EKKO™ CD Microplate Reader
UV Cut Offs due to Volume and Solution components

I – INTRODUCTION

Generally, the differential absorption between left and right circularly polarized light (CD) is used for determining enantiomeric purities in asymmetric syntheses, assigning the secondary structures of proteins and other chiral analysis. Each of these can benefit from the ability to do the measurements in a high-throughput fashion[1,2]. With standard technologies, these determinations are accelerated with the addition of robotic liquid handling systems that fail to remove the time-intensive processes of loading the samples and washing the cuvette between measurements.

By rotating the light path 90°, The EKKO™ CD Microplate Reader uses a vertical light path allowing the CD measurements to be read directly from a well plate, eliminating sample transfer and cuvette cleaning. This significantly increases productivity, as much as 100-fold with respect to standard CD's coupled to a robot[1,2,3].

In a well plate, complications arise that do not exist with standard technologies though. At a minimum, the combination of the volume dependent variable path length and solution properties dependent meniscus effects have the potential to alter CD determinations.

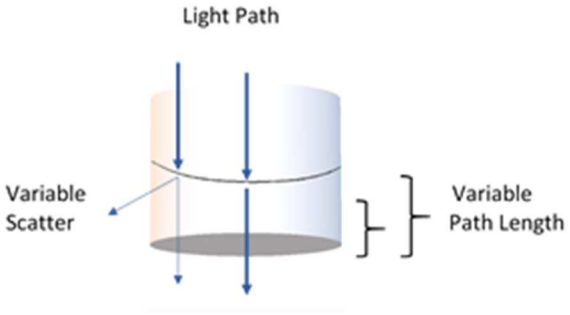

Fig. 1. Presumed effects on the light path as a function of volume and the meniscus in a well.

It is well known that buffer composition and path length significantly limit the UV cut off for CD measurements. In this note, we present circular dichroism measurements of water, buffer, (α)-lactoglobulin and Cytochrome C to address the volume and buffer dependent effects on the UV cut off wavelength for the EKKO™ CD Microplate Reader.

II – RESULTS & DISCUSSION

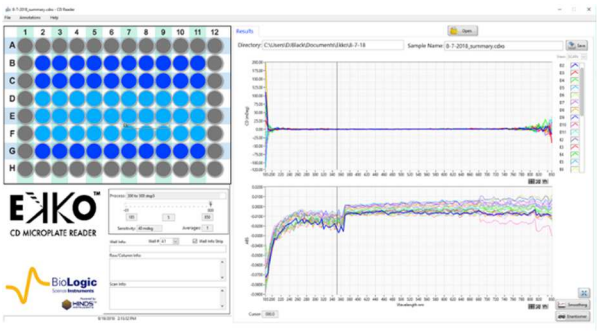

Fig. 2. CD spectra 10 mM K_2PO_4. 200 µl of K_2PO_4 pH 7.4 (Sigma) at 20 to 100 µg/ml was loaded into wells #D2 to #G11 of a solid fused silica 96 well plate (Hellma) to get a base line deviation of the CD signal. No effort to reduce the noise was used to collect the raw data.

Figure 2 is a representative data set of the CD and Absorbance measurements obtained for 75 to 300 µl volume loads of either water or some other spectrally harmless buffer in a well plate. Regardless of volume used, between 250 and 800 nm, the CD signal had an average deviation of 1.8 ± 0.4 mDeg for 10 mM K_2PO_4 and 1.2 ± 0.2 mDeg for H_2O (data not shown). The combined observed baseline deviations with multiple plate types and innocuous buffers is 1.6 ± 0.2 mDeg (n=208 spectra).

