# AN EVALUATION STUDY FOR FIBRE ANALYSIS BY UV-VISIBLE MICROSPECTROPHOTOMETRY

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## INTRODUCTION

Colour can be a highly discriminating feature in forensic fibre comparison. The advent of visible (VIS) microspectrophotometry increased the forensic scientist's ability for colour discrimination over and above microscopic comparison, and provided a more objective analysis. Suzuki, et al. (2001) and Grieve, et al. (2001) have reported that further discrimination can be obtained in the ultraviolet (UV) spectral range. However, there has been little published data on validation of the analytical parameters and protocols for ultraviolet-visible microspectrophotometry in forensic fibre analysis.

In this study, various aspects of UV-VIS microspectrophotometry were assessed to evaluate its use in a forensic laboratory for routine fibre analysis. Instrumental stability, and interferences from mounting materials and fibre polymers were tested. Its relative value over visible microspectrophotometry was also evaluated. A wide variety of fibres were selected to represent generic types and colours commonly encountered in forensic casework.

## METHODS AND MATERIALS

### Instrumentation

The Zeiss MPM 800 microspectrophotometer system was used for the transmittance analysis of all the samples in this study. The visible range analyses were done with the 40X Neofluar objective from 380-770nm. The UV-VIS range analyses were done with the 40X Ultrafluar objective from 240-770nm. All measurements were made with a 2.5nm measuring step and 5.0nm bandwidth, and an optimization limit of 15. System backgrounds

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were run under the same conditions as the sample scans for the non-dyed and coloured fibres, and were factored out of these analyses. Wavelength performance checks were done with a didymium oxide filter (VIS) and a holmium oxide filter (UV-VIS).

**UV-VIS Instrumental Stability**

Same-day fibre analysis reproducibility was tested by measuring the absorbance of an evenly dyed green acrylic fibre mounted on quartz, six times in approximately the same location over an eight-hour period. Day-to-day fibre analysis reproducibility was tested by measuring the absorbance of the same green acrylic fibre mounted on quartz, six times in approximately the same location over a period of 28 days.

**UV-VIS Absorbance Interferences**

*Analyses of Mounting Materials*

UV-VIS absorbance was measured through a glass slide, a quartz slide, glycerine on quartz, and XAM™ on quartz. A smear of XAM™ mounting medium was dried on a glass slide and the film was scraped off for analysis on the quartz slide. The system background absorbance was not factored out from these analyses.

*UV-VIS Analysis of Non-Dyed Fibres*

UV-VIS absorbance was measured for white, non-dyed fibres of different polymer types mounted in glycerine on quartz slides and cover slips to determine polymer interferences (see Table 1). A drop of glycerine on the cover slip was also used as an immersion medium for the Ultrafluar objective during all UV-VIS analysis. When absorbance was seen, several samples of the fibre type were run to confirm that the absorbance was due to the polymer. The polymer composition of the fibres was verified by FTIR microspectroscopy.

**Visible Analysis versus UV-VIS Analysis with Coloured Fibres**

Absorbance from 240-770nm was measured with coloured fibres (see Table 1) mounted

*TABLE 1  Fibres Used in Spectral Analyses*

| | | Non-Dyed Fibres | | |
|---|---|---|---|---|
| Cotton | Di-acetate | Nylon 6 | PET polyester | Acrylic PAN/MA[1] |
| Ramie | Triacetate | Nylon 66 | PCDT polyester | Acrylic PAN/MMA[1] |
| Wool | Viscose | Polyethylene | Modacrylic PAN/VC[1] | Acrylic PAN/VA[1] |
| Silk | | Polypropylene | Modacrylic PAN/VDC[1] | Acrylic PAN/PVP[1] |
| | | | Modacrylic PAN/VDC/MMA[1] | Acrylic PAN/MA/MVP[1] |
| | | | Modacrylic PAN/VDC/VBr/ARS[1] | Acrylic PAN/VA/MVP[1] |

| Dyed Fibres |
|---|
| <u>Wool</u>: red |
| <u>Viscose</u>: yellow, orange, red, pink, purple, green, blue, brown, and black |
| <u>Di-acetate</u>: yellow, tangerine, red, pink, violet, green, blue, brown, and black |
| <u>Nylon 66</u>: yellow, red burgundy, aqua, blue, brown, and black |
| <u>Acrylic PAN/MMA</u>: yellow, orange, rust, fuschia, purple, green, blue, brown, and charcoal |

