Lawrence Berkeley National Laboratory
Recent Work

Title
THE CONTRIBUTION OF LIGHT SCATTERING TO THE CIRCULAR DICHROISM OF DNA FILMS, DNA-POLY-LYSINE COMPLEXES AND DNA PARTICLES IN ETHANOLIC BUFFERS

Permalink
https://escholarship.org/uc/item/7950n39r

Author
Maestre, M.F.

Publication Date
1978-07-01

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

THE CONTRIBUTION OF LIGHT SCATTERING TO THE CIRCULAR
DICHROISM OF DNA FILMS, DNA-POLYLYSINE COMPLEXES AND
DNA PARTICLES IN ETHANOLIC BUFFERS

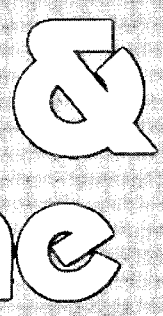Marcos F. Maestre and Charles Reich

July 1978

Donner Laboratory

DISCLAIMER

THE CONTRIBUTION OF LIGHT SCATTERING TO

THE CIRCULAR DICHROISM OF DNA FILMS,

DNA-POLYLYSINE COMPLEXES AND DNA PARTICLES

IN ETHANOLIC BUFFERS

by

Marcos F. Maestre* and Charles Reich

Donner Laboratory and Space Science Laboratory
University of California
Berkeley, California   94720

THE CONTRIBUTION OF LIGHT SCATTERING TO

THE CIRCULAR DICHROISM OF DNA FILMS, DNA-

POLYLYSINE COMPLEXES AND DNA PARTICLES

IN ETHANOLIC BUFFERS

by

Marcos F. Maestre and Charles Reich

ABSTRACT

The contribution of scattering to the circular dichroism (CD) of

DNA films with twisted structures, DNA-polylysine complexes, and condensed

DNA aggregates in ethanolic buffers of defined salt concentrations has

been studied by the use of novel measuring techniques. These techniques

include Fluoroscat cuvettes, fluorescence detected circular dichroism

(FDCD) methods, back scattering capturing devices and beam mounted

goniometer detectors. The result of the experimental measurement is that

DNA films can be made which have very large ellipticities or CD at

sharp specific wavelengths. The sign of these ellipticities is related to

the handedness of the twists, with a right handed twist producing large

positive rotations, and a left handed one producing negative rotations. The

film shows nodal angles at which the interaction with light is minimal. The

scattering patterns of both films, DNA-polylysine particles, and DNA-EtOH

condensates show that the main interaction is light scattering produced by

a resonance phenomenon similar to that produced in cholesteric liquid crystals

and twisted nematic liquid crystals. From this result we propose that the

so-called psi type CD spectrum is a manifestation of a side by side

packing of DNA molecules with a long range twisting order whose helical

parameters match the helical parameter of circularly polarized light

at specific resonance or critical wavelengths. Application of the Bragg

law for cholesteric liquid crystals gives the periodicity of the long

range ordered structures.

INTRODUCTION

As can be seen in the first paper in this series (Reich et al., 1979) the perturbations due to scattering of the CD signals on a variety of particles can be divided into two general types: a) that signal which can be corrected by recapturing the light deviated away from the detection system and b) that CD signal which is not corrected by increasing the efficiency of light collection and which may even be enhanced by increasing the envelope of light capture and/or the efficiency of measurement of the CD signal about the scattering particle.

The first type of perturbation is the best understood (Holzwarth et al. (1974), Gordon (1972), and Gordon and Holzwarth (1971). Reasonable approximations to certain types of optically active particles by the application of Mie scattering theory of a sphere have had a measure of success in explaining the absorbance spectrum and some of the so-called Duysens flattening manifested by large membranes. (Schneider and Harmatz, 1976). However, it was found in the case of bacteriophage T2 that the Mie theory was not successful in predicting the CD of the condensed nucleic acids inside the head of the virus (Holzwarth et al., 1974). In fact, the CD scattering components of the intact virus as measured by Dorman and Maestre (1973) show properties very similar to those measured in the optical activity dispersion curve of cholesteric liquid crystals (Holzwarth et al., 1974).

