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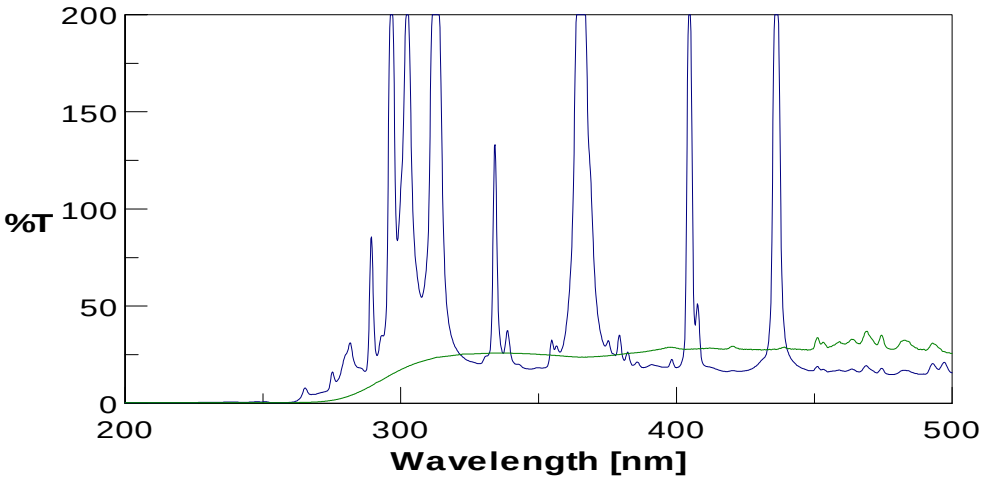
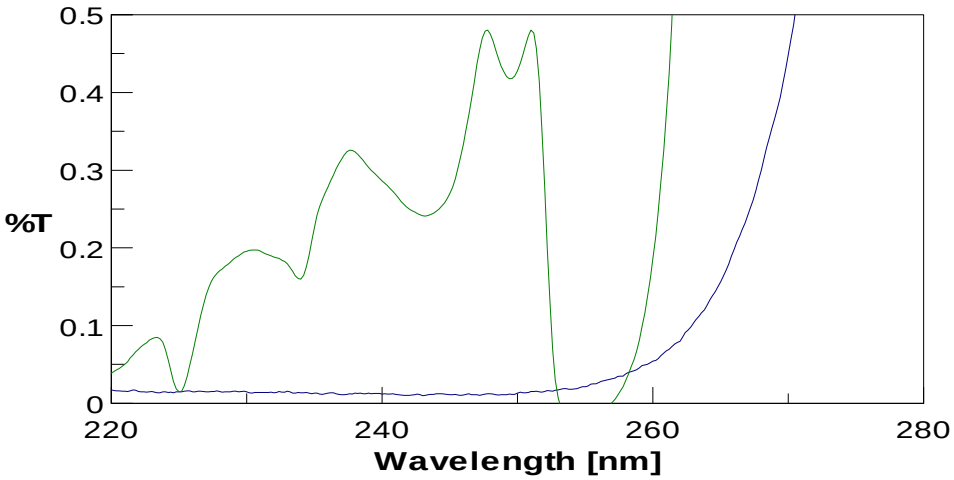
USE OF CD SPECTROMETER AS EXCITATION SOURCE FOR FLUORESCENCE

Today it's a common practice to use CD spectrometers as optical benches generating monochromatic light for fluorescence excitation, particularly when a stopped-flow device is fitted and users may be interested in stopped-flow fluorescence in addition to stopped-flow CD.
In a previous report (T.R. 62) we quickly evaluated the use of emission monochromator and compared sensitivities from a bench-top fluorometer. We used this time an early J-710 (converted into J-715) for the tests. First thing we checked was energy output at different wavelengths and bandpasses using a home made Si diode/preamplifier assembly and collecting the DC output.
Next figure shows the spectra obtained using standard Xe source (Osram XBO-150W/4) and Hg/Xe source (Hamamatsu L2482); both lamps are physically and electrically interchangeable. Bandpass used was 1nm in both runs.



