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VALIDATION OF CD SPECTROMETERS

We are enclosing copy of the poster presented at CD 2001 8th International Conference on Circular Dichroism, Sendai (Japan) Sept 23-28, 2001.
It's not an easy subject, poster was arranged to raise the problem.

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CD IN EUROPEAN (AND BRITISH) PHARMACOPOEIA BENEFITS AND DRAWBACKS

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It is exactly of the same nature as the Hindu's view, that the world rested upon an elephant and the elephant rested upon a tortoise; and when they said, "How about the tortoise?" the Indian said, "Suppose we change the subject."

Bertrand Russell
Why I am not a Christian

Process of validation of scientific instruments dictated by FDA, Eur. Pharm. or other regulatory organizations call for the use of defined, *traceable*[1], standards.
The path ends up typically to Certified Reference Materials traceable or directly obtained from USA National Institute of Standard and Technology (NIST).

Circular Dichroism is now in European Pharmacopoeia, this will soon mean the need of accurate and traceable testing procedures.

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1 from the "International Vocabulary of Basic and General Terms in Metrology (ISO 1993) traceability is the property of the result of a measurement of the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties

Experimental

All measurements were carried in different dates using various Jasco J-700/800 spectrometers.

The following jigs (not commercially available) were used:

- a Si diode detector and related preamplifier to monitor, where convenient, but mainly above 800 nm where standard PM tube looses sensitivity, intensity of beam
- a low pressure Hg source (UVP CP series) with its own power supply and customized holder
- a Neon source (RS lamp) powered directly from mains line with dedicated holder

Also:

- glass filters used were not certified (but certified ones are available from NIST while other suppliers can provide traceable ones)

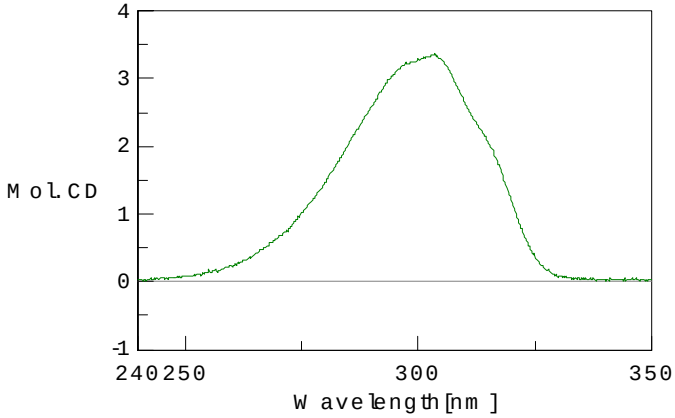
- same is true for the chemicals, but no CD certified standard is commercially available, only way to get traceability is to pass through polarimetric measurement with a validated polarimeter

Pharmacopoeia Calibration Procedures

Accuracy of absorbance scale

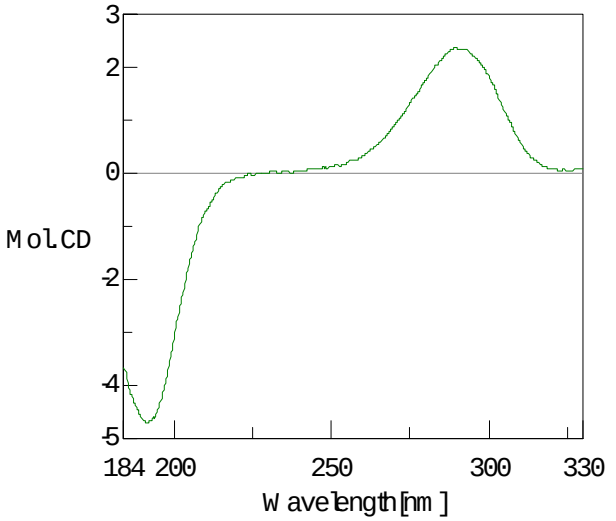
Dissolve 10.0 mg of *isoandrosterone* in *dioxan* and dilute to 10.0 ml with same solvent. Record the circular dichroism spectrum of the solution between 280 and 360 nm. Measured at the maximum of 304 nm Δε is 3.3