[1] PAN – polyacrylonitrile, VC – vinyl chloride, VDC – vinylidene chloride, MMA – methylmethacrylate, VBr – vinyl bromide, ARS – aromatic sulphonate, MA – methylacrylate, VA – vinyl acetate, MVP – methylvinylpyridine, PVP – polyvinylpyrrolidone

on quartz as above, and mounted on glass with XAM™. Absorbance from 380-770nm was measured with the visible system for coloured fibres mounted on glass with XAM™, to compare with information gathered from UV-VIS analysis.

**RESULTS AND DISCUSSION**

**UV-VIS Instrumental Stability**

Instrumental stability was demonstrated by the data obtained over six months of performance checks with the holmium oxide filter (see Table 2). The spectral results from same-day (Fig. 1(A)) and day-to-day (Fig. 1(B)) analyses of the green acrylic fibre also demonstrated no observable change in peak locations and negligible differences in absorbance.

**UV-VIS Absorbance Interferences**

*Mounting Materials*

For the mounting materials tested with the UV-VIS system, 240-770nm, glycerine and quartz showed no absorbance in addition to the background spectrum (see Fig. 2). Glass and XAM™ absorb substantially in the UV range beginning at about 310nm (glass) and

*TABLE 2  Mean Wavelength and Absorbance of Kopp Holmium Oxide Filter Glass (3131)*
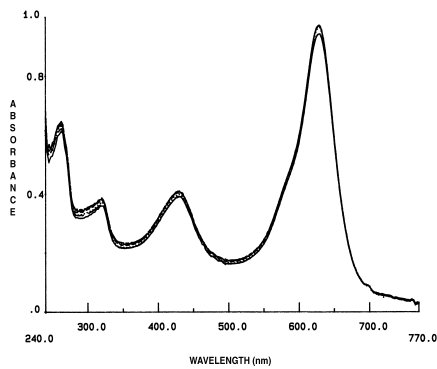
| PEAK* | 279nm | | 361nm | | 418nm | | 460nm | | 536nm | | 637nm | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\lambda$ (nm) | A.U. | $\lambda$ (nm) | A.U. | $\lambda$ (nm) | A.U. | $\lambda$ (nm) | A.U. | $\lambda$ (nm) | A.U. | $\lambda$ (nm) | A.U. |
| MEAN^ | 277.5 | 0.400 | 360.0 | 0.323 | 417.5 | 0.143 | 457.5 | 0.618 | 535.0 | 0.236 | 637.5 | 0.143 |
| STD DEV^ | 0 | 0.005 | 0 | 0.004 | 0 | 0.006 | 0 | 0.007 | 0 | 0.006 | 0 | 0.006 |

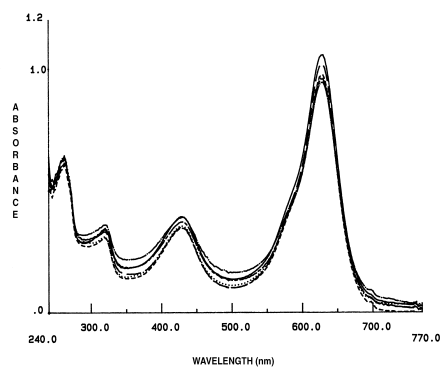* Peak values read from spectrum provided with the Kopp Holmium Oxide Filter Glass (3131)
^ Measurements made by Zeiss MPM800 microspectrophotometer;
240-770nm, Xenon lamp, 2.5nm Step, 5.0nm Bandwidth, n=21

