DR-CD accessory for the determination of enantiomeric ratio

I – Introduction

The preparation of pure enantiomers for pharmaceutical industry is part of mandatory safety requirements. Circular Dichroism is one technique to determine enantiomeric purity and it can be applied directly on solid samples.
Circular Dichroism measurements on solid samples have been done for years on pellets when sample proved to be insoluble or was showing structural change when changing media. The preparation of pellets is not straightforward and is also time consuming. To use an integration sphere for measuring Diffuse Reflectance (DR-CD) is an alternative to pellets that can be implemented in few minutes on MOS-500. This application note illustrates the use of DR-CD accessory to determine enantiomeric purity of some alanine preparation (powder mixture of L and D forms).

II– Principle of DR-CD accessory

The integration sphere is installed directly at the exit of MOS-500 optical bench so the incident light illuminates directly the solid sample. The detector is installed at 90° to sample protected from direct reflection using a diffusion baffle. The internal coating of the integration sphere was carefully chosen to offer the highest reflectance from far-UV to NIR region. The detector collects diffuse reflectance continuously so MOS-500 can generate DR-CD and DR-LD according to user requirements.

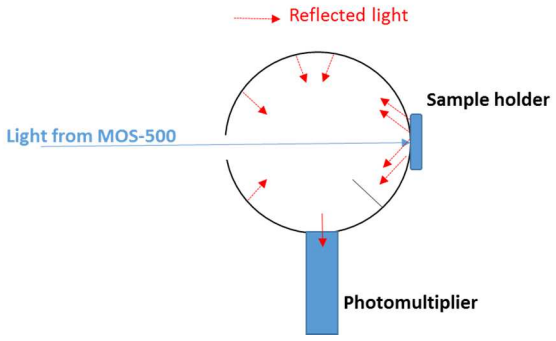

Fig. 1 : DR-CD principle

DR-LD (Diffuse Reflectance Linear Dichroism) is used as a quality measurement to check that quartz window of sample holder and integration sphere have no polarization effects that could distort DR-CD signal.

III–Experimental conditions

All spectra are recorded from 200 nm to 300 nm and without any purge of optics with N2 as it is not useful on this wavelength range so running cost is minimum in this configuration. Spectra are measured using a 0.25 nm data interval and a 4 nm bandwidth. A single spectrum is done for each powder sample and only raw data are shown below (no smoothing).
Pure D-Alanine and L-Alanine powders are obtained from Sigma-Aldrich and are used for reference and for the preparation of different enantiomeric mixtures.
All powders are grounded using a mortar and a pestle to have a thin and homogeneous powder. Powder is then packed easily in the solid sample holder.

III–CD and LD on pure enantiomers

CD spectra of pure enantiomers are first measured to check quality of sample preparation and integrity of CD signal measured. The spectra of L-alanine and D-alanine are shown in figure 2.

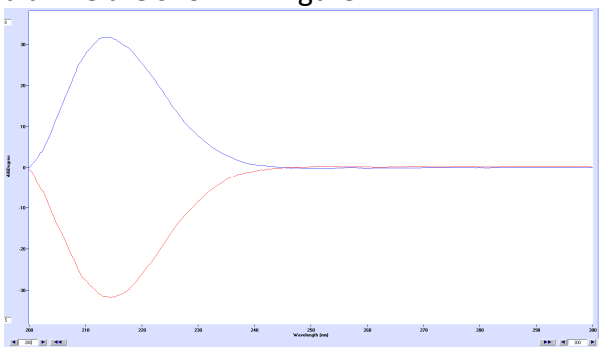

Fig. 2 : CD spectrum of L-alanine (blue) and D-alanine (red)

The two spectra are mirror images which match theoretical expectations. Peak is measured at 214 nm with a ± 31.8 mdeg amplitude.
DR-LD spectra are also recorded with sample in place to check level of linear dichroism and are shown in figure 3.

