**Starna scientific**

# Reference materials
# for
# Molecular Fluorescence
# Spectrophotometry

# Reference materials for Molecular Fluorescence Spectrophotometry

These materials may be used on a routine basis to calibrate and monitor the performance of Fluorescence Spectrophotometers. They are very stable and convenient to use and with reasonable treatment, will remain usable for many years. Normally the reference materials are used in a comparative mode; the instrument is adjusted to give a useful reading for a standard of known concentration, this may be directly in concentration or an arbitrary figure, eg. one corresponding to a test limit. A suitable standard is then measured under identical conditions and the intensity noted. For future assays, it is no longer necessary to prepare a standard each time the test is performed, as the reference material may simply be inserted, using the original instrument settings. Any deviation from the original reading is removed by adjusting the gain, making the instruments' read out identical to when it was calibrated.

The reference material should be selected so that there is reasonable overlap between the excitation and emission bands with that of the sample, as this will provide the best match between the readings, so that only small adjustments to the instrument gain will be needed in order to obtain suitable readings and reproducibility will be maximized.

The reference materials may also be used to monitor instrument performance and for standardization. If readings for the reference material are recorded on a regular basis, using the original instrument settings, ie. before adjusting the gain to standardize the instrument, they may be used to construct a Quality Control (QC) chart. Such a chart may be used to detect variations in instrument performance, such as long term drift due to deterioration of the source or optical surfaces, also to eliminate errors that arise from any incorrect setting up or an instrument malfunction. The materials are solid, so they do not contribute any noise to the measurements caused by convection currents or striations. Multiple readings may therefore be taken and used to provide an accurate estimate of instrument precision, an important requirement for many QC situations.

The fluorescent materials are dissolved in methylmethacrylate and the solution is then polymerized to produce a polymethyl methacrylate (PMMA) matrix which provides a stable environment for the fluorescent compounds. Being solids, there can be no evaporation of solvents or any significant photo-decomposition of the material, thus producing long term stability. This high stability ensures that any observed variation in readings is due to instrumental variation, rather than chemical or physical effects which may affect liquid fluorescence standards.

**Some advantages of solid reference materials**

No significant photo chemical degradation

Easy to store

No chemicals to mix and dilute

No evaporation problems

Long term stability

Low overall costs

## 6BF Fluorescent Reference Materials Set

This consists of six polymer blocks containing seven fluorescent compounds whose spectra cover a broad spectral range with emission maxima from 330 to 582nm and excitation maxima from 290 to 562 nm. This wide spectral range enables the user to select a reference material with broadly similar spectral properties to those of the analyte, ensuring a reasonable spectral overlap and measurable signals without changing important parameters such as slit widths and wavlength settings.

Each block is produced with optically polished surfaces on six sides, with dimensions of 12.5 x 12.5 x 45mm to fit the standard cell holders used in spectrofluorimeters. Starna is able to produce these materials in other sizes and shapes such as rods, cylinders and discs to fit other instruments and will quote for such requirements. Certain other materials can also be produced in similar form for specific studies such as phosphorescence, or singlet and triplet life time measurements and enquiries are welcome.

## Fluorescence Spectra

Fluorescence spectra are not absolute in the sense that absorption spectra are and the appearance of the spectra will depend upon the particular instrument and the mode used to record it. Most modern instruments have a choice of operating mode and this needs to be selected according to the analytical requirement.

Direct measurement of fluorescent intensity produces very distorted spectra. The effects of the varying intensity of the source, which falls off considerably with decreasing wavelength, the sensitivity of the detector which normally has a maximum in the near UV region and the light throughput of the monochromators which generally have a maximum in the UV region and whose efficiency falls off rapidly at shorter wavelengths and more gradually at longer wavelengths, are not compensated for. The advantage of this mode is that as the minimum number of optical components are used and no signal processing is required, it can produce the highest sensitivity for quantitative analysis, providing that the spectra are simple and the bands of interest are well resolved.

The *Ratio Mode* allows the instrument to monitor the intensity of the excitation source by means of a reference photomultiplier and presents the output in terms of a ratio to this reference signal, thus freeing the recorded spectrum of effects due to the varying energy of the source. This produces a good signal to noise ratio and therefore high sensitivity, as well as monitoring and correcting for drift. For quantitative analysis, it is usually the mode of choice, particularly for samples which produce more complex spectra.

The *Corrected Spectra Mode* produces a spectrum in which many of the above effects are compensated for, usually by spectral manipulation with a computer, although some older instruments may still use electo-optical methods to achieve the adjustments. This mode produces an excitation spectrum which has similar characteristics to that of the UV-Visible absorption spectrum. The sensitivity of the fluorescence spectra is much higher than that of the absorption spectrum, but direct comparisons can still be made, as the shapes are similar. It is also possible to make quantum yield measurements and energy yield calculations from the corrected spectra. This feature may be ofassistance if the instrument is being used to assist with the identification of the analyte, as these properties are known for a large number of substances. This mode also permits the comparison of data from different instruments which should show similar wavelengths for excitation maxima and minima, as well as similar values for ratios of the intensity of different bands and similar shapes for the spectral bands. Such spectra are particularly useful for publication and inter-laboratory studies, as uncorrected spectra, even those run on similar types of instrument, will include the various distortions introduced by the particular apparatus.

## Wavelength Calibration

The spectra of the materials provided in this set of reference materials are well established and their maxima and minima may be used to check the wavelength calibration of instruments which operate with narrow spectral band widths. Materials which produce narrow bands, such as anthracene and ovalene are particularly suitable for this application. However, it is important to ensure that such calibrations are carried out in the corrected mode at small spectral bandwidths, or errors can be introduced by peak broadening or apparent wavelength shifts due to instrumental effects.

The illustrated spectra were obtained using a Perkin Elmer LS50B spectrofluorimeter and the spectral band widths 2.5nm for all spectra. These spectra are for guidance only and will vary in shape if different spectral band widths are used. The peak wave lengths are only comparable for instruments operating in the corrected spectra mode. If this mode is not available, it is important that all instrument settings are reproduced exactly on each occasion, in order to ensure that subsequent comparisons are valid.

## Selectivity

Molecular Fluorescence Spectrometry is a more selective technique than UV-Visible Spectrophotometry. Material No.1 contains a mixture of Anthracene and Naphthalene and may be used to demonstrate the greater selectivity of the fluorescence technique. By exciting at two different wave lengths, the emission spectrum of either substance can be obtained free of interference from the other.