*FIGURE 1  UV-VIS Spectral Reproducibility of a Green Acrylic Fibre*

(A)  Same day, repeated six times      (B) Day-to-day, repeated six times
  over eight hours                         over 28 days



290nm (XAM™). UV transmission was cut off altogether at about 280nm for glass and at about 265nm for XAM™. This implied that there was potential for glass/XAM™-mounted fibres to be analysed between 300nm and 380nm, a range that cannot be scanned with the visible system. Fibres mounted on quartz slides and coverslips in glycerine immersion medium can be analysed in the UV range without any interferences.

Scanning glass/XAM™ mounted fibres may be of some interest, given that quartz slides and cover slips have been quite expensive, and glycerine has not provided a permanent mount. Although they have been ideal for use in UV-VIS microspectrophotometry, they have not been practical materials for the initial microscopic examination of numerous

*FIGURE 2  Absorbance Spectra of Mounting Materials*
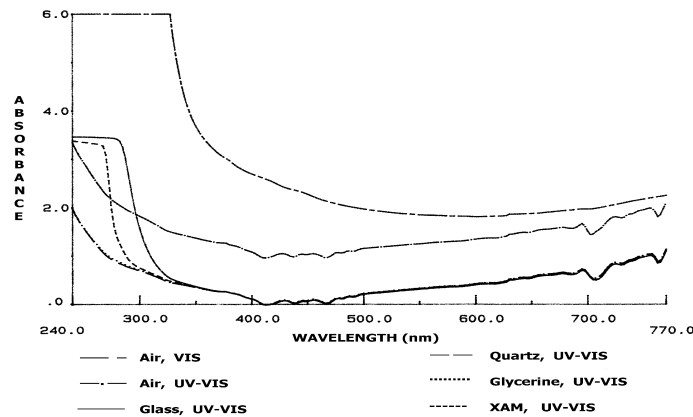


fibres. Unmounting fibres from XAM has been both laborious and time-consuming, risking the loss or damage of fibres in the process.

*Fibre Polymers*

The spectral results for the white, non-dyed fibres are shown in Table 3 and Figures 3-10. Fibre polymers have no inherent absorbance in the visible range but may exhibit absorbance below 300nm. The UV absorbance has been grouped into none, low, moderate, and high absorbance peaks/increases. These were defined as:  low absorbance < 0.25AU (absorbance units), moderate absorbance 0.25 – 1.0AU, and high absorbance > 1.0AU. Fibre polymers with no to very low absorbance at < 300nm were cotton, ramie, and viscose. Low absorbance was demonstrated by di- and tri-acetate, and nylon 6 and nylon 66 polyamides at < 300nm. Silk, wool, and polyesters had a high absorbance at < 300nm.

For olefins, acrylic, and modacrylic fibres, the absorbance differed depending on the polymer composition. Some co-polymers for the acrylic fibres had no absorbance in the UV range (PAN/MA, PAN/MMA, and PAN/VA). However, PAN/PVP had low absorbance below 280nm, while PAN/MA/MVP and PAN/VA/MVP showed substantial absorbance below 290nm. Amongst the modacrylic fibres, the types with VDC had low

absorbance below 300nm, which increased to moderate absorbance with the added presence of VBr and/or ARS. Different PAN/VC's gave variable spectra (one gave no absorbance below 300nm). The source of these peaks was not obvious upon examination of the FTIR spectra. Generally, olefins had no UV-VIS absorbance, but some samples had peaks varying in location and position.

**Visible Analysis versus UV-VIS Analysis with Coloured Fibres**

In general, the peak shapes in the visible range, 380-770nm were the same and no trend in sensitivity was seen with regards to VIS vs UV-VIS analysis of coloured fibres.

