Varying flow in linear dichroism cells

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The effect of varying fluid flow in different linear dichroism cells

Abstract

The usefulness of linear dichroism (LD) in structure identification depends strongly on the orientation of the molecule in the linear dichroism cell. Hence, the fluid flow inside the linear dichroism cell which performs this act of orientation is of great importance. As such, this paper records the steps taken to identify the varying flow states in the cell, with changing rotation speed, and the resulting effects on the orientation of the molecule, as deduced from the LD spectra obtained. The previously known stiffening effects of ethidium bromide (EB) on DNA are reproduced to a certain extent, the change appearing for high concentrations of EB, but, significantly, at the same point at which Taylor vortices appear in the flow. The inability to scale up the results obtained from the micro-volume LD cell to match those from the larger cell suggest a dependence of the orientation on the flow profile in the cell, which are different in the two cells.

Introduction

Linear dichroism (LD) is a very useful tool in the identification of structure of biological molecules. With the invention and implementation of the new micro-volume Couette flow cell (2), it has become even more suitable for use in biological spectroscopy due to the need for only a very small quantity of sample. With the possibility of expansion to more applications, it is necessary to understand the exact workings of the LD cell and its effect on the molecule under investigation.

The accuracy and reliability of LD, as a spectroscopic tool, depends on the appropriate orientation of the molecule inside the LD cell which is done by the Couette flow inside the cell. However, this flow changes with the set-up used and the rotation speed. The introduction of the micro-volume (MV) cell has bought this sharply into focus since it uses a set-up which would result in a different flow profile from the large volume (LV) cells used previously. The research described in this paper investigates the different results obtained from varying the LD cell used and rotation speed and attempts to connect these to the fluid dynamics in the cell.

The section detailing the materials and methods used in the experiments is followed by a brief background to LD and an introduction to Couette flow and other related flow properties. The results obtained will then be presented and a discussion follows with a look at possible future work.

Materials and Methods

Three different LD cells were used. These were the large volume cell (LV1) which has a volume of 2 ml and an irregular annular gap ranging from 0.5 mm – 1 mm, the more accurate large volume cell (LV2) with a volume of about 1.5 ml and an annular gap of 0.5 mm and the micro-volume cell (MV1) with an annular gap of 0.225 mm and a volume ranging from 40 µl to 60 µl. The key difference between these cells is that the larger ones have a stationary outer cylinder and a rotating quartz inner cylinder while the MV1 has a stationary quartz inner rod and a rotating outer capillary tube. For more details see references (1) and (2).

DNA was used in concentrations of 500 µM, 250 µM and 125 µM. The data presented are those from the 250 µM DNA concentration, unless otherwise stated. The solutions of DNA and ethidium bromide were made up by adding the appropriate volumes of 250 µM DNA and 250 µM to make up DNA:EB molar ratios of 50:1 and 25:1.

The spectroscopic scans for each sample at different voltages were conducted continuously without stopping the rotation between scans and the increasing of the voltage. Scans were conducted for wavelengths from 600 nm – 190 nm with 4 accumulations.

To determine the relation between the rotation rate and the voltage, the time scans of the high tension spectra were observed. The irregularity of the LV1 cell provides a periodicity which allows the determination of the rotation speed, while the MV1 cell required a marking along the length of the capillary. Unfortunately, the LV2 cell did not provide any suitable method in which to determine the rotation rate. Also, an unexpected malfunctioning in the LV2 cell half-way through the research meant that the data obtained was not considered in the analysis.

The images of the fluid flow were obtained with the help of Mr. James Sinfield and his digital camera using the 'super-macro' option. The AQ-1000 rheoscopic fluid used to obtain this imaging was obtained from the Kalliroscope gallery (264 Main Street, P.O. Box 60, Groton, Massachusetts 01450, USA. http://www.kalliroscope.com) and was used at a concentration of 5%. These flakes align along the direction of fluid flow, providing an insight into the actual fluid flow in the cell. A light source was shone onto the cell from above to illuminate the flakes.

Linear Dichroism

Linear dichroism is a valuable spectroscopic method used in determining the structure of biological molecules. The molecule is oriented using a range of methods including gel squeezing, electromagnetic alignment and thin film orientation. However, since the natural state of most biological molecules is in fluid, the most appropriate method would be shear flow orientation. This is accomplished by placing the sample in the small gap between the inner and outer cylinder and then rotating one of these cylinders. The resulting fluid flow shears the molecule into the appropriate orientation.

