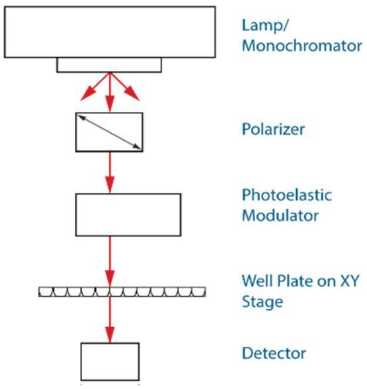
EKKO™ CD Microplate Reader
Well Plate Optical Characteristics Variation Effects on CD Measurements

I – INTRODUCTION

The differential absorption between left and right circularly polarized light is a commonly used technique. Circular dichroism (CD) is often used for assigning the secondary structures of proteins and determining enantiomeric purities in asymmetric syntheses, both of which benefit from the ability to do the measurements in a high-throughput fashion [1,2].



The primary advantage of the EKKO™ CD Microplate Reader is its speed resulting from the use of well plates allowing for the highest throughput possible. It accomplishes this by turning the light path from the horizontal to vertical, allowing for a computer controlled XY stage so that CD signals are read directly from a well plate.

This removes the time-consuming steps of 1) transferring the contents from each well of a well plate into a cuvette, and 2) cleaning the cuvette between measurements. As a result, it takes only two minutes to measure the CD signal in all 96 wells of a standard well plate at any given single wavelength and needs less than 90 minutes to measure all 96 CD spectra over 50 wavelengths in a standard well plate. This results in a significant increase in productivity, as much as 100-fold with respect to conventional CD systems coupled to liquid handling robotics[1,2,3].

However, unlike conventional CD systems, the optical features of the glass or silica is not guaranteed to be indistinguishable between each of the wells. This could lead to variations in the replicate measurements due to the potential heterogeneity of the glass making up the bottom of the wells in a 96 well plate.

In this technical note, we address this possible effect on the data obtained with the EKKO™ CD Microplate Reader with CD measurements of Insulin in two different solid fused silica 96 well plates obtained from Hellma.

II – RESULTS & DISCUSSION

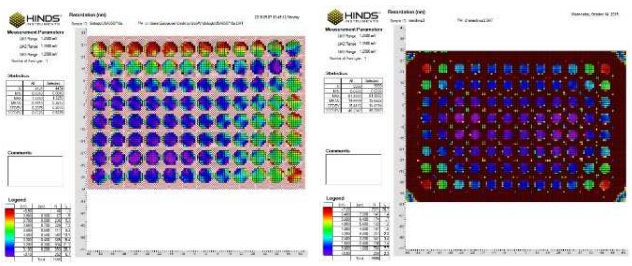


Fig. 1. Birefringence scanning of different fused silica well plates. In blue the spots with the lowest retardation.

Figure 1 illustrates the difference in the optical quality of the well plates that can be obtained for CD measurements from Hellma. Both are solid fused silica, yet they had significantly different retardation characteristics in nm/cm at 633 nm. The left plate with clear side walls (8% of the Wells ≤ 1 nm/cm) demonstrated an order of magnitude more retardation than the right plate with black side walls (97% of the Wells ≤ 1 nm/cm) and neither plate was homogeneous in its properties across the plate. This variable retardation has the

potential to alter the measurements across and between each individual well plate.

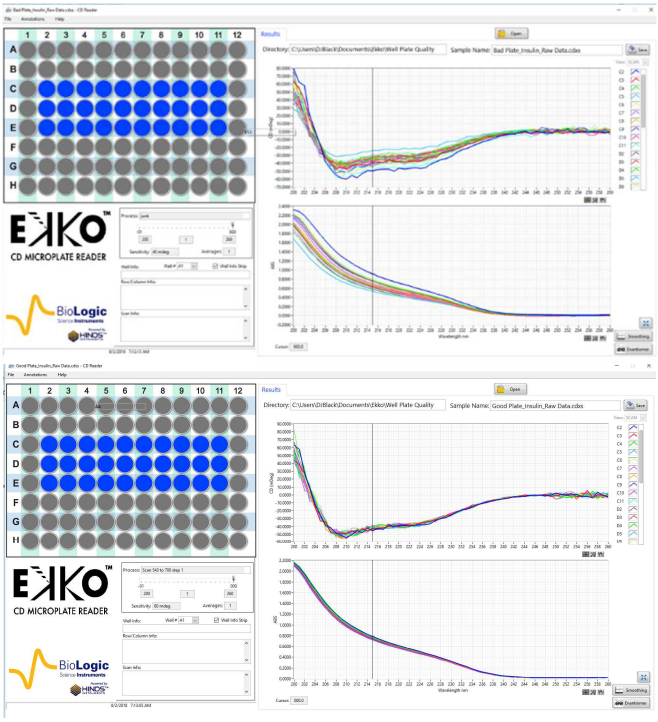


Fig. 2. Raw CD spectra of Insulin in 8% Well Plate (top) and 97% Well Plate (bottom). The CD spectrum of 200 µl of Insulin (Sigma) at 50 µg/ml in wells C2-E11 of both solid fused silica 96 well plates (Hellma) were recorded from 200 to 260 nm. Raw spectra were collected with no effort to minimize the noise or evaporation at a room temperature of 25°C.