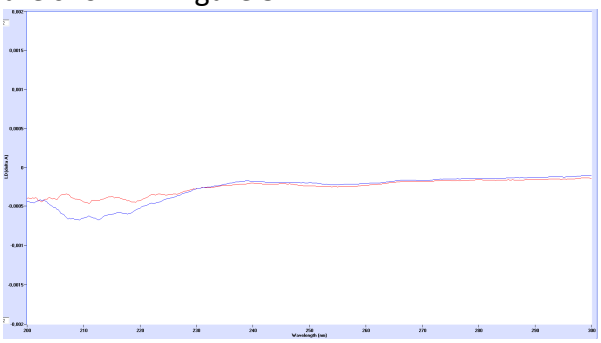

Fig. 3 : LD spectrum of L-alanine (blue) and D-alanine (red)

The Intensity of LD signal is below 0.0008 ΔA for L-alanine and below 0.0005 ΔA for D-alanine which shows the negligible effect of polarization effect on CD measurements. This LD artifact is also 4-5 times smaller compare to values reported by other manufacturers which show quality of our design.

IV–CD and LD on known prepared sample

Two powders are prepared from different mixture of D-alanine and L-alanine. Sample A is prepared by mixing 50% of L-alanine and 50% of D-alanine. Sample B is prepared by mixing 75% of L-alanine with 25% of D-alanine. Powder is well mixed before grounding.
CD spectra are shown in figure 4. Sample A is a racemic mixture showing no CD signal: the two enantiomers contribute equally in a opposite way (mirror effect shown in figure 2) way to CD. Sample B shows a major contribution of L-alanine, with a maximum CD signal at 15.9 mdeg. This value exactly matches the theoretical value obtained from amplitude measured on pure enantiomers.

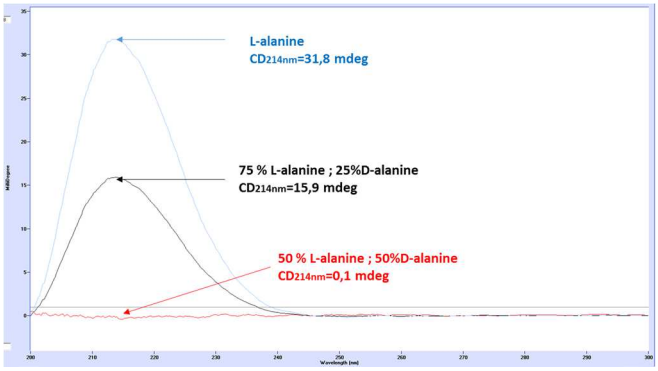

Fig. 4: CD spectrum of pure L-alanine (blue), racemic mixture A (red) and sample B (black)

V–Unknown powder composition

A sample C with unknown L and D composition is prepared the same way by grounding. CD spectrum of sample C is shown in figure 5.

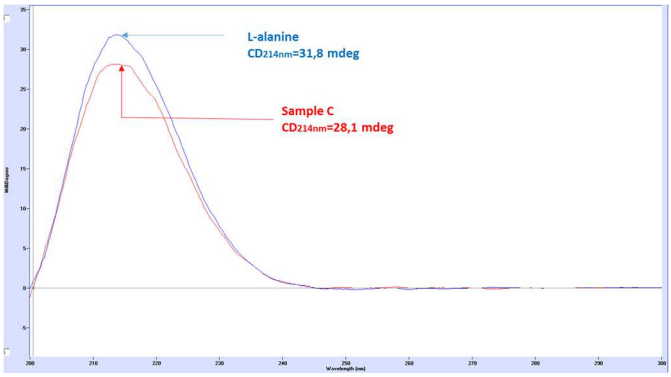

Fig. 5: CD spectrum of pure L-alanine (blue) and unknown mixture C (red)

Maximum CD signal for sample C is 28.1 mdeg. This CD spectrum would correspond to a mixture 94.2% of L-alanine and 5.8% of D-alanine.

VI–Conclusion

DR-CD accessory (integration sphere) of MOS-500 offer possibilities to determine enantiomeric ratio of some solid samples. It shows negligible contribution of polarization artifact and reliability of sample preparation. This application note also shows performance of DR-CD accessory down to 200 nm and without the need for purging optics with nitrogen.