The solution of *(1S)-(+)-camphorsulfonic acid* may also be used



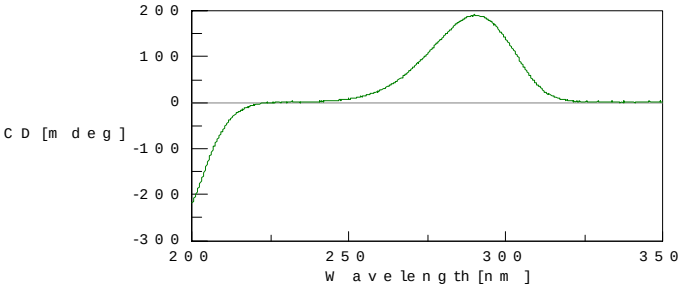
Linearity of modulation

Dissolve 10.0 mg of *(1S)-(+)-10 canphorsulphonic acid* in *water* and dilute to 10.0 ml with the same solvent. Determine the exact concentration of camphorsulphonic acid in the solution by ultraviolet spectrophotometry (2.2.25) (Appendix 2B), taking the specific absorbance to be 1.49 at 285 nm. Record the circular dichroism spectrum between 185 and 340 nm. Measured at the maximum at 290.5 nm, Δε is +2.2 to +2.5. Measured at the maximum at 192.5 nm, Δε is –4.3 to –5. *(1S)-(+)-* or antopodal *(1R)-(-) 10-camphorsulphonate* can also be used.

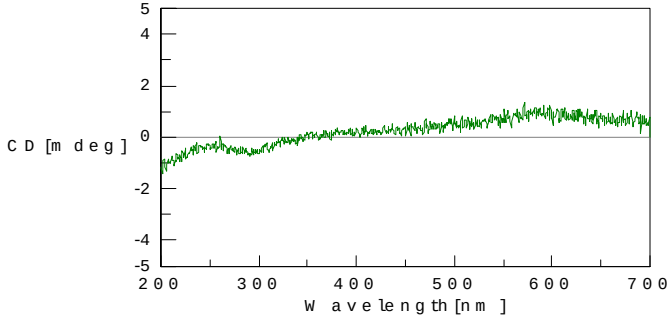


Manufacturer Validation Procedures

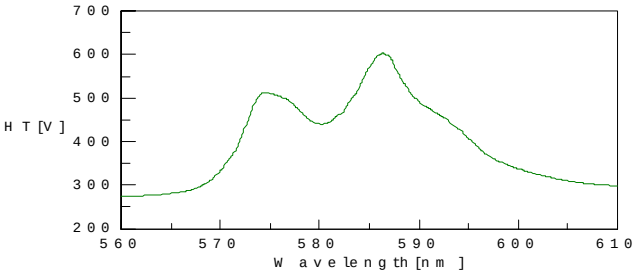
Photometric accuracy test
Fill a 10mm path cell with 0.06% aqueous solution of ammonium d-10-canphorsulfonate. Scan the range 350-200 nm, the peak value at about 290 nm should be of 190.4 +/-1 mdeg



Baseline flatness test
Scan an air baseline from 700 to 200 nm, the flatness should be within +/-5 mdeg



Wavelength accuracy test
It's performed scanning a *neodymium glass* filter between 610 and 560 nm. The absorption maximum on CH2 should be at 586 +/-0.8 nm



Wavelength repeatibility test
Baseline stability test
RMS noise test

Pharmacopoeia Calibration Procedures

Positive points:
- at last CD is listed as a method
- PEM linearity test at two wavelengths

Negative points:
- no indication about standards traceability
- no criteria to test wavelength accuracy
- wavelength range limited to the UV
- no method to test sensitivity
- no criteria for baseline flatness
- no stability check

