). The samples comprising MSP Group D also showed a high percentage of fluorescence with one of the three samples fluorescing.

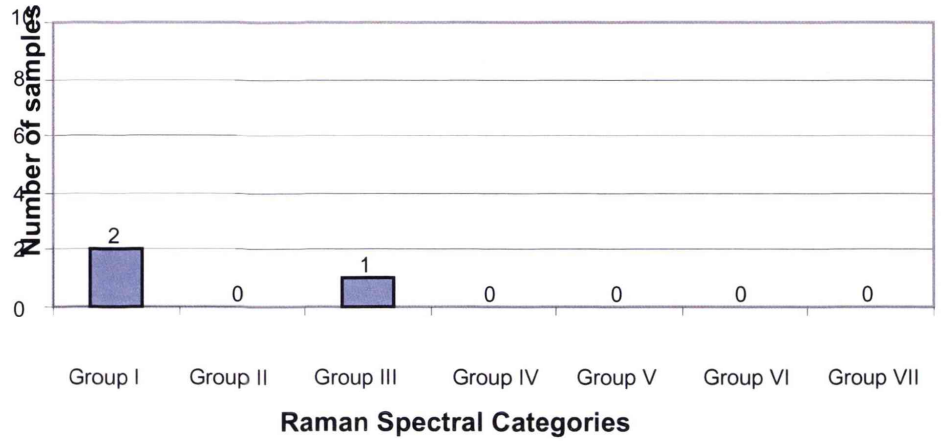


Figure 5-48 Raman spectral sub-classes in MSP Group D

Analysis by UV-Vis MSP allowed for organisation of 14 of the 91 samples into Group E-type spectra. Subsequent analysis by Raman spectroscopy was able to further sub-categorise these samples into four groups (refer to Figure 5-49).

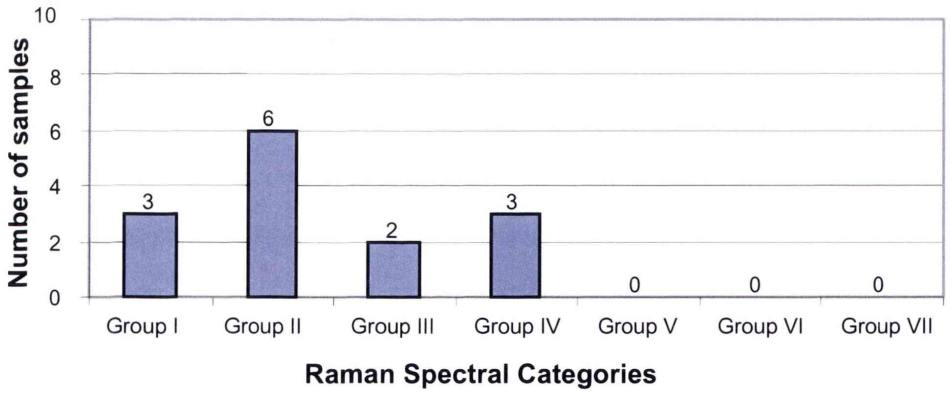


Figure 5-49 Raman spectral sub-classes in MSP Group E

Analysis by UV-Vis MSP allowed for organisation of 12 of the 91 samples into Group F-type spectra. Subsequent analysis by Raman spectroscopy was able to further sub-categorise these samples into five groups (refer to Figure 5-50). The

samples comprising MSP Group E also showed a high percentage of fluorescence with three of the 12 samples fluorescing.

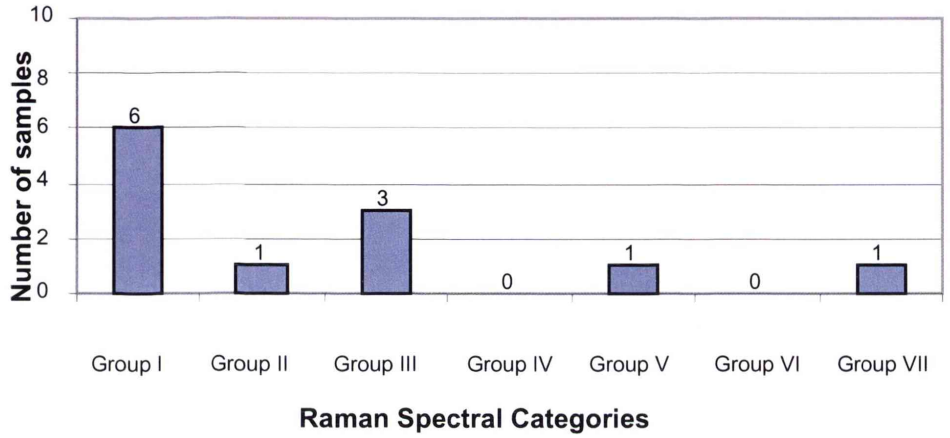


Figure 5-50 Raman spectral sub-classes in MSP Group F

Analysis by UV-Vis MSP allowed for organisation of two of the 91 samples into Group G-type spectra. Subsequent analysis by Raman spectroscopy was able to further sub-categorise these samples into two groups (refer to Figure 5-51).

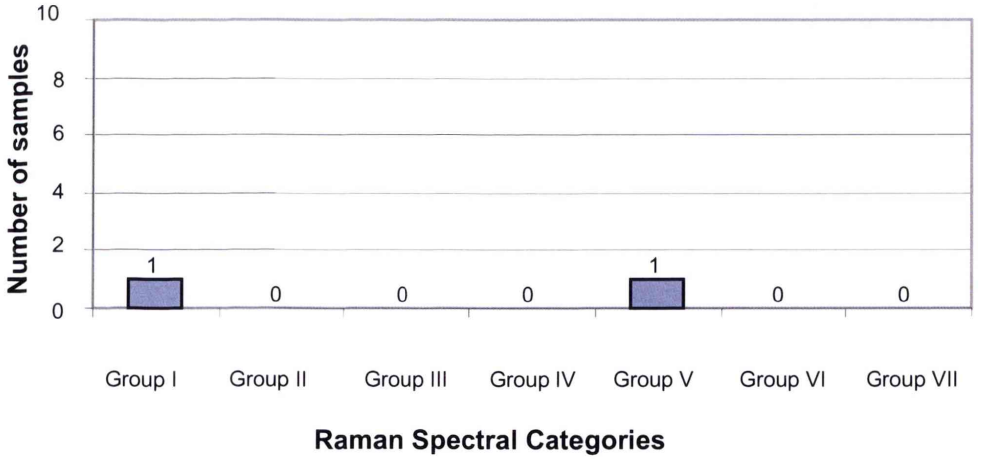


Figure 5-51 Raman spectral sub-classes in MSP Group G

Group H was already discriminated into discreet samples at the UV-Vis MSP stage. However, Raman spectroscopic analysis was conducted and resulted in five groups for the six individual samples (refer to Figure 5-52).

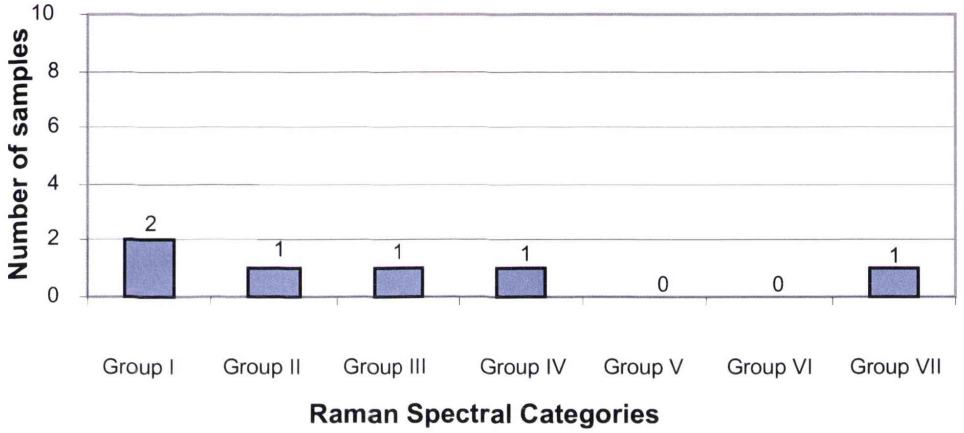


Figure 5-52 Raman spectral sub-classes in MSP Group H

Incorporating Raman spectroscopy may not be valid in all fibre case circumstances. However, if the technique is to be used, incorporating Raman spectroscopy after the MSP step would be recommended for two reasons:

1. for best results the sample will need to be demounted, and
2. the high percentage of samples that fluoresce and cannot be discriminated at this stage

Samples that fluoresced (in both the known and the unknown sample sets) were easily analysed with MSP. Using the Raman spectroscopy technique after that of MSP would reduce the number of samples that would have to be analysed unnecessarily with Raman spectroscopy.

In forensic analysis, the general rule of order is to analyse samples with techniques from least discrimination to highest discrimination (i.e. from stereomicroscopy to MSP in the case of natural fibres). If this rule was to be adhered to with Raman spectroscopy the technique would fall before that of MSP (as it has a lower level of discrimination). However, it is not recommended in this instance as the best results for Raman spectroscopy are obtained with direct laser impingement on the sample requiring the samples to be demounted before Raman spectroscopy analysis. This is a delicate procedure that can result in the loss or destruction of the fibre (both highly undesirable). Whilst it has been shown that spectral subtraction can be utilised to achieve a Raman spectrum of the sample this introduces a level of subjectivity that is undesirable.

A recommended procedure for the analysis of black/blue cotton fibres is detailed below in Figure 5-53.

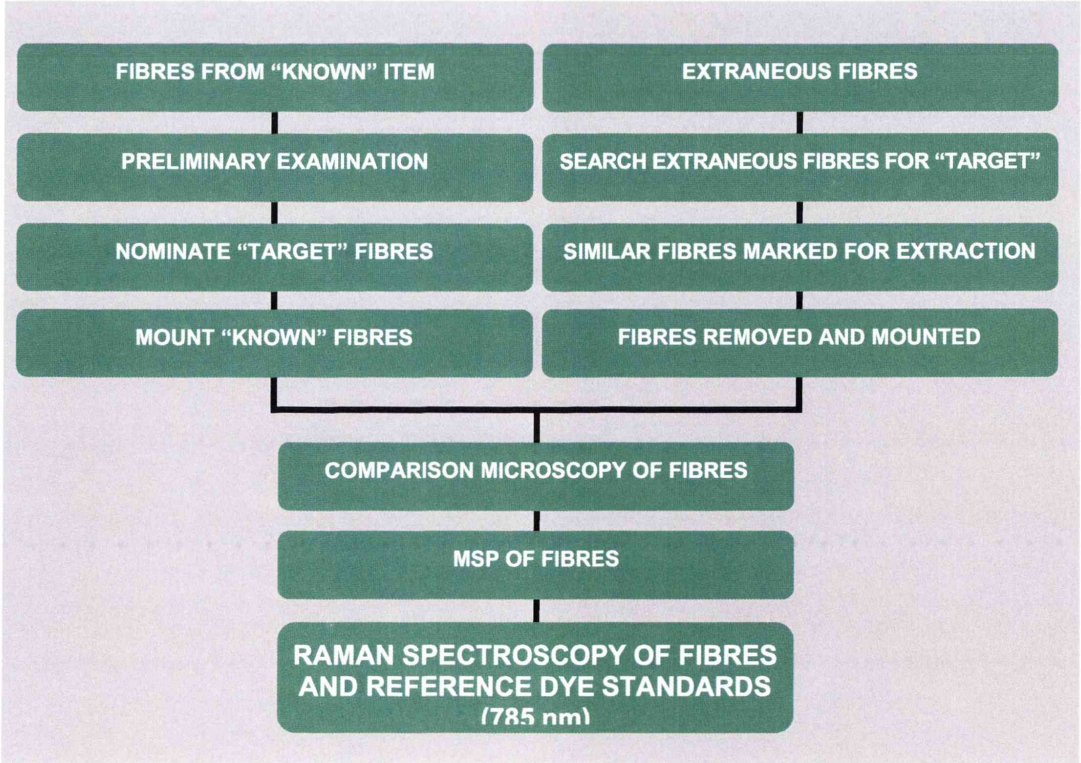


Figure 5-53 Suggested analysis sequence of known and extraneous fibres; incorporating Raman spectroscopy

5.5 Conclusions

Black/grey and blue cotton samples (in both the known sample set and the unknown sample set) were analysed with microspectrophotometry and Raman spectroscopy to determine the discriminatory power of the individual techniques as well as the techniques combined.

When analysing by Raman spectroscopy it was observed that samples with the same major dye component generally exhibited the same spectral characteristics even when minor dye components varied. This appeared to be due to the high concentration of the black or blue used to achieve the shade as samples of the grey samples (using lower concentrations of the black or blue dye) showed spectral contribution from the orange dye.

In analysis of both sample sets, Raman spectroscopy showed a lower discriminatory power than microspectrophotometry. However, combining the two techniques significantly increased the discriminatory power thereby highlighting the usefulness of Raman spectroscopy as an addendum to the current analysis sequence.

Chapter 6: Chemometrics of Raman Spectral Data

6.0 Summary

The purpose of investigating chemometrics for the discrimination of the Raman spectra was to assist in determining if visual comparison would provide better discrimination than that provided by chemometrically aided discrimination.

Chemometrics of the Raman spectral data of the known and unknown sample sets (from previous chapters) was investigated to determine if this mathematical technique could aid in the evaluation of spectral results. Principal Components Analysis (PCA) and Discriminant Analysis (DA) were the common chemometric techniques used to discriminate samples.

For the known sample set, visual comparison of the Raman spectra provided a discrimination power of 0.873, utilising Principal Component Analysis (PCA) and Discriminant Analysis (DA) increased the discrimination power to **0.982**. The 11 samples produced 55 pair-wise comparisons and after PCA and DA there was only 1 pair left not discriminated. Chemometrics could not be applied to the samples that fluoresced resulting in the one pair not being discriminated. A sub-set of the unknown sample set was used as an example and utilising chemometrics was able to further sub-classify samples.

6.1 Introduction

Modern automatic analysis methods provide opportunities to amass a large quantity of data in relatively short periods of time. A number of

chromatographic and spectroscopic methods can provide data on multiple components of a single specimen. Situations like this, where several variables are measured for each sample, yield **multivariate data** [184]. It would be possible to analyse and compare each variable in turn, but modern computers incorporating statistical methods can analyse multiple variables simultaneously.

One problem with multivariate data is that its sheer volume may make it difficult to see patterns and relationships. For example, a spectrum would normally be characterised by several hundred intensity measurements. Thus the aim of many methods of multivariate analysis is data reduction.

Chemometrics is the broad term that encompasses measurements via a mathematical or statistical application. Hibbert and James [185] define chemometrics as "...the use of computational and mathematical methods to extract information from analytical data". Whilst chemometrics is relatively new it has already had a huge impact on the field of spectroscopy [186]. Chemometrics has been successfully applied to a diverse range of problems such as:

- multivariate calibration
- reaction monitoring
- pattern recognition, classification and discriminate analysis, and
- multivariate process modelling[187].

When analysing samples, each sample is characterised by a set of measurements. When only two variables are present this can be characterised graphically as seen in Figure 6-1 [184], where the co-ordinates of the point give the values taken by the two variables (x_i, y_i). The point can also be defined as a vector, called a **data vector**, drawn to it from the origin ($\underline{r}_i$). Objects that have similar properties will have similar data vectors (they will be close to each other in the space defined by the variables). Such a group is called a **cluster**.

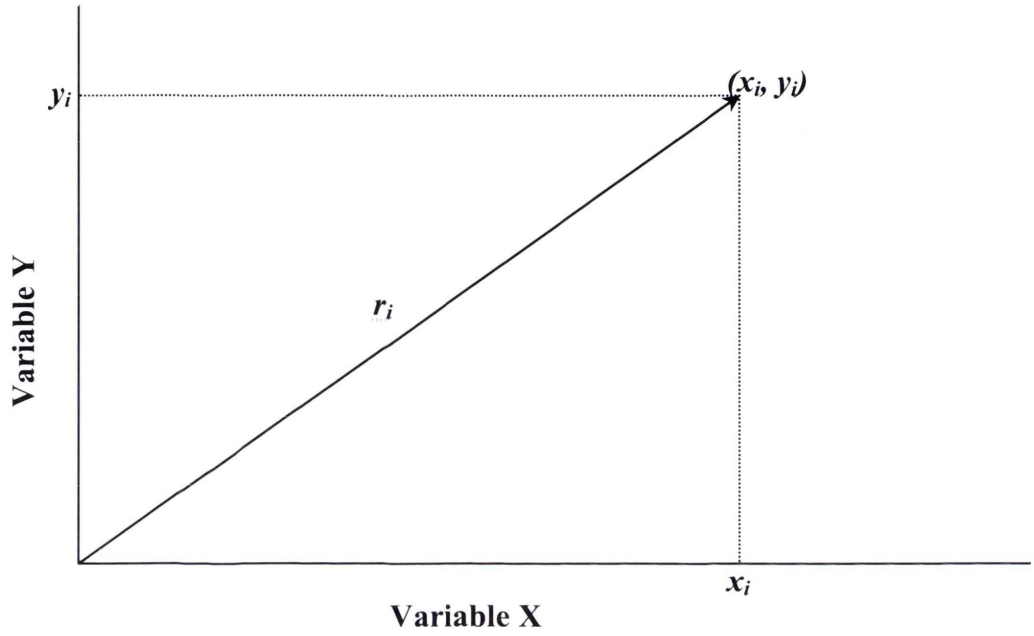


Figure 6-1 Illustration of a data vector, r_i [184]

A graphical representation is more difficult for three variables and impossible for four or more variables. It is in these situations that computer analysis is particularly valuable in finding patterns and relationships.

One chemometric tool that is widely applied to multivariate chemical data is principal components analysis (PCA). PCA has been described as "probably the oldest and best known of the techniques of multivariate analysis" [188]. PCA is a technique used to reduce the amount of information when there is correlation present.

6.1.1 Principal Components Analysis (PCA)

Principal components analysis reduces the dimensionality of a data set by sequential linear transformations of the data, where the first few transformations retain most of the variation of the original variables. Calculation of the principal component scores allows organization of results in lower dimensional space for

quick interpretation of results. The purpose of PCA is to obtain an overview of the dominant "pattern" in a data set [189].

The fact that PCA examines the variance in the original data set [188] is an important concept. Wold et al. [189] stated that in the past data sets were typically analysed one variable at a time, generally by examining each variable's mean and standard deviation. Given a data set of m variables this would yield $2m$ parameters. Introducing principal components analysis increases the number of available parameters by adding the covariance[1] between the variables to yield a total of $\{m(m\text{-}1)/2\}$ parameters. For m=25 variables there will be 300 parameters available for analysis, with only 50 of the variables coming from the mean and standard deviations. Including the covariance data significantly increases the information obtained from the analysis.

Geladi [186] states that "...there are different goals that require different chemometric technique". He defines these three goals as being: data exploration, supervised or unsupervised classification and curve resolution.

Data exploration involves looking at the data without prior information to find interesting phenomena. Classification involves placing spectra into groups and curve resolution is a strict form of data analysis that seeks to have the calculated spectra resembles the spectra of the pure constituent [186]. In this document data exploration and classification will be utilised for the sample sets. A method commonly used for classifying data is linear discriminant analysis (LDA).

[1] The terms *covariance* and *correlation* refer to the interrelation between two variables 190. Martens, H. and T. Naes, *Multivariate Calibration*. 1996, Chichester: John Wiley & Sons..

6.1.2 Linear Discriminant Analysis (LDA)

Linear discriminant analysis is a way of data classification when the classes are known (this is known as supervised classification). An observation (in this case the Principal Component scores of a Raman spectrum) is classified into a group if the squared distance (also called the Mahalanobis distance) of observation to the group centre (mean) is the minimum. An assumption is made that covariance matrices are equal for all groups. There is a unique part of the squared distance formula for each group and that is called the linear discriminant function for that group. For any observation, the group with the smallest squared distance has the largest linear discriminant function and the observation is then classified into this group.

Several studies have utilised chemometrics for data exploration and classification of Raman spectral data. Table 6-1 is a summary of the pre-processing and chemometric methods used. The studies are discussed in further detail.

Table 6-1 Sample types, pre-processing and chemometric methods that have been investigated for Raman spectroscopy

Reference	Sample Type	Pre-processing	Chemometric methods
Liu et al. [191]	cotton fibres	truncation, column & row mean centred	PCA
De Groot et al. [192]	dye labelled DNA fragments	glass peak correction, standard normal variate (SNV)	PCA
Kokot et al. [110]	cotton fibres	truncation, column & row mean centring	PCA and PLS
Svensson et al. [193]	ethyl acetate (reaction monitoring)	standard normal variate (SNV)	PCA, PLS
Brody et al. [194]	ivory	selected spectral regions, baseline correction, area normalisation	PCA

Liu et al. [191] studied seven types of raw, naturally coloured and white cotton cellulose fibres by Fourier transform Raman spectroscopy with the aid of chemometrics, namely principal component analysis (PCA). The PCA scores plot showed that PC2 discriminates the spectra of the naturally coloured cotton fibres and the white fibres. Further, it was shown that it was possible to differentiate some of the different types of white cotton fibres on this PC.

De Groot et al.[192] used Raman spectroscopy to yield indirect information on DNA-fragments. The authors found that it was possible to distinguish local differences in the sample composition and to correlate them with the sample morphology using principal component analysis (PCA). PCA facilitated the detection of three neighbouring spectra, clearly deviating from the others.

Kokot et al. [110] used FT-Raman spectroscopy for undyed poplin cotton fabric and the same fabric differently dyed with a bi-functional reactive dye. Four series of the dyed samples each contained the dye in a different form unfixed, ammonia-treated/unfixed, fixed, and ammonia treated/unfixed. The spectra were dominated by the dye, but the different states of the dye were not obviously differentiated. Application of principal component analysis showed that the spectral groups of the four different dye states could be discriminated from each other and from that of the undyed cotton. Further, for each series of the dyed fabrics, which contained samples with different amounts of dye, the individual dye concentration subgroups were distinguished.

Svensson et al. [193] studied the synthesis and hydrolysis of ethyl acetate using Raman spectrometry (785-nm laser). In the Raman spectra, the signals were seen to vary in proportion to the concentrations of ethanol, acetic acid and ethyl acetate. The rate constants were determined either by using the peak height at one single wavelength, or by using score values from principal components analysis PCA or partial least squares (PLS). It was shown that the single wavelength and the PCA/PLS approaches gave similar reaction profiles, where the standard normal variate SNV transformation was used for spectral pre-treatment.

Brody et al. [194] used Fourier-transform (FT) Raman spectroscopy and chemometrics as a non-destructive means of discriminating dentine from six mammalian species. The spectra were divided into eleven wavenumber regions and the area in each determined. Normalisation was achieved by dividing through by the values for one of the regions. Principal components and discriminant analysis of these values allowed for classification of the six different species. The dentine spectra could also be differentiated from those of bone and cementum.

The above studies demonstrate that chemometrics (specifically PCA and DA) can be used for Raman spectral data. It has been shown that it can be a useful aid in discriminating between samples that exhibit similar Raman spectra.

It is important to note that pre-processing of the data is necessary for chemometric analysis and that the above studies utilised various means of treating data prior to PCA. The above studies highlight that pre-treatment of data is dependent on both the data set obtained and the final chemometric analysis performed on the data. It is therefore, necessary to determine the pre-treatment best suited for the results obtained in the previous chapter (Chapters 4 and 5).

6.2 Aims

Raman analysis produces a spectrum of the sample being investigated. The spectra can be discriminated visually where the differences are obvious (and consistent within the sample) and where the sample set is of a sufficiently small size (to allow for direct comparison of all spectra). In instances where the spectra are not so easily discriminated or the sample set is of a large size visual comparison becomes difficult.

It is under such circumstances that chemometrics can be used as a valuable aid in discriminating the sample set. This chapter aims to use chemometrics for discriminating samples within the sample sets used in previous chapters. It will

focus on samples that result in visually similar Raman spectra but which are known to have different dye components.

The techniques of PCA and Liner Discriminant Analysis (LDA) will be used for data exploration and classification.

6.3 Materials and Methods

6.3.1 General

Nine of the eleven black/grey and blue cotton samples analysed in the previous chapters were used for data exploration and classification with PCA and LD. A sub-set of the unknown sample set (UV/VIS MSP group A/Raman sub group I) was used as an example of the application and discrimination of chemometrics. Data was truncated and pre-processed prior to chemometric analysis.

6.3.2 Samples

The sample set consisted of nine dyed black/grey and blue cotton fibres of different (reactive) dye combinations from Rocklea Spinning Mills Pty Ltd, Melbourne (Australia). The dyed fibres were Australian cotton with shade names and colours attributed by the manufacturer. The sample numbers and shades are shown in Table 5-5 of Chapter 5. The samples that fluoresced (samples 18 and 20) were not viable for chemometric analysis. Samples were run 5 times.

Samples from MSP group A (Langdon [25]) and Raman sub group I were used. Information for these samples is provided in Chapter 5. This sub-set was selected as it produced Raman spectra that were visually similar to that of CI Reactive Black 5.

6.3.3 Raman Microprobe Conditions

Renishaw System RM1000 (Renishaw Plc, Wotton-under-Edge, UK) equipped with a Leica microscope and GRAMS software (Galactic Industries Corporation). A 50x objective (numerical aperture of 0.75 and a working distance of 0.37 mm) was used for measurements. The microspectrometer was operated non-confocally at excitation wavelengths of 785nm with laser output powers of 300mW. Analysis was performed using either 100% laser power or 50% laser power depending on intensity of spectra and also any evidence of spectral degradation. The accumulated exposure time of the CCD detector was between 10 seconds and 120 seconds and the instrumental spectral resolution ranged from 1.5 to 2.5 cm^{-1}. Five repeats of each sample were taken at different points along the fibre (rather than ten due to time constraints).

6.3.4 Experiments

6.3.4.1 Chemometric analysis of known sample set

The nine samples (from the known sample set) were compared visually and the final groupings were then analysed with PCA and DA. The discrimination of the technique was then calculated (refer to Equation 7) and compared to that of visual discrimination.

6.3.4.2 Chemometric analysis of unknown sample set

The sub-set of the unknown sample set was analysed with PCA and LDA. The discrimination provided by chemometrics was then investigated.

6.3.5 Data Treatment

The spectra were exported from GRAMS 32 (Galactic Inc.) as ASCII XY data. This data was then imported into an Excel 4.0 spreadsheet. The spectral range of 1000-1700 cm^{-1} was selected for investigation (fingerprint region of samples is shown in Figure 6-2 and samples after spectral truncation is shown in Figure 6-4). This range corresponded approximately to the fingerprint region of the dyes that had previously been investigated by Raman spectroscopy.

One hundred and eighty-three points were sampled at ~4 cm^{-1} intervals from this range for chemometrics interpretation. Such spectral truncation is necessitated by the 256 column limit of the Excel software. This truncated data matrix was double centred (column and row mean-scaled) in Excel. This process removes the effect of multiplicative and baseline components (these effects are seen in Figure 6-3).