## The Fluorescent Materials

| Sample Number | Compound | Approximate Molar Conc. | Excitation Wavelength | Emission Wavelength |
|---|---|---|---|---|
| 1 | Anthracene | $1 \times 10^{-5}$ | 360 | 402 |
| | Napthalene | $6 \times 10^{-5}$ | 290 | 330 |
| 2 | Ovalene | $2 \times 10^{-7}$ | 342 | 482 |
| 3 | *p* -Terphenyl | $5 \times 10^{-7}$ | 295 | 338 |
| 4 | Tetraphenylbutadiene | $3 \times 10^{-7}$ | 348 | 422 |
| 5 | Compound 610 | $1 \times 10^{-6}$ | 440 | 475 |
| 6 | Rhodamine | $2 \times 10^{-7}$ | 562 | 573 |

### Care of the Reference Materials

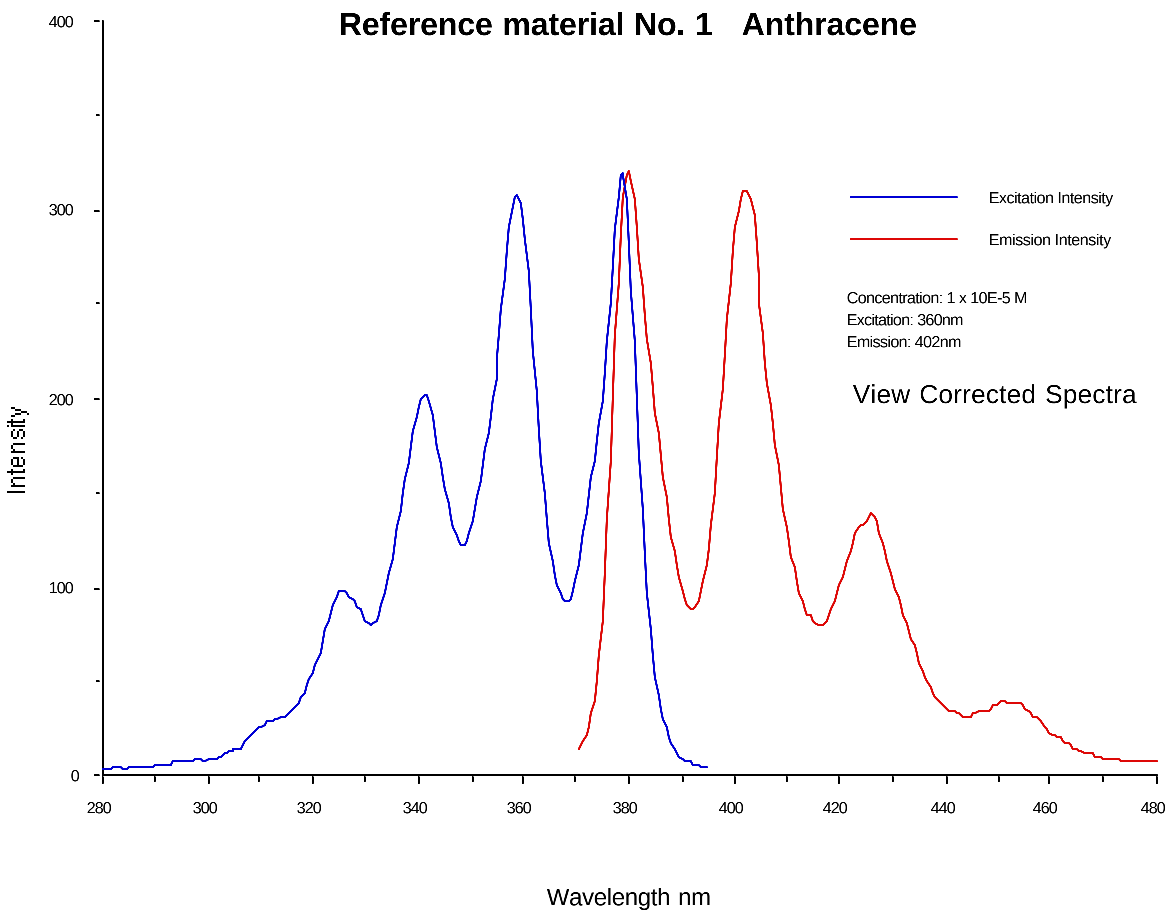
The blocks are supplied in a velvet lined case to protect the fine surface finish produced at manufacture. Providing that they are handled with care (cotton gloves are recommended) and always stored in the case provided, they should give many years of satisfactory service. Exposure to organic solvents can cause severe fogging of the surface and must be avoided, as should any attempt to clean the block with abrasive cleaners.

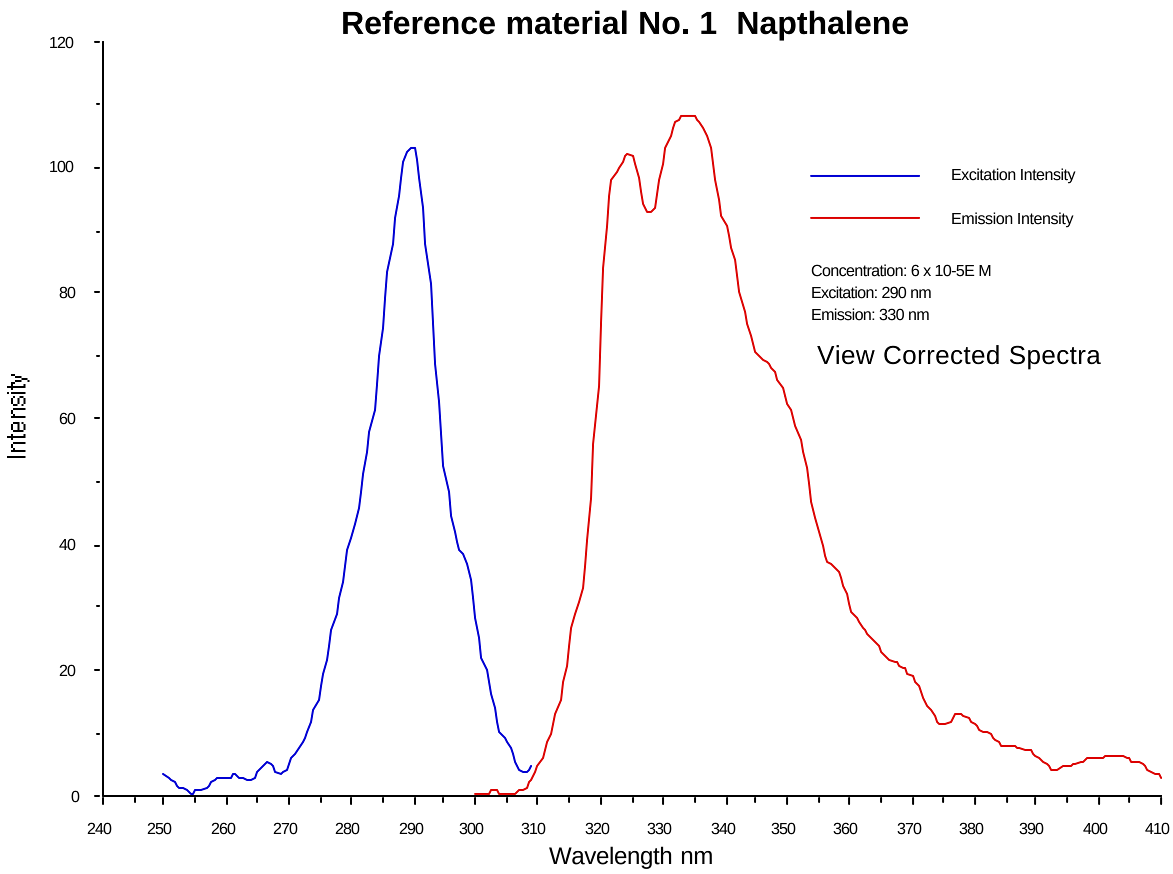
In the event of the surface of a block becoming soiled, it should be immersed in dilute neutral detergent solution which may be warmed to 40°. Low power ultrasonic vibration may also be used to remove the contamination. It should then be rinsed thoroughly with deionized water and dried carefully with a soft lint-free cloth.