Linearly polarised light, polarised either vertically or horizontally, is then shone through the sample of primarily horizontally aligned molecules. At the correct wavelengths, the light is absorbed by the molecule to initiate electron transitions in the molecule. The measured LD signal is the difference between the absorption of light polarised parallel to and perpendicular

to the orientation direction: $LD = A_{//} - A_{\perp}$. However, since perfect orientation cannot be obtained in practice, it is modified to $LD = {}^{3}/_{2} \mathbf{S} A_{iso}(3 \cos^{2}\alpha - 1)$, where α is the angle between the transition moment within the molecule and the preferred orientation direction of the molecule, $A_{iso} = {}^{1}/_{3} [A_{//} + 2A_{\perp}]$ and $\mathbf{S} = 3(\langle\cos^{2}\theta\rangle - \frac{1}{2})/2$ is the orientation factor of the sample with θ being the angle between the orienting axis and the z-axis of the molecule.

Obviously, a randomly oriented sample would give a $LD = 0$ since the absorptions would average out. Hence, the correct orientation of the sample is essential. When the linearly polarised light hits the oriented molecule, an electron transition occurs if the light is polarised in the same direction as the transition. If this is the case, a maximum LD signal is observed. However, if the light is polarised perpendicular to the transition, there will be no signal. In this manner, information is obtained as to the structure of the molecule.

In the case of DNA, the transitions which occur are all $\pi - \pi^{*}$ transitions of the DNA bases and α is known to be 86°. The maximum LD signal is obtained at a wavelength of 259 nm. With the addition of ethidium bromide, the magnitude of the signal at 259 nm increases and smaller signals are also obtained at 330 nm and 520 nm. This is due to the EB in the solution which provides the additional electron transitions and binds between the base pairs of the DNA, thus making the molecule more rigid and hence better aligned. See Figure 1.

Fluid Dynamics

The spinning of either cylinder in a co-centric cylinder system as in the LD cells causes the fluid in the annular gap to flow in a specific manner. However, this pattern depends on many parameters in the system, the more obvious being those of rotation speed of the cylinder, the viscosity of the fluid and the potential for interaction between the surface of the cylinder and the fluid or molecules in the fluid. With the introduction of the MV1 cell, it has become necessary to consider the effects of another parameter in the system: whether it is the inner or outer cylinder which is rotating.

As stated above, in the MV1 cell, it is the outer cylinder which rotates whereas in the LV1 and LV2 cells it is the inner. The differences in the flow profiles are shown in Figure 2. Although the direction of the flow is the same, the variation of the speed across the gap could have implications on the orientation of the molecules, depending on the shear force and the likelihood of an interaction between the biological molecules and the cylinder surface. This is relevant because DNA can adhere to quartz, especially if the speed at which it is moving is conducive.

Although the above is an interesting point to note, there is not much that could be done during this project to calculate specifics. Therefore, attention was placed more on the stability of the Taylor-Couette flow arising in the cell. This stability is dependent on the

Reynolds Number (R_e) of the system, where $R_e = \dfrac{\omega_1 r_1 \delta}{\nu}$ with ω_1 and r_1 the angular velocity

and radius of one rotating cylinder, δ is the gap width and ν is the kinematic viscosity of the fluid. At high R_e, the flow changes from the simple Couette flow to a series of more complex states. The first of these is the appearance of Taylor vortices which are vertically stacked toroids of flow around the inner cylinder as shown in Figure 3. Also in Figure 3 is the image of wavy vortex flow (WVF) which is the result of an even higher R_e than that for

Taylor vortex flow (TVF). Further increase in R_e leads to spiral vortex flow, twisting vortex flow and turbulent vortex flow.(3)

Although it appears that TVF would not affect the orientation of the molecules since the flow is essentially horizontal as in Couette flow, the slow twisting rotation of the individual toroids is likely to have an effect on the alignment. The relevance of this depends largely on the relative speed of the rotation speed of the toroid. It is fairly clear that WVF would definitely hinder the perfect orientation we are aiming for, although the extent to which this occurs remains to be explored. Similarly, the other fluid flow states would also affect the orientation. The research conducted was aimed at trying to observe these varying states within the cells. However, it was only possible in the LV1 cell as the MV1 cell was too small and the LV2 cell too fast to clearly examine these states.

Results

Fluid Flow

In order to observe the changing fluid states inside the LV1 cell, a 5% concentration of AQ-1000 rheoscopic fluid was placed in the gap and the voltage increase gradually. The increase in voltage leads to an increase in the rotation speed and, hence, the R_e number. The important results are as shown in Figure 4.