This new matrix was then imported into Minitab (Minitab Inc.) version 13.1. PCA (correlation) and DA were then used for analysis of the data.

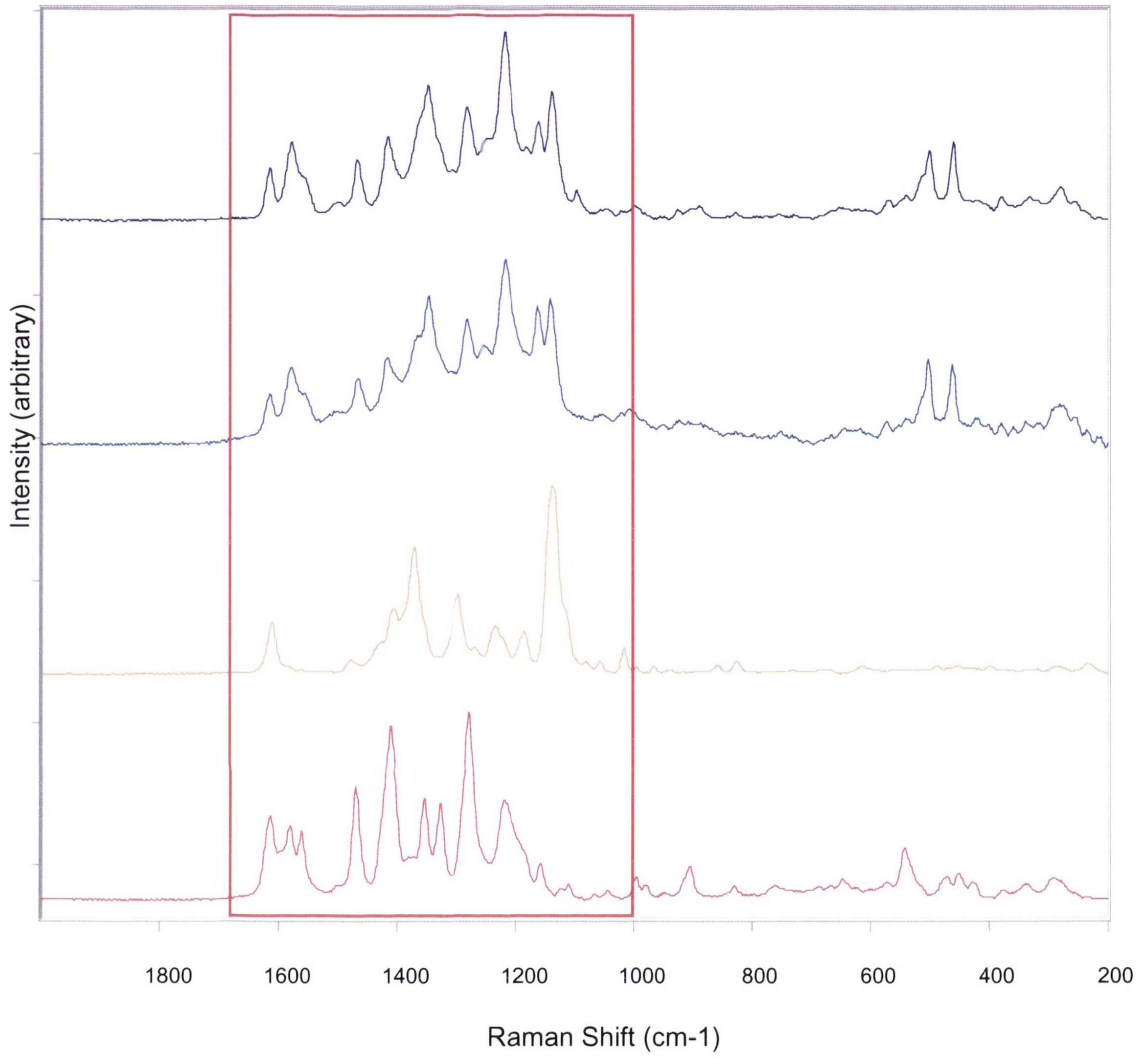


Figure 6-2 Raman spectra of 785nm analysis of (from top) dyed fibre - Moroccan Blue, Sumifix Navy dye, Synazol Gold-Yellow dye and Synazol Red dye. Red box indicates approximate area of interest after spectral truncation

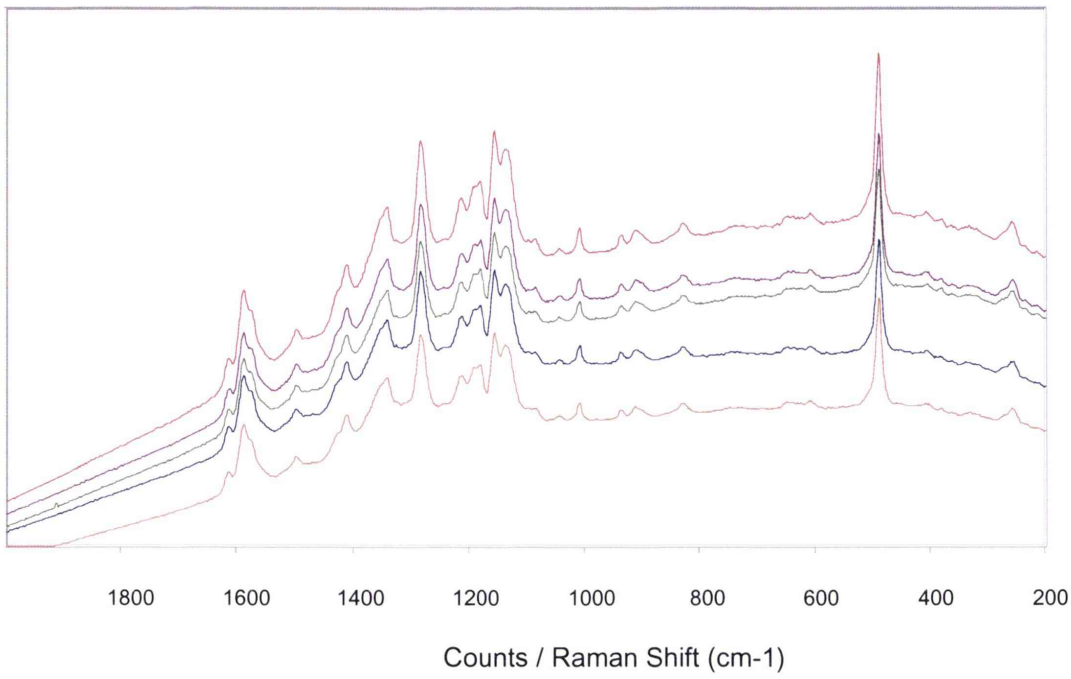


Figure 6-3 Raman spectra (five repeats) of Sample 14 showing multiplicative and baseline effects

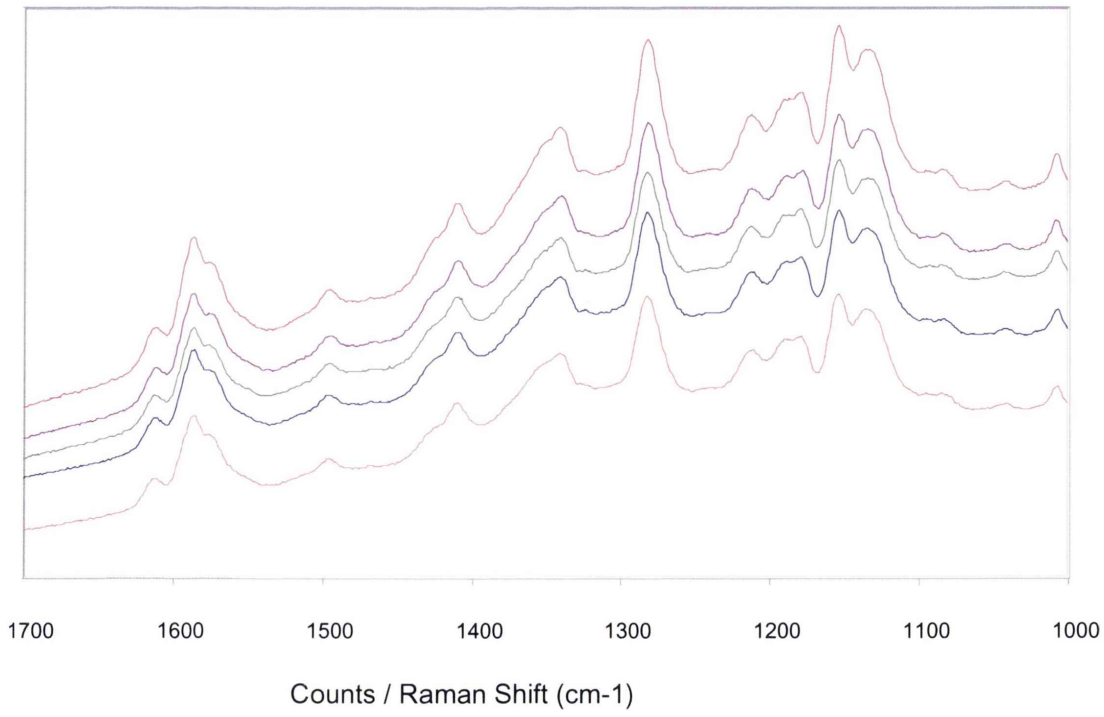


Figure 6-4 Raman spectra from sample 14 after spectral truncation

Discrimination Power

The discriminatory power (DP) of Raman spectroscopy for the known sample set (after chemometrics) was determined by applying the following calculation (Equation 6):

$$DP = \frac{\text{(number of discriminated pairs)}}{\text{(number of possible pairs)}}$$

Equation 6 Calculation for Discrimination Power

6.4 Results and Discussion

6.4.1 Known Sample Set

6.4.1.1 Samples visually discriminated using Raman spectroscopy (samples 32 and 35)

Even though samples 32 and 35 could be discriminated visually by Raman spectroscopy it was necessary to see if these samples could be discriminated after spectral truncation (as the truncation removed area of discrimination provided by cotton peaks). The Raman spectra of samples 32 and 35 are shown in Figure 6-5. The red boxed area shows the section of interest after truncation (note that samples were discriminated visually due to presence of cotton peaks in sample 35 in the 300-600nm region). This section was chosen as it contained the majority of the fingerprint region of the dyes used in the colouration of the fibres.

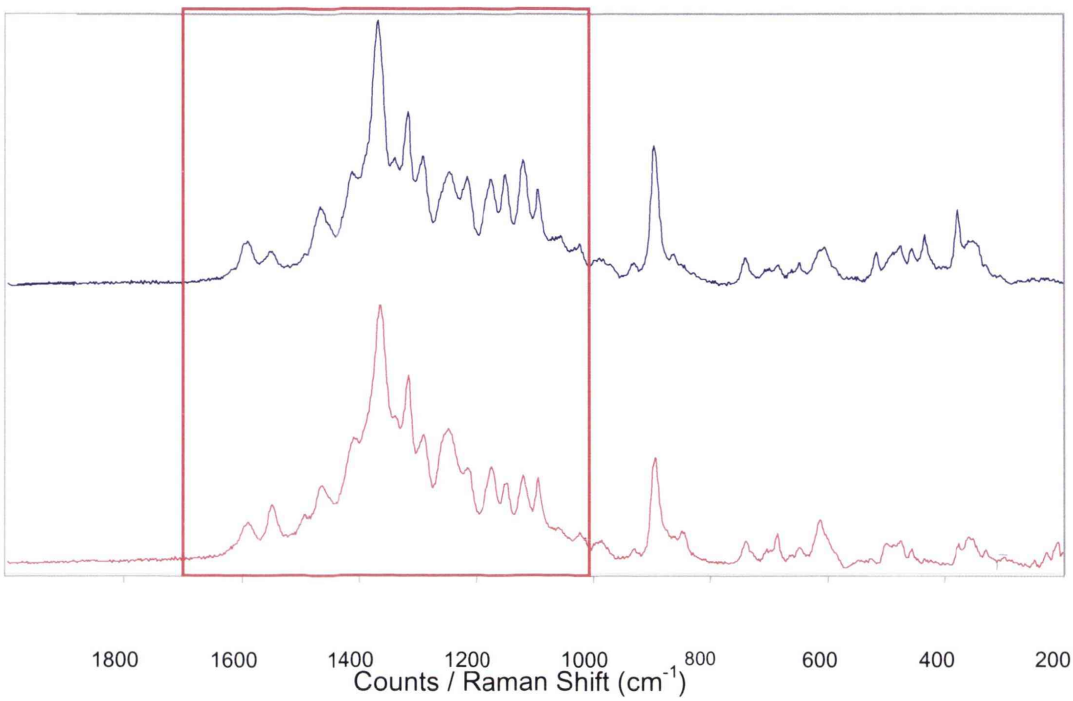


Figure 6-5 Spectra of (top) sample 35 and (bottom) sample 32, from Chapter 5. Red box indicates truncated area used for PCA analysis.

PCA was conducted on the truncated data and Figure 6-6 shows the variation attributed to each principal component. It is seen from this scree plot that the majority of variation is contained with the first four principal components.

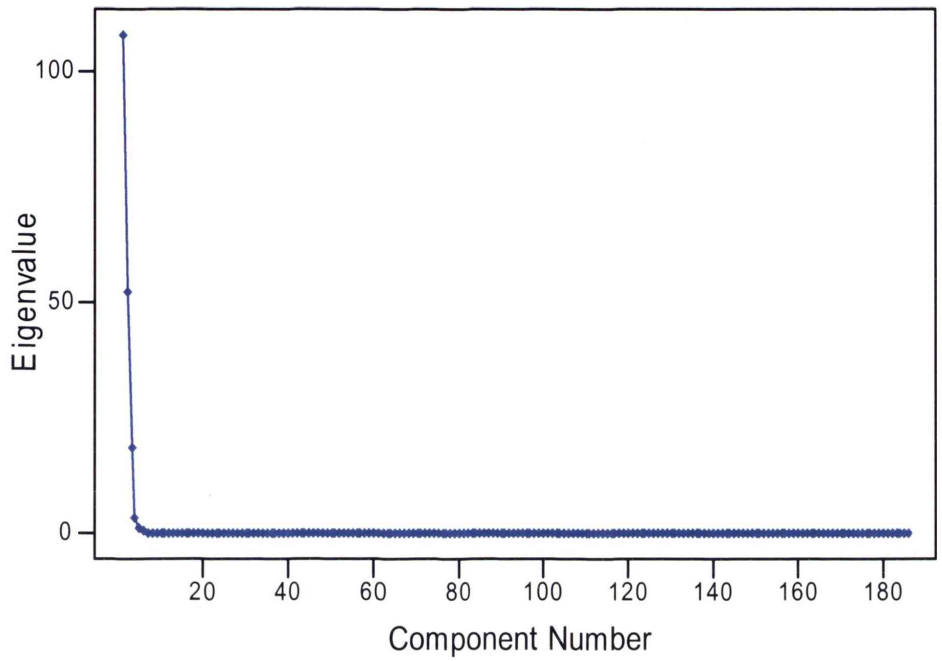


Figure 6-6 Scree plot of Principal Components for samples 32 and 35 showing majority of variation is accounted for in the first four Principal Components (PC1-PC4)

When determining how many principal components to extract it is generally an arbitrary decision based on when there is very little "random" variability left. However, there are two guidelines that can be used:

• *Kaiser's criterion:* According to Kaiser's criterion it is only meaningful to extract factors which account for more variance than a single perfect variable. Therefore, factors are extracted until their *eigenvalues* fall below 1. This is one of the most common guidelines used for extracting components An example scree plot showing the Kaiser criterion cut-off is shown in Figure 6-7.

• *Cattell's scree test:* This scree test is slightly more conservative than Kaiser's criterion. The maximum number of factors extracted is indicated by the point before a plot of *eigenvalues* flattens out (i.e. where the smooth decrease of eigenvalues appears to level off to the right of the plot). That is the point preceding the elbow of the curve. An example of the Cattell's scree test is shown in Figure 6-7.

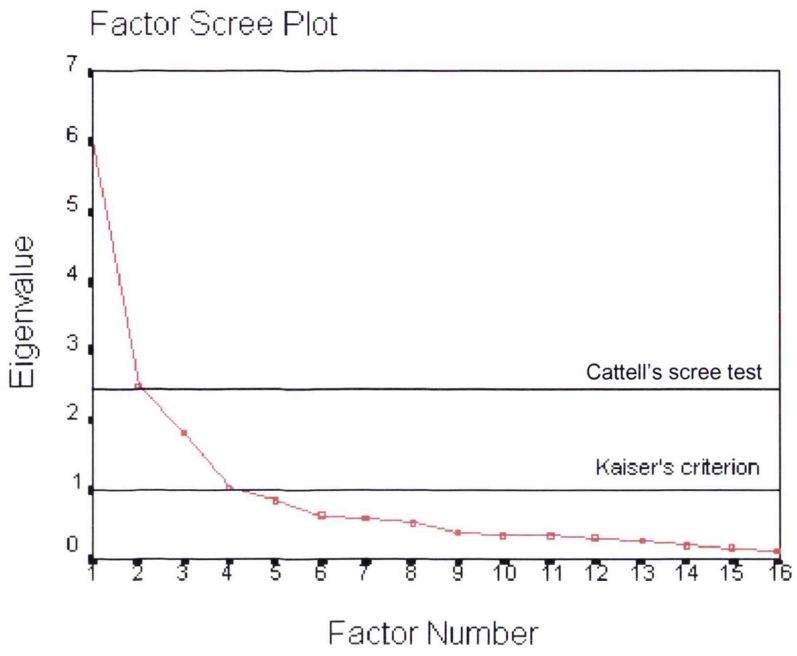


Figure 6-7 Scree plot showing the Kaiser Criterion cut-off (at the *eigenvalue* of 1, thereby using 4 Factors) and the Cattell's scree test (cut-off at 2.5 *eigenvalue*, thereby using 2 factors)

Both of these guidelines were considered when extracting relevant factors for further analysis. For the scree plot from samples 32 and 35 (refer to Figure 6-7 above) the eigenvalues for the first five factors are shown in Table 6-2. Using Kaiser's criterion, components one to five could be used as these all have an *eigenvalue* greater than one. Using the Cattell's scree test, components one to four could be used as these all occur before the curve of the elbow in the scree plot (Figure 6-6). However, using only the first three components the two samples are readily separated (refer to Figure 6-8 Comparison of Principal Component 1-3 (PC1 – PC3) for samples 32 and 35).

Table 6-2 Factor number and corresponding *eigenvalues* (plus proportion) for samples 32 and 35

Component Number	Eigenvalue	Proportion (%)
1	108.31	58.2
2	52.56	28.3
3	18.95	10.2
4	3.71	2.0
5	1.34	0.7
6	0.42	0.2

By plotting the principal components against each other (refer to Figure 6-8) clear separation of the samples 32 and 35 can be observed (when comparing PC2 and PC3).

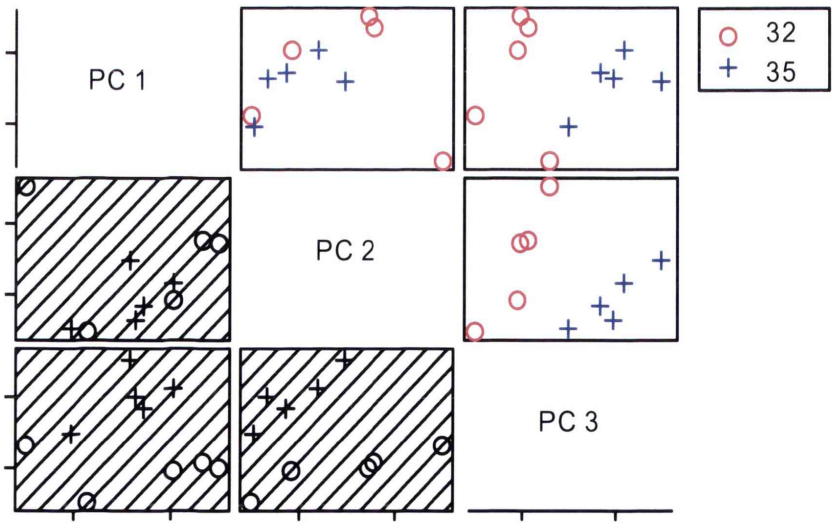


Figure 6-8 Comparison of Principal Component 1-3 (PC1 – PC3) for samples 32 and 35

If the plot of PC2 against PC3 is expanded (refer to Figure 6-9) it is noted that the PC3 values are positive for sample 35 and negative for sample 32.

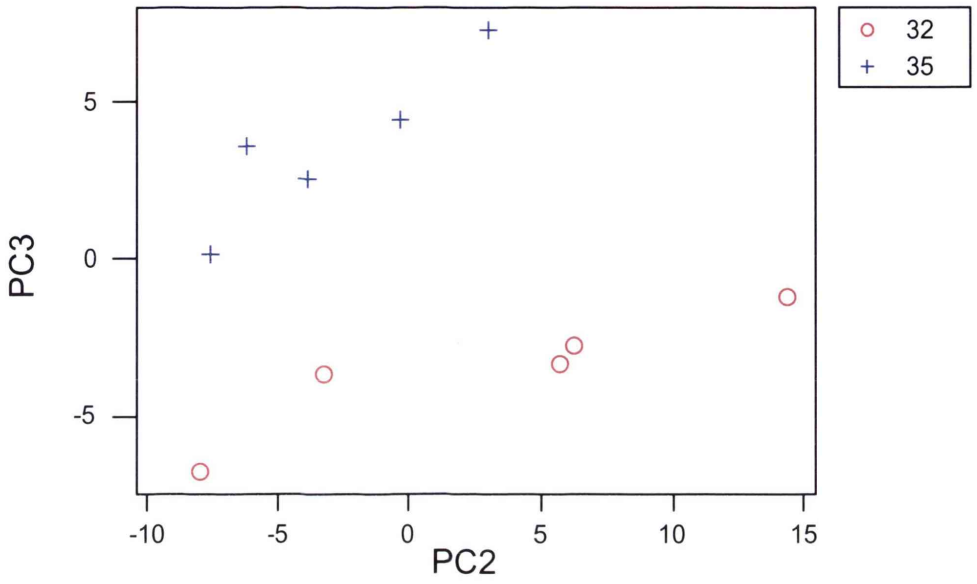


Figure 6-9 Principal Component 2 against Principal Component 3

By plotting the first principal component loadings against the wavelength region of interest (1000-1700 cm^{-1}) the areas contributing to variation can be observed. The loading plot for PC3 (refer to Figure 6-10) shows that the majority of the variation of this third principal component is attributed to variation in the peaks in the region of ~1085 cm^{-1} (seen as positive loadings) and ~1270 cm^{-1} (seen as negative loadings).

When these two areas of interest are examined in the Raman spectra from samples 32 and 35 (refer to Figure 6-11) variations are observed in the relative peak heights. It is these variations that the samples are being discriminated by.

These results highlight the value of using chemometrics. Determining if these variations are intra-sample or inter-sample can be time consuming (if the pattern is noticed at all) and the problem is multiplied with an increasing sample set size.

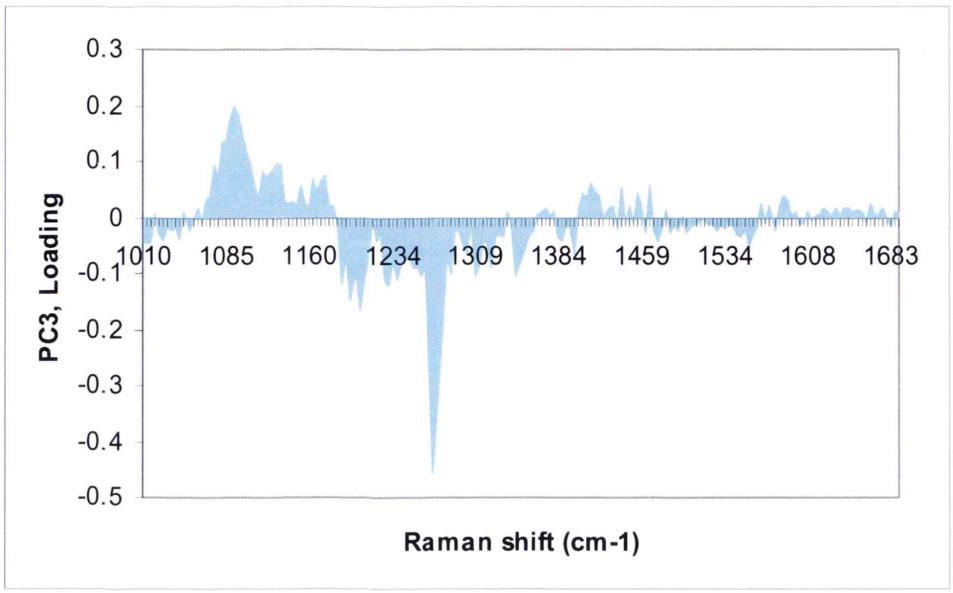


Figure 6-10 Principal Component 3 loading plot for Samples 32 and 35

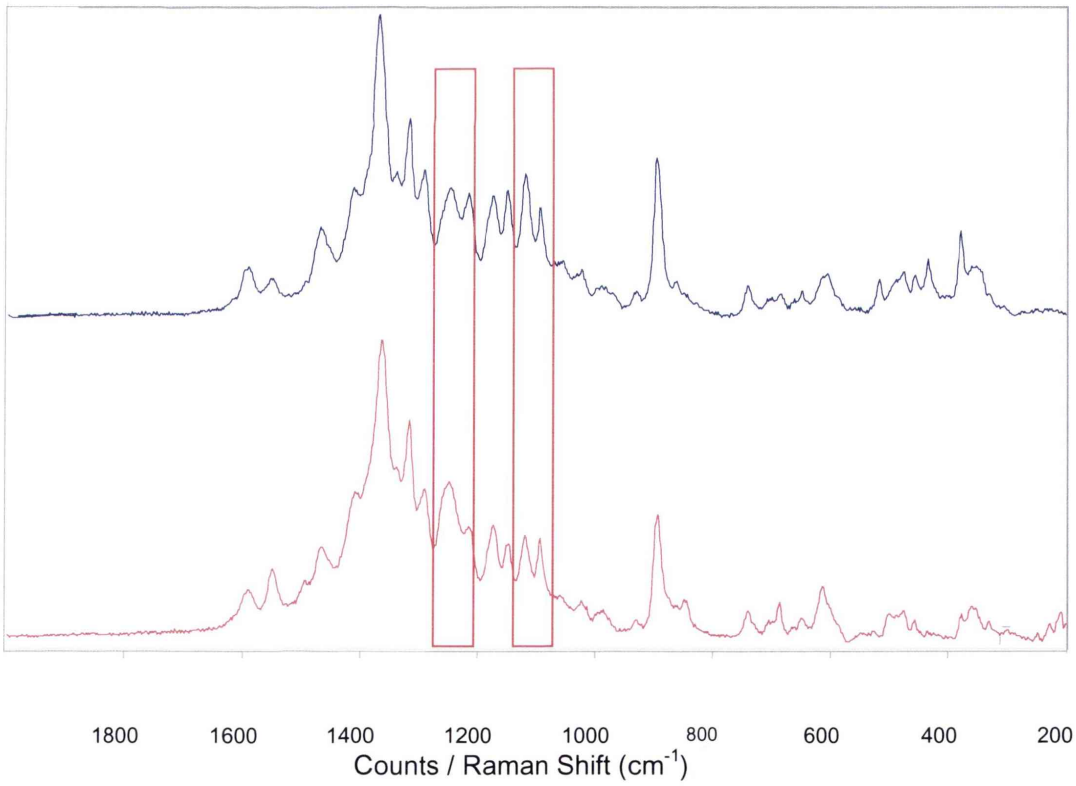


Figure 6-11 Spectra of (top) sample 35 and (bottom) sample 32, from Chapter 5. Red boxes indicate areas of interest from the PC3 loading plot

Performing linear discriminant analysis on the resultant principal components (first 3 PC's) for samples 32 and 35 are correctly classified (refer to Table 6-3).

