

FLUORESCENCE APPLICATIONS



CORRECTION OF EMISSION SPECTRA USING THE PERKINELMER MODEL LS-50 LUMINESCENCE SPECTROMETER

Spectra collected on luminescence spectrometers consist of true spectral information superimposed on instrumental artifacts such as grating reflectivity and photomultiplier response characteristics (1). It is very important to remove these artifacts to allow comparison of excitation spectra to absorbance spectra, to compare fluorescence spectra to examples quoted in the literature, and to obtain accurate quantum yield measurements (2,3). The excitation light path of the LS-50 is split to produce a ratioed optical system, which is coupled with automatic correction against a rhodamine 101 spectrum to remove excitation artifacts.

Excitation spectra collected using the LS-50 are thus automatically corrected so that they are superimposable on absorption spectra (4).

Emission spectra are not corrected automatically because the emission characteristics of the instrument depend on hardware options, for example the selection of the standard or red sensitive photomultiplier.

Two correction methods are described herein, to allow correction using either photomultiplier.

CORRECTION OF EMISSION SPECTRA COLLECTED USING THE STANDARD PHOTOMULTIPLIER

ABSTRACT

Correction of emission spectra over the spectral range 280-630 nm is carried out by comparison of an emission spectrum of quinine sulphate collected using the LS-50 to a National Bureau of Standards (NBS) standard spectrum of quinine sulphate.

The correction factor spectrum produced by this division represents the sum of the instrumental emission artifacts and is used to correct spectra recorded subsequently.

INTRODUCTION

A software routine is included in the LS-50 software to automatically collect spectral data and produce the correction factor spectrum. The routine is split into three parts:

1. Generation of the emission response over the range 280-400 nm using a diffuser plate
2. Collection of a quinine sulphate spectrum over the range 400-630 nm
3. Collection of the background spectrum for quinine sulphate

Since the emission spectrum of quinine sulphate shows little response below 400 nm, the first part of the routine is carried out by synchronously scanning both monochromators from 280-400 nm with a delta wavelength of 0 nm using a diffuser plate to scatter light through the instrument. Since the excitation light path is automatically corrected over this region, then the observed spectrum is due solely to emission artifacts and thus represents the emission correction factor spectrum over this range.

Above 400 nm, the correction factor spectrum is obtained by arithmetic division of an observed, background subtracted spectrum of quinine sulphate by a standard spectrum of quinine sulphate. The diffuser and quinine sulphate spectra are merged to produce one correction factor spectrum over the range 280 - 630 nm.

MATERIALS

Quinine sulphate and perchloric acid (A.C.S. reagent) were obtained from BDH Limited, Poole, UK. Triple deionized water was used throughout the experiment. 0.1 N perchloric acid was prepared by addition of 12 mL of reagent to 188 mL of triple deionized water.

Quinine sulphate stock was prepared by dissolving 2.3 mg of reagent in 10 mL 0.1 N perchloric acid. Working quinine sulphate reagent was prepared by dilution of stock to give an optical density at 350 nm of 0.05 in 0.1 N perchloric acid. Note that the maximum optical density should be 0.06 absorbance units and that the overriding criterion is that the concentration should be such that the fluorescence intensity of the sample should be between 200 and 500 using the following conditions:

10 mm pathlength cuvette

Excitation and emission wavelengths 350 nm and 450 nm

Slits Ex 5 nm Em 5 nm

The concentration of quinine sulphate used for the generation of the correction factor spectrum will depend on the sensitivity of the LS-50 being used, although a suitable concentration of quinine sulphate should be found within the range 1.0×10^{-8} M to 1.0×10^{-6} M. Diffuser plate and attenuators were used as supplied in the LS-50 shipping kit.

METHOD

The emission correction routine was initiated by clicking on the instrument menu item followed by the emission correction option. The prompts produced by the routine were followed, using the following methods:

Diffuser scan 280 - 400 nm

1. Two attenuators, 1 % T and 2% T, were inserted into the emission filter bracket inside the sample area. In addition to these two attenuators, the attenuator on the emission filter wheel was automatically inserted into the beam.
2. The diffuser plate was inserted into the cuvette holder.
3. On clicking OK, a synchronous scan was automatically performed over the spectral range 280-400 nm, slits Ex 5 nm, Em 20 nm, scan speed 1500 nm/min, delta wavelength 0.
4. On completion of the scan, the diffuser plate was removed, followed by the external and built-in attenuators.