### Recorded Spectra

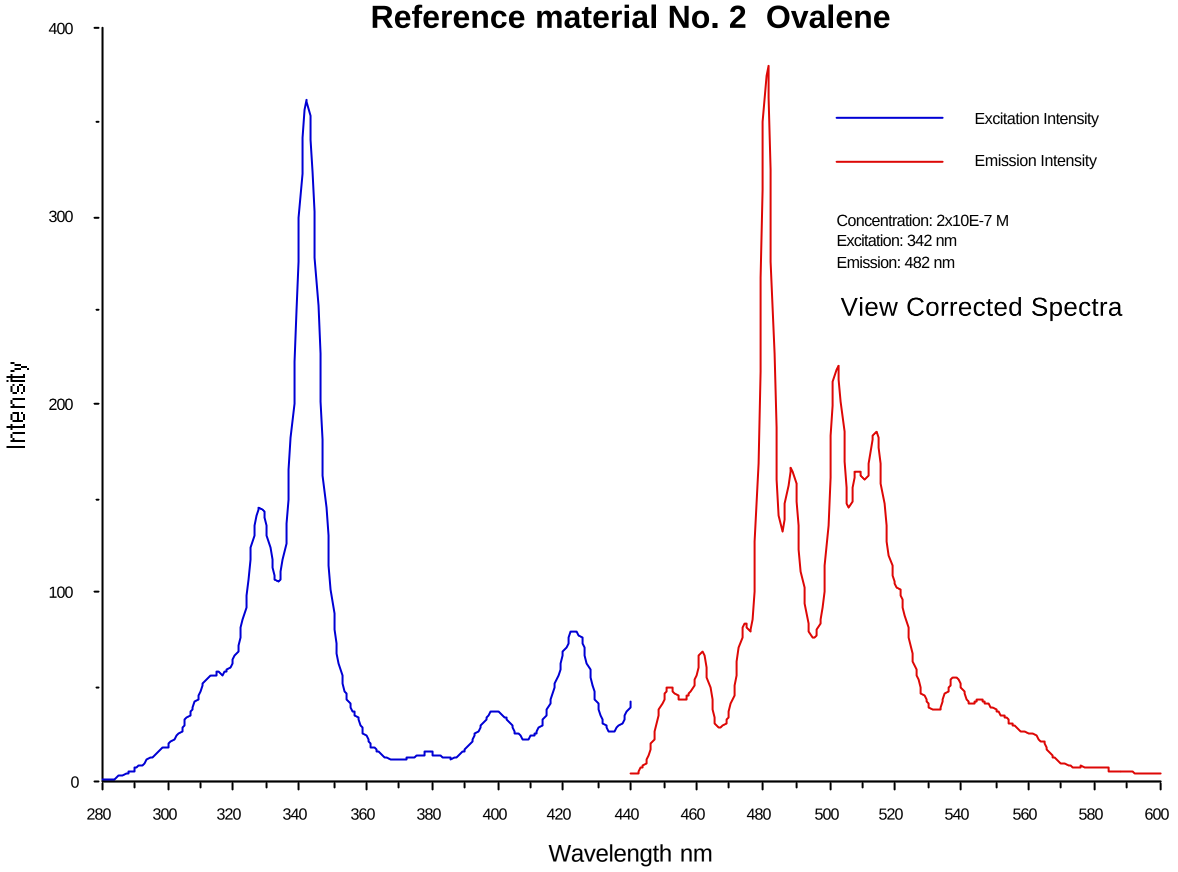
The following spectra were recorded in the ratio mode, using a Perkin Elmer LS50B Luminescence Spectrometer. The spectral band width for both excitation and emission monochromators, was 2.5 nm. Data points were recorded at 0.5 nm intervals. These spectra are specific to this instrument and the operating conditions used. Other instruments, or different operating conditions will produce spectra with maxima which may vary both in terms of relative magnitude and wavelength.

The corrected Spectra were recorded using a Perkin Elmer MPF44-B and should relate to others recorded in a similar manner.

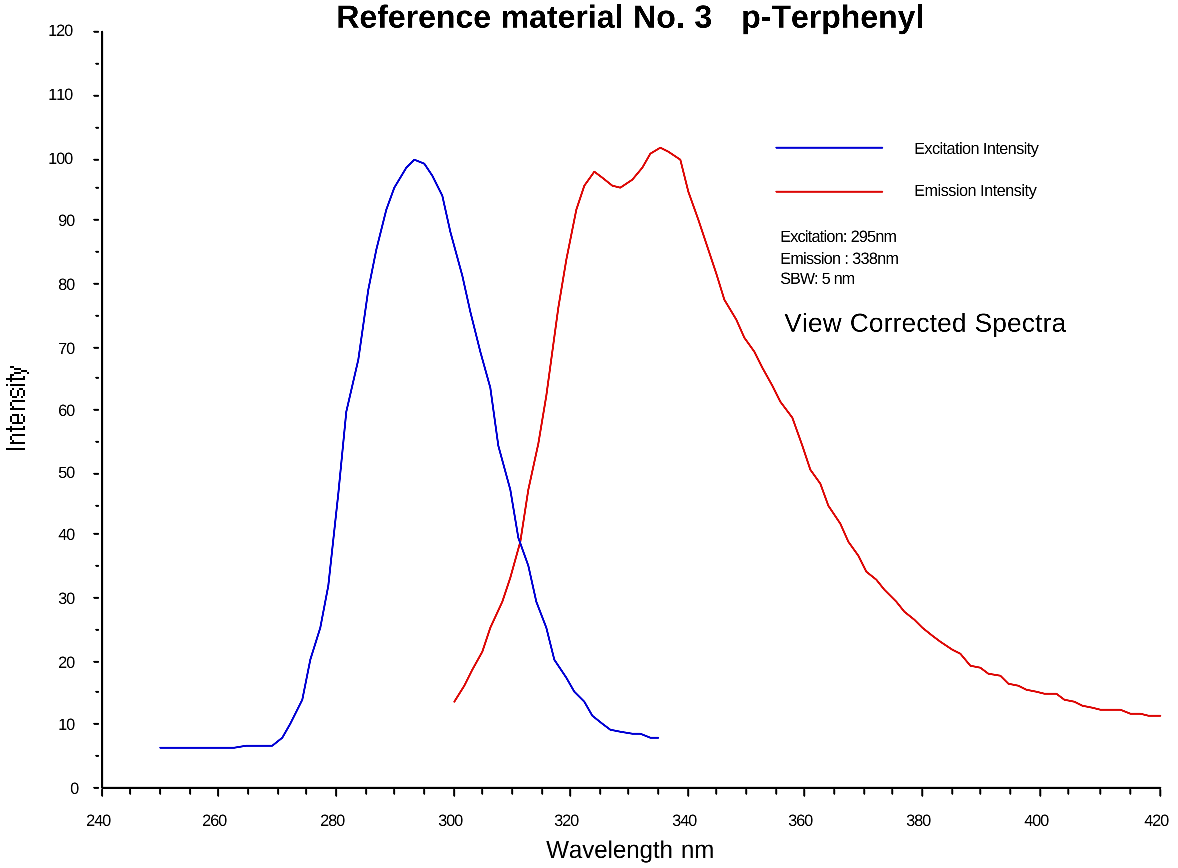

Reference material No. 1 Anthracene
Intensity
400
300
200
100
0
280
300
320
340
360
380
400
420
440
460
480
Wavelength nm
Excitation Intensity
Emission Intensity
Concentration: 1 x 10E-5 M
Excitation: 360nm
Emission: 402nm
View Corrected Spectra