The optical properties of liquid crystals with a well-defined chiral structure (i.e. cholesteric liquid crystals and twisted nematic liquid crystals) are indeed remarkable in their nature. They have been extensively studied (see de Gennes, 1974 and Chandrasekhar, 1977 for

excellent treatises on the analysis of the optical properties of liquid crystals) and since these optical properties are produced by a phenomenon of macroscopic dimensions they can be easily analyzed by the use of macroscopic dielectric tensors in combination with Jones matrix calculus and Stokes vectors (see Shurcliff "Polarized Light" and Chandrasekhar 1977). We shall mention some of the most pertinent properties as follows:

1. Cholesteric liquid crystals have bands at critical wavelength
    at which strong interactions occur with impinging light of the
    correct polarization. That is, a cholesteric liquid crystal of
    right handed helical structure will interact strongly with a right
    circularly polarized wave impinging upon it if the periodicity of
    the wave matches that given by a Bragg reflection law, to wit

Eq. 1. $n\lambda = 2L \cos r'$ (de Gennes, Chapter 6, 1974) where n = order,
    λ = wavelength, L = repeat period, r' = internal angle of
    refraction.

2. If at that wavelength there exist chromophores absorbing light,
    the phenomenon of anomalous transmission occurs (Nitayananda, 1973
    and Luresh, 1976) in which an apparent excess transmission or loss
    of absorbers is measured. This is analogous to the Borrmann
    effect (1941) for x-ray diffraction when a crystal is set for
    Bragg reflection.

3. The polarization of the reflected waves is the same as that of
    the impinging wave, which is the opposite case to that of ordinary
    reflection in which there is a phase shift of 180°. Thus, for
    a cholesteric array at the critical or resonance wavelength, the
    scattered light has the same sense of polarization as the incoming

wave. However, it reflects the helical sense of the scattering structure and it is related to the pitch by Eq. no. 1. In this property it also mimics the scattering behavior of an array of helical wire antennas for circularly polarized radio waves (Krauss' Antennas, 1950). These properties were used by Freeman and Tinoco (1957) to model the interaction of light with optically active chromophores by the use of arrays of wire coils and a radar transmitter with detectors in the 1.4 cm wavelength range.

4. The most remarkable property of all is the strength of the interaction. The rotational strengths measured for cholesteric liquid crystals or twisted nematics are 10^4 times (i.e. four orders of magnitude) larger than that of the optically active components. This is because of the very strong resonance coupling with the light beam at the critical wavelength. Thus the large magnitude of the CD signals provide a strong clue to the explanation for the so-called psi type (ψ) spectra measured in DNA aggregates or particles (Jordan et al., 1972).

$\psi(\pm)$ type CD spectra have been reported with very large specific circular dichroism in films of DNA by Tunis-Schneider and Maestre (1970) and by Brunner and Maestre (1974). Magnitudes for $\varepsilon_\ell - \varepsilon_r = -300$ for films of poly [d(A-T)·d(A-T)] and values as large as -70 for films of calf thymus DNA have been measured by these workers. Large values have also been reported for polylysine-DNA aggregates ($\Delta\varepsilon = -28$) and polyuridine-polylysine complexes or aggregates, ($\Delta\varepsilon = +120$) by Carroll (1972). The paper preceding this one showed very large values for DNA-particles produced by specific concentrations of salt-EtOH buffers.

What is most remarkable about these nucleic acid complexes,
films, and aggregates is that the large values are not corrected by
large angle integrating sphere methods and in the case of the DNA-EtOH
particles the magnitudes are substantially increased. The scattering
components of the CD of the particles that are correctable by FDCD
or fluorescent methods also show remarkable directional properties which
are not typical of the ordinary scattering behavior of non-optically
active particles and of some optically active particles. For DNA-EtOH-
salt condensates or particles the main CD (correctable) scattering components
are at right angles to the incoming light beam and also in the back direction
with very little measured in the forward scattering cone.

All the above-mentioned experimental characteristics lead us to
believe that what we are measuring are manifestations of optically
active behavior of long range order of the type seen in cholesteric
or twisted nematic liquid crystals or alternatively that of helical
antennae arrays.