The voltage was increased from 2.0 V, where rotation begins, to 6.0 V. Taylor vortices, in the form of dark and light streaks, appear at the top and bottom of the cell at 2.8 V and fill the entire cell at 3.0 V. The thickening of the streaks represents WVF, which can be seen at 3.2 V, and the tilted streaks are spiral vortex flow and 3.3 V. Turbulent flow begins at about 3.6 V and continues to get worse with increasing voltage. These conclusions were obtained by comparing the images to similar experiments done on a large scale by D. Coles (4) and are summarised in Table 1.

Spectroscopic results

The first set of experiments was conducted to ascertain the relation between the spectra and the rotation voltage. The magnitude of the spectra increased with voltage, almost linearly with a levelling out at the higher voltages. Figure 5 shows the relation between the rotation rate (in rpm), which was related to the voltages, and the minimum LD signal (for each sample in each cell) obtained for all the experiments conducted. The graphs show an increase in the magnitude of the minimum LD signal for all cells and samples, with the increase seen in the MV1 cell being more gradual than that of the LV1 cell. The magnitude of the LD signal from the LV1 cell tends to level out at about 3500 rpm.

Next, a comparison within the same cell using the different samples gave graphs as shown in Figures 6 and 7 for MV1 and LV1, respectively. The signal for the sample containing 50:1 DNA:EB is always larger than that for the pure DNA in both cells. However, the signal for 25:1 DNA:EB is larger than the pure DNA signal for voltages less than 1.5V, but is less than the pure DNA for all voltages greater than 1.5V in the MV1 cell and the same in the LV1 cell but at 3.0V. This is definitely unexpected as the EB is expected to make the DNA more rigid, thus giving a larger signal. However, the magnitude of the peak at 520 nm, which gives a smaller signal, increased with the addition of more EB.

Finally, an investigation was carried out into the difference between the spectra for the same sample in the different cells. Since the MV1 cell is smaller and has a smaller volume, one would naturally expect a smaller signal to that from the LV1 cell. Therefore, the signal from the MV1 cell was scaled up accordingly in an attempt to overlay it on the signal from the LV1 cell. This did not prove a problem for the pure DNA samples, but the samples containing DNA:EB of 50:1 ratio did not scale up very well. Surprisingly, the samples containing DNA:EB of 25:1 ratio scaled much better, almost as well as the pure DNA spectra. Figure 8 provides such an overlaying for both the ratios of DNA:EB.

Discussion and Conclusion

In the course of the analysis, we can conclude that increasing the voltage, and hence rotation rate, does not affect the LD spectra except to increase the magnitude of the peaks obtained. This would seem to imply that the varying fluid flow states do not affect the molecule alignment in any negative manner and only provides a better orientation and, as a result, a larger signal.

This is, however, belied by the unexpected behaviour of the spectra from the DNA:EB ratio of 25:1 samples, as these suggest that a change occurs in the orientation of these more rigid molecules between 1.2 V and 1.5 V in the MV1 cell and between 2.7 V and 3 V in the LV1 cell. For the LV1 cell, the fluid flow states, as seen in Figure 4, show that the TVF sets in approximately between 2.8 V and 3 V. This suggests that TVF hinders more rigid molecules from reaching a perfect orientation. To consider this observation as concrete is not possible at present since more detailed experiments would need to be conducted. Also, if it were possible to obtain proof as to the point at which TVF sets in in the MV1 cell, and if this were to correspond to the voltages shown above, it would be a solid claim. However, this does provide grounds for further investigation into the behaviour of the molecules under different flow states.

When considering the difference between the two cells used, the inability to effectively scale up the spectra from the MV1 cell to match the LV1 spectra suggests that the differing flow profile, due to the rotation of different cylinders, could be a key factor. Again, the scalability of the 25:1 ratio sample is anomalous and not easily explained or understood.

In conclusion, this research has been illuminating in terms of the various fluid flow states arising through the range of voltages used. The unexpected data raises questions which can only be answered with further study of the subject and using novel methods to investigate the effect of shear flow on molecules.

References

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7. Timothy R Dafforn and Alison Rodger. "*Linear dichroism of biomolecules: which way is up?*". www.sciencedirect.com

8. http://www.princeton.edu/~gasdyn/Research/T-C_Research_Folder/Intro_to_T-C_Flows.html