Table 6-3 Linear Discriminant analysis results for samples 32 and 35 – using first three principal components (PC1-PC3)

Discriminant Analysis: Samples 32 and 35 using PC1, PC2, PC3

```
Linear Method for Response:   Labels
Predictors:  PC1  PC2  PC3

Group        32        35
Count         5         5

Summary of Classification

Put into      ....True Group....
Group            32        35
32                5         0
35                0         5
Total N           5         5
N Correct         5         5
Proportion    1.000     1.000

N =   10     N Correct =   10     Proportion
Correct = 1.000

Squared Distance Between Groups
                 32        35
32           0.0000   53.2813
35          53.2813    0.0000

Linear Discriminant Function for Group
                 32        35
Constant   -6.6602   -6.6602
PC1         0.1317   -0.1317
PC2         0.8969   -0.8969
PC3        -2.9489    2.9489
```

6.4.1.2 Samples not visually discriminated using Raman spectroscopy

Samples 14, 25, 31 and 40 were not discriminated visually from each other when using Raman spectroscopy. This was attributed to the spectral dominance of the major dye used in colouration (a dye common to all four samples).

The same spectral truncation was applied to these samples as was applied to samples 32 and 35. Once again this was due to this region containing the majority of the fingerprint data from the dyes. PCA was conducted on the truncated data and Figure 6-12 shows the variation attributed to each principal component. It is seen from this scree plot that the majority of variation is contained with the first three principal components.

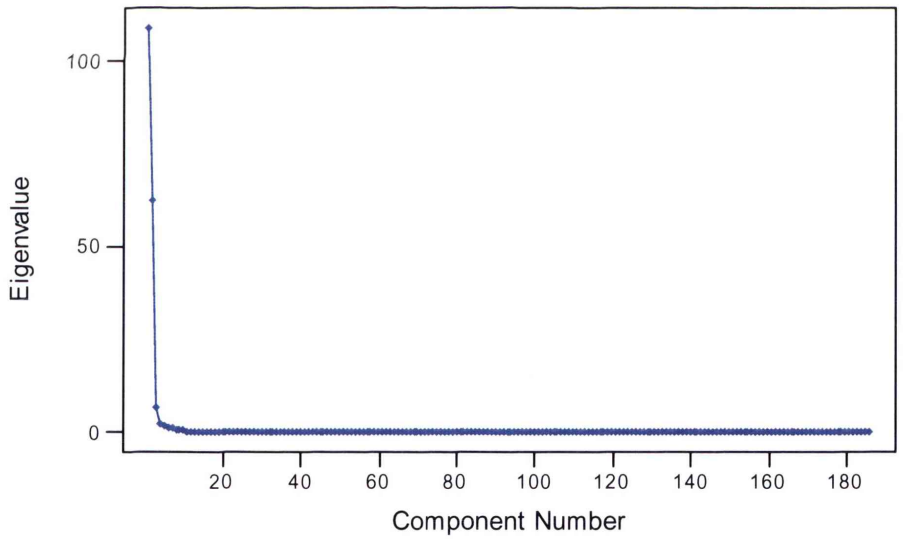


Figure 6-12 Scree plot of Principal Components for samples 14, 25, 31 and 40 showing majority of variation is accounted for in the first three Principal Components (PC1-PC3)

Table 6-4 lists the *eigenvalues* associated with each component number. Utilising Kaiser's criterion, the first six components can be used for further analysis. Using Cattell's scree test the first three components can be used (as these components fall before the curve of the elbow).

Table 6-4 Factor number and corresponding *eigenvalues* (plus proportion) for samples 14, 25, 31 and 40

Component Number	Eigenvalue	Proportion (%)
1	109.15	58.7
2	62.40	33.5
3	6.40	3.4
4	2.06	1.1
5	1.62	0.9
6	1.07	0.6
7	0.87	0.5

By plotting the principal components against each other (refer to Figure 6-13) for samples 14, 25, 32 and 40 separation of the samples cannot be clearly discerned. It is observed that sample 25 does show tighter clustering than the other samples.

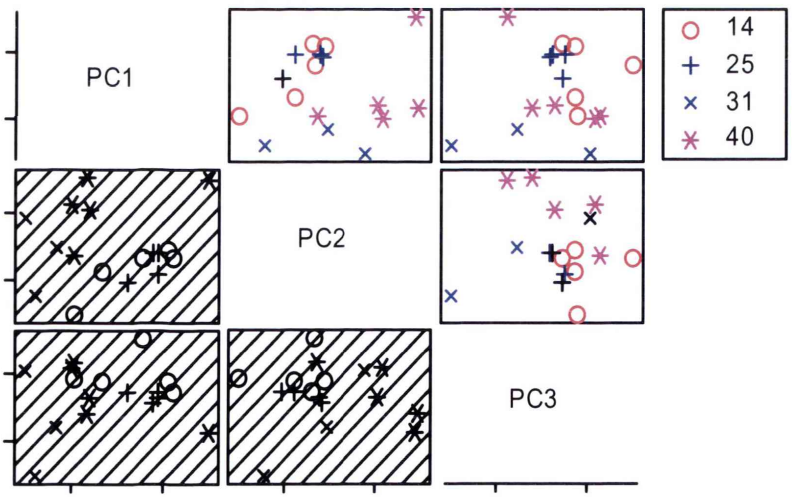


Figure 6-13 Comparison of Principal Component 1-3 (PC1 – PC3) for 14, 25, 31 and 40

Utilising the more conservative Cattell's scree test the first three principal components were used for discriminant analysis. Table 6-5 lists the results from linear discriminant analysis and shows that samples 14, 25 and 40 could not be discriminated (due to misclassification of some samples). However, sample 31 could be discriminated from the other three.

Table 6-5 Linear Discriminant analysis results for samples 14, 25, 31 and 40 – using first three Principal Components (PC1-PC3) – note incorrect classification of samples (highlighted in red)

Discriminant Analysis: Samples 14, 25, 31 and 40 using PC1, PC2, PC3

Linear Method for Response: C1
Predictors: PC1 PC2 PC3

Group	14	25	31	40
Count	5	5	4	5

Summary of Classification

Put into	....True Group....			
Group	14	25	31	40
14	2	0	0	1
25	3	5	0	0
31	0	0	4	0
40	0	0	0	4
Total N	5	5	4	5
N Correct	2	5	4	4
Proportion	0.400	1.000	1.000	0.800

N = 19 N Correct = 15 Proportion Correct = 0.789

Squared Distance Between Groups

	14	25	31	40
14	0.0000	0.5385	30.2990	19.0623
25	0.5385	0.0000	27.4157	16.7257
31	30.2990	27.4157	0.0000	5.2392
40	19.0623	16.7257	5.2392	0.0000

Linear Discriminant Function for Group

	14	25	31	40
Constant	-2.7667	-2.1558	-5.2817	-2.2587
PC1	0.5158	0.4862	-0.8041	-0.3588
PC2	-1.4650	-1.2935	1.5626	1.5085
PC3	1.6525	1.0726	-2.1210	-1.0283

Utilising four components (i.e. falling within the Kaiser criterion) all samples were correctly classified during linear discriminant analysis (refer to Table 6-6).

Table 6-6 Linear Discriminant analysis results for samples 14, 25, 31 and 40 – using first four Principal Components (PC1-PC6) – note incorrect classification no longer occurring

Discriminant Analysis: Labels versus PC1, PC2, PC3, PC4

Linear Method for Response: Group Labels
Predictors: PC1 PC2 PC3 PC4

Group	14	25	31	40
Count	5	5	4	5

Summary of Classification

Put into Group	....True Group.... 14	25	31	40
14	5	0	0	0
25	0	5	0	0
31	0	0	4	0
40	0	0	0	5
Total N	5	5	4	5
N Correct	5	5	4	5
Proportion	1.000	1.000	1.000	1.000

N = 19 N Correct = 19 Proportion Correct = 1.000

Squared Distance Between Groups

	14	25	31	40
14	0.0000	2.5451	32.2015	20.7484
25	2.5451	0.0000	35.2326	24.0973
31	32.2015	35.2326	0.0000	5.2457
40	20.7484	24.0973	5.2457	0.0000

Linear Discriminant Function for Group

	14	25	31	40
Constant	-2.8003	-3.5600	-5.9089	-2.7987
PC1	0.5384	0.6323	-0.9017	-0.4493
PC2	-1.5316	-1.7234	1.8499	1.7751
PC3	1.6845	1.2791	-2.2590	-1.1564
PC4	0.2305	1.4900	-0.9958	-0.9239

The same spectral truncation was applied to samples 38 and 39 as was applied to samples 32 and 35. PCA was conducted on the truncated data and Figure 6-14 shows the variation attributed to each principal component. It is seen from this scree plot that the majority of variation is contained with the first three principal components.

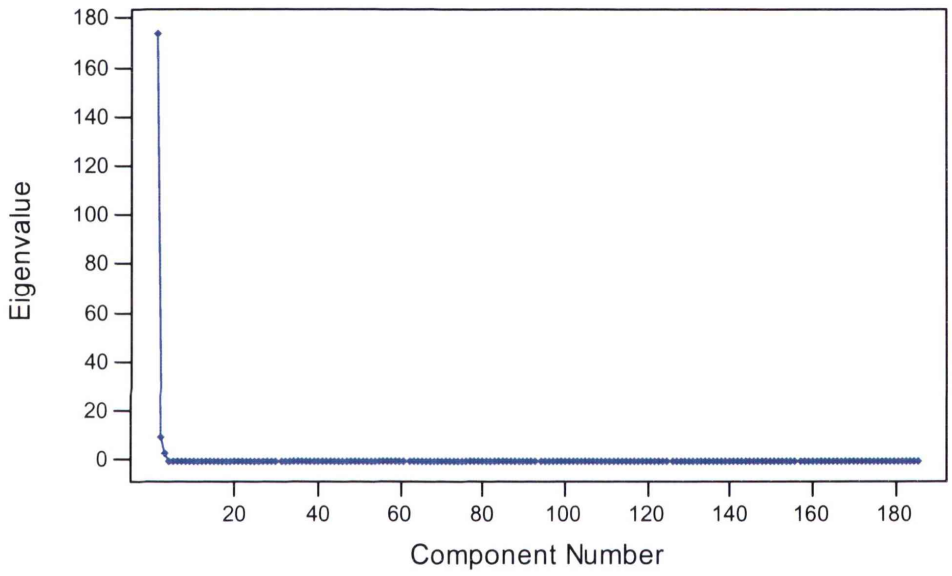


Figure 6-14 Scree plot of Principal Components for samples 38 and 39 showing majority of variation is accounted for in the first three Principal Components (PC1-PC3)

Table 6-7 lists the *eigenvalues* associated with each component number. Utilising Kaiser's criterion, the first three components can be used for further analysis. Using Cattell's scree test the first three components can be used (as these components fall before the curve of the elbow).

Table 6-7 Factor number and corresponding *eigenvalues* (plus proportion) for samples 38 and 39

Component Number	Eigenvalue	Proportion (%)
1	173.14	93.1
2	9.51	5.1
3	3.13	1.7
4	0.13	0.1
5	0.05	0.0
6	0.02	0.0

By plotting the principal components against each other (refer to Figure 6-15) for samples 38 and 39, separation of the samples can be clearly seen. However, the separation is not linear as was observed for samples 32 and 35. For this reason, Quadratic Discriminant Analysis (QDA) was employed.

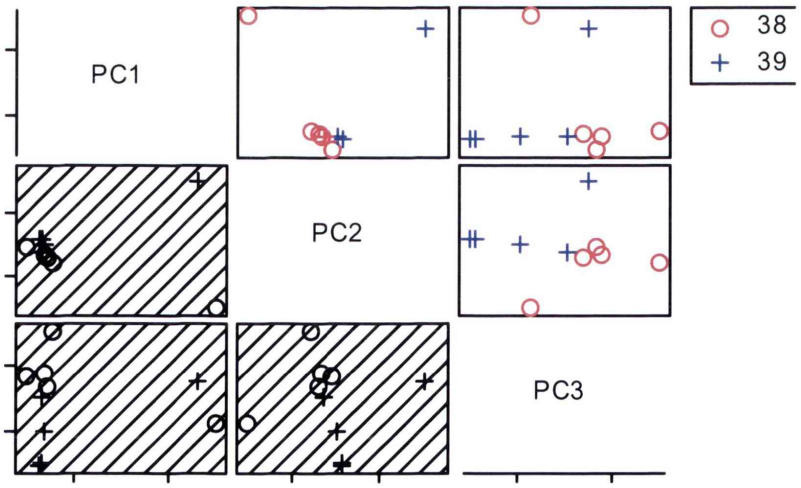


Figure 6-15 Comparison of Principal Component 1-3 (PC1 – PC3)

Utilising three components (i.e. falling within the Kaiser criterion and the Cattell's scree test) all samples were correctly classified during quadratic discriminant analysis (refer to Table 6-8).

Table 6-8 Quadratic Discriminant analysis results for samples 38 and 39 – using first three Principal Components (PC1-PC3)

Discriminant Analysis: Samples 38 and 39 using PC1, PC2, PC3,

```
Quadratic Method for Response:   Labels
Predictors:  PC1    PC2    PC3

Group         38         39
Count          5          5

Summary of Classification

Put into      ....True Group....
Group            38         39
38                5          0
39                0          5
Total N           5          5
N Correct         5          5
Proportion    1.000      1.000

N =   10      N Correct =   10      Proportion
Correct = 1.000

From     Generalized Squared Distance to Group
Group             38         39
38              1.06    3009.10
39            582.83      -0.62
```

Employing chemometrics (and the less conservative Kaiser's criterion) the discrimination of Raman spectroscopy significantly increases with only one pair of a possible 55 not discriminated (samples that fluoresced). The discriminatory power increased from 0.873 (visual comparison) to 0.982 (visual comparison then PCA/DA) making Raman spectroscopy (with PCA/DA) now more discriminatory than vis-microspectrophotometry (0.927) and equivalent to the combined techniques (0.982).

Care must be taken when selecting the number of components to utilise as there comes a point where all things are different. If the more conservative Cattell's

scree test is utilised, four of the 55 pair-wise comparisons are not discriminated making the discriminatory power of Raman spectroscopy (PCA/DA) 0.927 which is equivalent to the discrimination provided by Vis-microspectrophotometry. And if Vis-MSP and Raman spectroscopy (with chemometrics analysis) is used all samples are discriminated.

6.4.2 Unknown Sample Set

From the unknown sample set, the samples that could not be discriminated by Raman spectroscopy for the subset of UV/Vis MSP Group A / Raman Group I were investigated.

PCA was conducted on the truncated data and Figure 6-16 shows the variation attributed to each principal component. It is seen from this scree plot that the majority of variation is contained with the first three principal components.

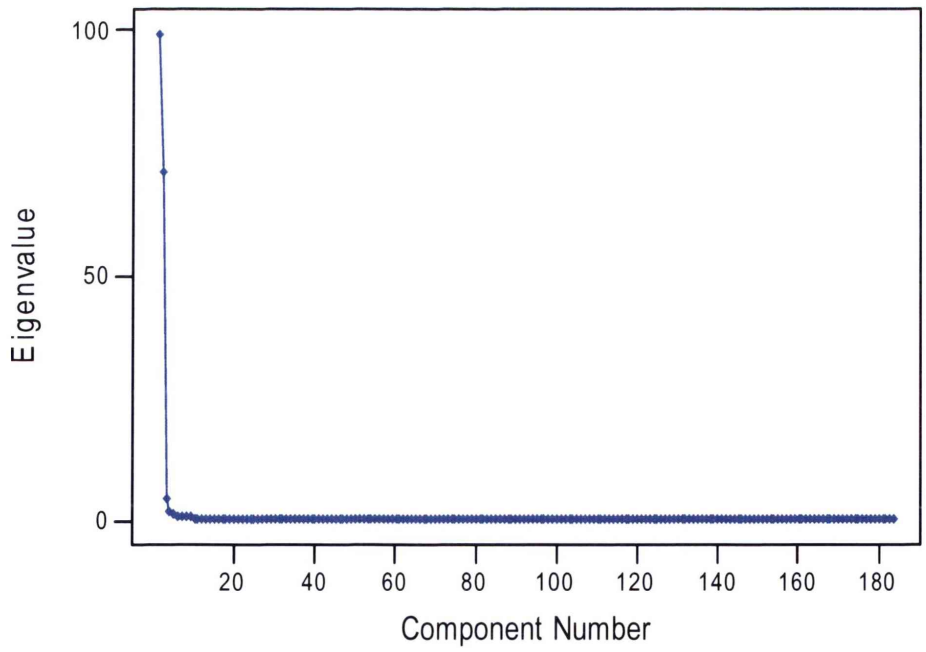


Figure 6-16 Scree plot of Principal Components for samples 5, 9, 10, 41, 43 and 47 (from unknown sample set) showing majority of variation is accounted for in the first three Principal Components (PC1-PC3)

For the scree plot from the unknown sample subset (refer to above) the *eigenvalues* for the first six factors are shown in Table 6-9. Using Kaiser's criterion, components one to five could be used as these all have an *eigenvalue* greater than one. Using the Cattell's scree test, components one to four could be used as these all occur before the curve of the elbow in the scree plot (Figure 6-16).

Table 6-9 Factor number and corresponding *eigenvalues* (plus proportion) for unknown sample set MSP Group A/Raman Group I

Component Number	Eigenvalue	Proportion (%)
1	99.53	54.1
2	71.08	38.6
3	4.50	2.4
4	1.93	1.0
5	1.30	0.7
6	0.78	0.4

By plotting the principal components against each other (refer to Figure 6-17) for the unknown sample subset, separation of the samples cannot be clearly discerned. It is observed that sample 10 does show tighter clustering than the other samples.

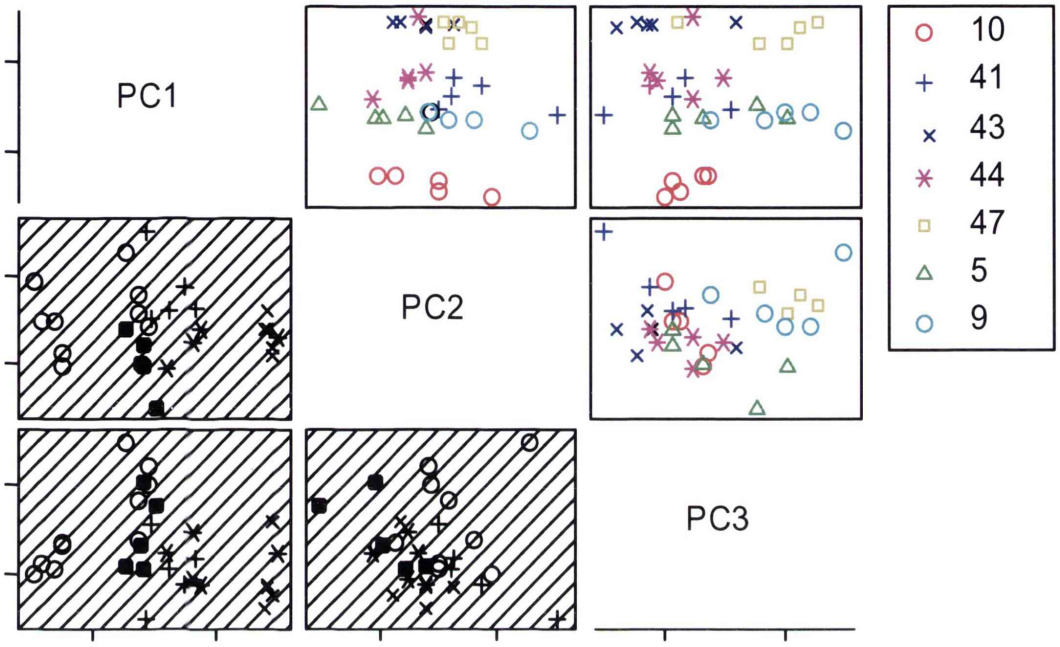


Figure 6-17 Comparison of Principal Component 1-3 (PC1 – PC3)

Utilising the more conservative Cattell's scree test the first four principal components were used for quadratic discriminant analysis. Table 6-10 lists the results from quadratic discriminant analysis and shows that all samples are correctly classified.

Table 6-10 Quadratic Discriminant analysis results for unknown sample set MSP group A/Raman group I – using first five Principal Components (PC1-PC5)

Discriminant Analysis: C1 versus PC1, PC2, PC3 AND PC4

Quadratic Method for Response: C1
Predictors: PC1 PC2 PC3 PC4

Group	10	41	43	44	47	5	9
Count	5	5	5	5	5	5	5

Summary of Classification

Put into	....True Group....						
Group	10	41	43	44	47	5	9
10	5	0	0	0	0	0	0
41	0	5	0	0	0	0	0
43	0	0	5	0	0	0	0
44	0	0	0	5	0	0	0
47	0	0	0	0	5	0	0
5	0	0	0	0	0	5	0
9	0	0	0	0	0	0	5
Total N	5	5	5	5	5	5	5
N Correct	5	5	5	5	5	5	5
Proportion	1.000	1.000	1.000	1.000	1.000	1.000	1.000

N = 35 N Correct = 35 Proportion Correct = 1.000

From Group	Generalized Squared Distance to Group 10	41	43	44	47	5	9
10	-2.4	789.0	29122.8	66.5	255.6	361.4	3943.7
41	725.9	2.9	4771.6	48.3	62.5	206.3	260.3
43	1886.8	99.7	-2.7	46.6	172.5	914.5	4088.4
44	1053.8	16.1	3528.4	3.4	211.2	401.2	2551.7
47	2332.1	464.0	206.7	74.2	2.3	1682.9	1995.3
5	270.5	345.3	9735.5	80.1	141.2	0.2	701.8
9	824.2	69.0	9722.2	110.3	71.5	118.5	-2.3

Employing chemometrics the discrimination of Raman spectroscopy significantly increases. Once again care must be taken when selecting the number of components to utilise as there comes a point where all things are different. Even with using the more conservative Cattell's scree test all samples in the sub-set are discriminated.

It is difficult to know how different (chemically) the samples are as they are a random population. The results do however; show that the production of CI

Reactive Black 5 does vary. Industry enquiries have found that whilst CI Reactive Black is the most popular reactive dye the manufacturing process is one that is difficult to control resulting in variations of the dye.

6.5 Conclusions

Black/grey and blue cotton samples (in both the known sample set and the unknown sample set) were analysed with Raman spectroscopy to determine the discriminatory power of the techniques through a visual comparison. The three groups that were not able to be visually discriminated were then analysed mathematically with PCA and DA. The use of chemometrics increased the discrimination power of Raman spectroscopy significantly for both the known sample set and the unknown sample subset used.

It is necessary to be aware that at some point all things can be discriminated and with chemometrics a certain subjectivity id required for determining the cut-off point for analysis. In this study the use of two techniques were used for determining the number of components to extract (Kaiser's criterion and the Cattell's scree test).

Chapter 7: Raman Spectroscopy for Forensic Fibre Examinations – Discussion, Conclusions and Future Directions

7.1 Discussion

In the preceding chapters various parameters of Raman spectroscopy were investigated to determine the optimal conditions for the analysis of the samples selected. The major variable investigated was laser wavelength. It has been observed in these studies and studies previously reported that the spectra of a sample (and in the case of this sample set: dyed cotton) can vary depending on the wavelength of the laser excitation. This variation is predominantly due to the resonance effect and the occurrence of fluorescence (generally with visible light lasers).

Theoretically resonance enhancement effects should not be significant when analysing samples with laser excitations in the near infra-red (NIR) region and NIR excitation has also been shown to reduce the problem of fluorescence of certain samples.

Specifically, the studies in this document have shown that analysis of black/grey and blue cotton fibres with the 785nm laser excitation (NIR) provided superior results with spectra being obtained quickly and with no degradation of the sample or the resultant spectra from photochemical or pyrolysis processes.

A larger sample set also incorporated analysis with the 632.8 nm laser excitation. This visible light laser was used to determine if resonance enhancement of the

particular sample set occurred and if this phenomenon could be utilised to further increase the discrimination between samples. It was observed, however, that this laser wavelength provided lower discrimination than that of the NIR laser. Whilst this laser wavelength was not useful for this sample set the incorporation of a range of laser excitations into analysis may provide more information than that obtained from a single laser wavelength particularly for samples that have a lower concentration of dyes.

A result from the study of these dark coloured cottons was that, at least, the major dye component could be identified using Raman spectroscopy. Initially this was considered to be a possible result of the resonance of the dark black or blue dye. Further investigation found that it was more likely to be due to the concentration of the dye on the fibre rather than resonance enhancement of the dye (as the dyes were found to have very little absorbance in the NIR region when analysing with microspectrophotometry). This was further clarified when analysis of the lighter coloured grey fibres (therefore lower concentration of dye) found that spectral contributions of the orange dye were observed.

When considering the incorporation of another technique as either an addition to an analysis sequence or as a technique to supercede a current technique it is vitally important to investigate the advantages and limitations of the new technique as well as the techniques "performance" against current techniques. It was deemed to be very important, therefore, to compare Raman spectroscopy with other techniques for analysing colour that are currently used in fibre examinations, primarily microspectrophotometry (MSP). This was achieved by comparing the discrimination of a sample set of known fibres with known dyes as well as a sample of randomly selected cotton fibres for each technique individually as well as the discrimination provided by combining the two techniques sequentially.

The discriminatory power of Raman spectroscopy was investigated for a sample set of 11 black/grey and blue cotton samples as well as a set of 91 black and blue cotton fibres of unknown dye types. This was then compared to the

discriminatory power of microspectrophotometry (MSP) for the same sample sets as well as the discriminatory power of the combined techniques. For the known sample set Raman spectroscopy provided a discrimination power of 0.873, MSP provided a discrimination power of 0.927 and the combined techniques provided a discrimination power of **0.982**. For the unknown sample set Raman spectroscopy provided a discrimination power of 0.799, MSP provided a discrimination power of 0.832 and the combined techniques provided a discrimination power of **0.962**.

The results from this study indicate that, as a single technique, MSP provides greater discrimination than Raman spectroscopy. However, because the samples were discriminated based on different aspects of the fibre (i.e. MSP-colour, Raman-molecular structure of the major dye) the combination of the two techniques provided the greatest discrimination. These results indicate that whilst Raman spectroscopy will not supercede MSP the introduction of the technique into the fibre examination sequence would be valid when analysing black/grey and blue cotton samples.