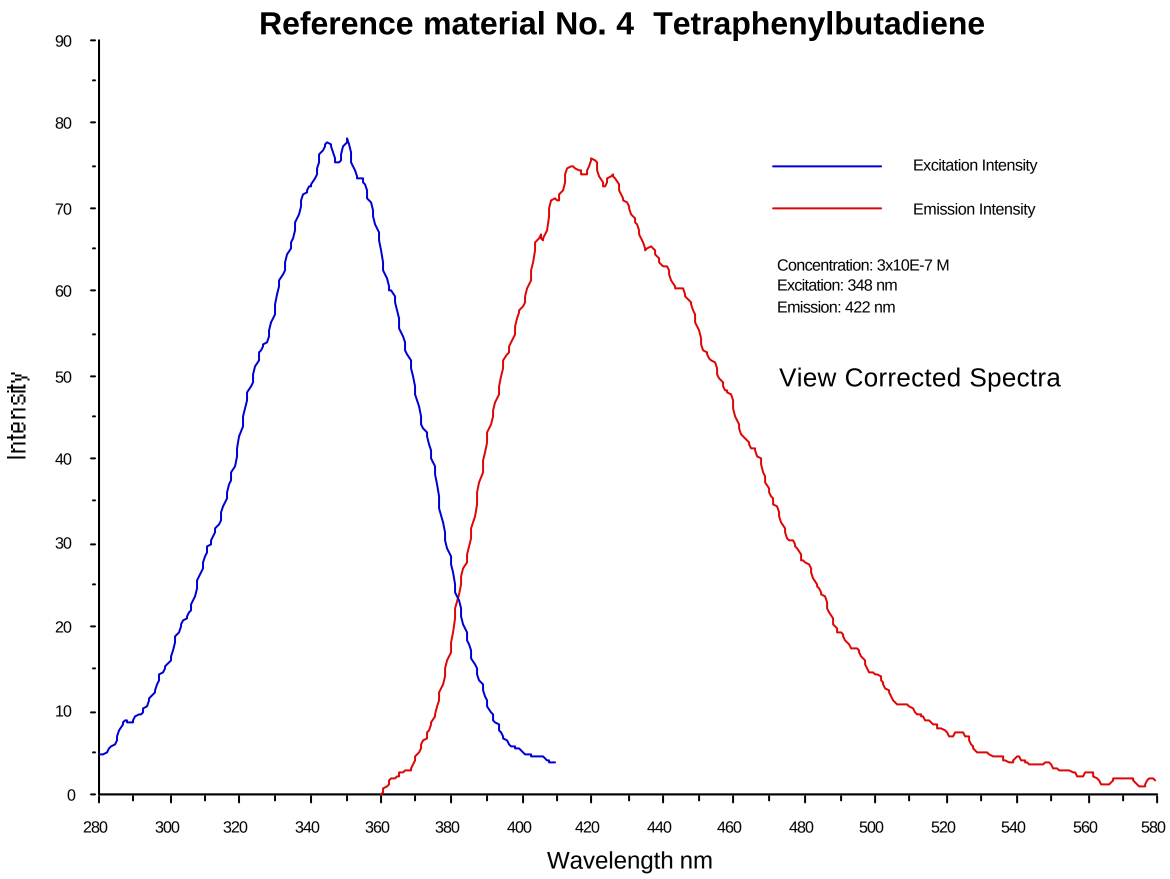

Reference material No. 1 Napthalene
Intensity
120
100
80
60
40
20
0
240
250
260
270
280
290
300
310
320
330
340
350
360
370
380
390
400
410
Wavelength nm
Excitation Intensity
Emission Intensity
Concentration: 6 x 10-5E M
Excitation: 290 nm
Emission: 330 nm
View Corrected Spectra