Table 1: **Fluid flow in the LV1 cell – variation with voltage**

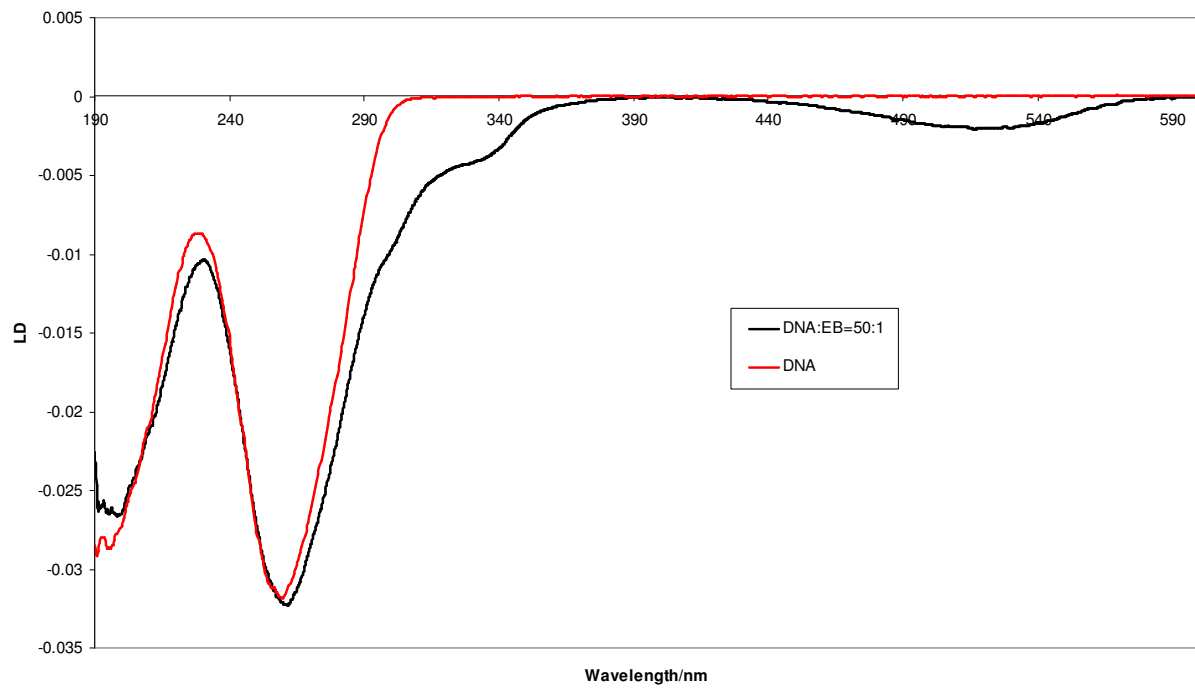| Voltage / V	Fluid state
2.0	Couette flow
2.8	Taylor vortices at top and bottom ends of cell
3.0	Taylor vortex flow
3.2	Wavy vortex flow
3.3	Spiral vortex flow
3.6	Onset of turbulent flow

Figure 1: **Comparison of LD spectra of DNA with and without EB at 3V**

The LD signal at 259 nm is larger for the EB-bound DNA sample than the pure DNA sample.

The EB-bound DNA sample also gives a signal at 520 nm.

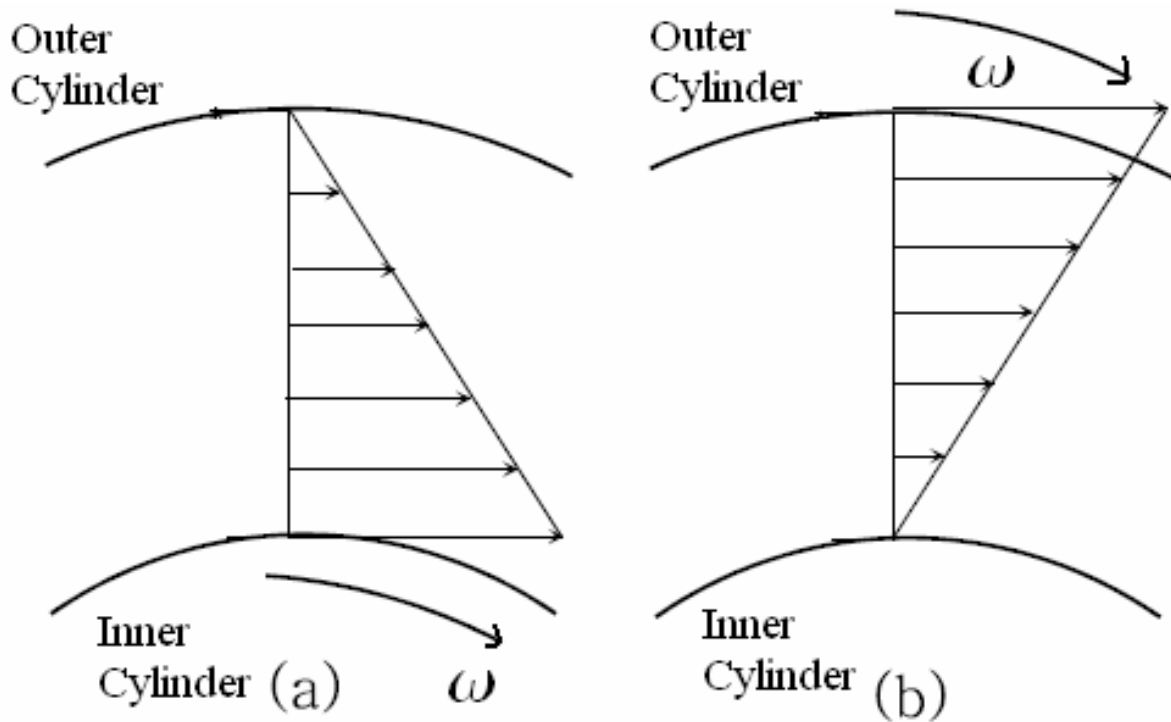Figure 2: **Fluid flow profiles for rotating cylinder set-up, viewed from above**