Whilst the advantages of Raman spectroscopy were evident during these studies one major limitation of the technique was also highlighted. The problem of fluorescence has long been regarded as the major drawback to using Raman spectroscopy for fibre examinations. The problem was overcome in some instances by changing the laser wavelength, but not all. In particular, during analysis of the unknown sample set fluorescence was observed in approximately 16% of the samples analysed.

Even with the occurrence of fluorescence it was shown that for the sample sets investigated Raman spectroscopy provided a level of discrimination not able to be achieved with microspectrophotometry alone. Therefore, Raman spectroscopy should be considered when undertaking analysis of these sample types.

Chemometrics is a well established field within mathematics. It has been utilised for pattern recognition and data classification for spectral data for many years and an investigation of its potential for discriminating between the studied samples was deemed to be important. Of particular interest was whether the technique could provide discrimination on the presence of the minor dyes that were not observed visually in the resultant Raman spectra.

In these studies the primary purpose of investigating chemometrics for the discrimination of the Raman spectra was to assist in determining if chemometrically aided comparison would provide better discrimination than that provided by visual comparison. Also of interest was when the technique was not a viable option for classifying and discriminating samples.

Chemometrics of the Raman spectral data of the known and unknown sample sets (from previous chapters) was investigated to determine if this mathematical technique could aid in the evaluation of spectral results. Principal Components Analysis (PCA) and Discriminant Analysis (DA) were the common chemometric techniques used to discriminate samples.

For the known sample set, visual comparison of the Raman spectra provided a discrimination power of 0.873, utilising Principal Component Analysis (PCA) and Discriminant Analysis (DA) increased the discrimination power to **0.982**. The 11 samples produced 55 pair-wise comparisons and after PCA and DA there was only 1 pair left not discriminated. Chemometrics could not be applied to the samples that fluoresced resulting in the one pair not being discriminated. A sub-set of the unknown sample set was used as an example and utilising chemometrics was able to further sub-classify samples. Incorporating chemometrics of the Raman results with Vis-MSP allowed for discrimination of the entire sample set.

What is interesting to note about the chemometric results is that the visual classification and the chemometric classification of the Raman results from the known sample set is equal to the discrimination provided from visual

classification of the combined techniques (Raman and MSP) for the same sample set.

7.2 Future Directions

Whilst this study has shown the usefulness of Raman spectroscopy for black, blue and grey reactively dyed cotton fibres (with previous studies having looked at other fibre types) there is need to further investigate more colour/substrate combinations. This is particularly important as it is evident that different laser wavelengths are optimal for different fibre types/colours.

It would also be of significance to study the population of Raman spectral types in a larger sample set of black, blue and grey fibres. This type of study would be further enhanced by the analysis of a larger random sample set by the current examination sequence and incorporating Raman spectroscopy.

As it was evident that dark coloured fibres were characterised by the Raman spectrum of the major dye component an increase in the spectral library would provide an important aid for Raman spectroscopic analysis of fibres. Incorporating industry enquiries into the use of particular dye types would contribute further to this resource.

Information pertaining to dye concentration used in the colouration of fibres would be helpful in the interpretation of Raman spectral data. Whilst it is often difficult to obtain this type of commercial information (particularly so in this study as the company went into receivership) it would be advantageous to collate this type of data in future studies.

Collaborative research especially that carried out by the European Fibres Group means that large amounts of data can be collated for a variety of instruments and laser wavelengths. The continuation of such collaboration would provide useful

information on not only different substrates and colours, but, also the variations that may be observed between instruments and laser wavelengths.

This study has shown that a higher degree of discrimination is achieved when conducting the analysis described in addition to current methods. However, in order to further clarify the statistical meaningfulness of this data, population and target fibre studies could be conducted to determine the number and types of dyes used in the population.

The use of Raman spectroscopy should also be investigated for samples that are "realistic" (i.e. samples that are encountered in forensic fibre practices) as it is generally observed that most cases involve only a small number of actual fibre types. Being able to link Raman spectroscopy with current practices would determine its ability/inability to be utilised in forensic laboratories.

7.3 Conclusions

This study has shown that Raman spectroscopy can be used to analyse reactively dyed cotton fibres without interference from the cotton substrate, thereby providing molecular information about the main dye used. When analysing by Raman spectroscopy it was observed that samples with the same major dye component generally exhibited the same spectral characteristics even when minor dye components varied. This appeared to be due to the high concentration of the black or blue used to achieve the shade as samples of the grey samples (using lower concentrations of the black or blue dye) showed spectral contribution from the minor dye component.

Utilising chemometrics for data classification and pattern recognition significantly increases the discrimination provided by Raman spectroscopy for the sample set. It is not, however, applicable for samples that exhibit fluorescence that swamps information from the dyed fibre.

Fluorescence was observed to be the major limitation of the technique with a minor one being that the best results (with least subjectivity) required the sample to be directly impinged with the laser. This necessitates the removal of the fibre from any permanent mountant which will add time to the analysis process and may result in the loss or destruction of a sample.

Incorporating the results observed and the limitations discussed, the previous studies have shown that the technique is a viable option when analysing black/grey and blue cottons.

The recommended procedure for the analysis of black/blue cotton fibres is detailed below in Figure 7-1. This analysis sequence incorporates Raman spectroscopy whilst minimising the need for demounting fibres.

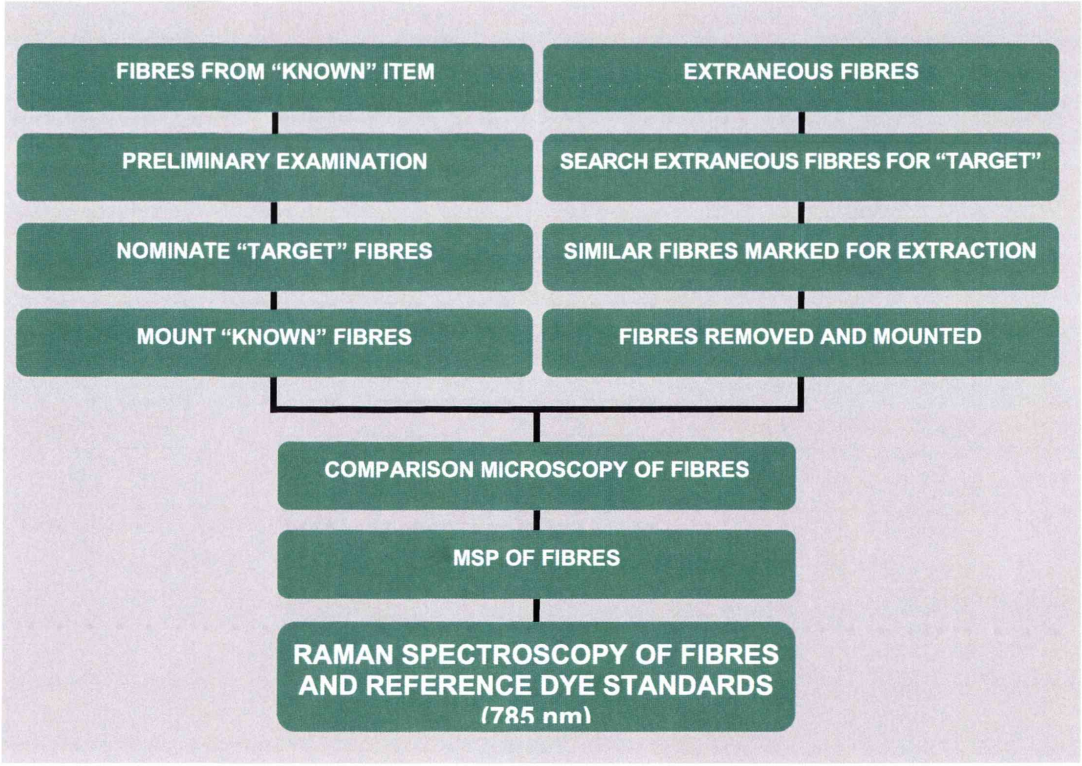


Figure 7-1 Suggested analysis sequence of known and extraneous fibres; incorporating Raman spectroscopy

Furthermore, the research has added to the argument against the perception that black and blue cotton have little to no evidentiary value. With the use of Raman

spectroscopy aided by chemometrics, very high levels of discrimination for these samples were achieved.

Appendix 1: Library of Reactive Dyes

Reactive dyes incorporated into the library are listed according to colour from manufacturers name (refer to Table A-1 to Table A-4). The spectra are then presented in raw format - and baseline corrected format if required (refer to Figure A-1 to Figure A-41)

Table A-1 Black dyes analysed by 785 nm showing manufacturer's name and CI number (if known).

Dye Name - Black	CI Number	Figure - Raw	Figure – Baseline Corrected
Cibacron Black C-NNHC		Figure A-2	Figure A-3
Cibacron Black LSN	Possibly Reactive Black 5	Figure A-4	Figure A-5
Cibacron Black LS-N HC	Possibly Reactive Black 5	Figure A-6	Figure A-7
Orthocron Black WNN	Possibly Reactive Black 5	Figure A-8	Figure A-9
Remazol Black B	Reactive Black 5	Figure A-10	Figure A-11
Synazol Black B	Reactive Black 5	Figure A-12	Figure A-13
Synazol Black HF-GRP	Reactive Black 5	Figure A-14	Figure A-15

Table A-2 Blue dyes analysed by 785 nm showing manufacturer's name and CI number (if known).

Dye Name - Blue	CI Number	Figure - Raw	Figure – Baseline Corrected
Cibacron Blue C-R		Figure A-16	
Cibacron Blue LS-3R HC		Figure A-17	
Cibacron Blue LSBR		Figure A-18	
Cibacron Brilliant Blue LS-G HC		Figure A-19	
Cibacron Navy LSG HC		Figure A-20	Figure A-21
Evercion Blue HERD		Figure A-22	
Everzol Bright Blue R		Fluoresced	
Lanasol Navy CE		Figure A-23	Figure A-24
Levafix Navy Blue E-BNA	Reactive Blue 225	Figure A-25	Figure A-26
Orthocron Blue HEGN	Reactive Blue 198	Figure A-27	
Remazol Turquoise Blue G	Reactive Blue 21	Figure A-28	
Sumifix Supra Blue BRF	Reactive Blue 221	Figure A-29	
Sumifix Supra Navy Blue BF	Reactive Blue 222	Figure A-30	

Table A-3 Red dyes analysed by 785 nm showing manufacturer's name and CI number (if known).

Dye Name - Red	CI Number	Figure - Raw	Figure – Baseline Corrected
Cibacron Red LSB		Figure A-31	
Lanasol Red CE		Figure A-32	Figure A-33
Procion Red HEXL		Figure A-34	
Procion BR Red HEGXL		Figure A-35	
Sumifix Scarlet 2GF		Figure A-36	Figure A-37
Synazol Red HF 6BN		Figure A-38	

Table A-4 Yellow and Orange dyes analysed by 785 nm showing manufacturer's name and CI number (if known).

Dye Name – Yellow/Orange	CI Number	Figure - Raw	Figure – Baseline Corrected
Cibacron Orange LSBR		Figure A-39	
Lanasol Yellow CE		Figure A-40	
Procion Yellow HEXL		Figure A-41	
Synazol Gold-Yellow HF 2GR		Figure A-42	

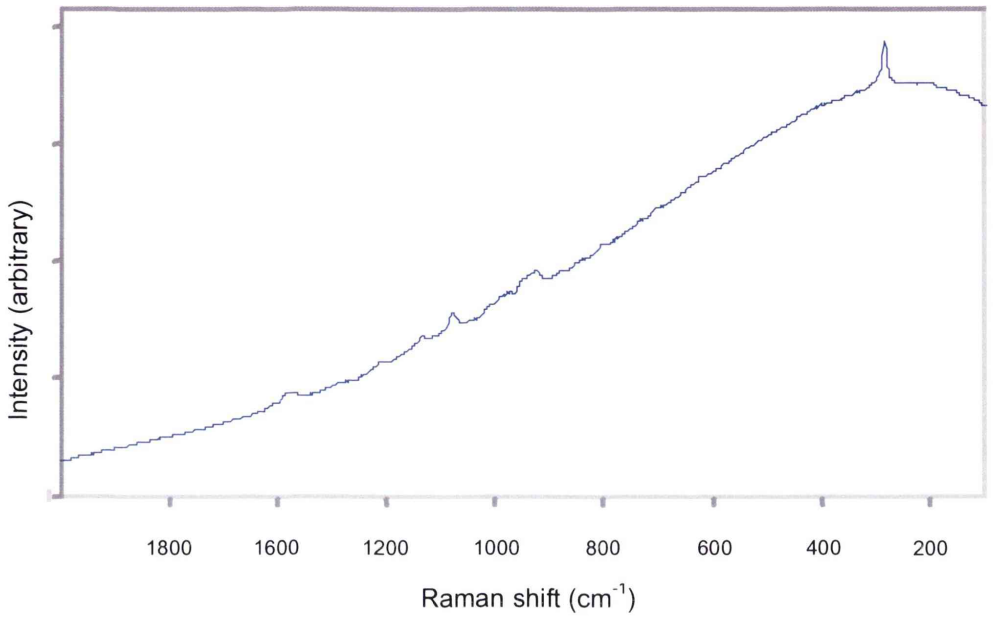


Figure A-2 Raman spectrum of Cibacron Black C-NN HC (raw)

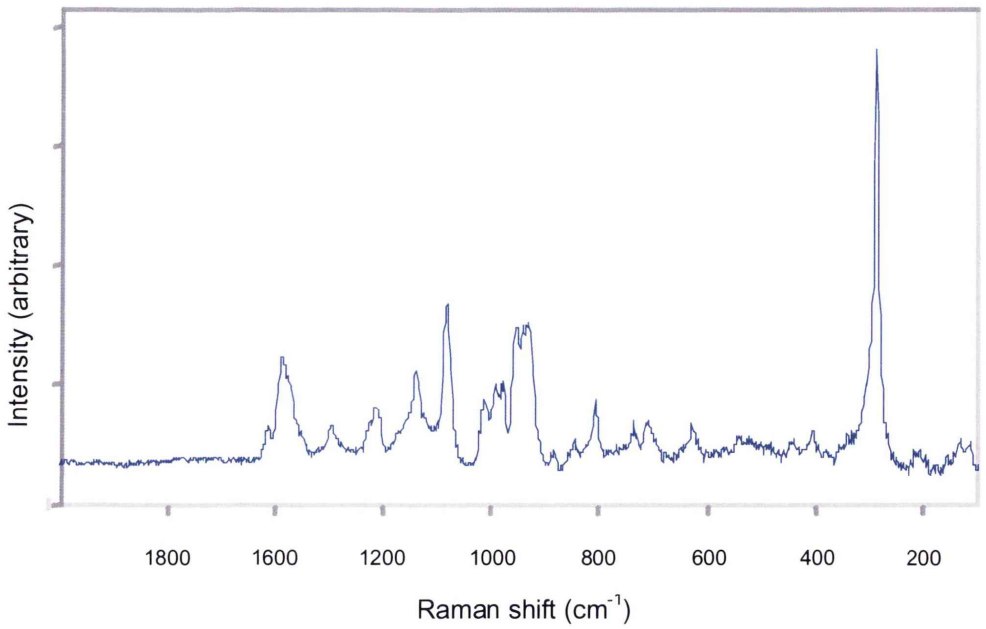


Figure A-3 Raman spectrum of Cibacron Black C-NN HC (baseline adjusted)

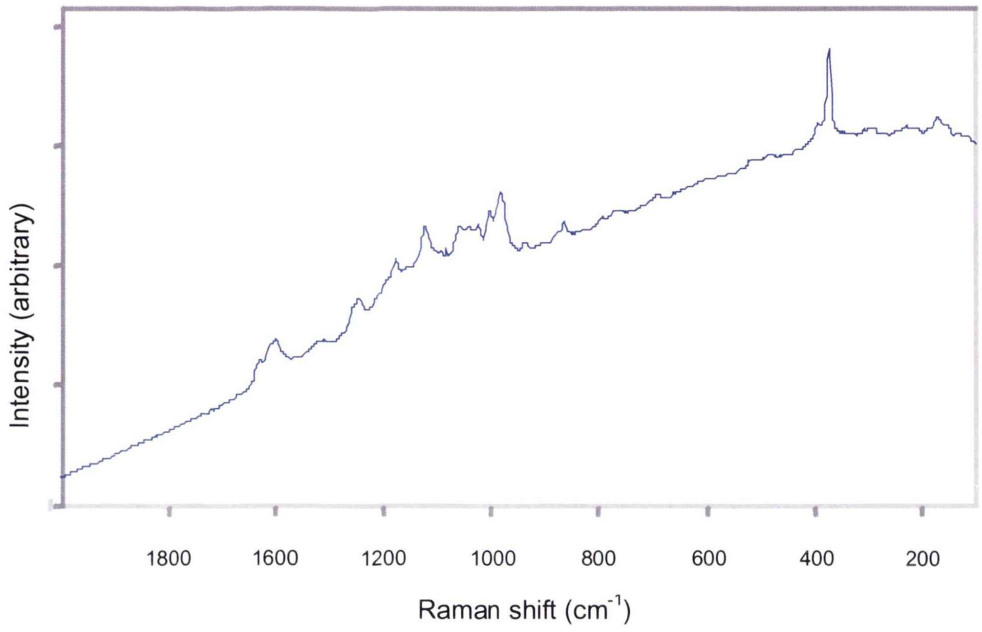


Figure A-4 Raman spectrum of Cibacron Black LSN (raw)

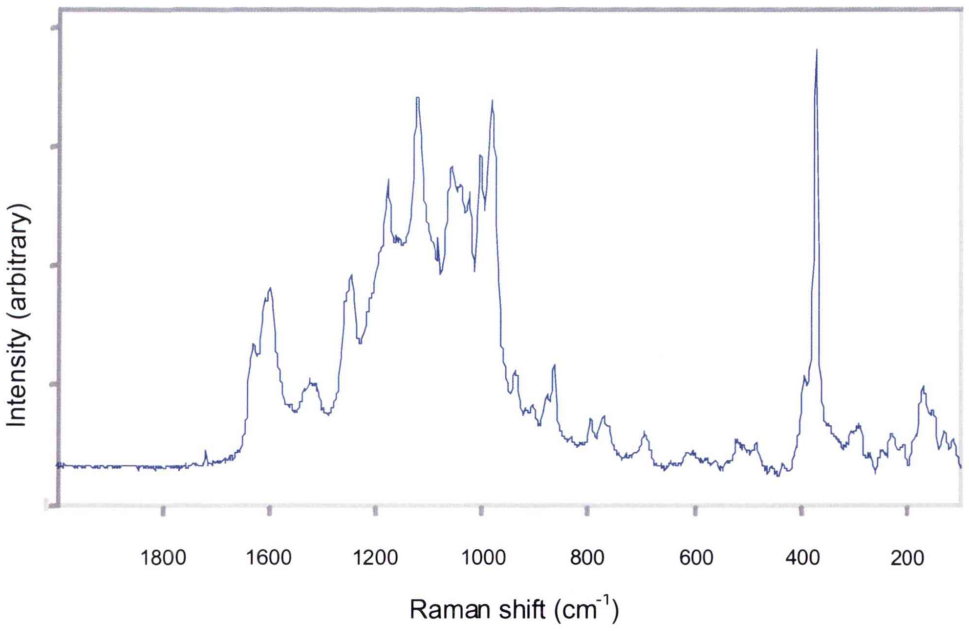


Figure A-5 Raman spectrum of Cibacron Black LSN (baseline adjusted)

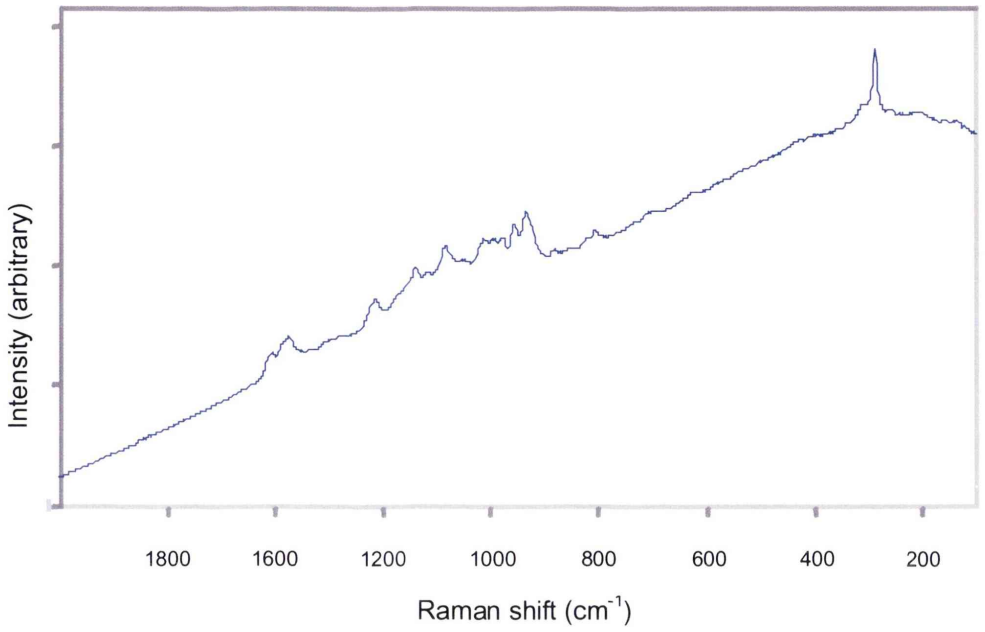


Figure A-6 Raman spectrum of Cibacron Black LS-N HC (raw)

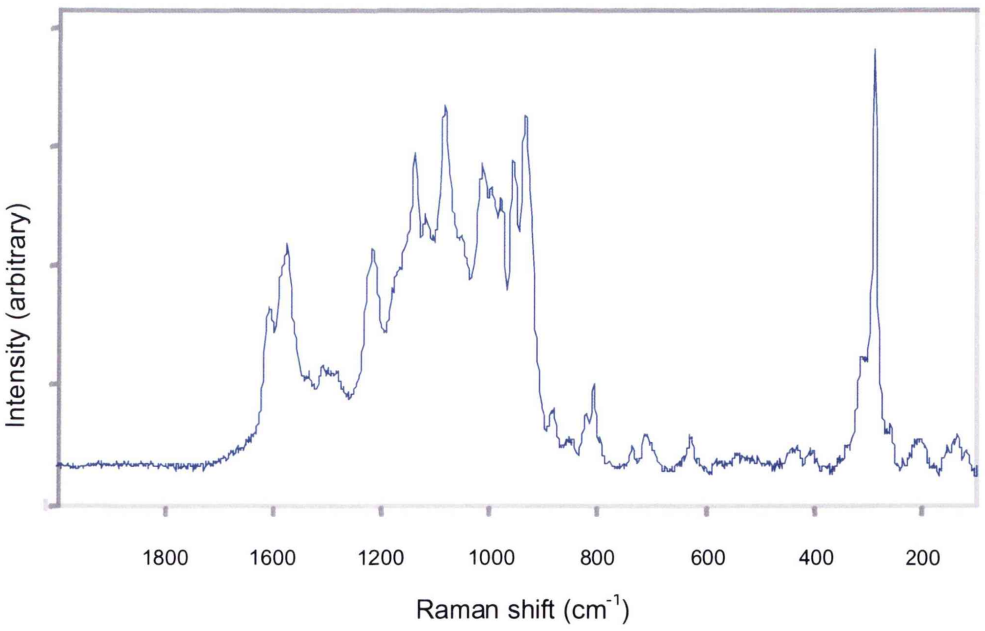


Figure A-7 Raman spectrum of Cibacron Black LS-N HC (baseline adjusted)

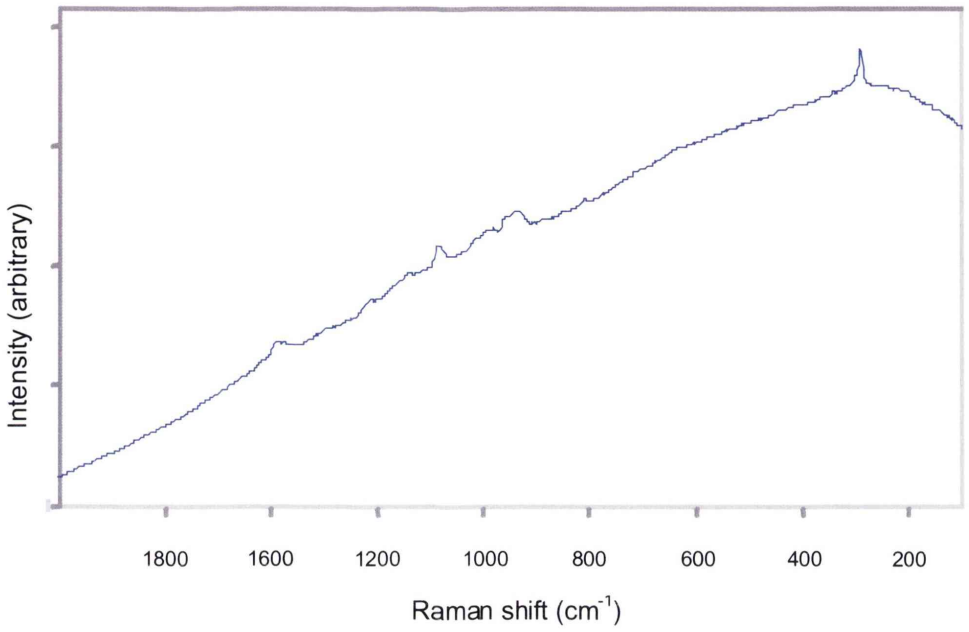


Figure A-8 Raman spectrum of Orthocron Black WNN (raw)

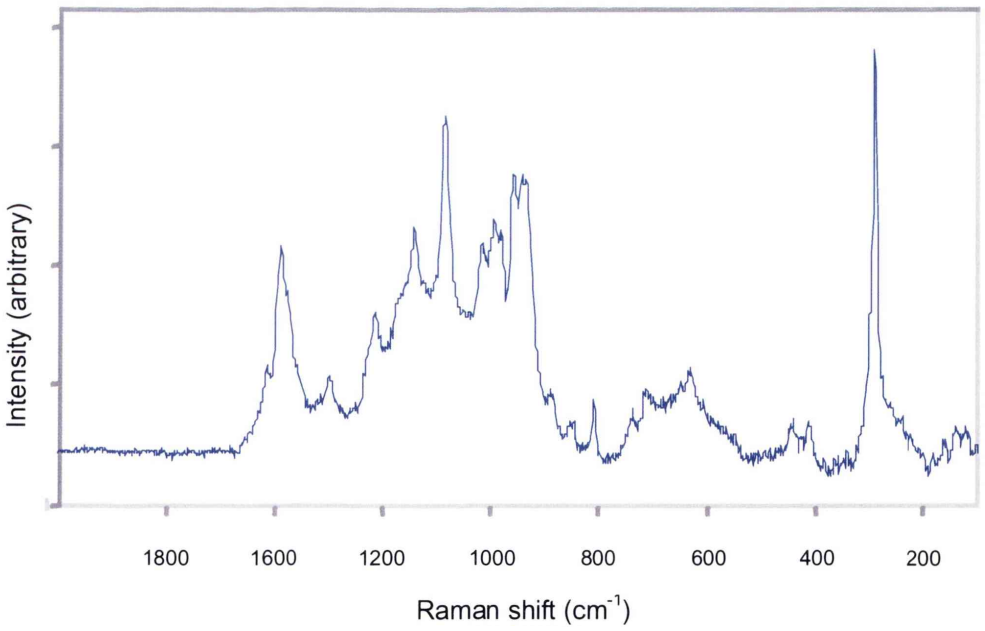


Figure A-9 Raman spectrum of Orthocron Black WNN (baseline adjusted)