Manufacturer Validation Procedures

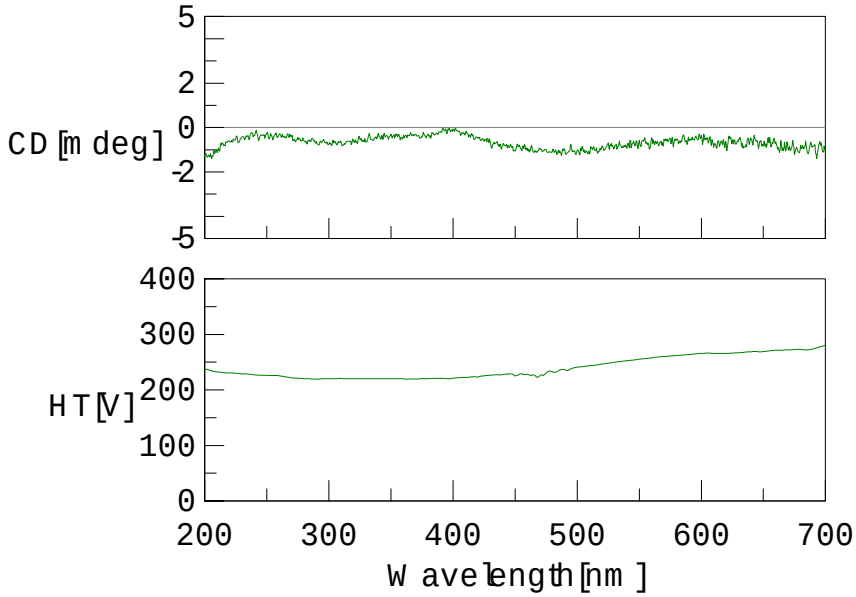
Positive points:
- well organized document to appeal FDA
- historical record of performances

Negative points:
- no traceability of standards
- single point scale check
- single point wavelength check
- no verification of PEM linearity

Proposed validation procedures

1 – baseline check

run a full baseline (800-190 nm) with no sample, but with the sampling accy you will use, at high scanning speed and fast response. Keep a record of CD baseline shape and HT profile. The latter is essential to verify proper monochromator alignment



2 – wavelength calibration check

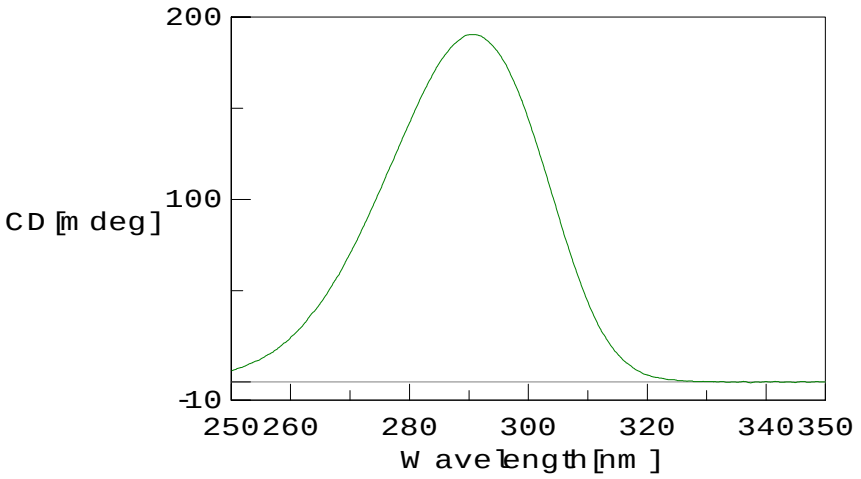
use neodymium glass for 586.5 nm band and holmium glass for 360.9 nm, to take at least two readings.

If data are not satisfactory or if you need traceable document use Hg source lines.

3 – CD scale calibration

Check CD calibration with 0.06% aqueous solution of ammonium-d-camphorsulfonate in a 10 mm cell.

If traceable results are necessary perform in advance polarimetric measurement of same solution with a validated polarimeter (i.e. certified with NIST sucrose or with traceable polarimetric plates). $[\alpha]_D^{25}$ +20.9°