## Reference material No. 2  Ovalene

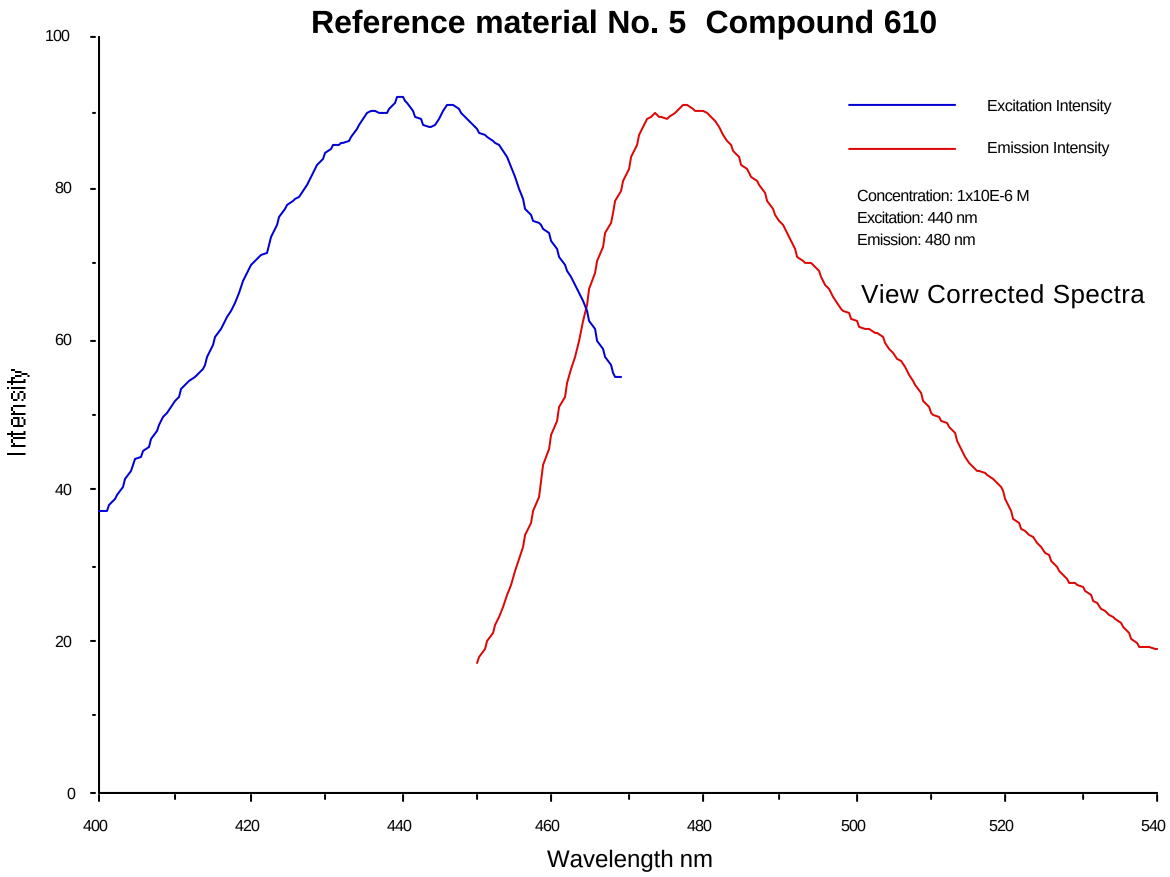


## Reference material No. 3   p-Terphenyl



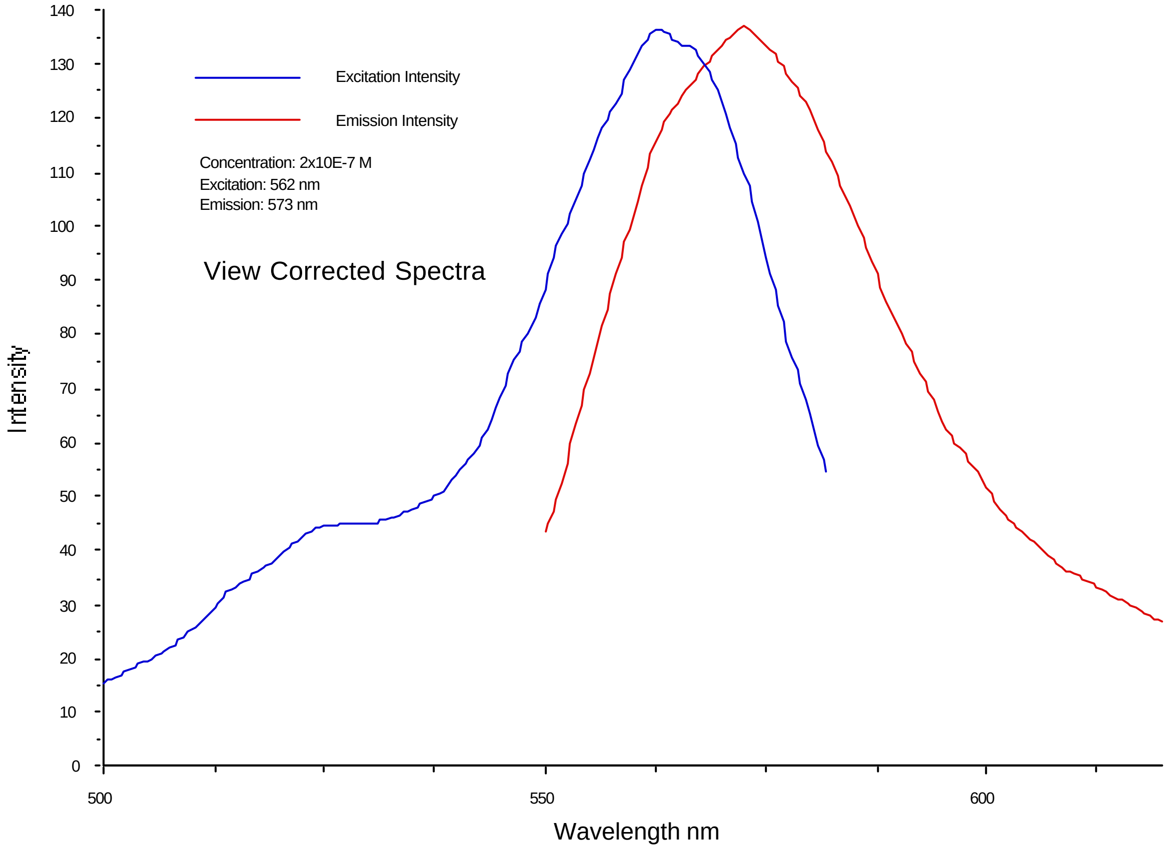


Reference material No. 4 Tetraphenylbutadiene

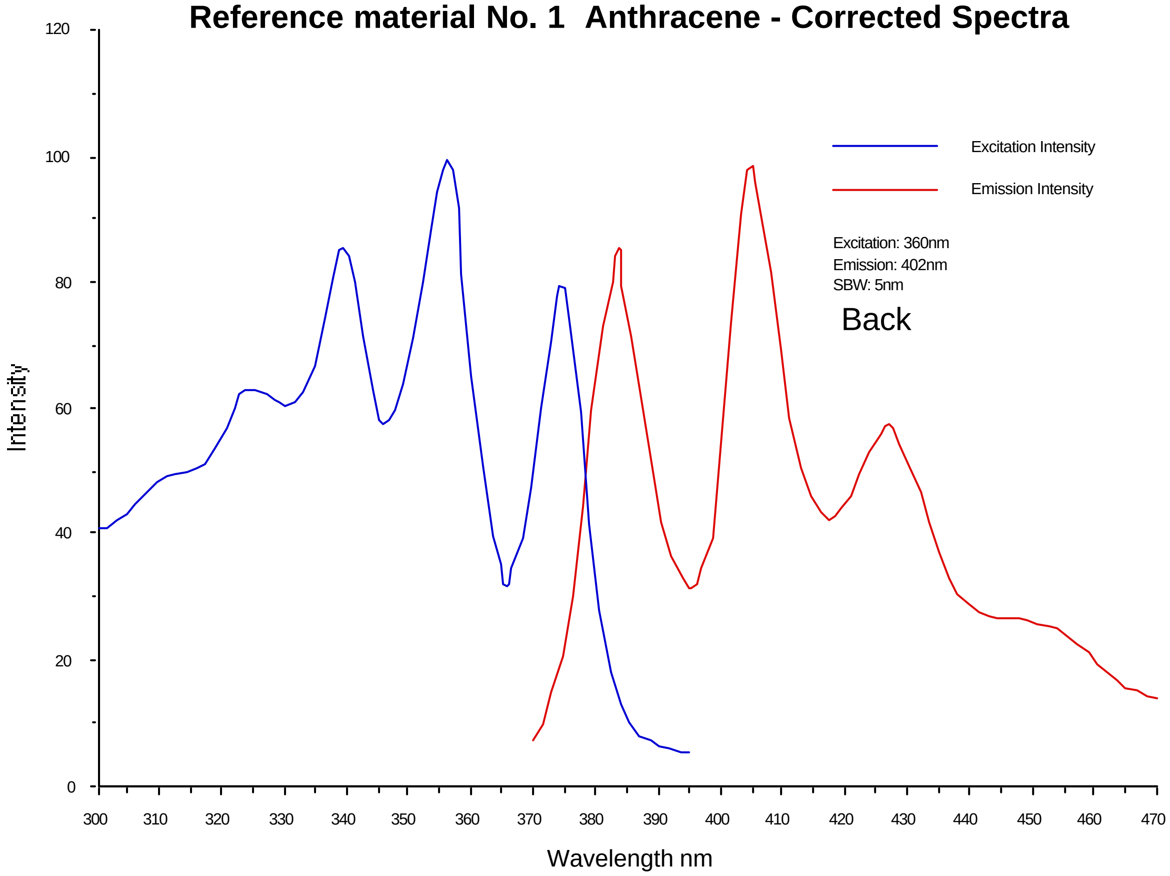


Reference material No. 5 Compound 610

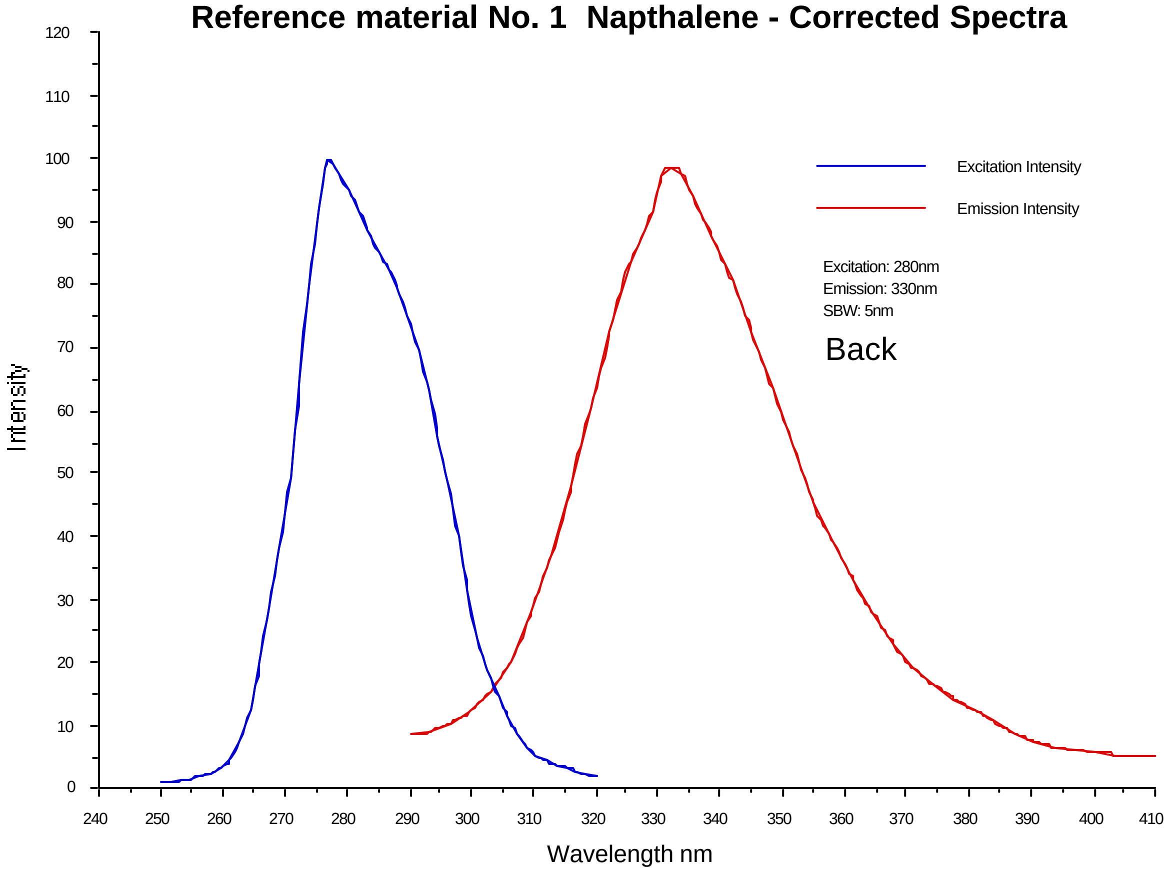
# Reference material No. 6  Rhodamine B

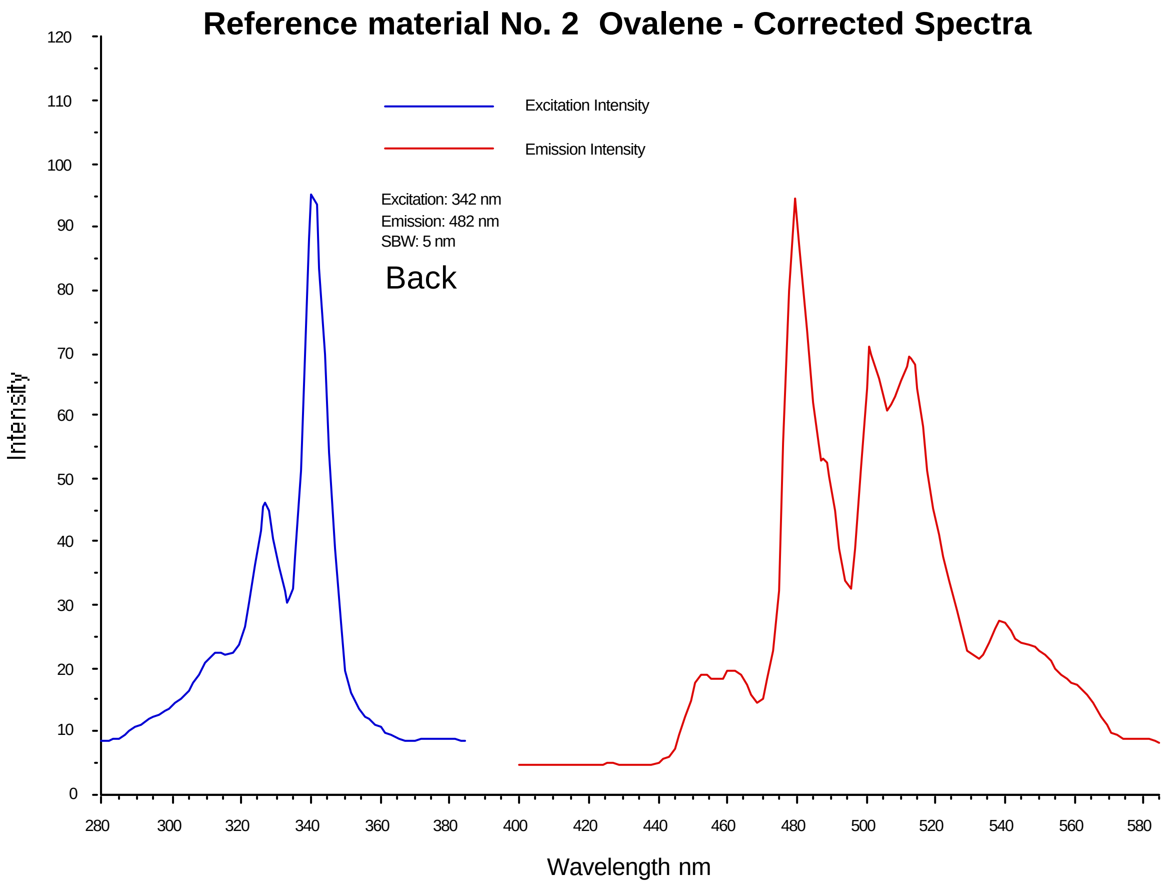


## Reference material No. 1  Anthracene - Corrected Spectra

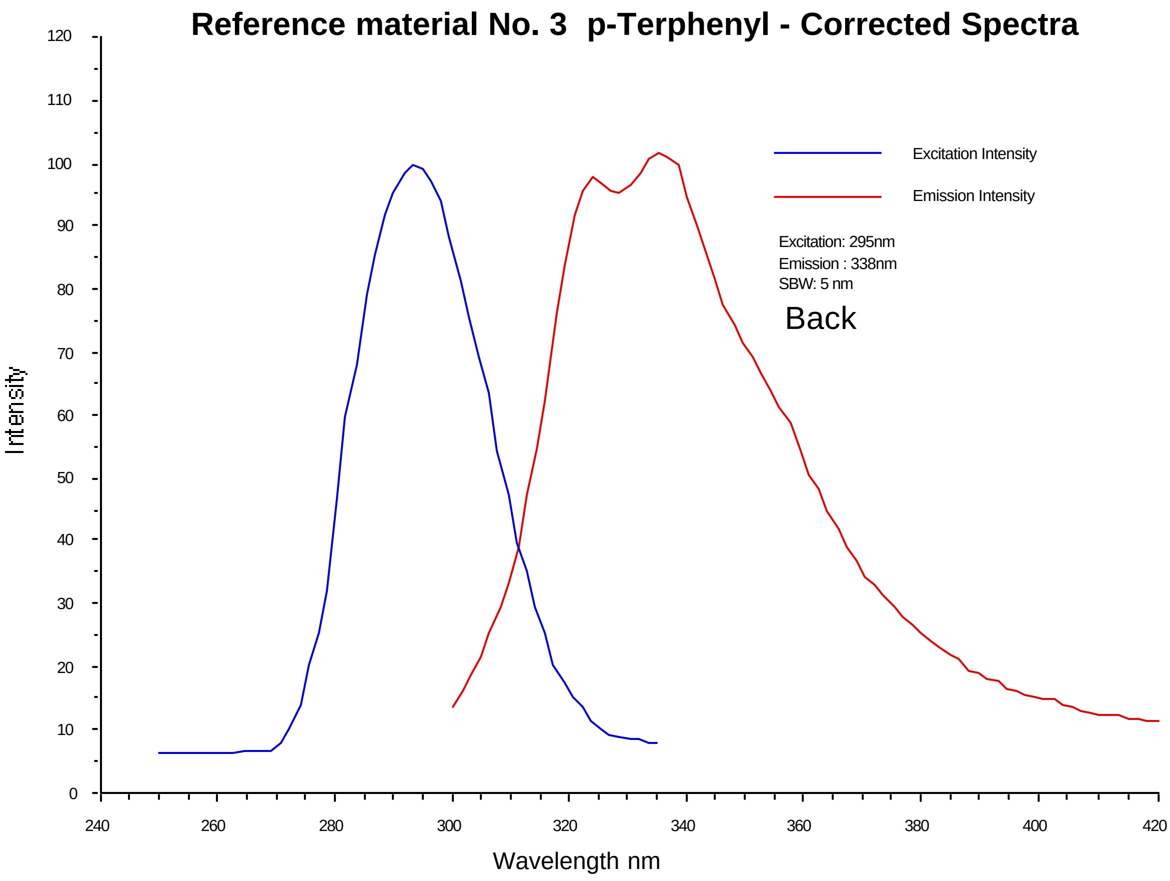