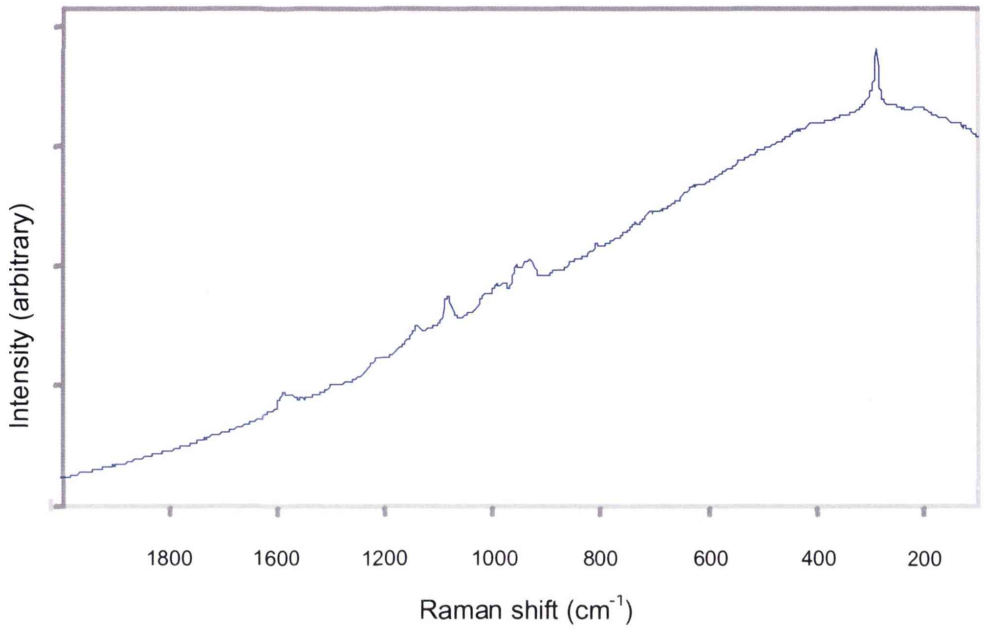


Figure A-10 Raman spectrum of Remazol Black B (raw)

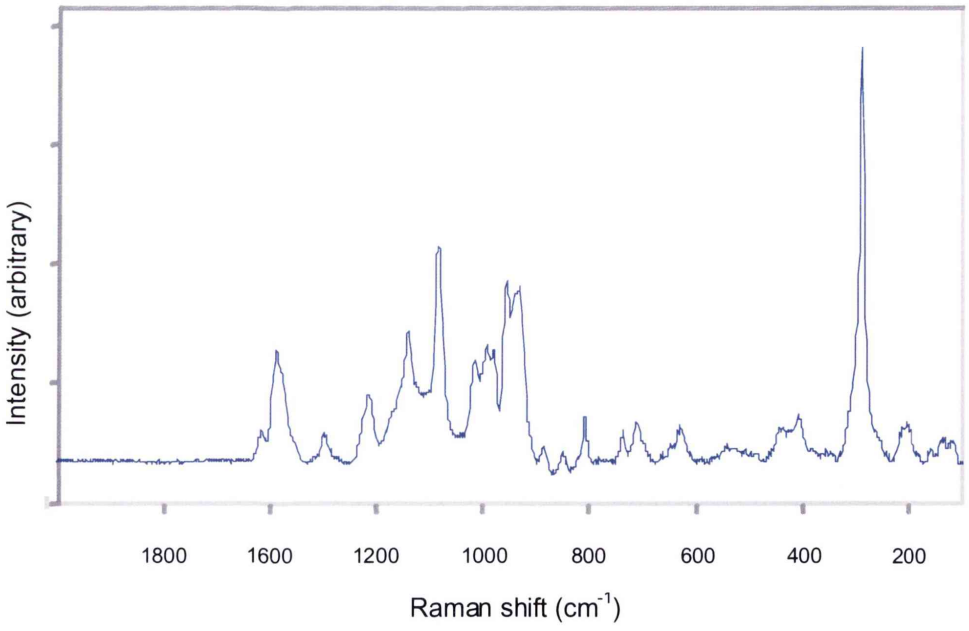


Figure A-11 Raman spectrum of Remazol Black B (baseline adjusted)

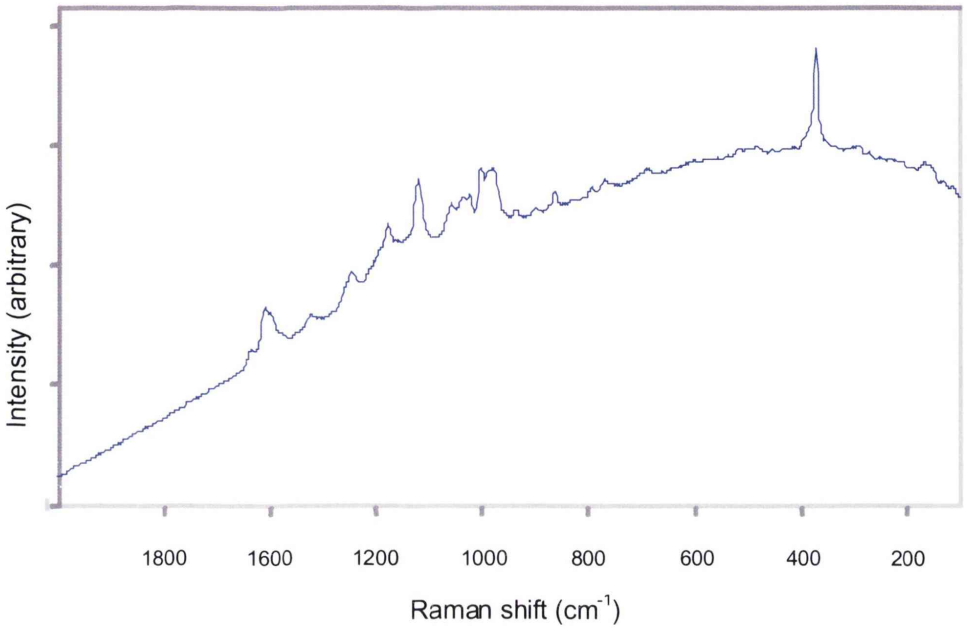


Figure A-12 Raman spectrum of Synazol Black B (raw)

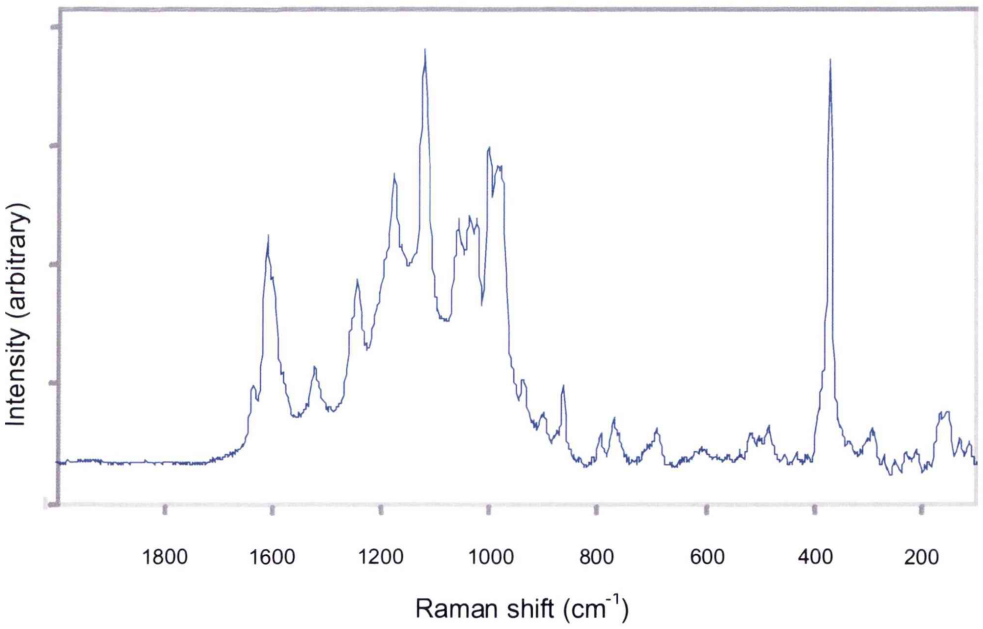


Figure A-13 Raman spectrum of Synazol Black B (baseline adjusted)

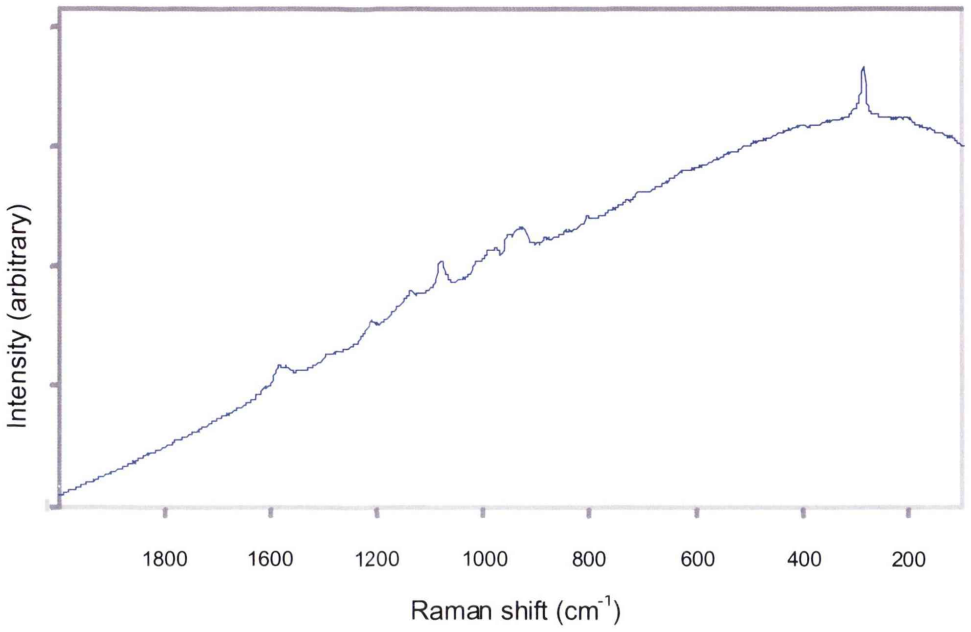


Figure A-14 Raman spectrum of Synazol Black HF-GRP (raw)

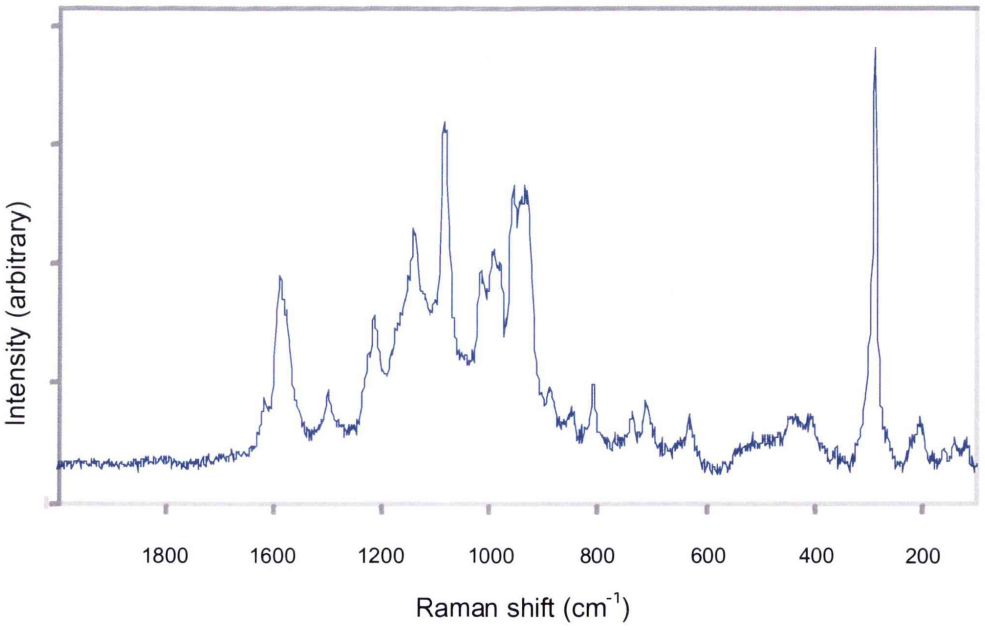


Figure A-15 Raman spectrum of Synazol Black HF-GRP (baseline adjusted)

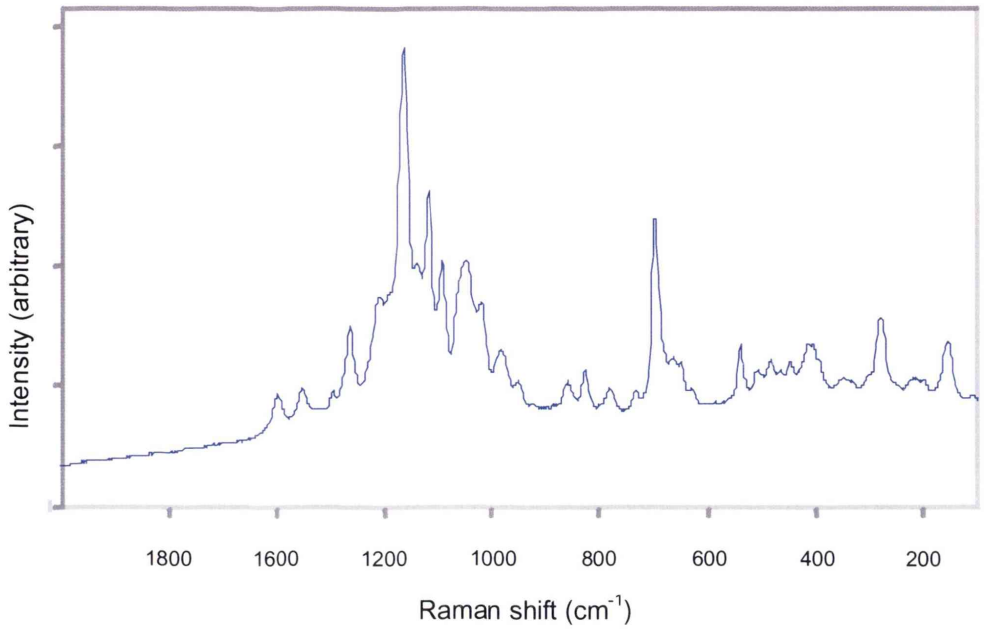


Figure A-16 Raman spectrum of Cibacron Blue C-R

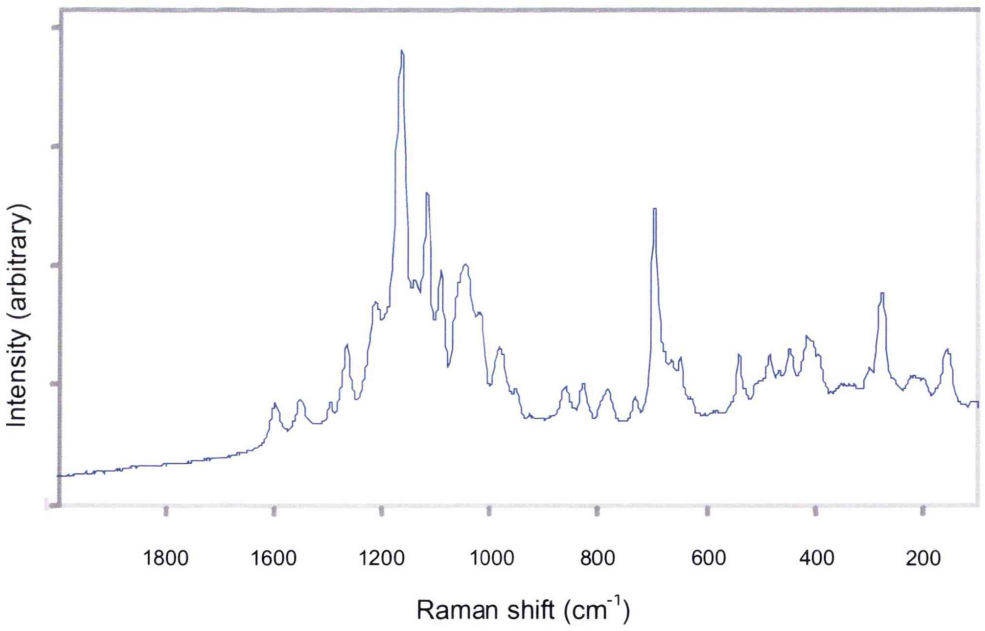


Figure A-17 Raman spectrum of Cibacron Blue LS-3R HC

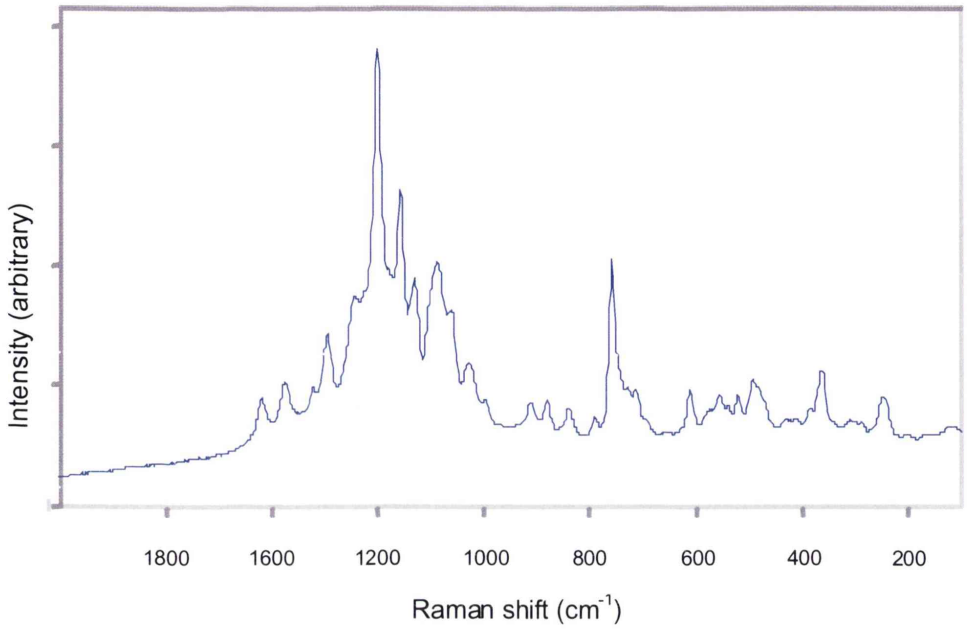


Figure A-18 Raman spectrum of Cibacron Blue LSBR

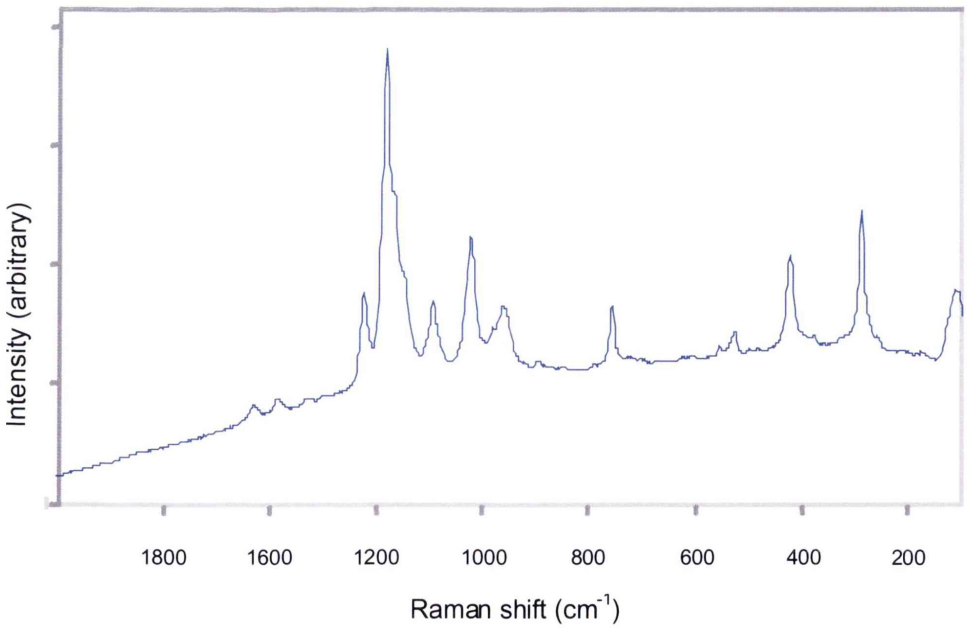


Figure A-19 Raman spectrum of Cibacron Brilliant Blue LSBR

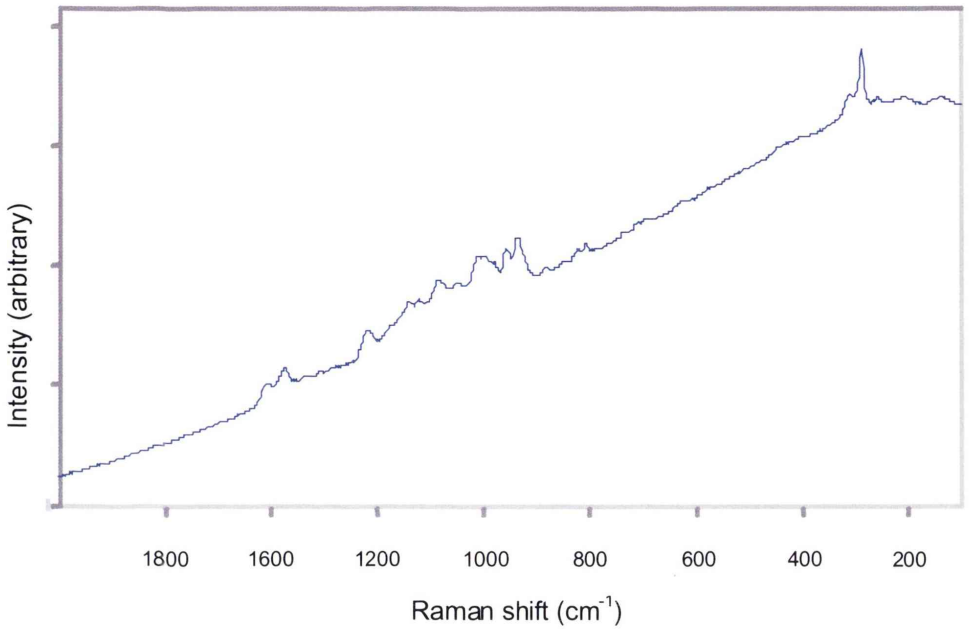


Figure A-20 Raman spectrum of Cibacron Navy LS-G HC (raw)

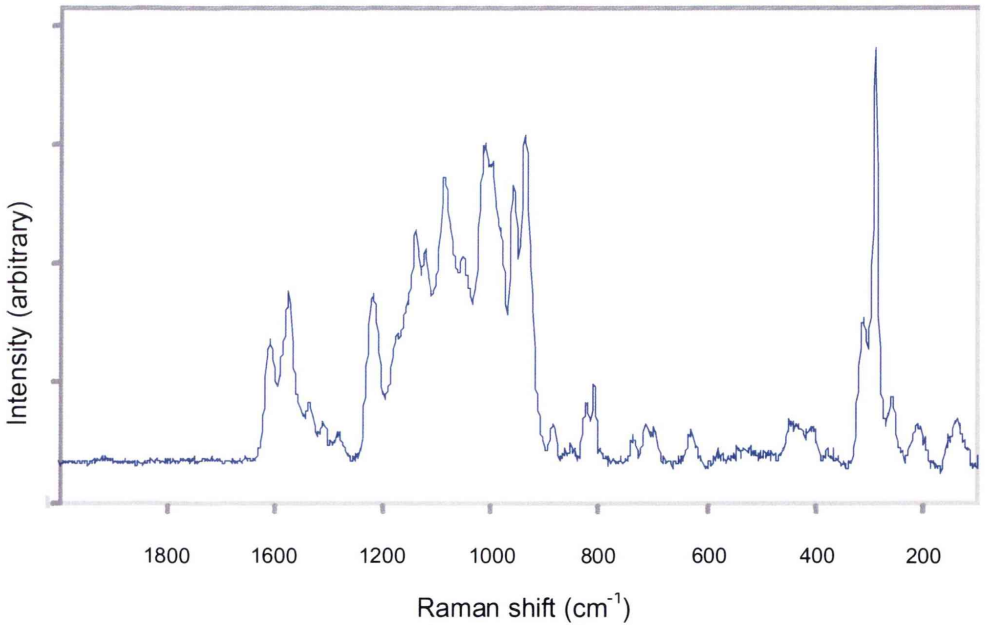


Figure A-21 Raman spectrum of Cibacron Navy LS-G HC (baseline adjusted)

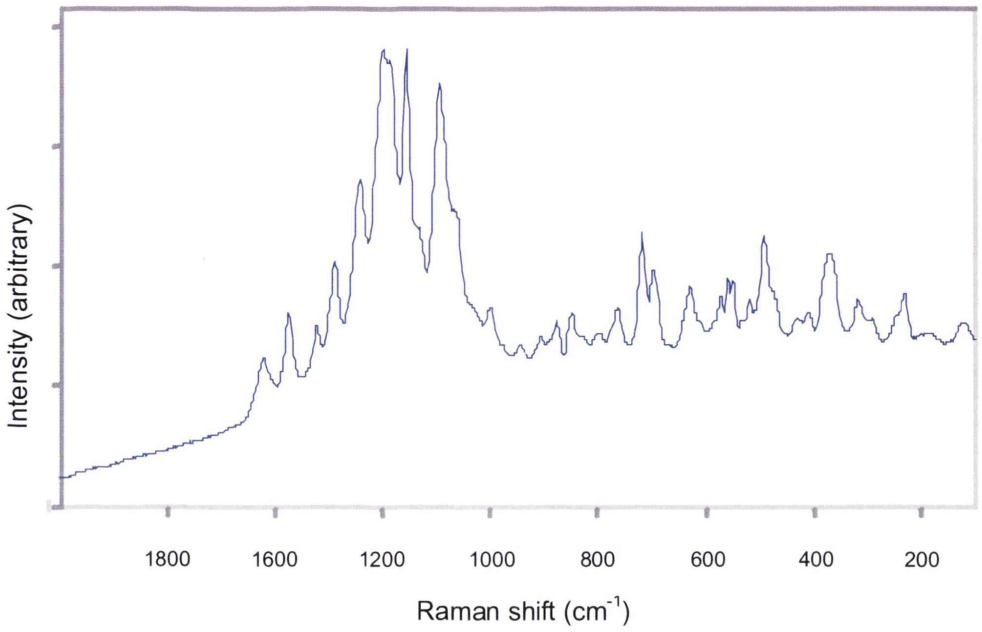


Figure A-22 Raman spectrum of Evercion Blue (HERD)