Note that this sequence of removal is critical: if the attenuators are removed first, then intense light may be scattered directly through the instrument causing permanent damage to the emission photomultiplier.

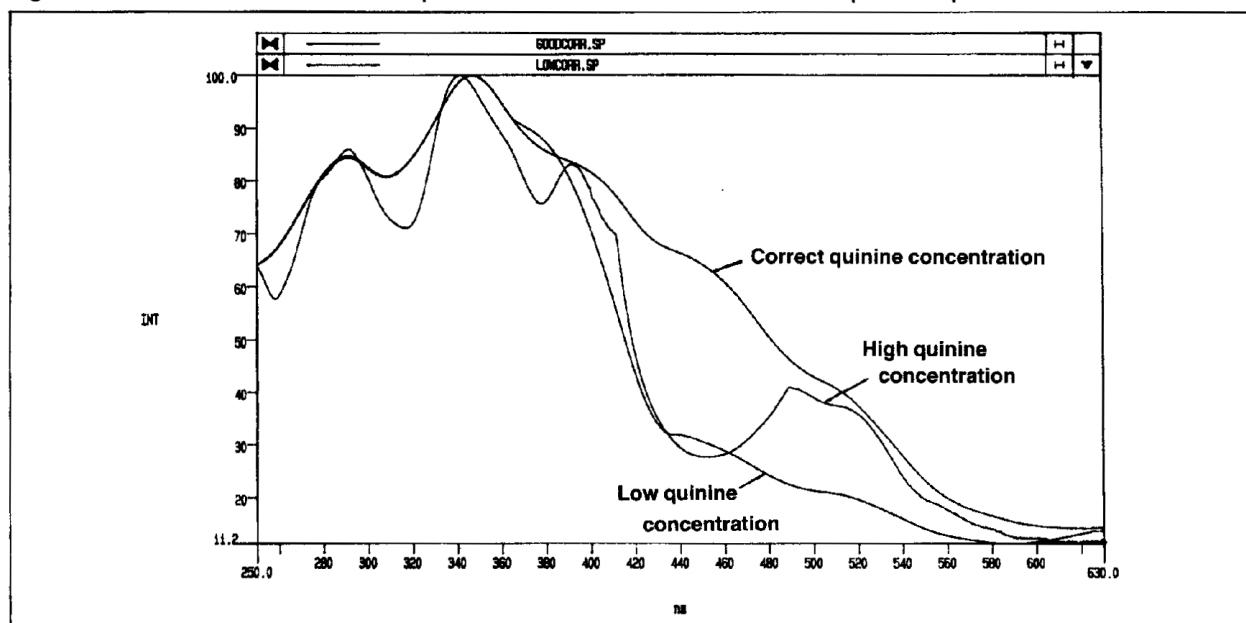
Background subtracted quinine sulphate scan

1. A quinine sulphate solution of concentration 1.0×10^{-6} M was inserted into a 10 mm pathlength cuvette located in the instrument cell holder. On clicking OK, an emission spectrum was collected over the wavelength range 400-630 nm using an excitation wavelength of 350 nm, slits Ex 5 nm, Em 5 nm, scan speed 120 nm/min.
2. On completion of the scan the quinine sulphate solution was replaced by a 0.1 N perchloric acid blank. The cuvette was rinsed thoroughly with 0.1 N perchloric acid to minimize carry over of quinine sulphate.
3. On clicking OK, the emission spectrum for this blank was collected under identical conditions as were used for the collection of the quinine sulphate spectrum.

On completion of the final scan, the routine automatically subtracts the blank spectrum from the quinine sulphate spectrum, then arithmetically divides this background subtracted spectrum by the standard spectral data (stored on the hard disk under the filename QDATA.DA). The resulting correction factor spectrum is then rescaled to merge with the spectrum obtained from the diffuser scan and the final correction factor spectrum for the wavelength range 280-630 nm stored on the hard disk as LS50000A.SP.