*TABLE 3  UV Absorbance of Fibre Polymers*

| POLYMER TYPE | UV ABSORBANCE* |
|---|---|
| CELLULOSIC (Fig. 3) | |
| Viscose | None |
| Cotton, ramie | Less than low at < 300nm |
| Di- and tri-acetate | Low at < 300nm |
| PROTEIN (Fig. 4) | |
| Silk | High at < 300nm |
| Wool | High at < 310nm |
| POLYESTER (Fig. 5) | |
| PET | High at < 310nm |
| PCDT | High at < 300nm |
| OLEFIN (Fig. 6) | |
| Polypropylene | None |
| Polyethylene | None |
| POLYAMIDE (Fig 7) | |
| Nylon 6, Nylon 6.6 | Low at < 300nm |
| POLYACRYLONITRILE | |
| *Acrylic* (Fig. 8) | |
| AcrylicPAN/MA, | Less than low at < 300nm |
| Acrylic PAN/MMA | None |
| Acrylic PAN/VA | Less than low at < 300nm |
| Acrylic PAN/PVP | Low at < 280nm |
| Acrylic PAN/MA/MVP | High at < 290nm |
| Acrylic PAN/VA/MVP | High at < 290nm |
| | |
| *Modacrylic* (Fig. 9 & 10) | |
| Modacrylic PAN/VC | Low at < 300nm |
| Modacrylic PAN/VDC | Low at < 300nm |
| ModacrylicPAN/VDC/MMA | Low at < 300nm |
| Modacrylic PAN/VDC/VBr/ARS | Moderate at < 300nm |

* Low absorbance < 0.25 AU
  Moderate absorbance between 0.25 – 1.0 AU
  High absorbance > 1.0 AU

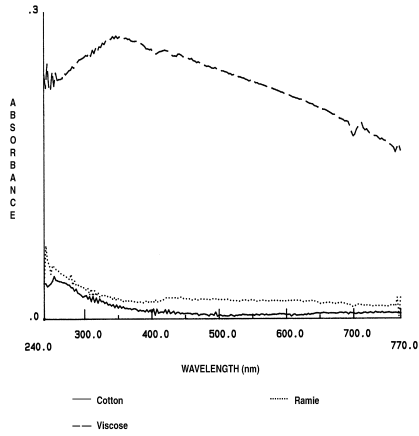## FIGURE 3  Cellulosic Polymer Types (acetates not included)

ABSORBANCE vs WAVELENGTH (nm)

— Cotton    ⋯⋯⋯ Ramie
–– Viscose

## FIGURE 4  Protein Polymer Types

ABSORBANCE vs WAVELENGTH (nm)

— Silk    ⋯⋯⋯ Wool
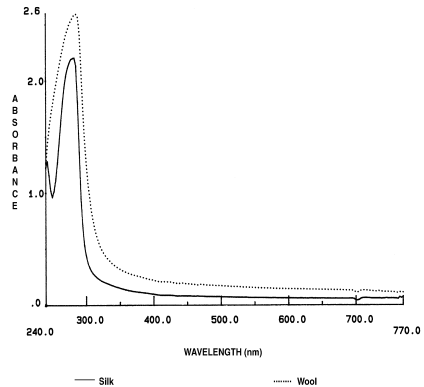
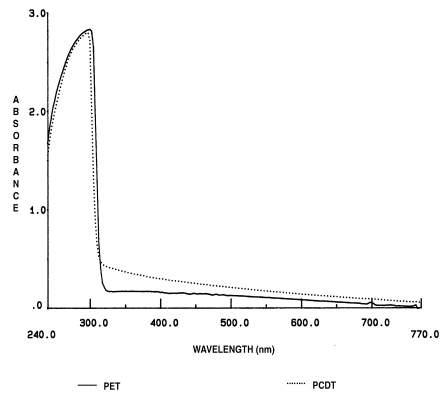## FIGURE 5  Polyester Polymer Types

ABSORBANCE vs WAVELENGTH (nm)

— PET    ⋯⋯⋯ PCDT

## FIGURE 6  Olefin Polymer Types

ABSORBANCE vs WAVELENGTH (nm)