Figure 2 demonstrates the effect of the variable optical characteristics of the plate can have on the raw CD spectra. As expected, the plate with the larger heterogeneity in its well to well optical features demonstrated the largest differences in the reproducibility between each of the spectra for identical loads of Insulin. It is interesting to note that the plate with the black side walls had higher reproducibility than was observed for the standard silica quartz walls. Presumably this is a function of removing the potential for inter-well scatter increasing the stray light observed by the photo multiplier tube.

Even though the effect of the heterogeneity of the optical quality of well plates was apparent in the raw scans, the averaged spectra from both plates were qualitatively similar.

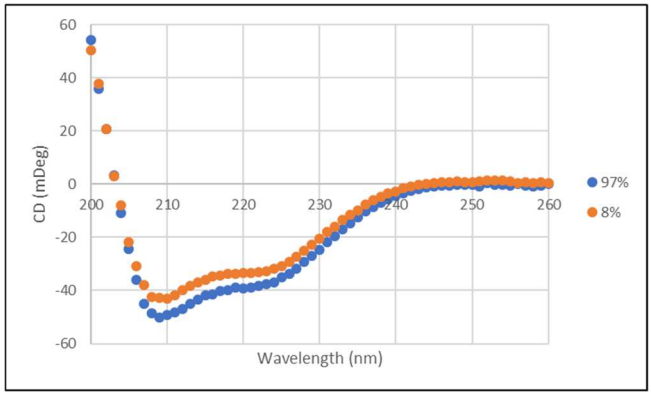


Fig. 3. Averaged (n=30) CD spectra of Insulin in the differing Solid Fused Silica Well Plates (Hellma). The CD spectrum of 200 µl of Insulin (Sigma) at 50 µg/ml in wells C2-E11 of both solid fused silica 96 well plates (Hellma) were recorded and averaged. The CD spectrums were over the wavelengths 200-260 nm. Raw spectra were collected with no effort to minimize the noise and were taken at a room temperature of 25°C.

Figure 3 establishes that even though the spectra obtained from each well were different, the overall effect on the population average was minimal over the course of the entire spectra and is only significant at shorter wavelengths occurring in the deep UV.

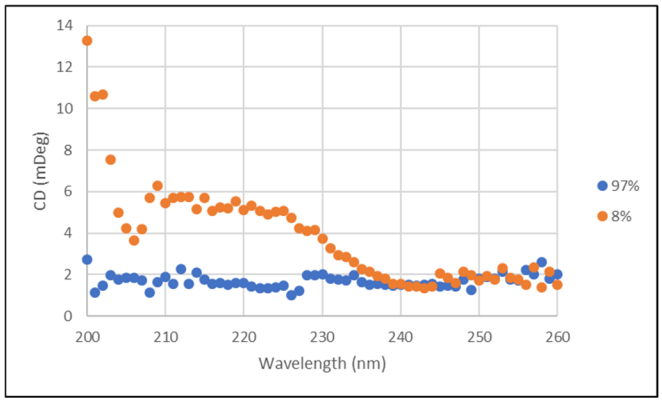


Fig. 4. Standard Deviation as a function of Wavelength for the Averaged CD spectra for the different Solid Fused Silica Well Plates (Hellma). The standard deviation of the n=30 CD spectrum of 200 µl of Insulin (Sigma) at 50 µg/ml in wells C2-E11 of both solid fused silica 96 well plates (Hellma) were recorded at 25°C with an integration time of 1 sec/nm.

Figure 4 verifies and demonstrates the effects of the difference in the optical quality of the two plates on the CD spectra is substantial only at the shorter wavelengths of the deeper UV. Above 240 nm, both plates behaved identically. Below 240 nm, in the region of interest for protein secondary structure prediction, the black side-walled well plate, which had 97% of its wells with retardations below 1 nm/cm, behaved as well as it did above 240 nm.

III – SUMMARY & RECOMMENDATIONS

1. For experimental regimes at wavelengths > 240 nm, heterogeneity in the optical characteristics of the well plate do not affect the results.

2. For qualitative determinations in experimental regimes ≤ 240 nm, having heterogeneity in the optical characteristics of the well plate has little noteworthy consequences for the data.

3. For quantitative determinations in experimental regimes ≤ 240 nm, the presence of significant heterogeneity in the optical characteristics causes significant deviations in the data obtained. For these experiments, it is recommended to have a plate that is as homogeneous as possible, ideally with < 1 nm/cm deviation across the entire plate.

REFERENCES

1) Metola, P., Nichols, S.M. Kahr, B., and Anslyn, E.V., Well plate circular dichroism reader for the determination of enantiomeric excess. *Chem. Science.* 5, 4278-4282 (2014).
2) Jo, H.H., Cao, X. You, L., Anslyn, E.V., and Krische, M.J., Application of high-throughput enantiomeric excess optical assay involving a dynamic covalent assembly: parallel asymmetric allylation and ee sensing of homoallylic alcohols. *Chem. Science.* 6, 6747-6753 (2015).
3) Fielder, S., Cole, L., and Keller, S., Automated Circular Dichroism spectroscopy for medium throughput analysis of protein conformation. *Anal. Chem.* 85, 1868-1872 (2013).