NOTE: This spectrum must be smoothed with an 8-point factor and saved on the hard disk. A typical smoothed correction factor spectrum generated by the LS-50 is shown in Figure 1(a). Note also that the quinine sulphate concentration is critical in the generation of an acceptable correction factor spectrum. Figures 1(b) and 1(c) show the effect on the correction factor spectrum of excessively low and high quinine sulphate concentrations respectively.

Figure 1: The emission correction factor spectrum for the LS-50 fitted with a standard photomultiplier



RESULTS

Correction of the spectrum is carried out by arithmetic division of the sample spectrum by the correction factor spectrum.

The accuracy of the correction routine was checked by correction of an emission spectrum of quinine sulphate collected for a 1.0×10^{-8} sample. The corrected emission spectrum for this sample was superimposed on the NBS standard spectrum (Figure 2).

Figure 3 shows the uncorrected and corrected fluorescence emission spectra of ovalene superimposed on the correction factor spectrum. Figure 4 shows a similar plot for terbium.

Figure 3 shows the uncorrected and corrected fluorescence emission spectra of ovalene superimposed on the correction factor spectrum. Figure 4 shows a similar plot for terbium.

CONCLUSION

The emission response of the PerkinElmer Model LS-50 luminescence spectrometer can be automatically corrected over the spectral range 280-630 nm. Corrected emission spectra produced are free from instrumental artifacts and are compatible with corrected emission spectra from other sources.

Figure 2: Corrected fluorescence emission spectrum and NBS standard spectrum of quinine sulphate