(a)Rotating inner cylinder; (b) Rotating outer cylinder

The longer the arrow describing the direction of the fluid flow, the faster the flow speed.
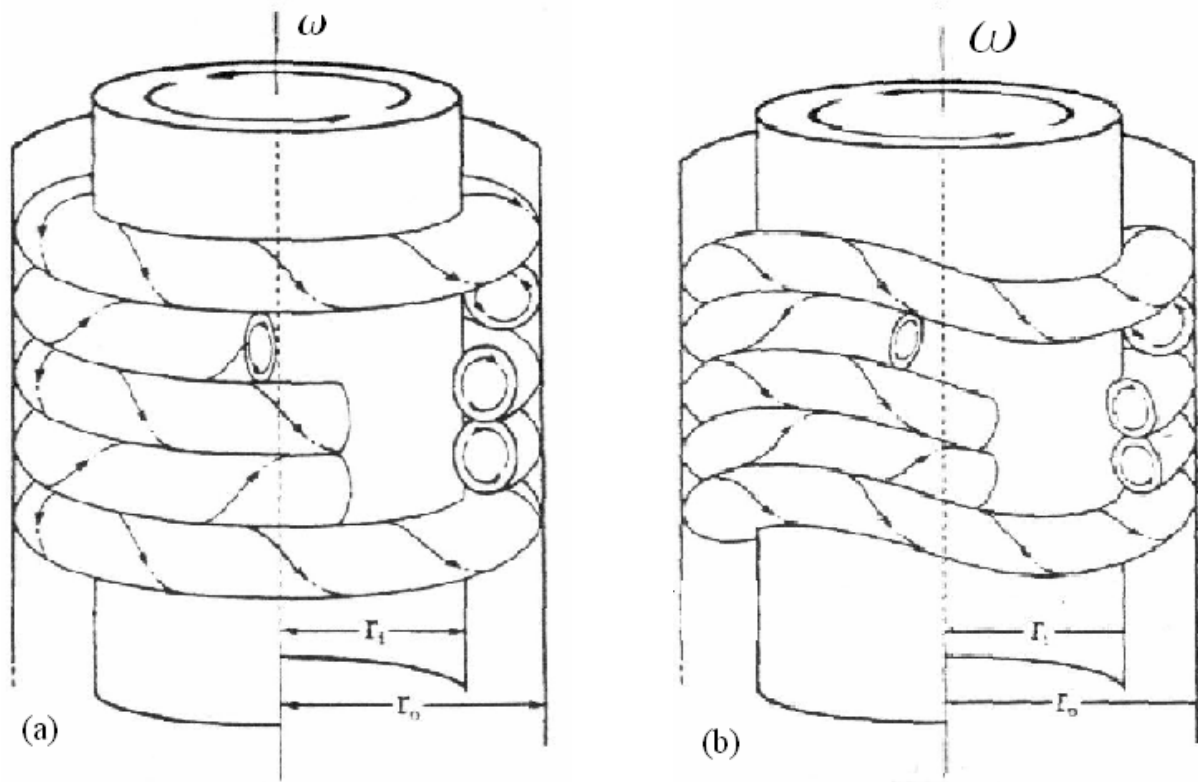
(Image from reference 5.)