Thus we first attempted to develop cholesteric or twisted nematic
films of DNA and then proceeded to measure the reflection or resonance
phemonena or scattering patterns by novel experimental techniques. In
the next section we will describe in detail how we measured every component,
and its direction, of DNA films of defined helical superstructure,
DNA-polylysine complexes of varying (DNA/polylysine) ratios and
of ethanol condensed DNA, or particles, of extremely large optical
rotations or dichroism.

We shall show by the use of these techniques and materials that

-5-

psi type spectra, both positive and negative, are a manifestation of superorganization of the DNA in these particles, films, and aggregates. Moreover, these CD signals occur at specific wavelengths reflecting the repeat periodicity of the helical superstructures and that such periodicities are related to the maximum and minimum CD signals by some type of reflection Bragg scattering law.

TECHNIQUES AND METHODS

We shall describe in some detail the variety of techniques used
in measuring the optical interaction of films, DNA-polylysine particles
and ethanolic particles. Some of these techniques have been reported
previously, having been used in the measurement of scattering optically
active particles (Dorman and Maestre, 1973, Dorman et al., 1973). It
is important to understand how and precisely what each of these techniques
is measuring and how the signal from the CD machine must be further
processed to obtain the true intensity and polarization values for the
light after interaction with the scattering object.

1. Circular Dichroism as measured by the Grosjean and Legrand (1960)
   technique. This technique uses the modulation of linear polarized
   light by a quarter wave plate to which is applied an oscillating
   variable voltage (Pockels' cell or electrooptic modulator) thereby
   rotating its optical axes by $90°$, back and forth, producing a
   positive or negative $90°$ phase shift on the x, y components of
   the incoming light beam. The result is that circularly polarized
   light is produced, first with one sense of polarization, followed
   by the opposite sense (i.e., left circularly polarized light followed
   by right circularly polarized). There are several ways of producing
   this effect including mechanical rocking of the $\lambda/4$ plate or the
   use of piezoelectric modulators (Morevue modulator). The circularly
   polarized light of intensity I_0 interacts with the material under
   investigation and after the interaction a quantity I is measured.
   The usual circular dichrograph, following the design of Grosjean
   and Legrand (1960), obtains by electronic means a ratio of intensities

$$\frac{I_\ell - I_r}{I_\ell + I_r} = \frac{(a.c.\ component)}{(d.c.\ component)} = Signal \qquad Eq.\ 2$$

If the material in question obeys a Beer-Lambert absorption law for each polarization we have for

$$I_\ell = I_{o\ell}e^{-c\varepsilon_\ell \cdot d} \quad , \quad I_r = I_{or}e^{-c\varepsilon_r \cdot d} \qquad Eq.\ 3$$

and signal = $k \cdot tanh\ (\varepsilon_\ell - \varepsilon_r)$

$$Signal = k \cdot (\varepsilon_\ell - \varepsilon_r)$$ for ellipticities of the order of $5°$ or less.

$$= k \cdot \Delta\varepsilon \qquad Eq.\ 4$$

ε_r = extinction coefficient for right circularly polarized light

ε_ℓ = extinction coefficient for left circularly polarized light

The above equation implies pure absorption phenomena. No scattering (anisotropic or otherwise), fluorescence or phosphorescence is assumed, and the measuring instrument is perfect (i.e., no stray light, linear birefringence in optics or detectors or linear dichroism or cicular dichroism in the optics or detectors is assumed).

For the case in which a particle large enough to produce long range effects among the interacting chromophores, for a given range of wavelength, the above equations must be modified to include these perturbations as follows:

$$Signal = k \cdot \frac{(I_\ell + S_\ell) - (I_r + S_r)}{(I_\ell + S_\ell) + (I_r + S_r)} \qquad Eq.\ 5$$

k = instrument constant and S_ℓ = scattered left circularly polarized

light S_r = scattered right, etc.

The terms S_ℓ, S_r can be defined formally to include: a) scattered light deviated isotropically from the main direction of the beam (ordinary scattering, $S_\ell = S_r$, $S_\ell > 0$), b) scattered light deviated anisotropically (i.e., differential circular dichroism scattering). Linear dichoric scattering can also occur and can be of importance in film work later. ($S_\ell \neq S_r$, S = function of orientation angles with respect to light beam.)

3. Stray light contributions from cuvette window reflections, wall reflections, dust in solutions and in cuvette windows and reflections from the optics.