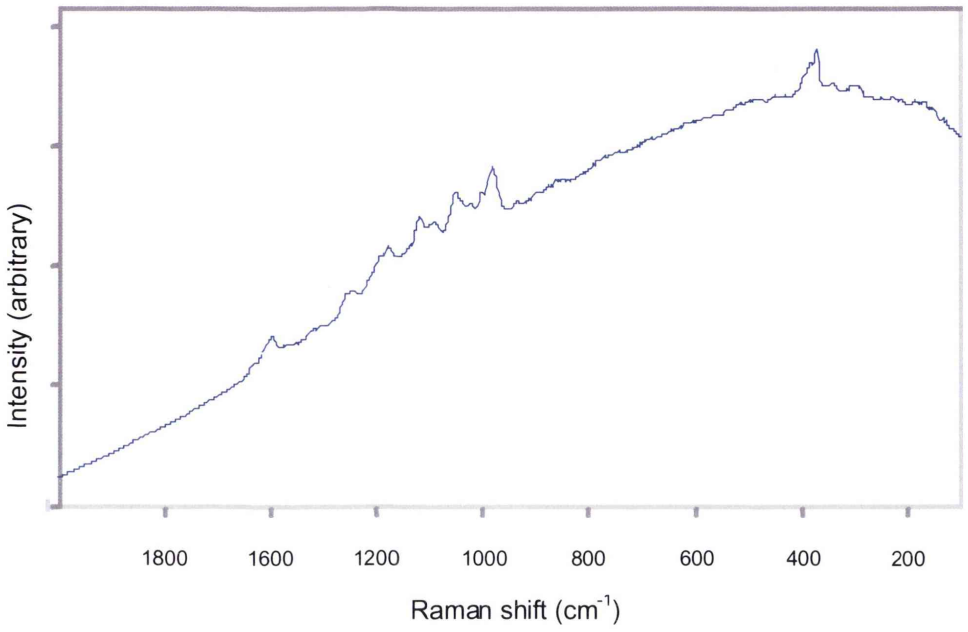


Figure A-23 Raman spectrum of Lanasol Navy CE (raw)

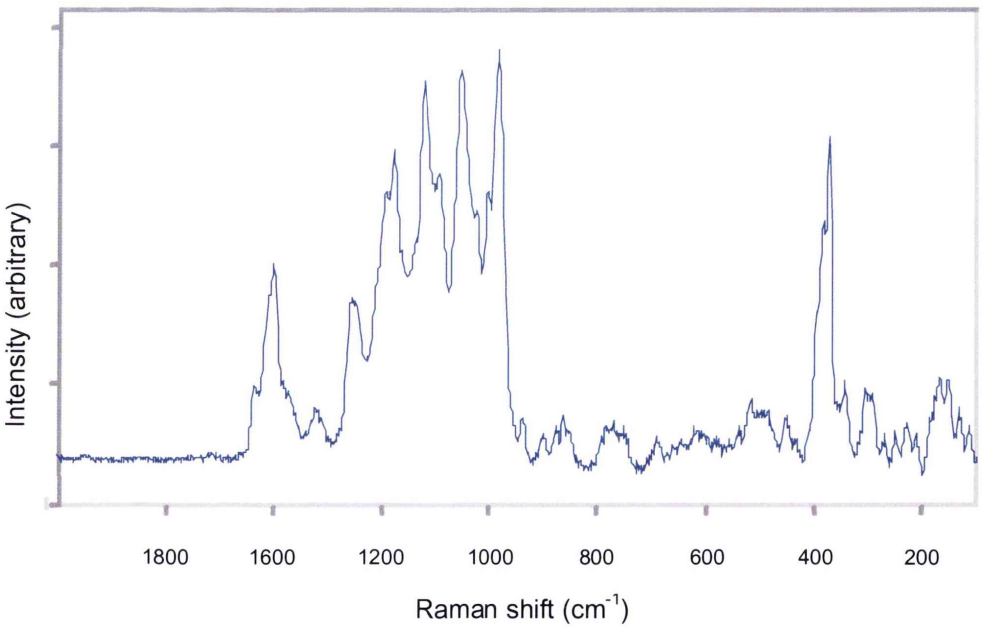


Figure A-24 Raman spectrum of Lanasol Navy CE (baseline adjusted)

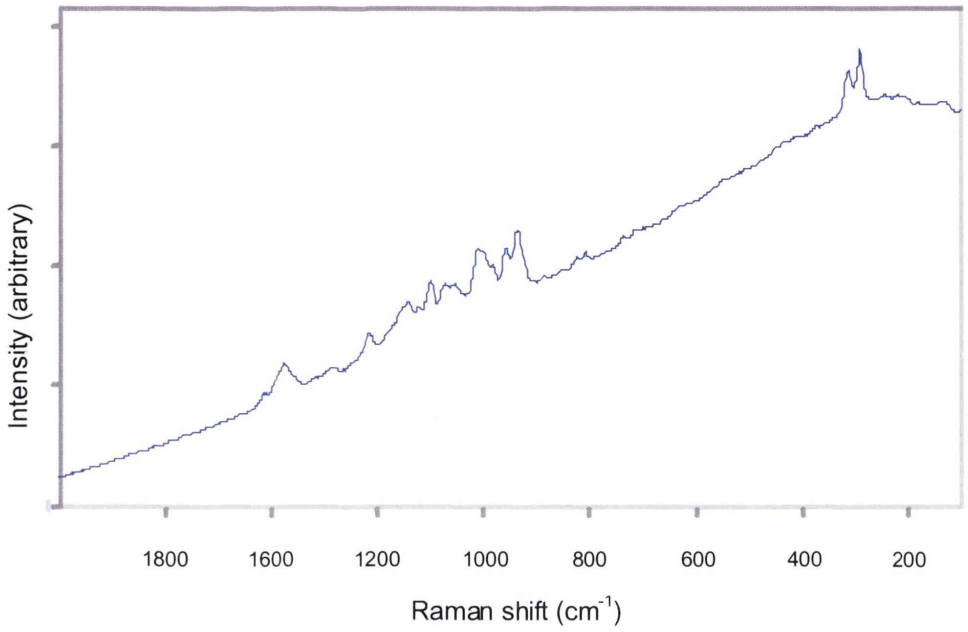


Figure A-25 Raman spectrum of Levafix Navy Blue E-BNA (raw)

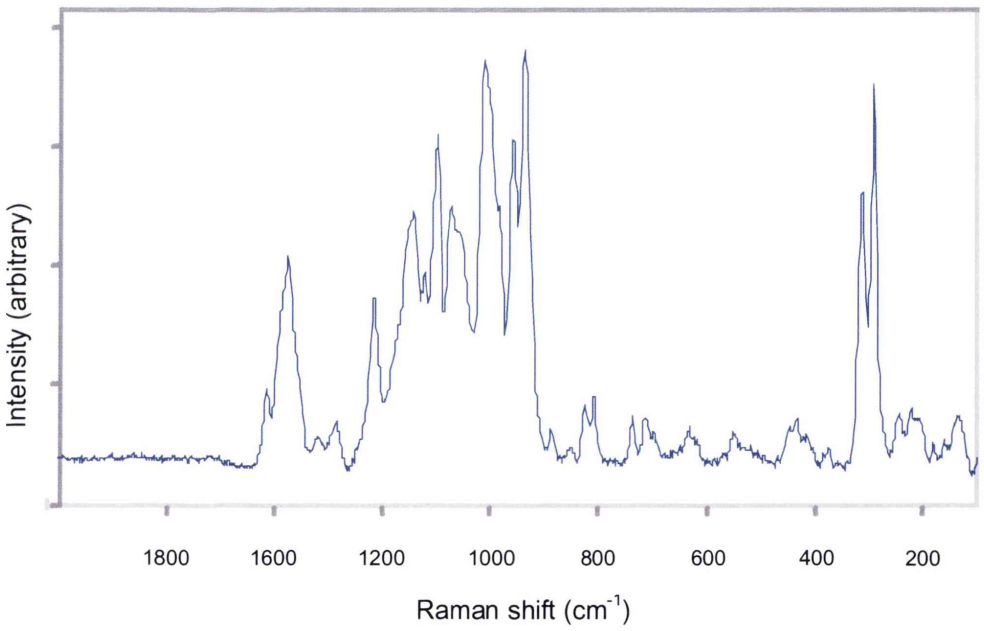


Figure A-26 Raman spectrum of Levafix Navy Blue E-BNA (baseline adjusted)

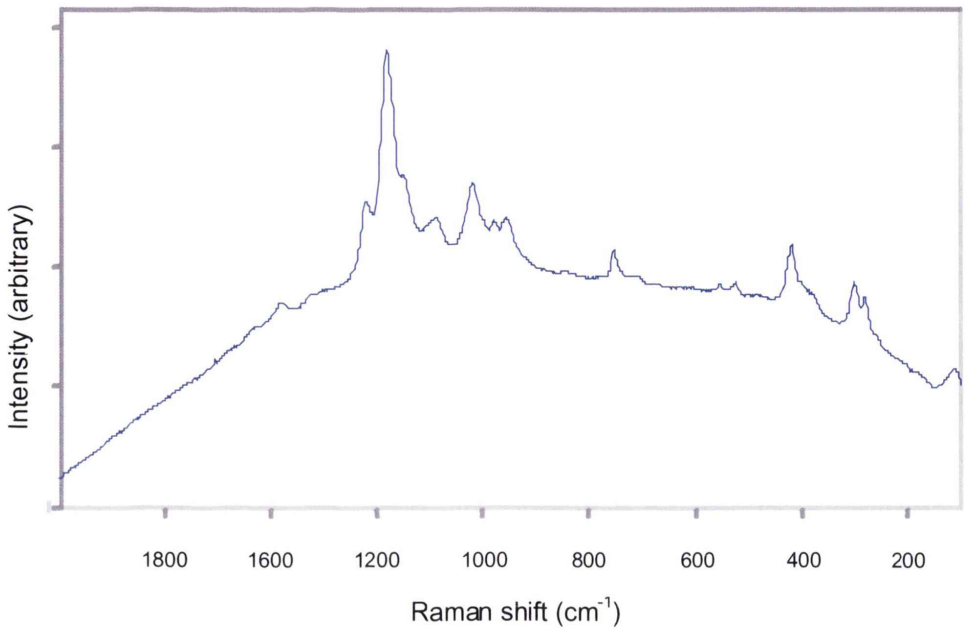


Figure A-27 Raman spectrum of Orthocron Blue HEGN (raw)

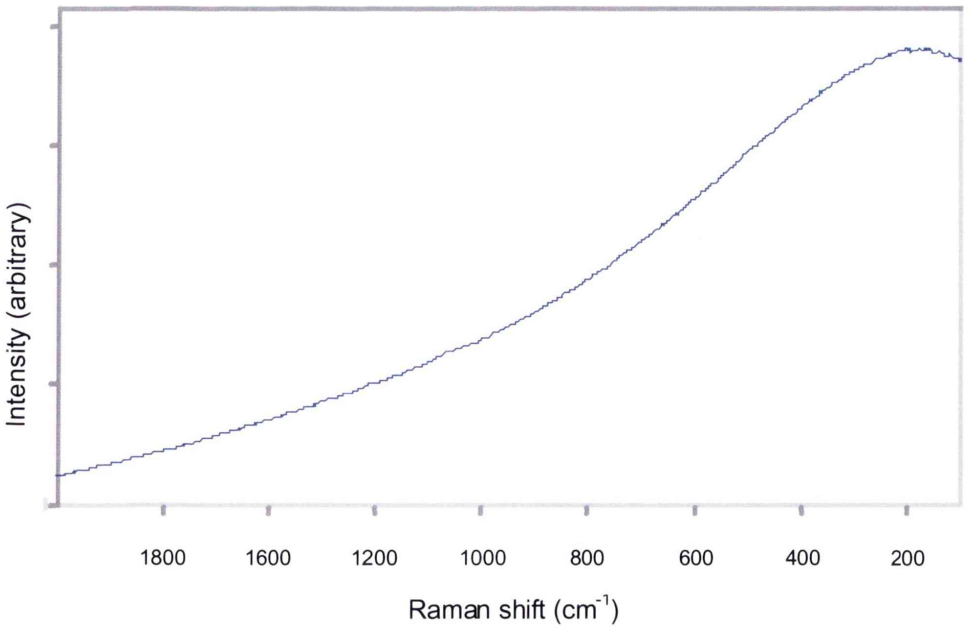


Figure A-28 Raman spectrum of Remazol Turquoise Blue G (fluoresced)

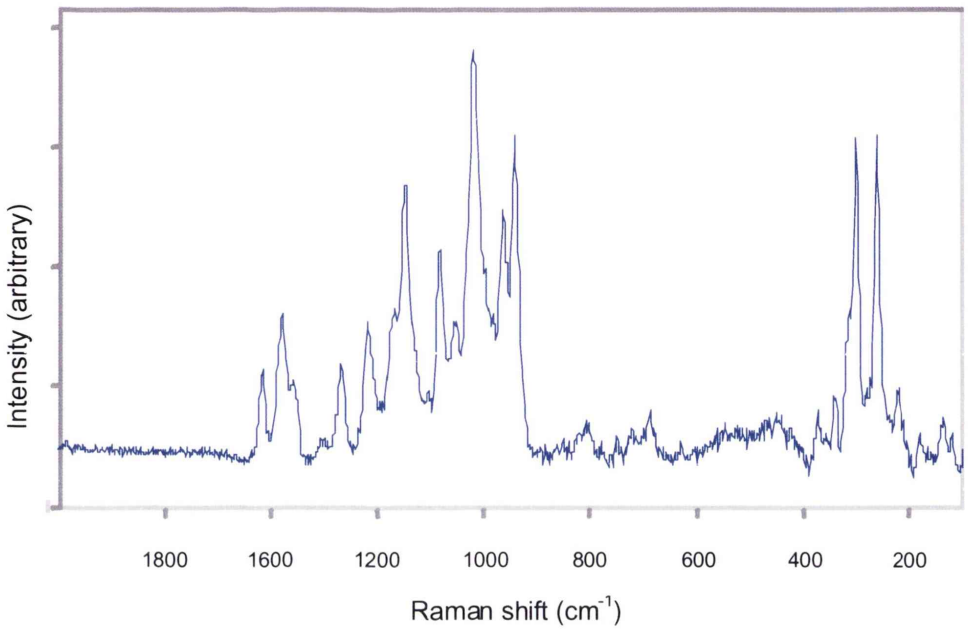


Figure A-29 Raman spectrum of Sumifix Supra Blue BRF

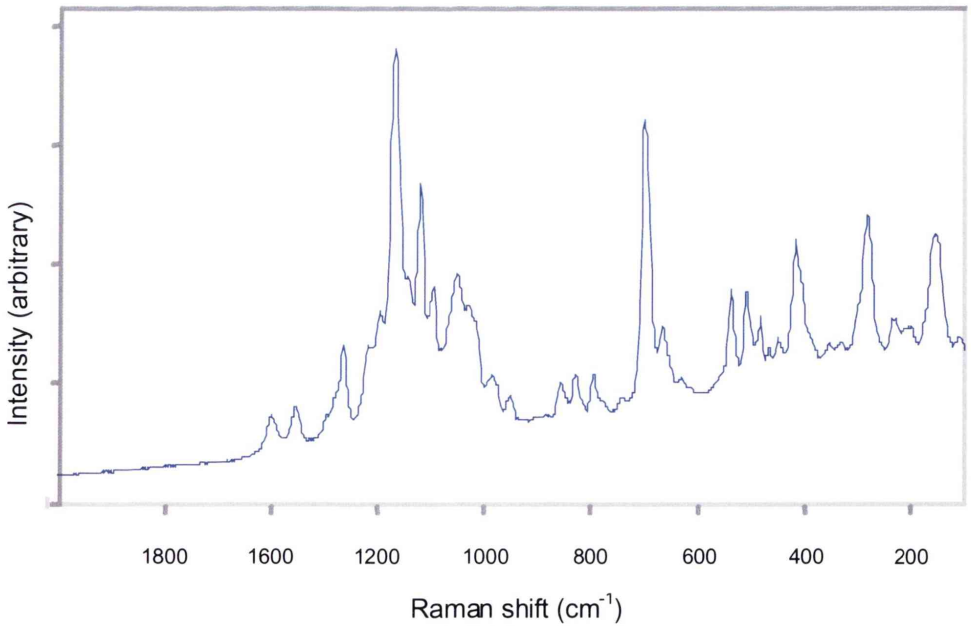


Figure A-30 Raman spectrum of Sumifix Supra Navy Blue BF

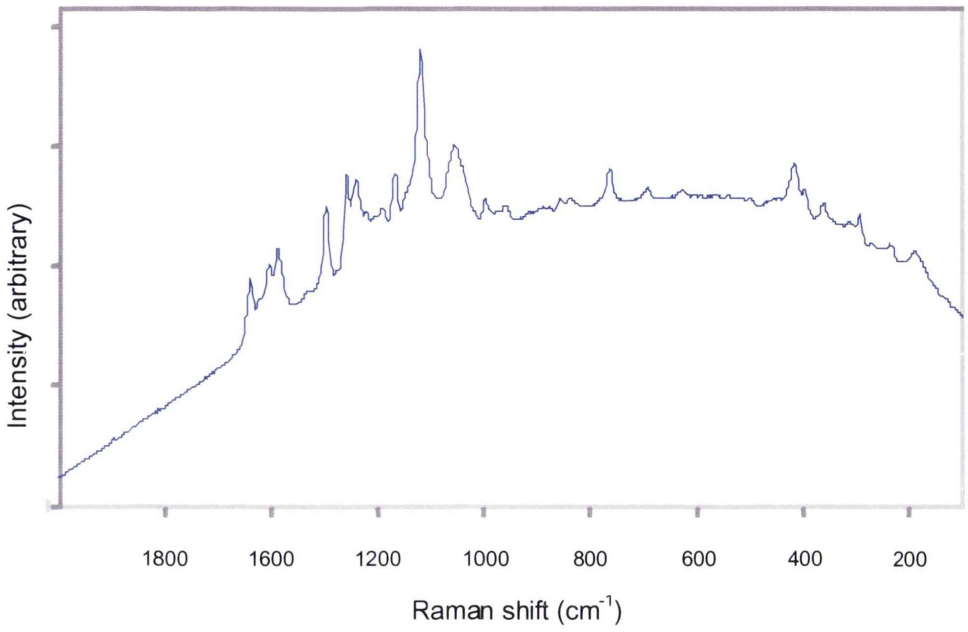


Figure A-31 Raman spectrum of Cibacron Red LSB

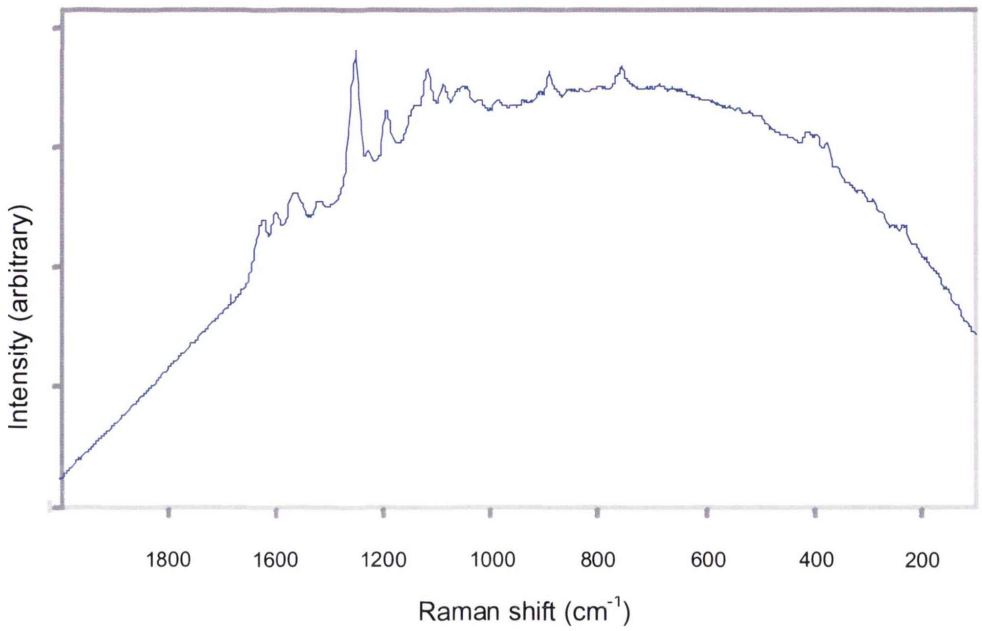


Figure A-32 Raman spectrum of Lanasol Red CE (raw)

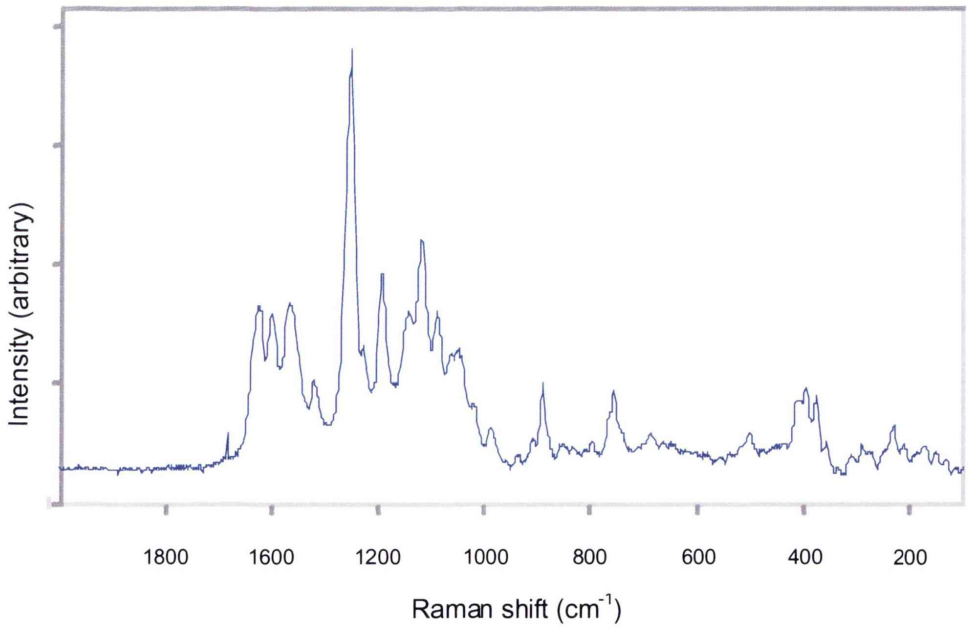


Figure A-33 Raman spectrum of Lanasol Red CE (baseline adjusted)

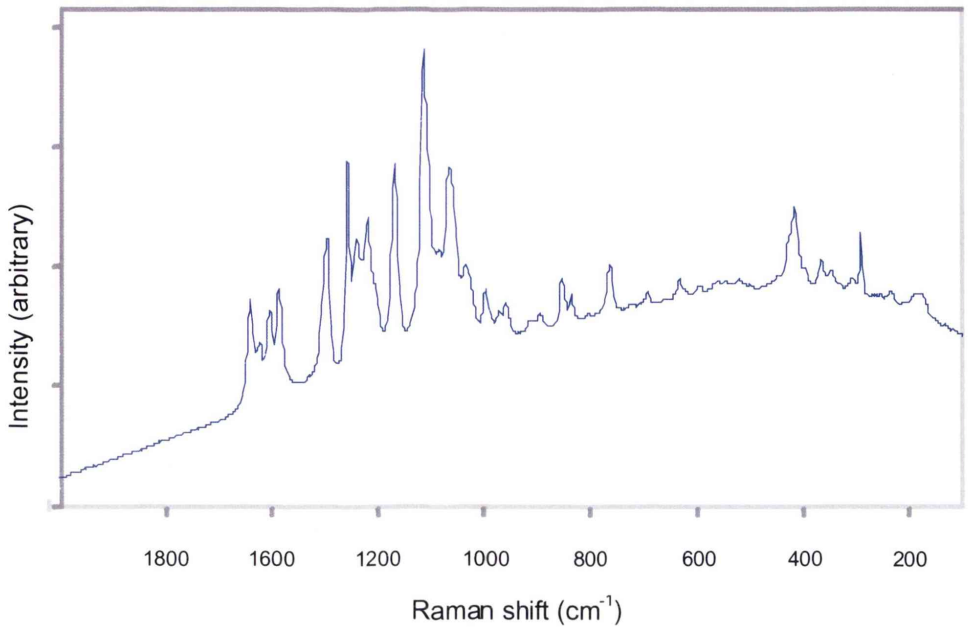


Figure A-34 Raman spectrum of Procion Red HEXL

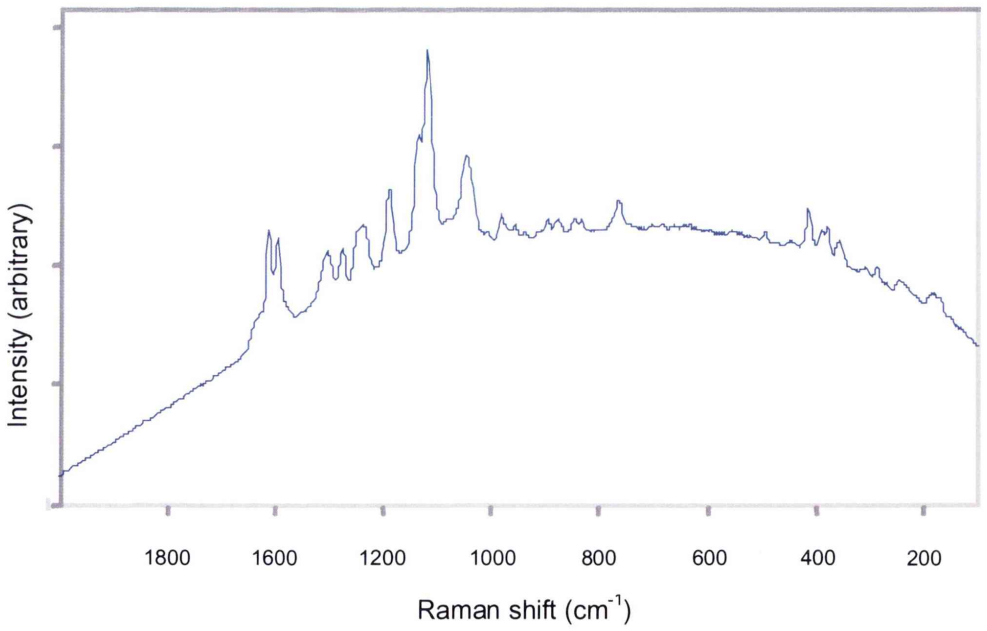


Figure A-35 Raman spectrum of Procion BR Red HEGXL

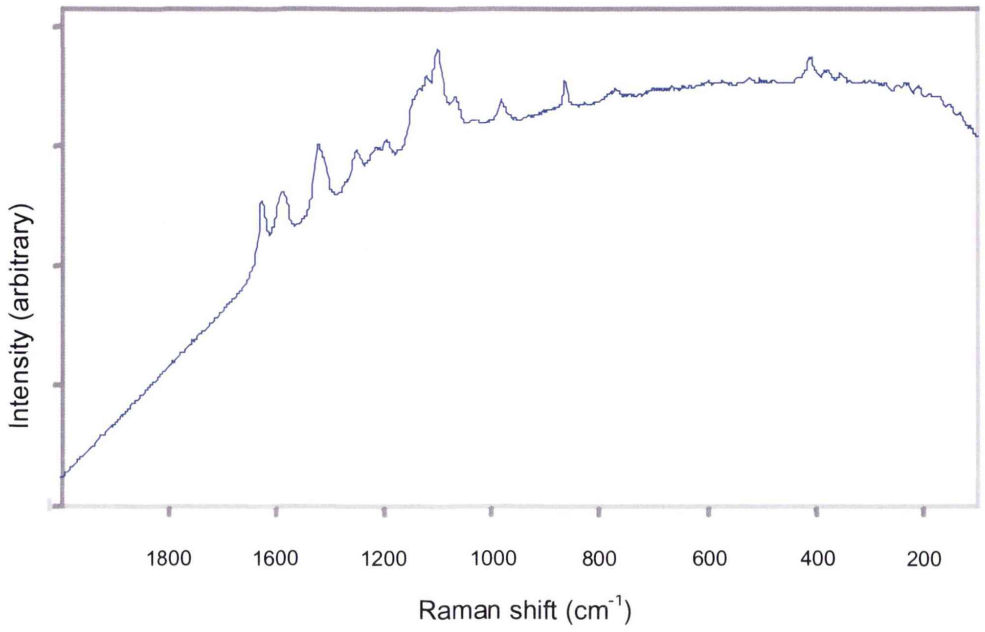


Figure A-36 Raman spectrum of Sumifix Scarlet 2GF (raw)