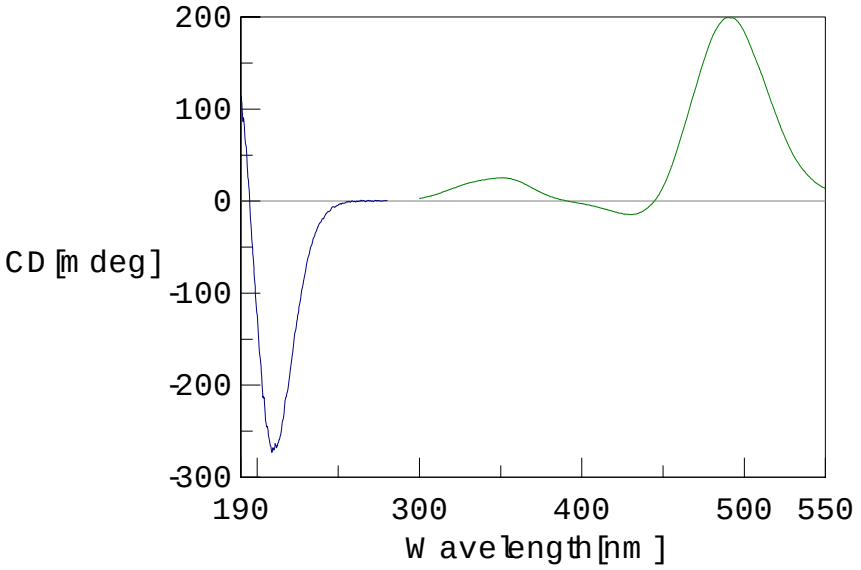


4 – PEM linearity of modulation

using $2(+)_D$-$[Co(en)_3]Cl_3$-NaCl-$6H_2O$ complex dissolved in water.

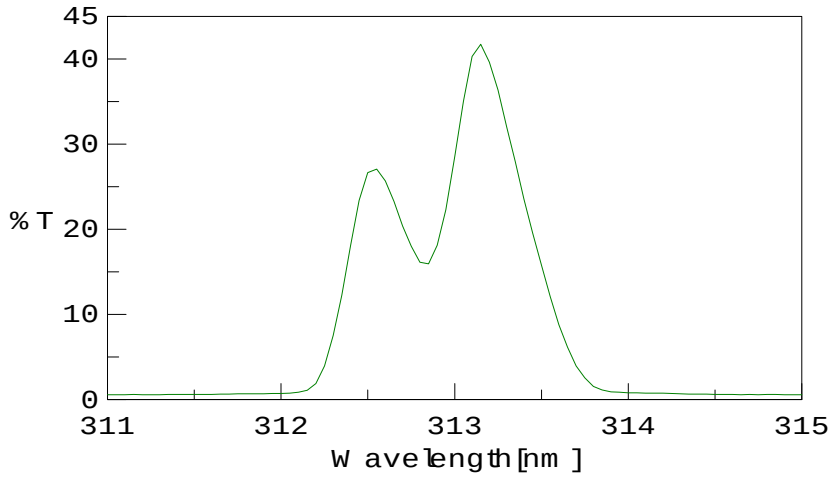
Two dilutions are necessary to get main bands in scale.

Check that band intensities at 490 and at 209 nm are maximized using manual PEM program



5 – Resolution

with low pressure Hg lamp check separation of 313.16 and 312.56 nm bands using narrow slits



6 – Noise

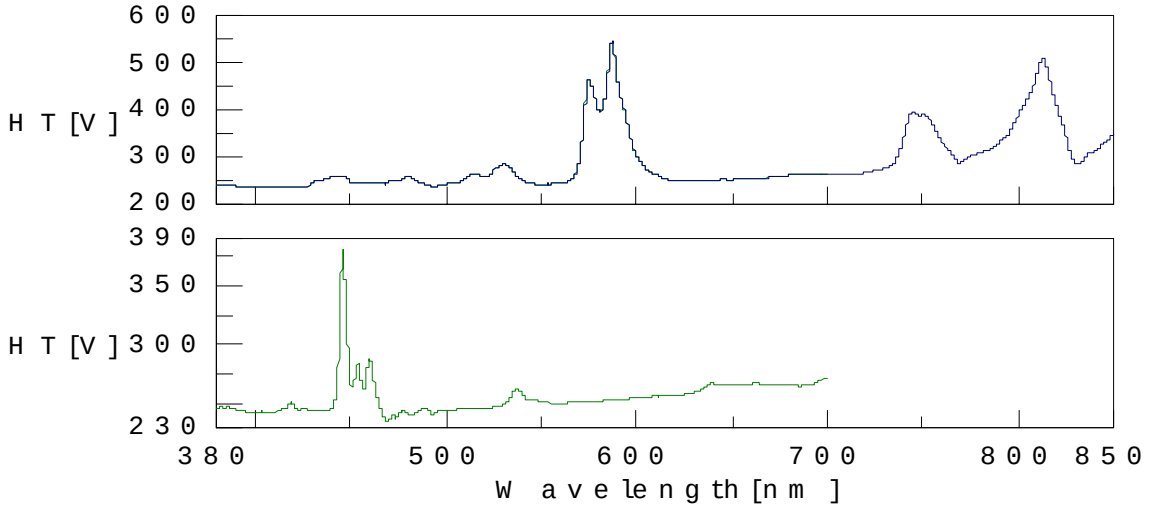
measure peak to peak or RMS noise with given bandpass and integration time at wavelengths of your interest