## Reference material No. 1  Napthalene - Corrected Spectra



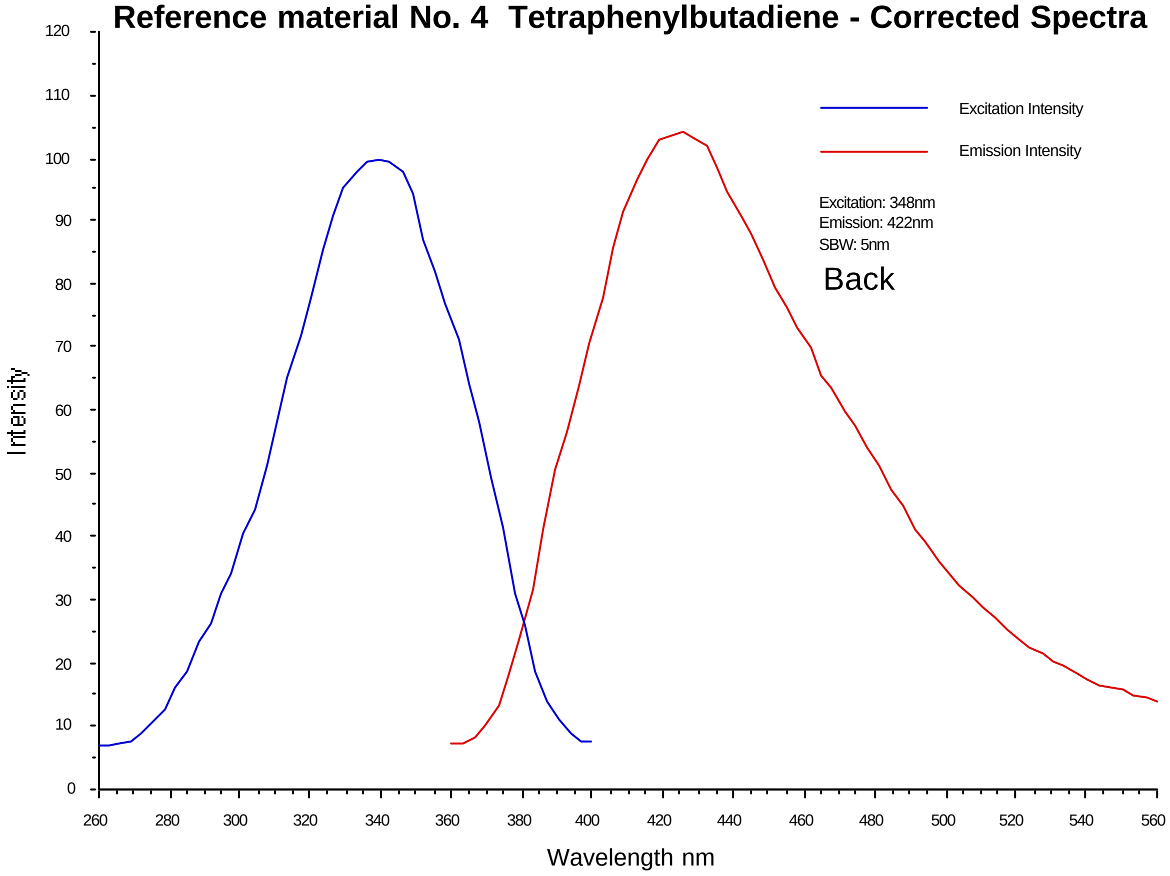


Reference material No. 2  Ovalene - Corrected Spectra

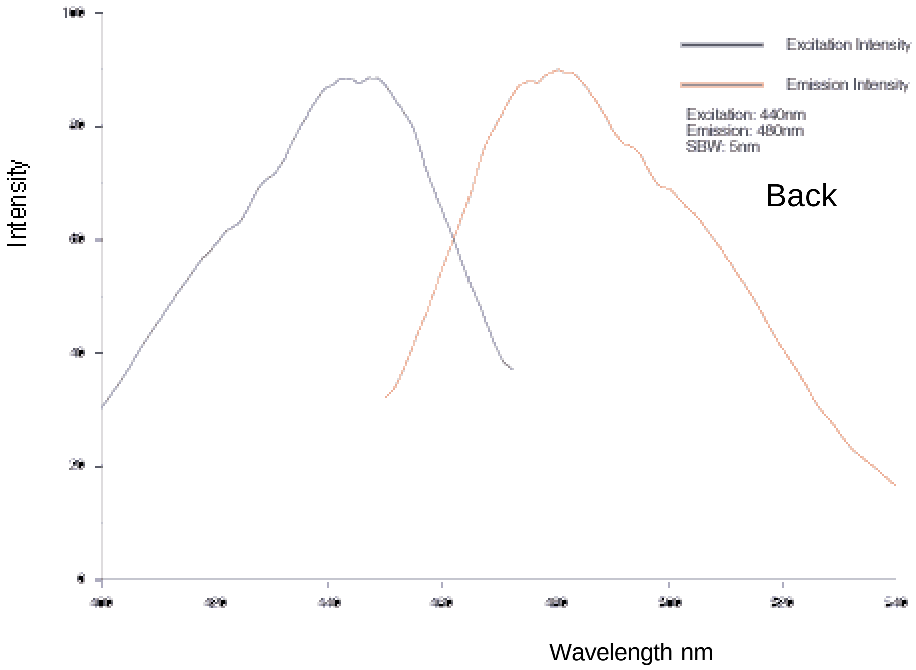


Reference material No. 3  p-Terphenyl - Corrected Spectra

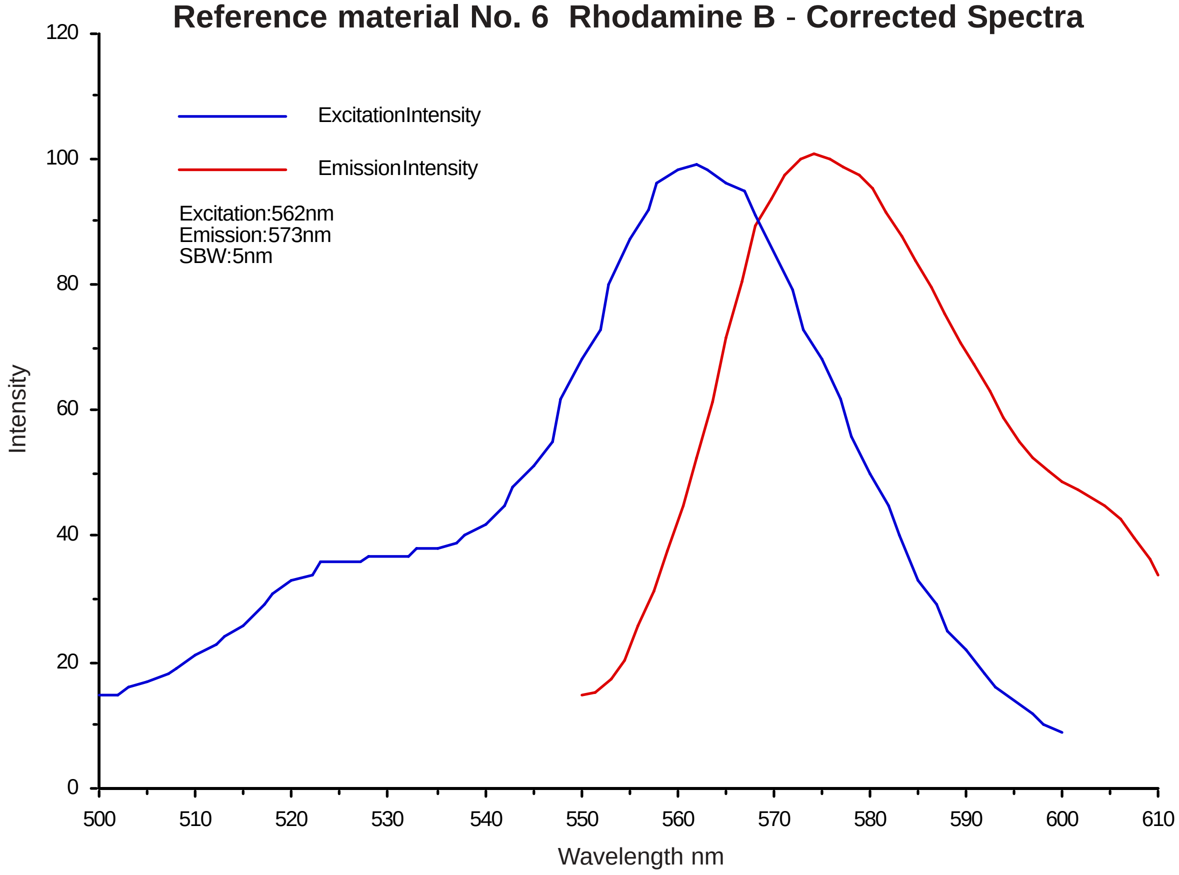
## Reference material No. 4  Tetraphenylbutadiene - Corrected Spectra



## Reference material No. 5  Compound 610 - Corrected Spectra



**Reference material No. 6 Rhodamine B - Corrected Spectra**



# Order