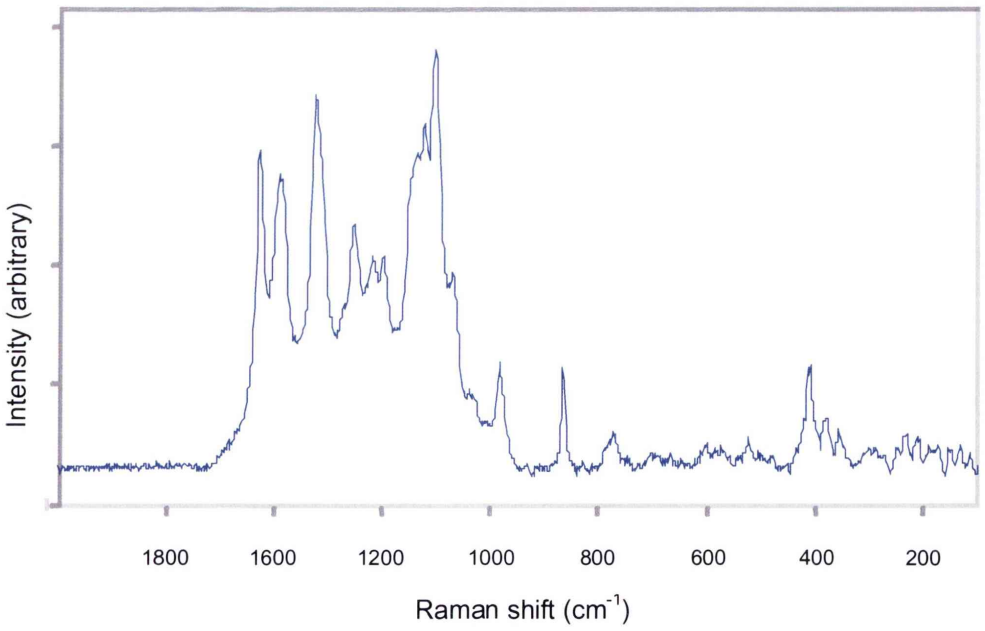


Figure A-37 Raman spectrum of Sumifix Scarlet 2GF (baseline adjusted)

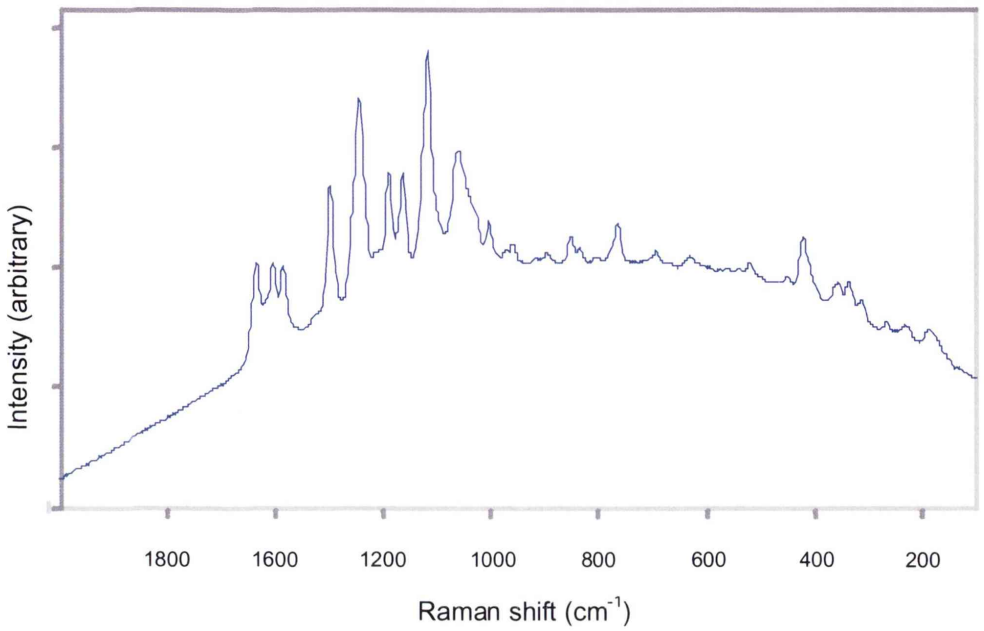


Figure A-38 Raman spectrum of Synazol Red HF 6BN

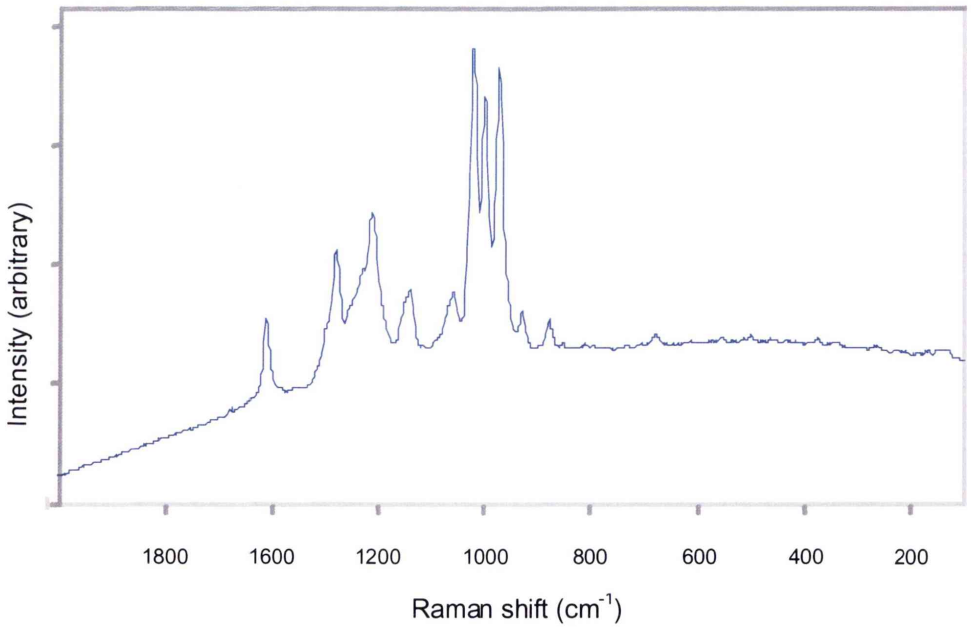


Figure A-39 Raman spectrum of Cibacron Orange LSBR

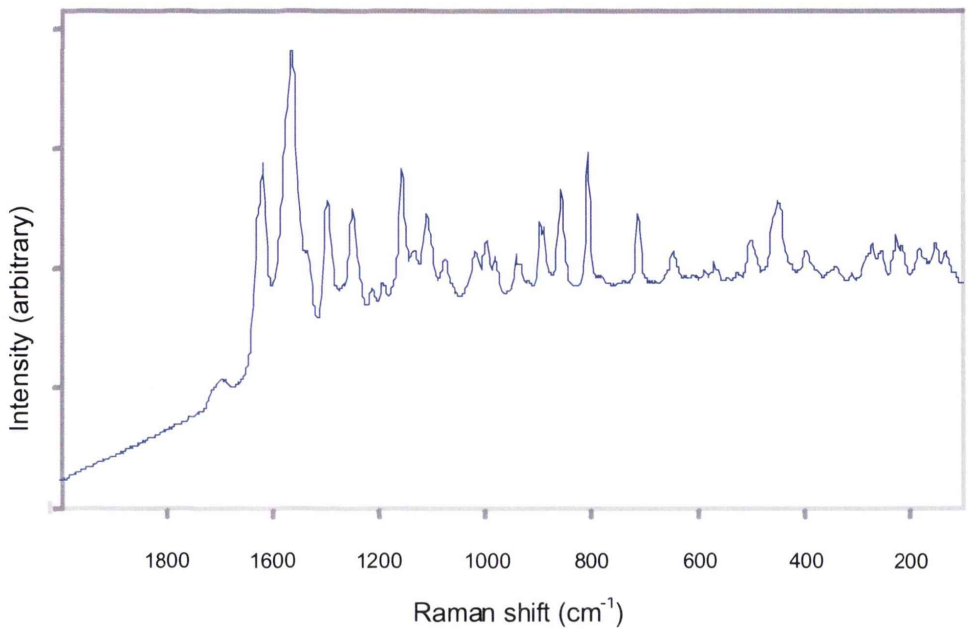


Figure A-40 Raman spectrum of Lanasol Yellow CE

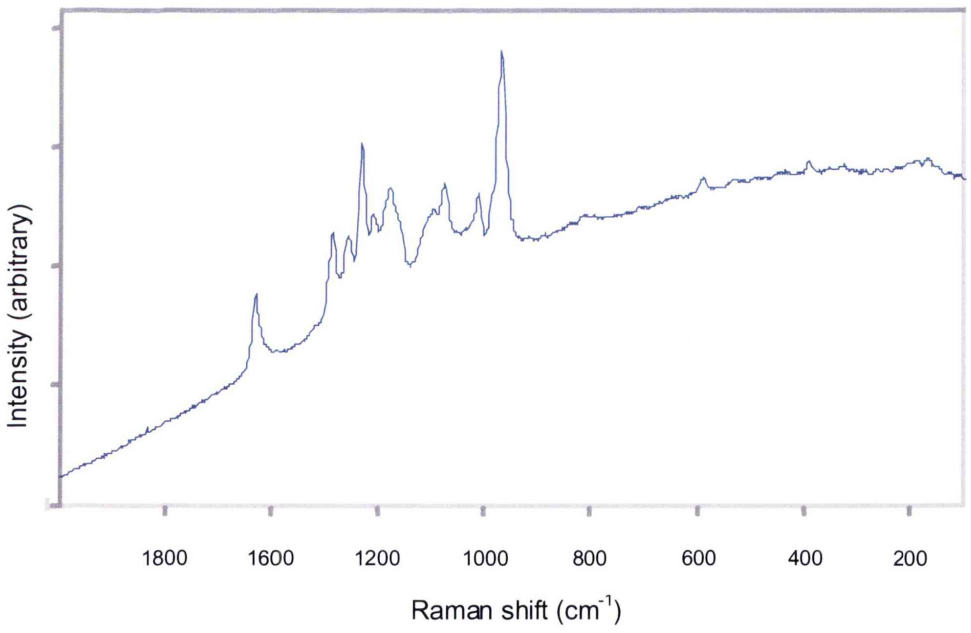


Figure A-41 Raman spectrum of Procion Yellow HEXL

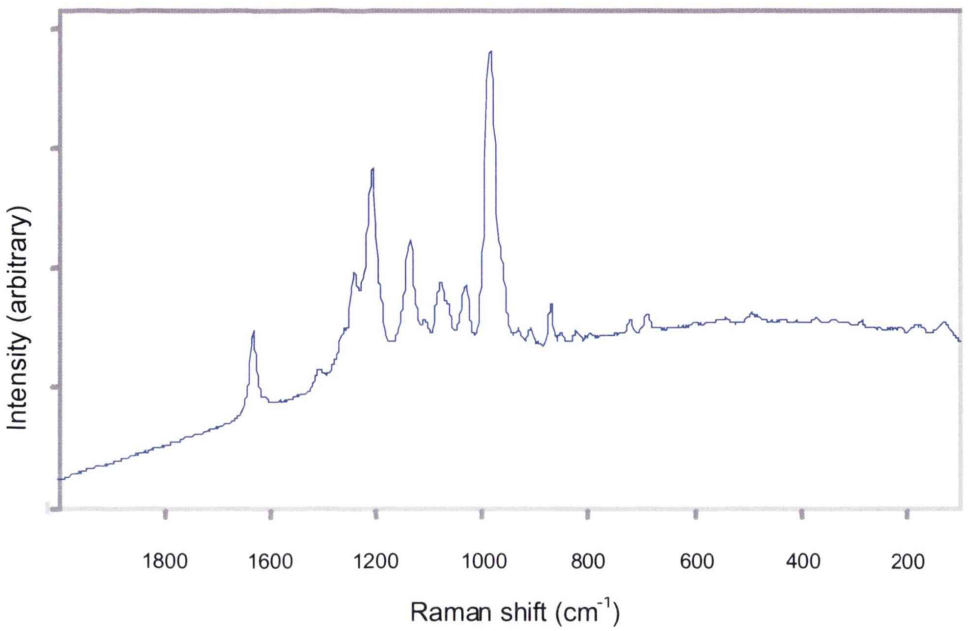


Figure A-42 Raman spectrum of Synazol Gold-Yellow HF 2GR

Chapter 8: References

1. *Textile Technology Catalogue*, Woodhead Publishing Ltd.: Cambridge, England.

2. *International Textile Bulletin*, in *International Textile Services*, ITS Publishing: Schlieren Zurich.

3. The European Network of Forensic Science Institutes (ENFSI) website, *http://www.enfsi.org*. Visited 21/02/2005

4. The INDUSTRIEVEREINIGUNG CHEMIEFASER E.V. (IVC) website. *http://www.ivc-ev.de*. Visited 25/05/2005

5. The Textiles From France website, *http://www.textile.fr*. Visited 22/04/2005

6. The Textile World website, *http://www.textileworld.it*. Visited 22/04/2005

7. The TextileWeb website, *http://www.textileweb.com*. Visited 22/04/2005

8. Adolf, F.P., *The Structure of Textiles: An Introduction to the Basics*, in *Forensic Examination of Fibres*, J. Robertson and M. Grieve, Editors. 1999, Taylor and Francis: London.

9. Cook, G.J., *Handbook of Textile Fibres*. 1968: Merrow Publishing Co. Ltd.

10. Anon., *The Identification of Textile Materials*. 7th ed. 1975, Manchester: the Textile Institute.

11. Bruschweiler, W. and M. Grieve. *State of the Art in the Field of Textile Fibre Examinations*. in *Proceedings of The 12th INTERPOL Forensic Science Symposium*. 1998: The Forensic Science Foundation Press.

12. Chabli, S. *Scene of Crime Evidence - Fibres: A Review 1998-2001*. in *13th INTERPOL Forensic Science Symposium*. 2001. Lyon, France,.

13. Robertson, J. and C. Roux. *Forensic Examination of Fibres - a Review: 2001-2004*. in *14th INTERPOL Forensic Science Symposium*. 2004. Lyon, France.

14. Robertson, J. and M. Grieve, *Forensic Examination of Fibres*. 2nd ed. 1999, London: Taylor and Francis.

15. David, S.K. and M.T. Pailthorpe, *Classification of Textile Fibres: Production, Structure and Properties*, in *Forensic Examination of Fibres*, J. Robertson and M. Grieve, Editors. 1999, Taylor and Francis Ltd.: London. p. 1-31.

16. Saferstein, R., *Criminalistics: An Introduction to Forensic Science*. 7th ed. 2001, New Jersey: Prentice Hall.

17. Grieve, M.C., *An Index of Textile Fibres Introduced During the Last Decade*. Journal of the Forensic Science Society, 1992. **32**(1): p. 35-47.

18. Houck, M.M. and e. al. *Poly(Trimethylene Terephthalate): A "New" Type of Polyester Fibre*. in *8th European Fibres Group Meeting*. 2000. Kracow.

19. The INDUSTRIEVEREINIGUNG CHEMIEFASER E.V. (IVC) website *http://www.ivc-ev.de/englisch/statistics2.htm*. Visited 12/02/2003

20. Grieve, M.C., T. Biermann, and M. Davignon, *The Evidential Value of Black Cotton Fibres.* Science & Justice, 2001. **41**: p. 245-260.

21. Wiggins, K.G., S.R. Crabtree, F.P. Adolf, and M.C. Grieve, *The Importance of Analysis of Reactive Dyes on Cotton Fibres.* Crime Laboratory Digest, 1995. **22**(3): p. 89.

22. Grieve, M.C. and T. Biermann, *The Population of Coloured Textile Fibres on Outdoor Surfaces.* Science & Justice, 1997a. **37**(4): p. 231-239.

23. Palmer, R. and S. Oliver, *The Population of Coloured Fibres in Human Head Hair.* Science & Justice, 2004. **44**(2): p. 83-88.

24. Roux, C. and P. Margot, *The Population of Textile Fibres on Car Seats.* Science & Justice, 1997a. **37**(1): p. 25-30.

25. Langdon, S., *The Evidential Value of Textile Fibres in Criminal Investigations*, in *Department of Chemistry, Materials and Forensic Science*. 2003b, University of Technology: Sydney.

26. Needles, H., *Textile Fibers, Dyes, Finishes, and Processes. A Concise Guide*. 1986, New Jersey: Noyes Publications.

27. Welham, A., *The Theory of Dyeing (and the Secret of Life).* Journal of the Society of Dyers & Colourists, 2000. **116**: p. 140-143.

28. Ferguson, M. *Dyes and Pigments*. in *RMIT*. 2005. Melbourne.

29. Gorensek, M., *Dye-Fibre Bond Stabilities of Some Reactive Dyes on Cotton.* Dyes & Pigments, 1999. **40**(2-3): p. 225-233.

30. Okada, Y., F. Fukuoka, and Z. Morita, *Environmental Effects of Oxygen on the Fading of Monochlorotriazinyl Reactive Dyes on Cotton Fabrics.* Dyes and Pigments, 1998. **37**(1): p. 47-64.

31. Ollgaard, H., L. Frost, G. Galster, and O.C. Hansen, *Survey of Azo-Colourant in Denmark: Consumption, Use, Health and Environmental Aspects*. 1998, Ministry of Environment and Energy: Denmark.

32. Taupin, J.M., F.P. Adolf, and J. Robertson, *Examination of Damage to Textiles*, in *Forensic Examination of Textiles*, J. Robertson and M. Grieve, Editors. 1999, Taylor and Francis: London.

33. Nehse, K. *Colour and Shade.* in *Young Persons Workshop (EFG)*. 2004. Prague.

34. Jochem, G. *Fibre Plastic Fusions.* in *Young Persons Workshop (EFG)*. 2004. Prague.

35. Wiggins, K.G., *Ropes and Cordage*, in *Forensic Examination of Textiles*, J. Robertson and M. Grieve, Editors. 1999c, Taylor and Francis: London.

36. Morley, J. *Comparison of Clothing to Video Images.* in *11th European Fibres Group (EFG) meeting*. 2003. Istanbul.

37. Jochem, G. and H. Pabst. *Fibre-Plastic Fusions in Traffic Accidents Reconstruction.* in *8th European Fibres Group Meeting*. 2000. Cracow.

38. Robertson, J. and C. Roux, *From the Crime Scene to the Laboratory*, in *Forensic Examination of Fibres*, J. Robertson and M. Grieve, Editors. 1999, Taylor and Francis: London.

39. Grieve, M.C., *Fibers and Forensic Science - New Ideas, Developments, and Techniques.* Forensic Science Review, 1994. **6**(1): p. 59-77.

40. Locard, E., *Dust and Its Analysis.* Police Journal, 1928: p. 177-192.

41. Palenik, S., *Microscopy and Microchemistry of Physical Evidence*, in *Forensic Science Handbook*, R. Saferstein, Editor. 1988, Prentice Hall: New Jersey.

42. Deadman, H., *Fibre Evidence and the Wayne Williams Trial: Part 2.* FBI Law Enforcement Bulletin, 1984b: p. 10 -.

43. Deadman, H., *Fiber Evidence and the Wayne Williams Trial: Part 1.* FBI Law Enforcement Bulletin, 1984a: p. 13 - 20.

44. Wiggins, K.G. and M.C. Grieve, *The European Fibres Group: 1993-1998.* Science & Justice, 1999b. **39**(1): p. 45-47.

45. SWGMAT, *Notes from Technical Working Group for Fiber Examination.* Crime Laboratory Digest, 1995. **22**(3): p. 77-89.

46. ENFSI European Fibres Group., *Manual of Best Practice.* 2001.

47. Wiggins, K., *The European Fibres Group (EFG) 1993-2002: Understanding and Improving the Evidential Value of Fibres.* Analytical and Bioanalytical Chemistry, 2003. **376**: p. 1172-1177.

48. *Proceedings of the 9th European Fibres Group meeting.* 2001. Vantaa, Finland.

49. *Proceedings of the 10th European Fibres Group meeting.* 2002. Rosny-sous-bois, France.

50. *Proceedings of the 11th European Fibres Group meeting*. 2003. Istanbul, Turkey.

51. Frei-Sulzer, M., *Die Sicherung Von Mikrospuren Mit Klebeband.* Kriminalistik, 1951: p. 190-194*.

52. Grieve, M.C., *Back to the Future - 40 Years of Fibre Examinations in Forensic Science.* Science & Justice, 2000b. **40**(2): p. 93-99.

53. Feeman, J.F., Canadian Textile Journal, 1970. **87**: p. 83.

54. Pounds, C.A. and K.W. Smalldon, *The Transfer of Fibres between Clothing Material During Simulated Contacts and Their Persistence During Wear. Part 1 - Fibre Transference.* Journal of the Forensic Science Society, 1975a. **15**: p. 17-27.

55. Pounds, C.A. and K.W. Smalldon, *The Transfer of Fibres between Clothing Material During Simulated Contacts and Their Persistence During Wear. Part 2 - Fibre Persistence.* Journal of the Forensic Science Society, 1975b. **17**: p. 29-37.

56. SWGMAT, *Forensic Fiber Examination Guidelines.* Forensic Science Communications, 1999. **1**(1).

57. AFP, *AFP Forensic Services Scientific - Hair and Fibres Procedures Manual*. 1996, Australian Federal Police.

58. SWGMAT, *Trace Evidence Recovery Guidelines*, (TWGMAT), Editor. 1998, US Department of Justice, FBI.

59. Roux, C., J. Huttunen, K. Rampling, and J. Robertson, *Factors Affecting the Potential for Fibre Contamination in Purpose Designed Forensic Search Rooms.* Science & Justice, 2001. **41**(3): p. 135-144.

60. Biermann, T. *The Advantages and Disadvantages of 1:1 Taping.* in *Proceedings of the 6th European Fibres Group Meeting.* 1998. Dundee, Scotland.

61. Robertson, J., *Protocols for Fibre Examination and Initial Preparation*, in *Forensic Examination of Fibres*, J. Robertson and M. Grieve, Editors. 1999, Taylor and Francis: London.

62. Palmer, R., *Fibers: Identification and Comparison*, in *Encyclopaedia of Forensic Science*, J.A. Siegel, P.J. Saukko, and G.C. Knupfer, Editors. 2000, Academic Press: London. p. 815-823.

63. Palenik, S., *Microscopical Examination of Fibres*, in *Forensic Examination of Fibres*, J. Robertson and M. Grieve, Editors. 1999, Taylor & Francis: London. p. 153-175.

64. Roux, C., P. Maynard, and M. Dawson, *FTIR Spectroscopy Applications in Forensic Science.* Chemistry in Australia, 1999a. **66**(2): p. 11-15.

65. Kirkbride, P. and M. Widmark Tungol, *Infrared Microspectroscopy of Fibres*, in *Forensic Examination of Fibres*, J. Robertson and M. Grieve, Editors. 1999, Taylor & Francis: London. p. 179-222.

66. Grieve, M.C., *Another Look at the Classification of Acrylic Fibres, Using FTIR Microscopy.* Science & Justice, 1995a. **35**(3): p. 179-190.

67. Adolf, F.P. and J. Dunlop, *Microspectrophotometry/Colour Measurement*, in *Forensic Examination of Fibres*, J. Robertson and M. Grieve, Editors. 1999, Taylor and Francis: London.

68. Wiggins, K.G., *Thin Layer Chromatographic Analysis for Fibre Dyes*, in *Forensic Examination of Fibres*, J. Robertson and M.C. Grieve, Editors. 1999a, Taylor and Francis: London. p. 291-310.

69. Wiggins, K.G., S.R. Crabtree, and B.M. March, *The Importance of Thin Layer Chromatography in the Analysis of Reactive Dyes Released from Wool Fibers.* Journal of Forensic Sciences, 1996. **41**(6): p. 1042-1045.

70. Cantrell, S., C. Roux, P. Maynard, and J. Robertson, *A Textile Fibre Survey as an Aid to the Interpretation of Fibre Evidence in the Sydney Region.* Forensic Science International, 2001. **123**(1): p. 48-53.

71. Salter, M.T. and R. Cook, *Transfer of Fibres to Head Hair, Their Persistence and Retrieval.* Forensic Science International, 1996. **81**(2-3): p. 211-221.

72. Ashcroft, C.N., S. Evans, and I.R. Tebbett, *The Persistence of Fibres in Head Hair.* Journal of the Forensic Science Society, 1988. **28**: p. 289-293.

73. Roux, C., J. Chable, and P. Margot, *Fibre Transfer Experiments onto Car Seats.* Science & Justice, 1996. **36**(3): p. 143-151.

74. Roux, C. and P. Margot, *An Attempt to Assess the Relevance of Textile Fibres Recovered from Car Seats.* Science & Justice, 1997b. **37**(4): p. 225-230.