Glass Filters wavelength standards

Visible range:

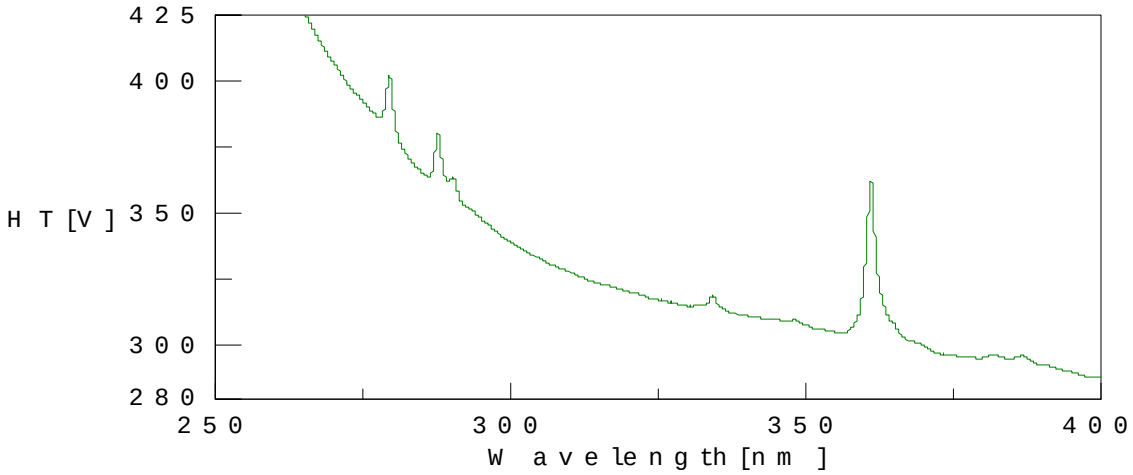
Didymium glass
807.5, **586.5**, 573.7 nm

Holmium glass
460.0, 453.2 nm



UV range:

Holmium glass
360.9, 287.5, 279.4 nm

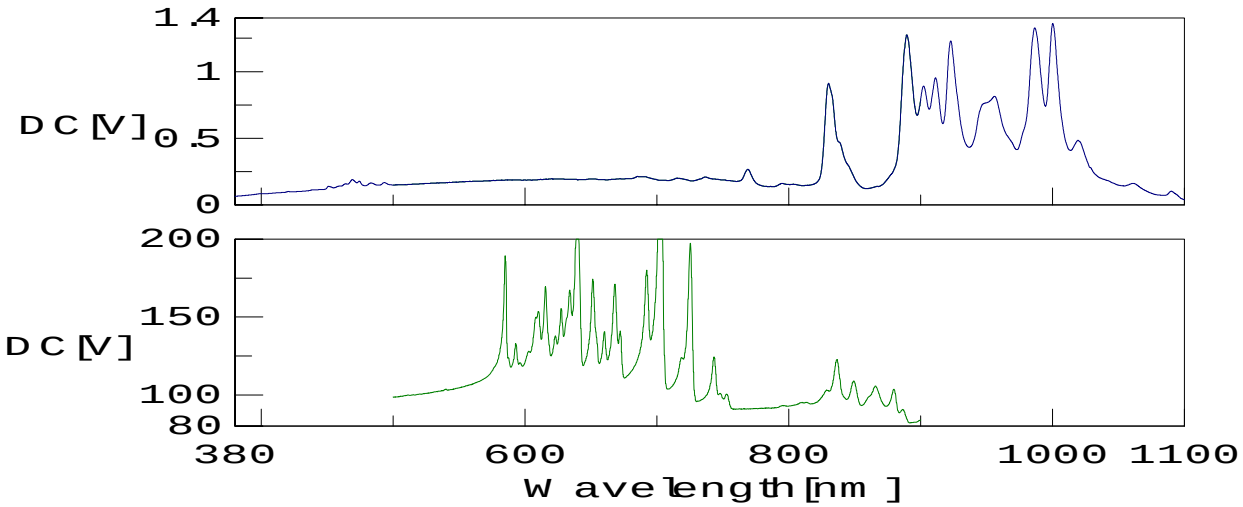


Gas Lines wavelength standards

VIS-NIR range:

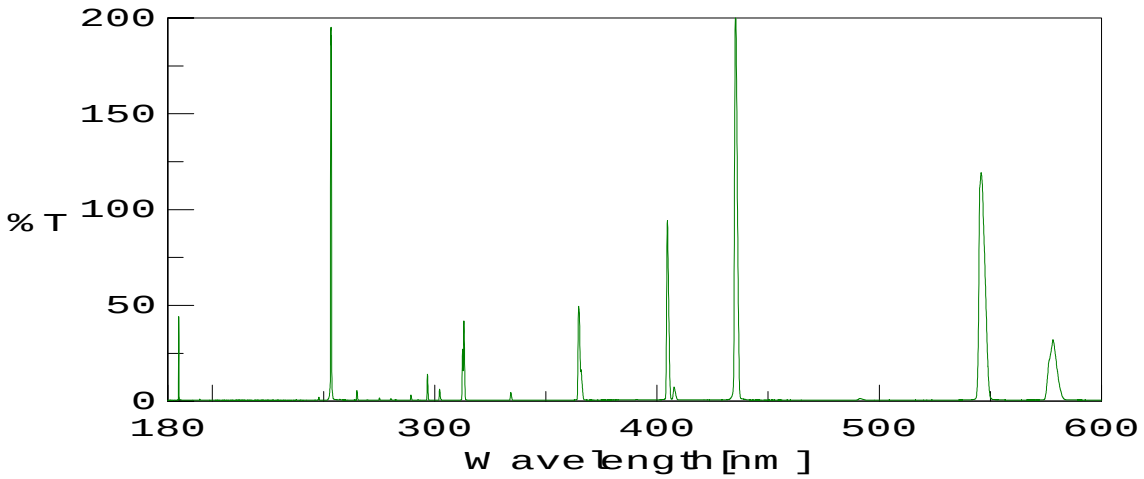
Xe lamp (built in)
881.9, **823.2**, 764.3 nm

Neon lamp
724.5, 692.9, 585.2 nm
(Neon has too many lines, pay attention not to get confused!)



UV-VIS range:

Hg low pressure
578.0*, 546.07, 435.83,
404.66, 313.16, **253.65**,
184.90 nm
* unresolved doublet



Conclusion

- Due to relative complexity: PEM not achromatic, prism monochromator, lack of certified intensity standards …., full check and validation of a CD spectrometer is a matter far more complex from what reported in Pharmacopoeia or even in manufacturer literature and software.

- Methods proposed for UV-VIS spectrophotometers can be applied, but these can cover only a part of the requirements.

- Proper testing criteria should be sorted out using common sense and skill in order to avoid, as far as possible, *traceable* expensive standards (Hg spectra is not a NIST property, it relies rather on tortoise ….).

- Different applications may call for the use of only a limited wavelength range: this may simplify substantially the matter and call for specific, relatively simple, procedures.

- Use of accessories (for example Peltier elements for temperature ramping) will call for further checking routines

References

- Ph. Eur. Method 2.2.41, 2001
- T. Takakuwa, T. Konno, H. Meguro, *Anal. Sciences*, 1, 1985, 215
- P.H. Schippers, H.P.J.M. Dekkers, *Anal. Chem.*, 53, 1981, 778
- J.P. Hennessey jr, W.Curis Johnoson jr, *Anal. Biochem.*, 125, 1982, 177
- T. Konno, H. Meguro, K. Tsuzimura, *Anal. Biochem.*, 67, 1995, 226
- C. Burgess, T. Frost, *Standards and Best Practice in Absorption Spectroscopy*, Blackwell Science, London, 1999
- E. Castiglioni, *Spettrofotometria UV-Vis Criteri di valutazione e controllo*, Morgan Ed. Tecniche, Milano, 1996
- E. Castiglioni, *lecture at CD'97*, Pisa, 1997
- Varian, *Cary 61 Manual*, Monrovia, 1970
- Jasco, *J-810 Service Manual*, Hachioji, 2001
- Jasco, *JWVAL-486 Operation Manual*, Hachioji, 1999
- Jasco UK, *J-700 Qualification Workbook*, Great Dunmow, 1999 (draft)