information for Fluorescent reference materials

## 6BF Set of six reference materials for Fluorescent spectrophotometry

Polymethylmethacrylate blocks containing seven materials with excitation maxima between 290 and 562nm emission between 330 and 573nm, packaged in a velvet lined case.

## Starna Scientific Ltd.

52-54 Fowler Road
Hainault Industrial Estate
Hainault
Essex IG6 3UT
England

*Starna products order Hot line:*
Tel : (44) 020 8501 5550
Fax : (44) 020 8501 1118

*Technical enquiries:*
Tel : (44) 020 8500 1264
Fax : (44) 020 8500 1955

## Starna Worldwide

**Starna Cells Inc.**
PO Box 1919
Atascadero
Ca 93423 USA
Tel : (800) 228 4482
(805) 466 8855
Fax : (805) 461 1575

**Starna Pty. Ltd.**
PO Box 6751
Baulkham Hills BC
NSW 2153
Australia

Tel: +61 (2) 9659 8088
Fax: +61 (2) 9659 8511
email: info@starna.com.au

**Starna GmbH**
Postfach 1206
D-64319 Pfungstadt
Germany
Tel : (06157) 2813
Fax : (06157) 85564

**Starna GmbH. Wien**
Oberneuberg 78
A-8225 Pollauberg
Austria
Tel : (02243) 85 009
Fax : (02243) 85 019