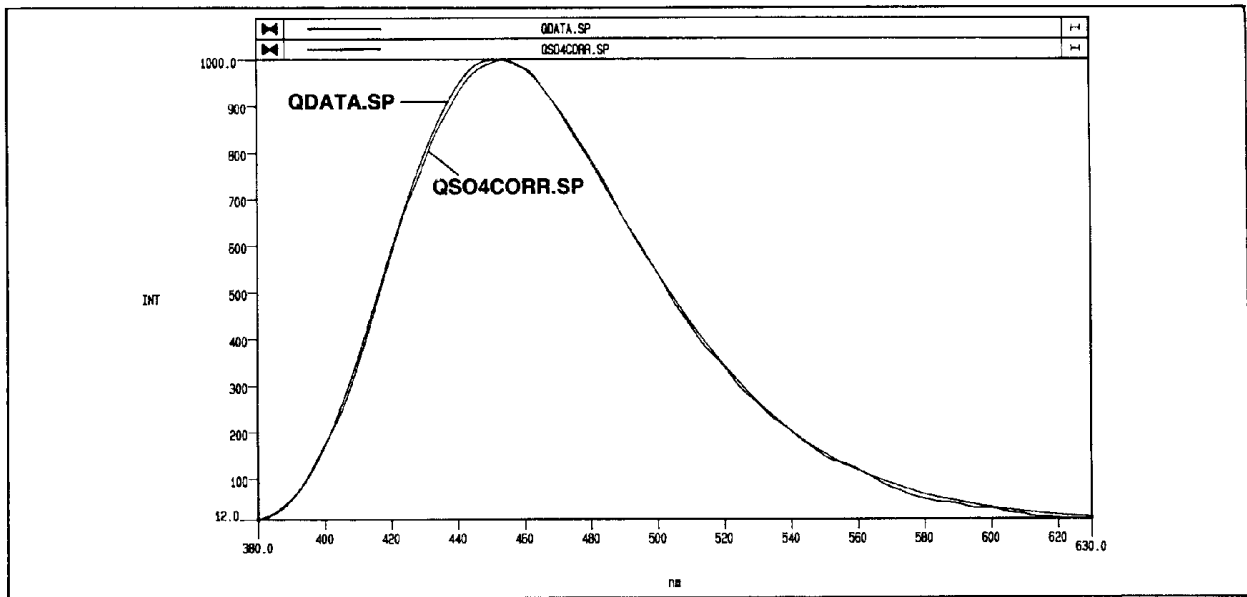
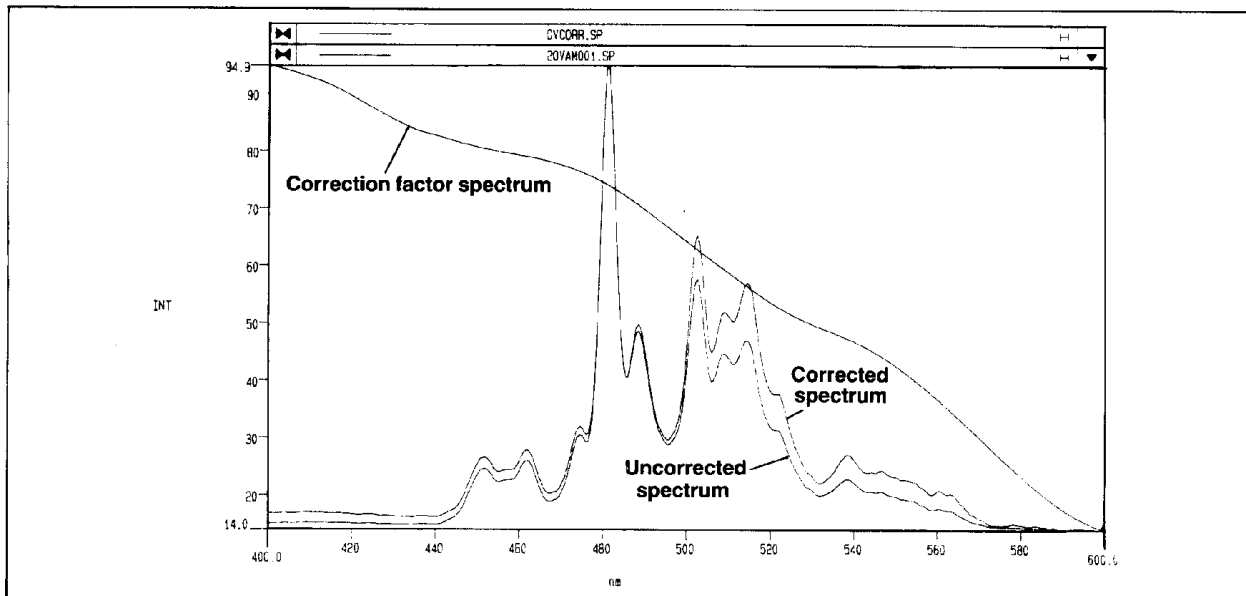


Figure 3: Uncorrected and corrected fluorescence emission spectra of ovalene



CORRECTION OF EMISSION SPECTRA COLLECTED USING THE RED SENSITIVE R928 PHOTOMULTIPLIER

ABSTRACT

For many applications the spectral response of the standard photomultiplier (i.e. 200 - 650 nm) fitted in the LS-50 is adequate. For a small number of specific applications, however, emission spectral information is required well above this range, for example porphyrins, chlorophylls and long wavelength laser dye analyses. For these applications the red sensitive R928 photomultiplier (Part No. 5212-4966) is fitted, extending the emission range to approximately 900 nm. Clearly in this case the emission correction routine using quinine sulphate is inadequate, so an alternative emission correction routine is employed, whereby the observed spectral emission from a calibrated tungsten lamp is compared to NBS standard data for the lamp emission.

INTRODUCTION

One of the many novel features of the LS-50 is the high degree of control which may be applied to the integration gating and source pulsing. Combination of these two features allows the user to measure light from a source external to the instrument's optical system, examples including bio- and chemi-luminescence, excitation using an external laser and in this case the emission from an external light source. Since the spectral emission characteristics of the tungsten lamp are known, then the difference between the observed and standard spectral data represents the emission correction factor spectrum over the emission range of the tungsten lamp.

As with the quinine sulphate method, the emission correction spectrum is generated by arithmetic division of the observed spectrum by the standard spectrum, emission spectra are corrected subsequently by arithmetic division of the spectrum by the correction factor spectrum.