Figure 3: **Flow at high R_e in an LD cell.**

(a) Taylor Vortex Flow; (b) Wavy Vortex Flow

 (Image from reference 6.)
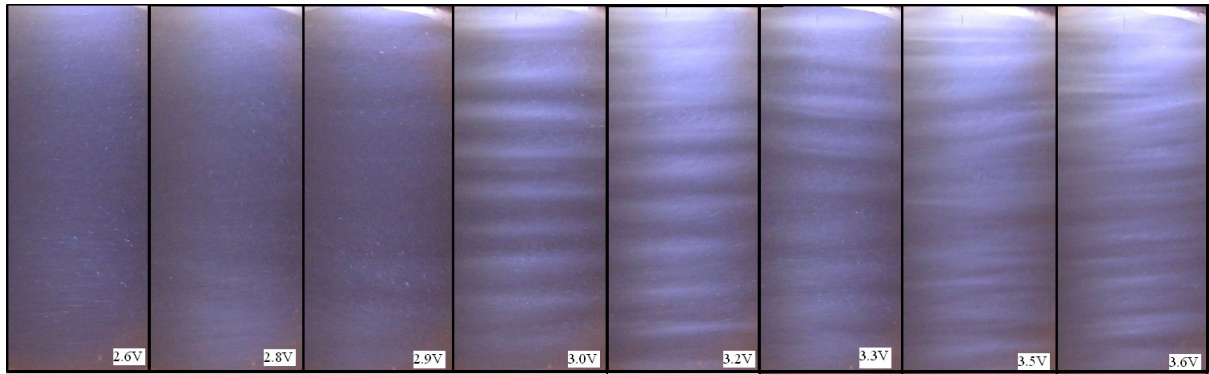
Figure 4: **Fluid flow in the LV1 cell**

A series of photographs of the fluid flow in the LV1 cell, as seen through the window in the cell, with increasing voltage from 2.6 V to 3.6 V. The images show Taylor vortices appearing at the two ends of the cell at 2.8 V and throughout the cell at 3.0 V in the form of dark and light streaks. The thickening of the streaks represents wavy vortex flow at 3.2 V and the tilted streaks at 3.3 V are spiral vortex flow with turbulent flow coming in at around 3.6 V.
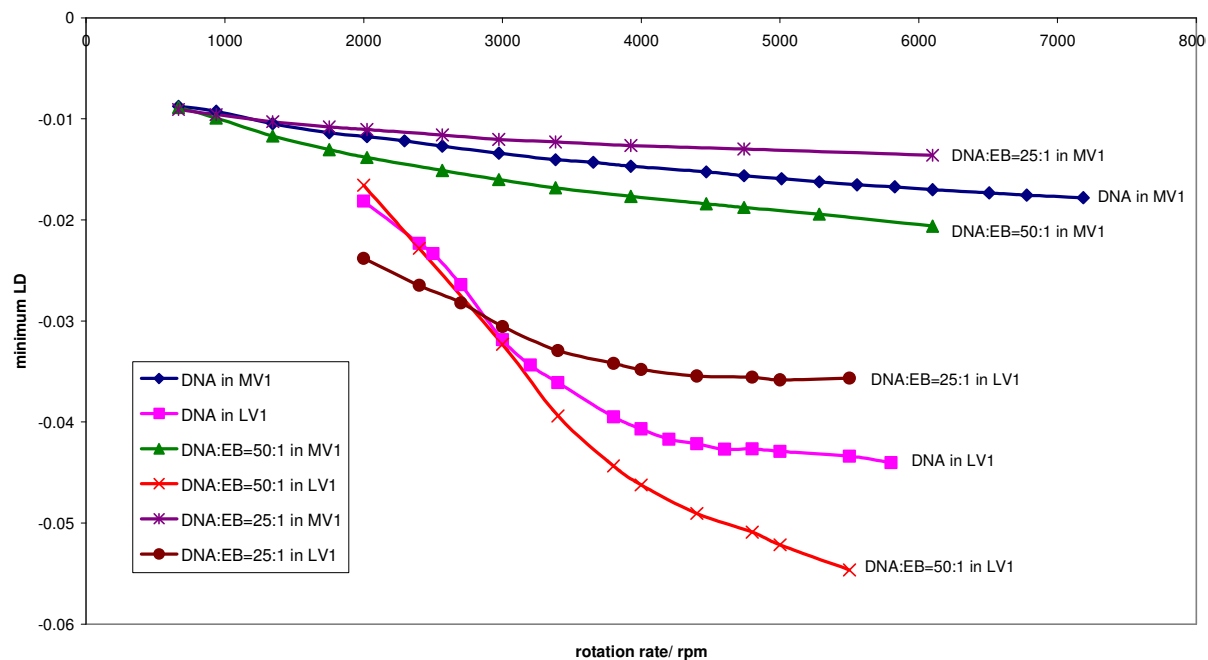
Figure 5: **Relation between negative maximum LD signal at 259 nm and rotation rate for each cell and sample**