75. Robertson, J. and M. Lim, *Fibre Transfer and Persistence onto Car Seats and Seatbelts.* Canadian Society of Forensic Science Journal, 1987. **20**: p. 140-141.

76. Grieve, M. and T. Biermann, *Wool Fibres - Transfer to Vinyl and Leather Vehicle Seats and Some Observations on Their Secondary Transfer.* Science & Justice, 1997b. **37**: p. 31-38.

77. Krau(ss), W. and U. Hildebrand. *Fibre Persistence on Garments under Open Air Conditions.* in *3rd meeting of the European Fibres Group.* 1995. Linkoping, Sweden.

78. Merciani, P., F. Monard Semier, P. Buzzini, G. Massonnet, and F. Taroni, *A Study of the Cross Transfer of Fibres.* Forensic Science International, 2003. **136**(1): p. 121-122.

79. Home, J.M. and R.J. Dudley, *A Summary of Data Obtained from a Collection of Fibres from Casework Materials.* Journal of the Forensic Science Society, 1980. **20**: p. 253-261.

80. Biermann, T.W. and M.C. Grieve, *A Computerized Data Base of Mail Order Garments - a Contribution toward Estimating the Frequency of Fibre Types Found in Clothing .2. The Content of the Data Bank and Its Statistical Evaluation.* Forensic Science International, 1996c. **77**(1-2): p. 75-91.

81. Biermann, T.W. and M.C. Grieve, *A Computerized Data Base of Mail Order Garments - a Contribution toward Estimating the Frequency of Fibre Types Found in Clothing .1. The System and Its Operation.* Forensic Science International, 1996b. **79**(1): p. 65.

82. Kelly, E. and R.M.E. Griffin, *A Target Fibre Study on Seats in Public Houses.* Science & Justice, 1998. **38**(1): p. 39-44.

83. Houck, M.M., *Inter-Comparison of Unrelated Fiber Evidence.* Forensic Science International, 2003. **135**(2): p. 146-149.

84. Taroni, F., C.G.G. Aitken, and P. Garbolino, *De Finetti's Subjectivism, the Assesment of Probabilities and the Evaluation of Evidence: A Commentary for Forensic Scientists.* Science & Justice, 2001. **41**(3): p. 145-150.

85. Robertson, B. and G.A. Vignaux, *Interpreting Evidence: Evaluating Forensic Evidence in the Courtroom.* 1997, Chichester: John Wiley & Sons.

86. Causin, V., S. Schiavone, A. Marigo, and P. Carresi, *Bayesian Framework for the Evaluation of Fiber Evidence in a Double Murder--a Case Report.* Forensic Science International, 2004. **141**(2-3): p. 159-170.

87. Champod, C. and F. Taroni, *The Bayesian Approach*, in *Forensic Examination of Fibres*, J. Robertson and M. Grieve, Editors. 1999, Taylor & Francis: London.

88. Aitken, C.G.G. and F. Taroni, *Interpretation of Scientific Evidence (Part II).* Science & Justice, 1997. **37**: p. 65.

89. Webb-Salter, M.T. and K. Wiggins, *Aids to Interpretation*, in *Forensic Examination of Fibres*, J. Robertson and M. Grieve, Editors. 1999, Taylor & Francis: London.

90. Taroni, F. and C.G.G. Aitken, *Fibres Evidence, Probabilistic Evaluation and Collaborative Test.* Forensic Science International, 2000. **114**(1): p. 45-47.

91. Taroni, F., A. Biedermann, P. Garbolino, and C.G.G. Aitken, *A General Approach to Bayesian Networks for the Interpretation of Evidence.* Forensic Science International, 2004. **139**(1): p. 5-16.

92. Grieve, M.C., *A Survey on the Evidential Value of Fibres and on the Interpretation of the Findings in Fibre Transfer Cases. Part 1 - Fibre Frequencies.* Science & Justice, 2000c. **40**(3): p. 189-200.

93. Grieve, M.C., *A Survey on the Evidential Value of Fibres and the Interpretation of the Findings in Fibre Transfer Cases. Part 2 - Interpretation and Reporting.* Science & Justice, 2000d. **40**(3): p. 201-209.

94. *Personal Communication* with Dr James Robertson and Prof. Claude Roux, May and June 2005

95. Grieve, M.C. *Striking a Balance in Fibre Examinations Today!* in *Proceedings NRIPS International Workshop Forensic Examination of Trace Evidence*. 1998b. Tokio (Tokyo).

96. Grieve, M. *The Perspective for Forensic Fibre Examinations in the Year 2000.* in *International Symposium on the Forensic Examination of Trace Evidence*. 1996. San Antonio, Texas.

97. Grieve, M. and K. Wiggins, *Fibres under Fire: Suggestions for Improving Their Use to Provide Forensic Evidence.* Journal of Forensic Sciences, 2001. **46**(4): p. 835 - 843.

98. Roux, C., S. Langdon, D. Waight, and J. Robertson, *The Transfer and Persistence of Automotive Carpet Fibres on Shoe Soles.* Science & Justice, 1999c. **39**: p. 239-251.

99. Tuinman, A.A., L.A. Lewis, and S.A.S. Lewis, *Trace-Fiber Colour Discrimination by Electrospray Ionization Mass Spectrometry: A Tool for the Analysis of Dyes Extracted from Submillimeter Nylon Fibers.* Analytical Chemistry, 2003. **75**: p. 2753-2760.

100. Huang, M., J. Yinon, and M.E. Sigman, *Forensic Identification of Dyes Extracted from Textile Fibers by Liquid Chromotography Mass Spectrometry (LC-MS).* Journal of Forensic Sciences, 2004. **49**(2): p. 238-249.

101. Morrison, W.H.I. and D.D. Archibald, *Analysis of Graded Flax Fibre and Yarn Spectrometry.* Journal of Agricultural and Food Chemistry, 1998. **46**(5): p. 1870-1876.

102. Wallace, C., C. Roux, D. Exline, and C. Lennard. *Forensic Applications of Chemical Imaging.* in *16th International Symposium for Forensic Science*. 2001. Canberra, Australia.

103. Payne, G., B. Reedy, C. Lennard, D. Exline, and C. Roux. *Evaluation of the CI Trace for Fibre Analysis.* in *17th International Symposium on the forensic sciences (Australian and New Zealand Forensic Science Society)*. 2004. Wellington, New Zealand.

104. Grieve, M., T. Biermann, and M. Davignon, *The Occurance and Individuality of Orange and Green Cotton Fibres.* Science & Justice, 2003. **43**(1): p. 5-22.

105. Suzuki, S., Y. Suzuki, H. Ohta, R. Sugita, and Y. Marumo, *Microspectrophotometric Discrimination of Single Fibres Dyed by Indigo and Its Derivatives Using Ultraviolet-Visible Transmittance Spectra.* Science & Justice, 2001. **41**: p. 107-111.

106. Massonnet, G., P. Buzzini, G. Jochem, M. Staub, T. Coyle, C. Roux, J. Thomas, H. Leijenhorst, R.M.E. Griffin, K.G. Wiggins, C. Russell, S. Chabli, and A. Rosengarten, *Evaluation of Raman Spectroscopy for the Analysis of Coloured Fibres: A Collaborative Study.* Journal of Forensic Sciences, 2004b.

107. Massonnet, G., P. Buzzini, G. Jochem, L. Fido, R. Beattie, S. Bell, M. Stauber, T. Coyle, C. Roux, J. Thomas, H. Leijenhorst, Z. Van Zanten, K.G. Wiggins, C. Russell, S. Chabli, A. Rosengarten, L. Marshall, E. Bartick, and M. Murphy. *Raman Group 2004 - Green Cotton.* in *12th EFG Meeting*. 2004a. Prague, Czech Republic.

108. Lang, P.L., J.E. Katon, J.F. O'Keefe, and D.W. Schiering, *The Identification of Fibers by Infrared and Raman Microspectroscopy.* Microchemistry. Journal., 1986a. **34**(3): p. 319-31.

109. Lang, P.L., J.E. Katon, D.W. Schiering, and J.F. O'Keefe, *Application of Infrared and Raman Microspectroscopy to Polymer Characterization*, in Polymer Material Science and Engineering. 1986b. p. 381-5.

110. Kokot, S., N.A. Tuan, and L. Rintoul, *Discrimination of Reactive Dyes on Cotton Fabric by Raman Spectroscopy and Chemometrics.* Applied Spectroscopy, 1997. **51**(3): p. 387-395.

111. Miller, J.V. and E.G. Bartick, *Forensic Analysis of Single Fibers by Raman Spectroscopy.* Applied Spectroscopy, 2001. **55**: p. 1729-1732.

112. Raman, C.V., *A New Type of Secondary Radiation.* Nature, 1928. **121**: p. 619.

113. Skoog, D.A. and J.J. Leary, *Principles of Instrumental Analysis*. 4th ed. 1992: Saunders College Publishing.

114. Otieno-Alego, V., *Raman Spectroscopy: A Useful Tool for the Archaeometric Analysis of Pigments*, in *Radiation in Art and Archaeometry*, D.C. Creagh and D.A. Bradley, Editors. 2000, Elsevier: Amsterdam.

115. Banwell, C.N., *Raman Spectroscopy*, in *Fundamentals of Molecular Spectroscopy*. 1983, McGraw-Hill Book Company: London. p. 124-153.

116. Smekal, A., *The Quantum Theory of Dispersion.* Naturwissenschaffen, 1923. **11**: p. 873.

117. Landsberg, G. and L. Mandelstam, *A Novel Effect of Light Scattering in Crystals.* Naturwissenschaffen, 1928. **16**: p. 557.

118. Laitinen, H.A. and G.W. Ewing, *A History of Analytical Chemistry*. 1977, Washington: American Chemical Society.

119. Jones, R.N., *Analytical Applications of Vibrational Spectroscopy ? A Historical Review.* European Spectroscopy News, 1987. **72**: p. 10.

120. Welsh, H.L., M.F. Crawford, T.R. Thomas, and G.R. Love, *Raman Spectroscopy of Low-Pressure Gases and Vapors.* Canadian Journal of Physics, 1952. **30**: p. 577.

121. Maiman, T.H., *Stimulated Optical Radiation in Ruby.* Nature,, 1960. **187**: p. 493.

122. Porto, S.P.S. and D.L. Wood, *Ruby Optical Laser as a Raman Source.* J. Opt. Soc. Am., 1962. **52**: p. 251.

123. Hirschfeld, T. and D.B. Chase, *FT-Raman Spectroscopy: Development and Justification.* Applied Spectroscopy, 1986. **40**: p. 133.

124. Cutler, D.J., *The Development of Fourier Transform Raman Spectroscopy.* Spectrochimica Acta Part A: Molecular Spectroscopy, 1990a. **46**(2): p. 123-129.

125. Cutler, D.J., *Fourier Transform Raman Instrumentation.* Spectrochimica Acta Part A: Molecular Spectroscopy, 1990b. **46**(2): p. 131-151.

126. Boyle, W.S. and G.E. Smith, *Charge Coupled Semiconductor Devices.* Bell System Tech. J., 1970. **49**: p. 587.

127. Dierker, S.B., C.A. Murray, J.D. Legrange, and N.E. Schlotter, *Characterization of Order in Langmuir-Blodgett Monolayers by Unenhanced Raman Spectroscopy.* Chemical Physics Letters, 1987. **137**: p. 453.

128. Williams, K., G. Pitt, B. Smith, A. Whiley, D. Batchelder, and I. Hayward, *Use of Rapid Scanning Stigmatic Raman Imaging Spectrograph in the Industrial Environment.* Journal of Raman Spectroscopy, 1994. **25**: p. 131-138.

129. Huong, P.V., *New Possibilities of Raman Micro-Spectroscopy.* Vibrational Spectroscopy, 1996. **11**(1): p. 17-28.

130. The Graz University of Technology " Infrared- & Raman Microscopes: Raman Microscope" website, *http://www.felmi-zfe.tugraz.at/research_raman3.html.* Visited 22/05/2004

131. Khalilov, A.K. and P.P. Shorygin, *Variation of the Intensity of Raman Lines with the Frequency of the Exciting Light.* Doklady Akademii Nauk SSSR, 1951. **81**: p. 1031-3.

132. Fleishman, M., P.J. Hendra, and A.J. McQuillan, *Raman Spectra of Pyridine Absorbed at a Silver Cathode.* Chem. Phys. Lett, 1974. **26**: p. 163-167.

133. Campion, A. and P. Kambhampati, *Surface-Enhanced Raman Scattering.* Chemical Society Reviews, 1998. **27**(4): p. 241-250.

134. Stacey, A.M. and R.P. VanDuyne, *Surface Enhanced and Resonance Raman Spectroscopy in a Non-Aqueous Environment - Tris (2,2'-Bipyridine) Ruthenium Ii Adsorbed on Silver from Acetonitrile.* Chem. Phys. Lett, 1983. **102**: p. 365-370.

135. Coupry, C. and D. Brissaud, *Applications [of Raman Microscopy] in Art, Jewellery and Forensic Science*, in *Raman Microscopy*, G. Turrell and J. Corset, Editors. 1996, Academic Press: London. p. 421-453.

136. Hodges, C.M. and J. Akhavan, *The Use of Fourier Transform Raman Spectroscopy in the Forensic Identification of Illicit Drugs and Explosives.* Spectrochimica Acta Part A: Molecular Spectroscopy, 1990. **46**(2): p. 303-307.

137. Suzuki, E.M. and M. Carrabba, *In Situ Identification and Analysis of Automotive Paint Pigments Using Line Segment Excitation Raman*

Spectroscopy: I. Inorganic Topcoat Pigments, in *J. Forensic Sci.* 2001. p. 1053-1069.

138. Harvey, S.D., M.E. Vucelick, R.N. Lee, and B.W. Wright, *Blind Field Test Evaluation of Raman Spectroscopy as a Forensic Tool.* Forensic Science International, 2002. **125**(1): p. 12-21.

139. Rodger, C. and D. Broughton, *The in-Situ Analysis of Lipsticks by Surface Enhanced Resonance Raman Scattering*, in *Analyst (Cambridge, U. K.)*. 1998. p. 1823-1826.

140. Best, S.P., R.J.H. Clark, and R. Withnall, *Non-Destructive Pigment Analysis of Artefacts by Raman Microscopy.* Endeavour, 1992. **16**(2): p. 66-73.

141. Clark, R.J.H., *Pigment Identification on Medieval Manuscripts by Raman Microscopy.* Journal of Molecular Structure, 1995. **347**: p. 417-427.

142. Clark, R.J.H., *Raman Microscopy-Application to the Identification of Pigments on Medieval Manuscripts.* Chemical Society Reviews, 1995. **24**: p. 187-196.

143. Clark, R.J.H., S.P. Best, M.A.M. Daniels, C.A. Porter, and R. Withnall, *Identification by Raman Microscopy and Visible Reflectance Spectroscopy of Pigments on an Icelandic Manuscript.* Studies in Conservation, 1995a. **40**: p. 31-40.

144. Clark, R.J.H., L. Burgio, and D.A. Ciomartan, *Pigment Identification on Medieval Manuscripts, Paintings and Other Artefacts by Raman Microscopy: Applications to the Study of Three German Manuscripts.* Journal of Molecular Structure, 1997a. **405**: p. 1-11.

145. Clark, R.J.H., L. Burgio, and D.A. Ciomartan, *Raman Microscopy Study of the Pigments on Three Illuminated Mediaeval Latin Manuscripts.* Journal of Raman Spectroscopy, 1997b. **28**: p. 79-83.

146. Clark, R.J.H., L. Cridland, B.M. Kariuki, K.D.M. Harris, and R. Withnall, *Synthesis, Structural Characterization and Raman-Spectroscopy of the Inorganic Pigments Lead-Tin Yellow Type-I and Type-II and Lead Antimonate Yellow-Their Identification on Medieval Paintings and Manuscripts.* Journal of the Chemical Society-Dalton Transactions, 1995b. **16**: p. 2577-2582.

147. Clark, R.J.H. and P.J. Gibbs, *Non-Destructive in Situ Study of Ancient Egyptian Faience by Raman Microscopy.* Journal of Raman Spectroscopy, 1997a. **28**: p. 99-103.

148. Clark, R.J.H. and P.J. Gibbs, *Identification of Lead(II) Sulfide and Pararealgar on a 13th Century Manuscript by Raman Microscopy.* Chemical Communications, 1997b: p. 1003-1004.

149. Clark, R.J.H. and P.J. Gibbs, *Analysis of 16th Century Qazwini Manuscripts by Raman Microscopy and Remote Laser Raman Microscopy.* Journal of Archaeological Science, 1998. **25**(7): p. 621-629.

150. Clark, R.J.H. and P.J. Gibbs, *A Non-Intrusive, in Situ Study of a 13th Century Byzantine/Syriac Gospel Lectionary.* Analytical Chemistry, 1998. **70**: p. 99A-104A.

151. Clark, R.J.H., P.J. Gibbs, K.R. Seddon, N.M. Brovenko, and Y.A. Petrosyan, *Non-Destructive in Situ Identification of Cinnabar on Ancient*

Chinese Manuscripts. Journal of Raman Spectroscopy, 1997. **28**: p. 91-94.

152. Coupry, C., A. Lautie, M. Revault, and J. Dufilho, *Contribution of Raman Spectroscopy to Art and History.* Journal of Raman Spectroscopy, 1994. **25**: p. 89-94.

153. Coupry, C., G. Sagon, and P. Gorguet-Ballesteros, *Raman Spectroscopic Investigation of Blue Contemporary Textiles*, in *J. Raman Spectroscopy* 1997. p. 85-89.

154. Gardiner, D.J., R. Davey, B.W. Singer, and M. Spokes, *Examples of Analysis of Pigments from Fine Art-Objects by Raman Microscopy.* Journal of Raman Spectroscopy, 1994. **25**: p. 53-57.

155. Gardiner, D.J., B.W. Singer, and J.P. Derow, *Analysis of White and Blue Pigments from Water Colours by Raman Microscopy.* The Paper Conservator, 1993. **17**: p. 13-19.

156. Andreev, G.N., B. Schrader, H. Schulz, R. Fuchs, S. Popov, and N. Handjieva, *Non-Destructive NIR-FT-Raman Analyses in Practice. Part 1. Analyses of Plants and Historic Textiles*, in Fresenius' Journal of Analytical Chemistry. 2001. p. 1009-1017.

157. Bell, I.M., R.J.H. Clark, and P.J. Gibbs, *Raman Spectroscopic Library of Natural and Synthetic Pigments (Pre- ~ 1850 Ad).* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 1997. **53**(12): p. 2159-2179.

158. Burgio, L. and R.J.H. Clark, *Library of FT-Raman Spectra of Pigments, Minerals, Pigment Media and Varnishes, and Supplement to Existing*

Library of Raman Spectra of Pigments with Visible Excitation. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2001. **57**(7): p. 1491-1521.

159. Burgio, L., D.A. Ciomartan, and R.J.H. Clark, *Pigment Identification on Medieval Manuscripts, Paintings and Other Artefacts by Raman Microscopy: Applications to the Study of Three German Manuscripts.* Journal of Molecular Structure, 1997. **405**(1): p. 1-11.

160. Barnes, A.J., M.A. Majid, M.A. Stuckey, P. Gregory, and C.V. Stead, *The Resonance Raman Spectra of Orange Ii and Para Red: Molecular Structure and Vibrational Assignment.* Spectrochimica Acta Part A: Molecular Spectroscopy, 1985. **41**(4): p. 629-635.

161. Edwards, H.G., D.W. Farwell, and D. Webster, *FT Raman Microscopy of Untreated Natural Plant Fibres.* Spectrochimica Acta. Part a, Molecular and Biomolecular Spectroscopy, 1997. **53A**(13): p. 2383-92.

162. Edwards, H.G.M. and M.J. Falk, *Investigations of the Degradation Products of Archaeological Linens by Raman Spectroscopy.* Applied Spectroscopy, 1997. **51**(8): p. 1134 - 1138.

163. Jahn, A., M.W. Schroder, M. Futing, K. Schenzel, and W. Diepenbrock, *Characterization of Alkali Treated Flax Fibres by Means of FT Raman Spectroscopy and Environmental Scanning Electron Microscopy.* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2002. **58**(10): p. 2271-2279.

164. Carter, E.A., P.M. Fredericks, J.S. Church, and R.J. Denning, *FT-Raman Spectroscopy of Wool--I. Preliminary Studies.* Spectrochimica Acta Part A: Molecular Spectroscopy, 1994. **50**(11): p. 1927-1936.

165. Hendra P.J., Maddams W.F., Royaud I.A.M., Willis H.A., and Zichy V., *The Application of Fourier Transform Raman Spectroscopy to the Identification and Characterisation of Polyamides - 1. Single Number Nylons.* Spectrochimica Acta Part A Molecular Spectroscopy, 1990. **46a**: p. 747-756.

166. Maddams, W.F. and I.A.M. Royaud, *The Application of Fourier Transform Raman Spectroscopy to the Identification and Characterisation of Polyamides - Ii. Double Number Nylons.* Spectrochimica Acta Part A Molecular Spectroscopy, 1991. **47a**: p. 1327-1333.

167. Kazarian, S.G., N.H. Brantley, and C.A. Eckert, *Applications of Vibrational Spectroscopy to Characterize Poly(Ethylene Terephthalate) Processed with Supercritical Co2*, in Vibrational Spectroscopy 1999. p. 277-283.

168. Bourgeois, D. and S.P. Church, *Studies of Dyestuffs in Fibres by Fourier Transform Raman Spectroscopy.* Spectrochimica Acta Part A: Molecular Spectroscopy, 1990. **46**(2): p. 295-301.

169. Keen, I.P., G.W. White, and P.M. Fredericks, *Characterization of Fibers by Raman Microprobe Spectroscopy.* Journal of Forensic Sciences, 1998. **43**(1): p. 82-89.

170. White, P.C., C.H. Munro, and W.E. Smith, *In Situ Surface Enhanced Resonance Raman Scattering Analysis of a Reactive Dye Covalently Bound to Cotton.* Analyst, 1996. **121**: p. 835-885.

171. Jochem, G. and R.J. Lehnert, *On the Potential of Raman Microscopy for the Forensic Analysis of Coloured Textile Fibres.* Science & Justice, 2002. **42**(4): p. 215-221.

172. Massonnet, G., P. Buzzini, G. Jochem, L. Fido, R. Beattie, S. Bell, M. Stauber, T. Coyle, C. Roux, J. Thomas, H. Leijenhorst, Z. Van Zanten, K.G. Wiggins, C. Russell, S. Chabli, A. Rosengarten, L. Marshall, E. Bartick, and M. Murphy. *Detection Limit of Raman Spectroscopy.* in *13th Meeting of the European Fibres Group.* 2005. Bled, Slovenia.

173. Phillips, D., *Environmentally Friendly, Productive and Reliable: Priorities for Cotton Dyes and Dyeing Processes.* Journal of the Society of Dyers & Colourists, 1996. **112**: p. 183.

174. Colthup, N.B., L.H. Daly, and S.E. Wiberley, *Introduction to Infrared and Raman Spectroscopy.* 3rd ed. 1990: Academic Press. 547.

175. White, P.C., C. Rodger, V. Rutherford, Y. Finnon, W.E. Smith, and M. Fitzgerald, *Surface Enhanced Resonance Raman Scattering (Serrs) Spectroscopy. A Powerful Technique for the Forensic Analysis of Colorants?* Proc. SPIE-Int. Soc. Opt. Eng., 1999a. **3576**(Investigation and Forensic Science Technologies): p. 77-86.

176. Cooke, P.M., *Chemical Microscopy*, in Analytical Chemistry 2000. p. 169-188.

177. Bouffard, S.P., A.J. Sommor, J.E. Katton, and S. Godber, *Use of Molecular Microspectroscopy to Characterize Pigment-Loaded Polypropylene Single Fibres.* Applied Spectroscopy, 1994. **48**: p. 1387-1393.

178. Pellow-Jarman, M.V., P.J. Hendra, and R.J. Lehnert, *The Dependence of Raman Signal Intensity on Particle Size for Crystal Powders.* Vibrational Spectroscopy, 1996. **12**(2): p. 257-261.

179. *http://www.iljinnanotech.co.*

180. Grieve, M., T. Biermann, and K.G. Wiggins, *Fibre Comparisons Using Microspectrophotometry.* Science & Justice, 1999. **39**(4): p. 273.

181. Cassista, A. and A.D. Peters, *Survey of Red, Green and Blue Cotton Fibres.* Canadian Society of Forensic Science Journal, 1997. **30**(4): p. 225-231.

182. Palmer, R. and L.D. Turnbull, *A Survey of Dye Batch Variation.* Science & Justice, 1995. **35**(1): p. 59-64.

183. Wiggins, K., J. Holness, and B.M. March, *The Importance of Thin Layer Chromotography and Uv Microspectrophotometry in the Analysis of Reactive Dyes Released from Wool and Cotton Fibres.* Journal of Forensic Sciences, 2005. **50**(2): p. 1-4.

184. Miller, J.N. and J.C. Miller, *Statistics and Chemometrics for Analytical Chemistry*. 4th ed. 2000, England: Prentice Hall.

185. Hibbert, D.R. and A.M. James, *MacMillan Dictionary of Chemistry*. 1987, London: Macmillan press.

186. Geladi, P., *Chemometrics in Spectroscopy. Part 1. Classical Chemometrics.* Spectrochimica Acta Part B, 2003. **58**: p. 767-782.

187. Wold, S. and M. Sjostrom, *Chemometrics, Present and Future Success.* Chemometrics and Intelligent Laboratory Systems, 1998. **44**(1-2): p. 3-14.

188. Joliffe, I.T., *Principle Component Analysis*. Springer Series in Statistics. 1986, New York: Springer Verlag.

189. Wold, S., *Chemometrics; What Do We Mean with It, and What Do We Want from It?* Chemometrics and Intelligent Laboratory Systems, 1995. **30**(1): p. 109-115.

190. Martens, H. and T. Naes, *Multivariate Calibration*. 1996, Chichester: John Wiley & Sons.

191. Liu, Y., *Vibrational Spectroscopic Investigation of Australian Cotton Cellulose Fibers Part 1. A Fourier Transform Raman Study*, in Analyst *(Cambridge, U. K.)*. 1998. p. 633-636.

192. De Groot, P.J., G.J. Postma, W.J. Melssen, L.M.C. Buydens, V. Deckert, and R. Zenobi, *Application of Principal Component Analysis to Detect Outliers and Spectral Deviations in near-Field Surface-Enhanced Raman Spectra*, in Analytica Chimica Acta. 2001. p. 71-83.

193. *Svensson*, O., M. Josefson, and F.W. Langkilde, *Reaction Monitoring Using Raman Spectroscopy and Chemometrics.* Chemometrics and Intelligent Laboratory Systems, 1999. **49**(1): p. 49-66.

194. Brody, R.H., H.G.M. Edwards, and A.M. Pollard, *Chemometric Methods Applied to the Differentiation of Fourier-Transform Raman Spectra of Ivories*, in Analytical Chimica Acta. 2001. p. 223-232.