

## Standard Operating Procedure

### Environmental Considerations:

Oxygen absorbs strongly below about 200 nm so very extensive purging with pure (oxygen-free) nitrogen (>16 liters/min) is necessary for these measurements.

### Buffer Selection:

A typical buffer used in CD experiments is 10mM phosphate, although low concentrations of Tris, perchlorate or borate are also acceptable. Potassium fluoride is preferred to NaCl for increasing the ionic strength as the chloride ion has a strong UV absorbance at low wavelengths.

### Acceptable:

1. Potassium Phosphate with KF, K<sub>2</sub>SO<sub>4</sub> or (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> as the salt.
2. Hepes, 2mM.
3. Ammonium acetate, 10mM.

Avoid: Tris; NaCl; Anything optical active, e.g. Glutamate

### Solvent selection:

The CD spectra of peptides have been reported in a number of solvent systems, for example: Trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP) may promote helix conformations, and may mimic an in vivo system (e.g.; Waterhouse & Johnson, 1994).

### Typical Conditions for protein CD:

1. Protein Concentration: 0.2 mg/ml
2. Cell Path Length: 1 mm

3. Volume 350  $\mu$ l
4. Need very little sample 0.1 mg
5. Concentration reasonable
6. Stabilizers (Metal ions, etc.): minimum
7. Buffer Concentration : 5 mM or as low as possible while maintaining protein stability

#### Protein CD spectra:

1. CD spectra in the far UV region (180 nm –250 nm) probes the secondary structures of proteins.
2. CD spectra in the near UV region (~250 and ~ 350) monitors the side chain tertiary structures of proteins.

#### Main CD features of protein 2<sup>ndary</sup>structures

	-band (nm)	+band (nm)
<b><math>\alpha</math>-helix</b>	222	192
	208	
<b><math>\beta</math>-sheet</b>	216	195
<b><math>\beta</math>-turn</b>	220-230 (weak)	205
	180-190 (strong)	
<b>Polypro II helix</b>	190	210-230 (weak)
<b>Random coil</b>	200	212

#### Summary

1. CD is a useful method for looking at **secondary structures** of proteins and peptides.
2. CD is based on measuring a very small difference between two large

signals must be done carefully

3. The Abs must be reasonable max between  $\sim 0.6$  and  $\sim 1.2$ .
4. Quartz cells path lengths between 0.0001 cm and 10 cm. **1cm and 0.1 cm common**
5. Have to be careful with buffers TRIS bad - high UV abs.
6. Measure cell base line with solvent
7. Then sample with same cell inserted same way around
8. Turbidity kills - filter solutions
9. Everything has to be clean
10. For accurate 2ndary structure estimation must **know concentration** of sample

## AVIV CD startup procedures

1. Open the valve on the liquid nitrogen dewar , check left pressure gauge indicate at 20 spi , the flow rate should be between 15-20 SCFH , waiting for 20 minutes to purge system.
2. startup the chiller at right side of the CD ( ON/OFF button at chiller control panel)
3. Switch on the switch “ **LAMP POWER & COOLING SYSTEM** “ on POWER box , the LED “**CONTROL POWER ON**” will be on
4. wait until “**LAMP READY**” lit , press red button “ **IGNITE LAMP**” to ignite the lamp , “**LAMP ON**” lit indicate the light source was on .
5. Switch on the switch “ **CPU & INSTRUMENT**“ at POWER box , the LED “**CPU/INST PWR ON**” will on .
6. power on the PC at down left side
7. startup the CDS program
8. Display → Data browser → set “default data path “ at the bottom of the window -→ return

## **AVIV CD shutdown procedures**

- 1. save your data and close CDS program**
- 2. shutdown PC**
- 3. Switch off the switch “ CPU & INSTRUMENT“ at POWER box**
- 4. Switch off the switch “ LAMP POWER & COOLING SYSTEM “ on POWER box**
- 5. shutdown the chiller at right side of the CD ( ON/OFF button at chiller control panel)**
- 6. waiting for 5 minutes and turn off(clockwise) the valve on liquid nitrogen tank**

**in case of power failure , please turn off both “ CPU & INSTRUMENT“ and “ LAMP POWER & COOLING SYSTEM “**