250 µM DNA and EB were used in these samples at the ratios shown. The graphs show an increase in the magnitude of the minimum LD signal (at 259 nm) for all cells and samples, with the increase seen in the MV1 cell being more gradual than that of the LV1 cell. The magnitudes of the LV1 cell tend to level out at about 3500 rpm.
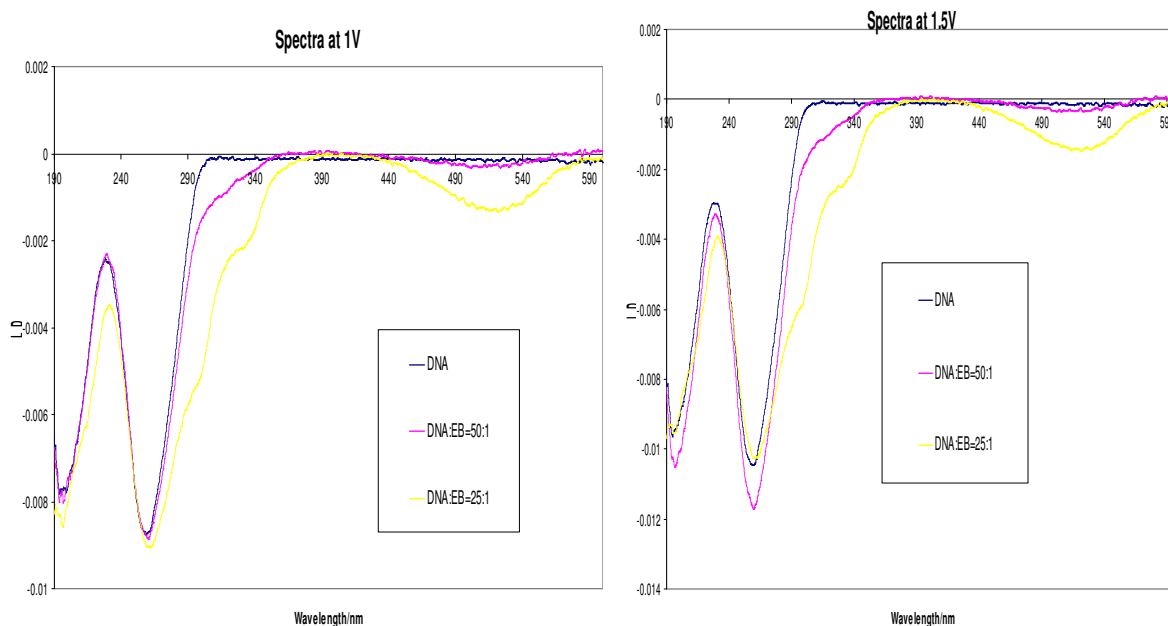
Figure 6: **Spectra for different samples in the MV1 cell at 1V and 1.5V**

250 µM DNA and EB were used in these samples at the ratios shown. The signal for the sample containing 50:1 DNA:EB is always larger than that for the pure DNA. However, the signal for 25:1 DNA:EB is larger than the pure DNA signal for voltages less than 1.5V, but is less than the pure DNA for all voltages greater than 3V.
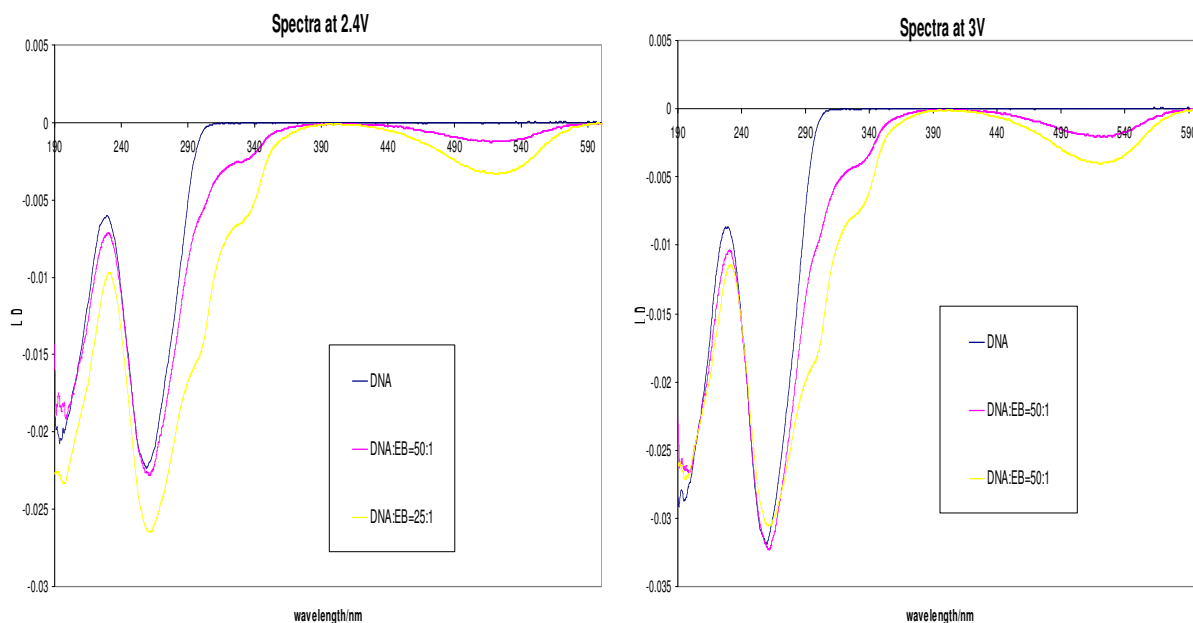
Figure 7: **Spectra for different samples in the LV1 cell at 1V and 3V**