— Spectra Polyethylene    ⋯⋯⋯ Pylen Polypropylene
---- Boltalen Polyethylene    ––– Herculon Polypropylene
–– Bexyarn Polyethylene
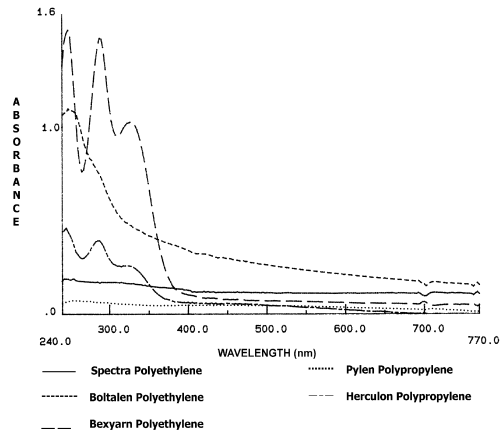
## FIGURE 7  Polyamide Polymer Types

ABSORBANCE vs WAVELENGTH (nm)

— Nylon 6    ⋯⋯⋯ Nylon 66
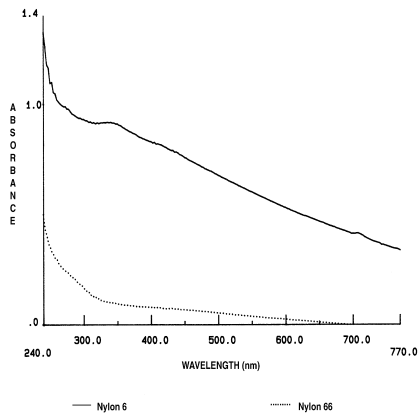
## FIGURE 8  Polyacrylonitrile Polymer Types - Acrylics

ABSORBANCE vs WAVELENGTH (nm)

–– Acrylic PAN/MA    ⋯⋯⋯ Acrylic PAN/MMA
–– Acrylic PAN/VA    —— Acrylic PAN/PVP
–– Acrylic PAN/MA/MVP    ––– Acrylic PAN/VA/MVP
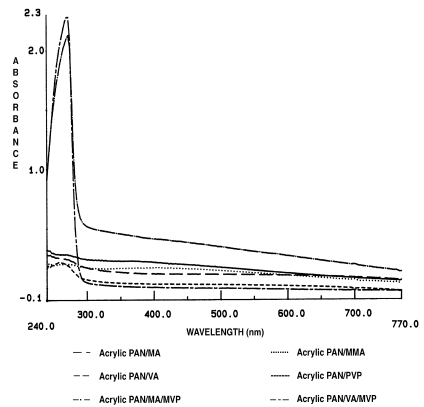
*FIGURES 9 and 10  Polyacrylonitrile Polymer Types - Modacrylics*



Modacrylic PAN/VC, Vinyon Noru ———  Modacrylic PAN/VDC, Dralon C100 ·······
Modacrylic PAN/VDC/MMA, Verel III –·–  Modacrylic PAN/VDC/VBr/ARS, SEF ———
Modacrylic PAN/VDC/MMA, Verel FB ·····  Modacrylic PAN/VDC/VBr/ARS, Acrilan SEF —·—