MATERIALS

Calibrated tungsten source, together with power supply and calibration data, was obtained from Hitachi, Part No. 018-0620.

Note that the process of digitizing and interpolating the standard NBS data has been carried out at PerkinElmer Limited, and the calibrated tungsten lamp standard spectrum can be obtained on floppy disk on demand.

Attenuators were used as supplied with the LS-50 shipping kit.

METHOD

The standard photomultiplier was replaced with the red sensitive 8928 photomultiplier. The built-in attenuator was inserted into the emission beam using the emission options page. Two attenuators (1 % T and 2% T) were manually inserted into the emission filter bracket inside the sample area.

Integration mode was set via the mode page of the Data Manager: the source was switched off and the mode set to phosphorescence with parameters cycle time 200, delay time 0, gate time 180, all units in milliseconds. The tungsten lamp was temporarily fitted into the sample area so that light from the source passed into the emission monochromator of the LS-50.

Note that it is very important to fit the automatic and manual attenuators before switching on the tungsten lamp - failure to do this could result in light from the lamp passing directly to the emission photomultiplier resulting in permanent damage.

A black cloth was used to cover the sample area to eliminate ingress of stray light from the laboratory.

Lamp spectral output data was recorded by scanning the emission monochromator over the range 400-900 nm, slit width 10 nm, scan speed 120 nm/min

RESULTS

Figure 5 shows the observed and standard spectra for the calibrated tungsten lamp over the range 450-900 nm. The correction spectrum was generated by arithmetic division of the observed lamp spectrum by the standard spectrum.

The uncorrected and corrected emission spectra for coproporphyrin in 0.1 N hydrochloric acid, together with the correction spectrum, are shown in Figure 6.