250 µM DNA and EB were used in these samples at the ratios shown. The signal for the sample containing 50:1 DNA:EB is always larger than that for the pure DNA. However, the signal for 25:1 DNA:EB is larger than the pure DNA signal for voltages less than 3V, but is less than the pure DNA for all voltages greater than 3V.
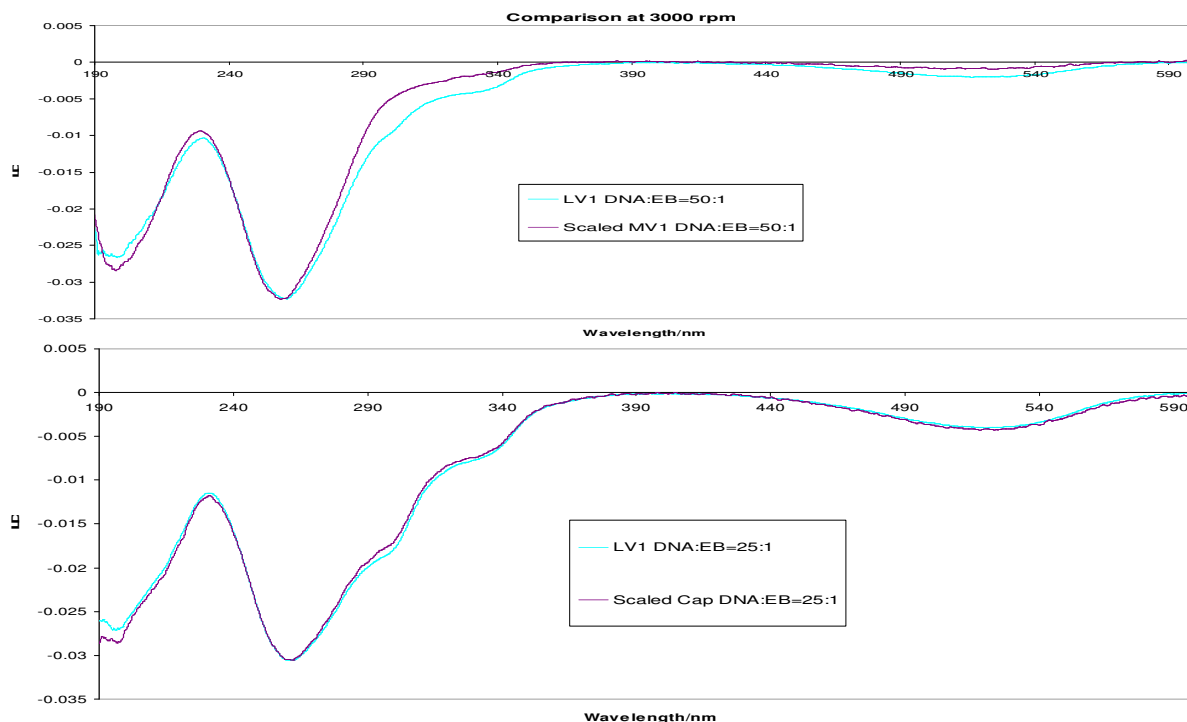
Figure 8: **Scaling up and overlaying of the spectra**

250 µM DNA and EB were used in these samples at the ratios shown. The scaled MV1 spectra of the 25:1 DNA:EB sample fits it corresponding LV1 spectra much better than that for the 50:1 DNA:EB sample.