Vinyon Noru, PAN/VC Modacrylic ———  Dynel 150, PAN/VC Modacrylic ———
Kanekalon K, PAN/VC Modacrylic ·······  Dynel 297, PAN/VC Modacrylic ·······
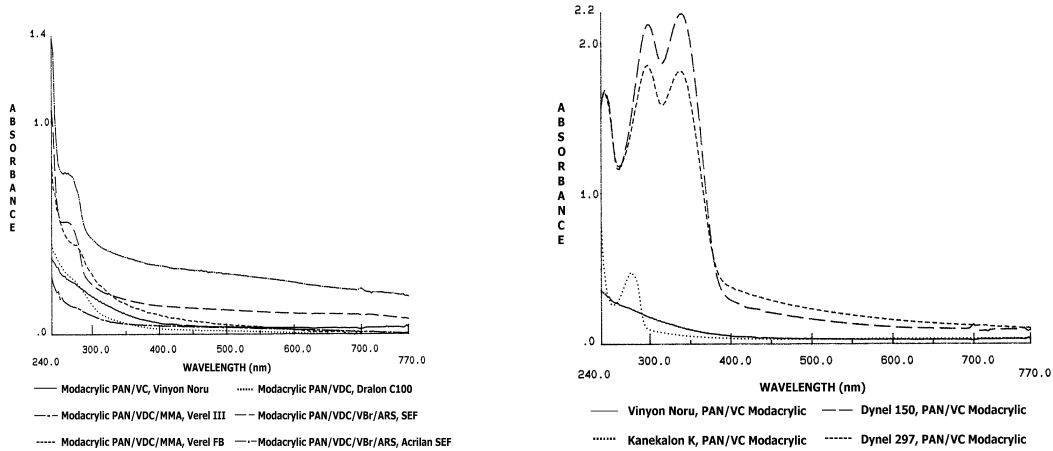
*Fibre polymers with high UV absorbance*

The red wool fibre spectra in Fig. 11 show a high absorbance peak below 300nm, due to the fibre polymer, and another peak at 330nm. This demonstrated that analysis between 300nm to 380nm gave pertinent information but there would be no discriminating value to remounting on quartz to measure below 300nm.

*Fibre polymers with moderate to low UV absorbance*

For di-acetate and nylon fibres, the inherent absorbance of the polymers made it difficult to interpret low absorbance for the coloured fibres below 300nm. In general, a peak below 300nm in a spectrum of a coloured fibre might be due to interference from the fibre polymer or from the fibre polymer and dye.

The coloured fibres spectra had peaks in the 300nm to 380nm range. In all the glass-mounted spectra where the absorbance slope just above 300nm was greater than that of the non-dyed polymer, there was an obvious peak or shoulder below 300nm on the quartz spectra. The absorbance spectra of  violet and  white di-acetate fibres illustrate this property (see Fig. 12).
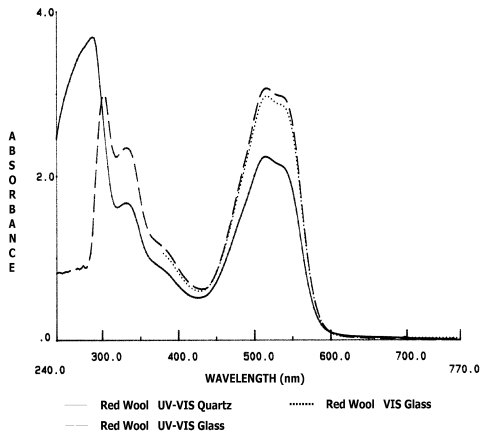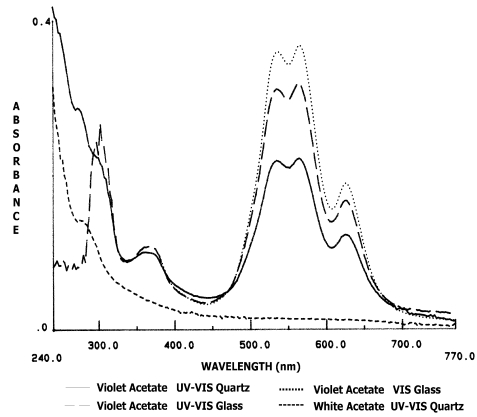
**FIGURE 11  Absorbance Spectra of a Red Wool Fibre**



Red Wool  UV-VIS Quartz    ......... Red Wool  VIS Glass
Red Wool  UV-VIS Glass

**FIGURE 12  Absorbance Spectra of Violet and White Di-acetate Fibres**



Violet Acetate  UV-VIS Quartz    ......... Violet Acetate  VIS Glass
Violet Acetate  UV-VIS Glass    ------ White Acetate  UV-VIS Quartz