Figure 5: Observed and standard emission spectra of a calibrated tungsten lamp

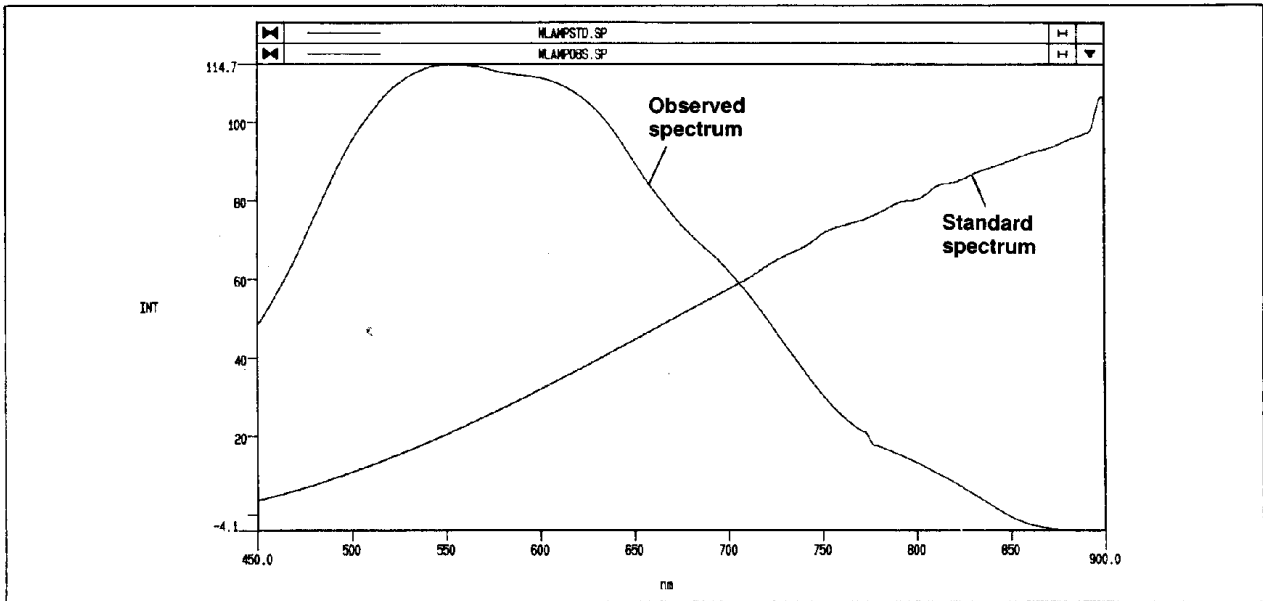
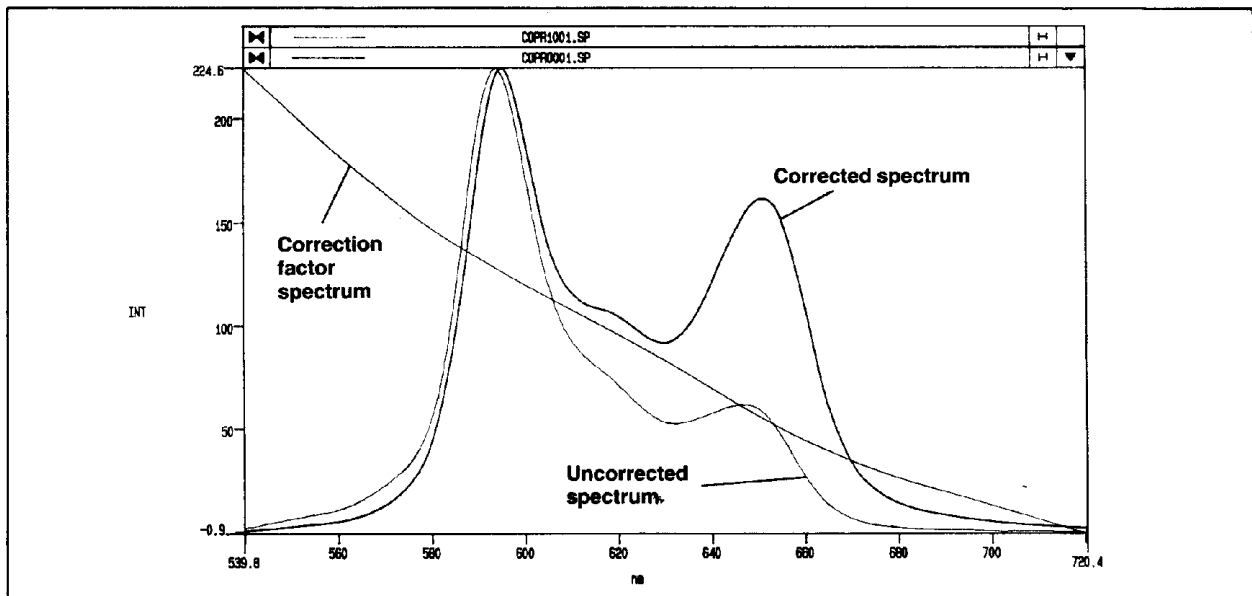


Figure 6: Uncorrected and corrected emission spectra of coproporphyrin collected with the R928 photomultiplier



CONCLUSION

The emission response of the LS-50 fitted with the red sensitive R928 photomultiplier can be corrected using a calibrated tungsten source.

REFERENCES

1. Parker, C.A., Photoluminescence of Solutions. Elsevier, New York (1968).
2. Demas, J.N., & Crosby, G.A., J. Phys. Chem. **75**, 99 (1971).
3. Melhuish, W.H., J. Res, Nat. Bur. Stand. **76A**, 547 (1972).
4. Lakowicz, J.R., Principles of Fluorescence Spectroscopy, Plenum, New York, pp 24-25, 39-40.



PerkinElmer Instruments:

761 Main Avenue, Norwalk, CT 06859-0243, U.S.A.

Tel: 1-800-762-4000 or 1-203-762-4000 Fax: 1-203-761-2882

Internet: <http://www.perkinelmer.com>

PerkinElmer Ltd:

Post Office Lane, Beaconsfield, Bucks, HP9 1QA, England

Tel: 44-1494-676161 Fax: 44-1494-679331/3

Copyright © 2000 PerkinElmer, Inc.

PerkinElmer is a registered trademark of PerkinElmer, Inc.