As expected the Hg/Xe source has much stronger emission than the standard Xe one and it may be best choice for fluorescence applications.
Even expanding the UV range the benefit of Hg/Xe is clear (apart from 254nm are where the Hg emission line is fully absorbed).



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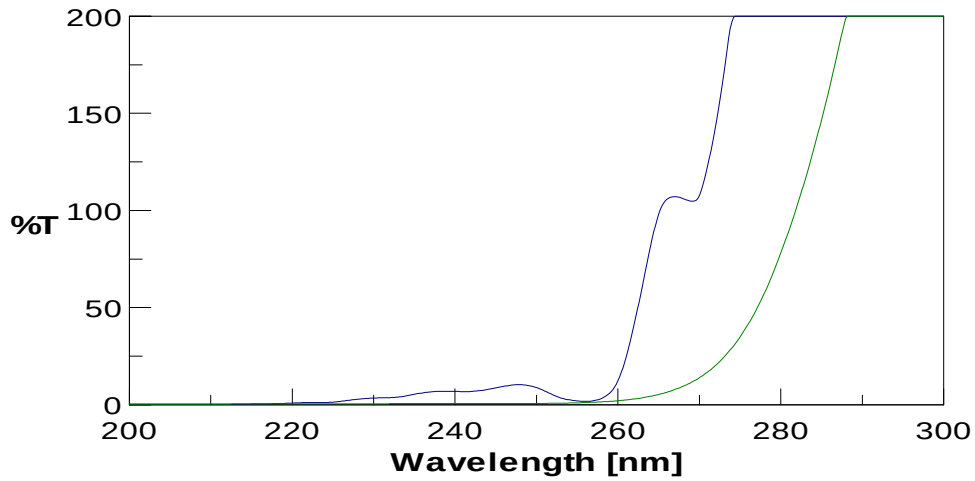
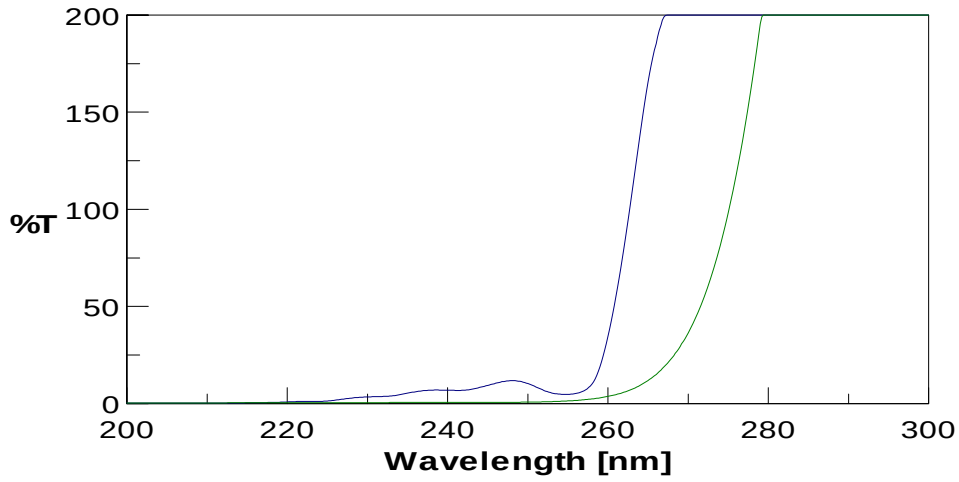
In fluorescence spectroscopy the excitation bandpass used is typically much larger than 1nm.
Here we report intensities with 5nm bandpass obtained with both sources:



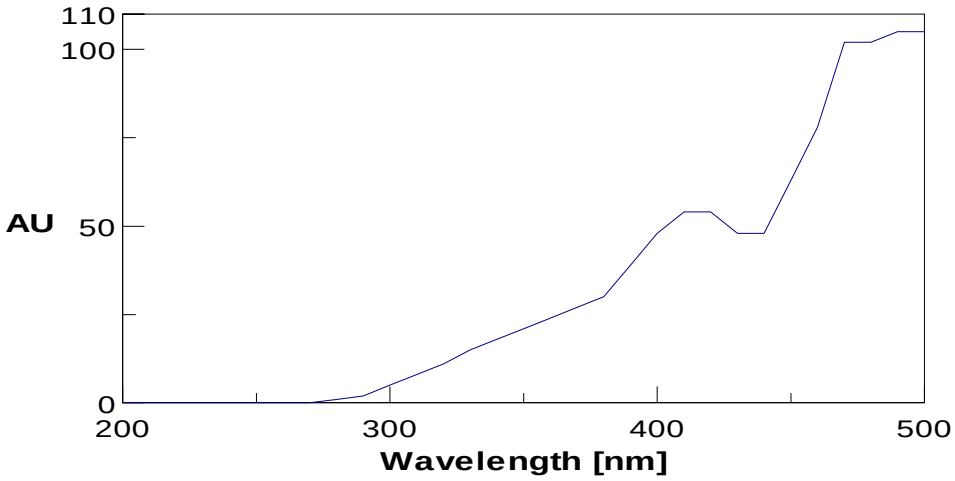
And with 10nm bandpass:



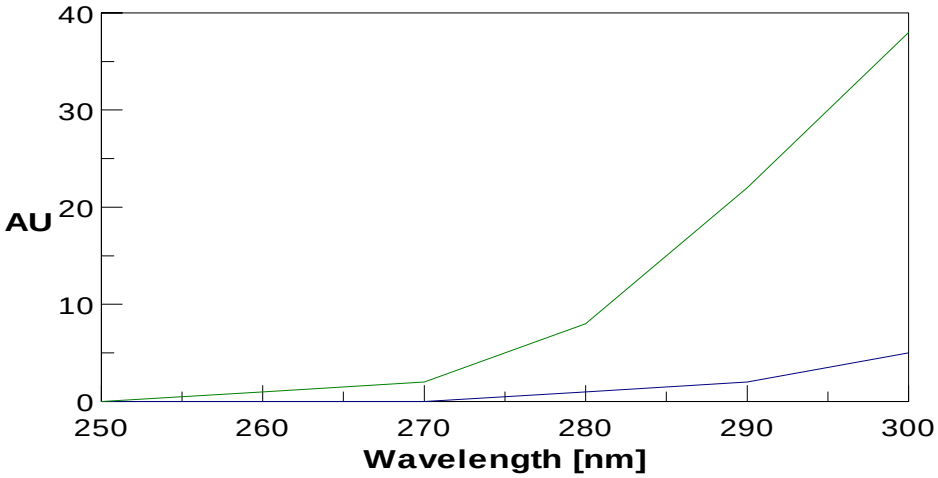
Very little can be gained in the UV since (see T.R. 17) slitwidth quickly reaches its maximum value (3mm) using large bandwidths. Actually with 10nm SBW slits full opening is reached at about 295nm, while with 5nm SBW this is reached at about 250nm.
So a conventional double prism monochromator CD spectrometer is somehow limited to relatively narrow bandpass in the UV range.

But how does it compare with a standard fluorescence spectrometer?
For comparison purpose we tested a Jasco FP-6200 (with 150W Xe lamp) equipped with a quartz optical fiber suitable to illuminate the cell of a stopped-flow apparatus.
We fed the optical fiber output into the sample compartment* of the J-715 and collected (manually) the DC output levels at different wavelengths.
** this coupling is far from ideal, sizeable light losses are present, but this would match situation when a fiber bundle will irradiate a stopped-flow cell*

First measurements have been carried on with the minimum bandpass available on this spectrometer (5nm):



Results is not so encouraging (vertical scale is always same despite what's written), also setting SBW to 20nm it's not so impressive, pls see results compared with the 5nm setting:



Clearly the loss caused by the quartz fiber optic light guide is a concern*, particularly in the low UV, so a directly interfaced CD spectrometer will outperform a much brighter spectrofluorometer when fibers are fitted.
** at least for the type used in this experiment*

The strong benefit of Hg/Xe source is however very evident and we'd strongly recommend this lamp whenever sensitivity is a concern.

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