*Fibre polymers with no UV absorbance*

For viscose and acrylic PAN/MMA, there were varying results. For the coloured viscose fibres, there were peaks between 300nm to 380nm, while for the acrylic fibres, there were no peaks within this range. In some cases of the viscose and acrylic fibres, the spectra gave a hint of a peak below 300nm, while others gave no indication. In Fig. 13 the glass absorbance spectrum  of an orange viscose fibre gives no indication of the peak that is seen below 300nm in the quartz absorbance spectrum. The increasing slope below 350nm of the rust acrylic fibre spectra, in Fig. 14, does indicate the peak at 290nm.

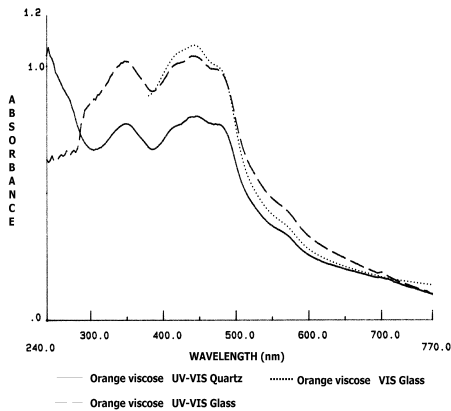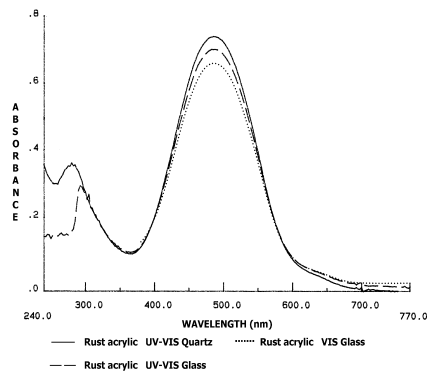**FIGURE 13  Absorbance Spectra of an Orange Viscose Fibre**



Orange viscose  UV-VIS Quartz    ......... Orange viscose  VIS Glass
Orange viscose  UV-VIS Glass

**FIGURE 14  Absorbance Spectra of a Rust Acrylic Fibre**



Rust acrylic  UV-VIS Quartz    ......... Rust acrylic  VIS Glass
Rust acrylic  UV-VIS Glass

Finding variable peaks with some non-dyed olefins and modacrylics PAN/VC indicated that UV absorbance may be due to the presence of additives as opposed to the polymers or dyes, and this may not be predictable even with the FTIR spectra. White fibres in casework may also have whitening additives. Therefore, some discrimination may be attainable even for white fibres, using UV-VIS microspectrophotometry. Further work may determine whether this technique could be applied towards the discrimination of white fibres.

**CONCLUSIONS**

The microspectrophotometry of all coloured fibres can initially be done with the XAM™-mounted glass slides from 300-770nm with the UV-VIS system, instead of the traditional visible range of 380-770nm. The need for re-mounting onto quartz slides for full UV-VIS analysis at 240-770nm depends on the fibre type and spectral results.

Wool, silk, polyester and acrylics with MVP are not suitable for UV analysis below 300-310nm, due to the polymer absorbance interference. Since this is also in the glass cut-off area, there is no advantage to remounting on quartz for full UV-VIS analysis. For these fibre types, analysis at 300 to 770nm with the glass mount will suffice.

Acetates, nylons, modacrylics and acrylics with PVP have low or moderate absorbance below 300nm. The spectra of coloured fibres should be compared to the reference spectra of non-dyed fibres to determine if any absorbance present could be due to the dye. Additional peaks may be visible with the glass mount between 300-380nm. If the coloured fibre spectrum just above 300nm has an increasing slope, which is greater than that of the non-dyed fibre, there may be a distinguishable dye peak in the lower UV region. Remounting the fibre on quartz for a full spectrum UV-VIS analysis may provide more discriminating information.