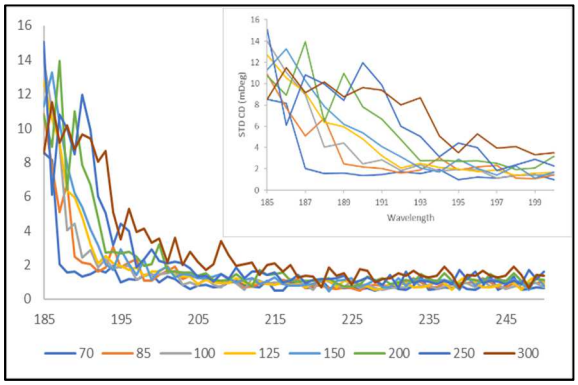

Fig. 3. STD of the CD signal as a function of wavelength. Increasing volumes of (70 - 300 µl) of K_2PO_4 pH 7.4 (Sigma) at 20 to 100 µg/ml was loaded into wells #A1 to #H12 of a solid fused silica 96 well plate (Hellma). Each trace is the standard deviation at the individual wavelength for n=12 spectra for the increasing volumes.

Figure 3 illustrates the effect of increasing the volume on the UV wavelength cut off as the volume is increased within a well plate. The inset clearly shows the red shift in the usable wavelengths with K_2PO_4, pH 7.4, as expected. Similar results were obtained regardless of solute added to the innocuous buffers.

The effective path length in a well can also be affected by the composition of the buffers because the physical properties of the solutes should alter the meniscus attributes. As such, we replotted the cut off wavelength for the various circular dichroism measurements obtained with the CD plate reader as a function of volume.

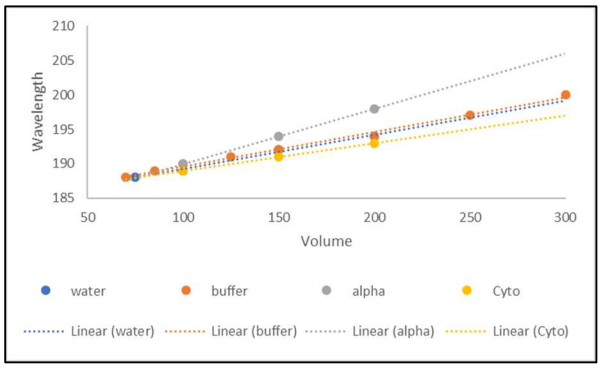

Fig. 4. Cut Off Wavelength as a Function of Volume. Replots of the cut off wavelength as a function of volume for H_20, K_2PO_4 (10 mM, pH 7.4 Sigma), α-lactoglobulin (40 µg/ml Sigma) and Cytochrome C (40 µg/ml Sigma). Cut Off wavelength is defined as the wavelength which is significantly different from the standard deviation (STD) of the spectra above 220 nm. Each point data point represents the STD of n ≥ 12 individual spectra.

Figure 4 demonstrates that, as feared, buffer and solute composition also effect the UV wavelength cut off. For the innocuous buffer, there was no distinguishable difference between its wavelength cut off dependence and that of water. Cytochrome C and α-lactoglobulin demonstrated unique volume relationships for the red shift of the UV wavelength cut off. Presumably, this is a function of the different shapes of the menisci present leading to variable scatter.

We have purposely used proteins to determine the sensitivity of the EKKO™ CD Microplate Reader as a function of volume because of the presumed variable surface tension effects on the meniscus of the solution in a well plate. Unlike conventional technologies, the wavelength cut off must be

empirically determined for each concentration and well plate type used with the EKKO™ CD Microplate Reader.

III – SUMMARY & RECOMENDATIONS

1. The EKKO™ CD Microplate Reader has similar UV cut off wavelengths as observed for standard CD Technologies as a function of pathlength for aqueous solute and innocuous buffer and solute components.
2. The EKKO™ CD Microplate Reader displays UV cut offs that are susceptible to the physical characteristics of the solutes due to the presence of the meniscus.
3. It is recommended that the cut off wavelength be empirically determined for each protein concentration, volume, plate and protein or solute type. Fortunately, this is a trivial task when replicates are taken.

REFERENCES

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