Coloured fibres with no polymer interference (cotton, ramie, viscose, olefin and acrylic with no PVP/MVP) may or may not give an indication of peaks or increasing absorbance below 300nm, but there may still be an absorbance peak in the lower UV area. Whether the fibres without the absorbance above 300nm should be remounted on quartz for a full spectrum analysis from 240-770nm may be left to the discretion of the case analyst.

Overall analysis in the UV-VIS range can provide increased discrimination potential for certain fibres. A flowchart decision tree was developed to guide the analyst in determining when to employ full UV-VIS microspectrophotometry for the analysis of coloured fibres (see Table 4). The evaluation study has demonstrated that UV-VIS microspectrophotometry can be employed for the routine analysis of fibre samples recovered in criminal investigations.

*TABLE 4  Protocol for UV-VIS Microspectrophotometry of Coloured Fibres*
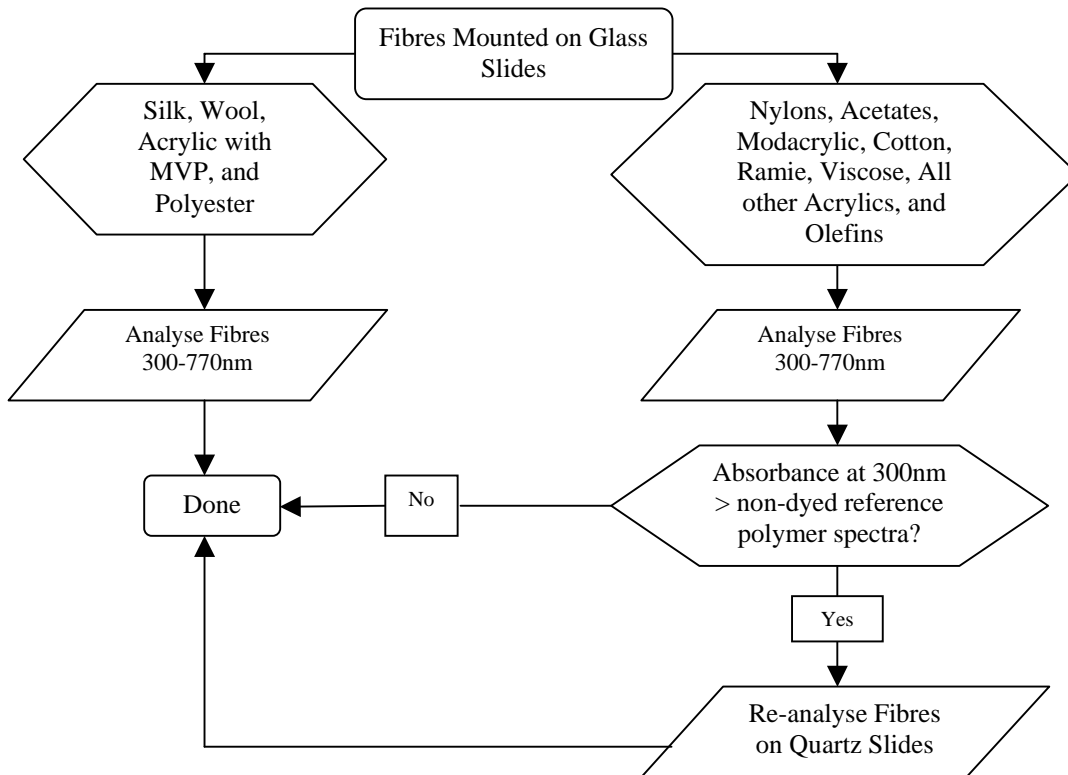
## ACKNOWLEDGEMENTS

## REFERENCES

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Grieve M.C., Biermann, T.W. and Davignon M. The Evidential Value of Black Cotton Fibres. Science and Justice. 2001; 41(4): 245-260.