4. The signal can also include non Beer-Lambert contributions from such particles whose effective extinction is almost total, in which case the effect is manifested as a Duysens flattening distortion for the circular dichroism signal. This has been seen in membranes (Schneider, 1973) whole red blood cells (Gitter-Amir et al., 1976) and in crystals of protein (in this last case reversibly) (Chiu and Maestre, in preparation).

5. Most importantly, the signal can include enhancement scattering due to a manifestation of anomalous transmission. $S_\ell \neq S_r$ and S_ℓ or S_r having a positive value so as to make it appear that less absorption occurs for a given set of chromophores (i.e., anisotropic hypochromicity) for a particular polarization and region of the spectrum. (See Chandrasekhar, Liquid Crystals 1977).

The above equations describe the case in which the detection system is centered about the incoming light beam and also measures the

incoming light plus all scattering components that lie within its acceptance measuring cone.

If the technique requires measurements outside the main beam (i.e., CD as a function of scattering angle by a beam mounted goniometer detector system) the terms I disappear and we have now

$$\text{Signal} = k \cdot \Delta\theta \cdot \frac{S_\ell(\theta) - S_r(\theta)}{S_\ell(\theta) + S_r(\theta) + \text{stray light components}} \qquad \text{Eq. 6}$$

$\Delta\theta$ = angle of acceptance of detection system.

Note: at a given angle θ the total scattering $S_\ell(\theta) + S_r(\theta)$ can be very small so that the stray light can make quite a large contribution. Conversely, if both stray light and total scattering are small at a given angle θ we may have a very large apparent CD at that angle. Thus the interpretation of these types of measurements should be done with caution and should be checked by differing techniques as we did in our measurements.

6. For the measurement of the CD of films and the scattering CD
    components the equations now become functions of the angles
    of incidence i (tilt angle), the angle of internal refraction
    r and the orientation angle about the axis of the incoming light
    beam as shown in Fig. 1a. It has been shown by Tunis-Schneider
    and Maestre (1970) that a film with linear orientation will
    introduce an apparent circular dichroism component to the intrinsic
    CD as given by

$$CD_{\text{apparent}} = CD_{\text{intrinsic}} - 0.298 \; p \cos 2\phi \qquad \text{Eq. 7}$$

where p = linear dichroism and ϕ = angle of rotation about the optical axis.

Therefore, it is important when measuring the CD of films that have been manipulated in their manufacture to check for possible linear dichroism components by rotating the film assembly about the optical axis.

NOMENCLATURE AND DESCRIPTION OF TECHNIQUES

1. Small angle of acceptance circular dichroism ("normal CD"). Acceptance angles range between $3°$ to $5°$. This is the standard CD machine configuration.

2. Large angle acceptance CD. This is usually measured with a large 2" diameter end window PM tube (Dumont no. 2706 or Hamamatsu R375) especially selected for low birefringence artifacts. The angle of acceptance is increased by bringing the tube close to the cuvette (see Dorman et al. (1973) for a complete discussion of this technique).

3. Fluorescence cuvette integrating methods. As described by Dorman et al (1973), these are two cuvettes, one inside the other. The outer one is filled with a fluorescent solution whose main absorbance band covers the region of the spectrum of interest, in this case sodium salycilate in absolute ethanol. The mechanism of correction utilizes the fact that all light that is not absorbed in the inner chamber eventually hits a fluorescent molecule in the outer chamber. What the photomultiplier sees is the fluorescence emission due to excitation by this light. The fluorescence cuvette covers most of the scattering envelope except for a region towards the back where the light beam impinges upon the first window of the inner cuvette containing the material of interest.

4. Fluorescence Detected Circular Dichroism (FDCD) (See Fig. 1b). As shown in the preceding article, this technique, properly used, corrects for all the differential scattering including the back scattering cone. It is probably the most efficient means of recovering information scattered in all directions. An interesting modification of this method is the one described in Fig. 1c where a DNA film is sandwiched

between two fluorescence cuvettes, a back cuvette covering the back
scattering cone and a front cuvette measuring the front scattering
cone. This technique allows the <u>individual</u> and <u>separate</u> measurement
of these two regions of the scattering envelope. This technique was
used to measure the back scattering lobes of the DNA films, the
DNA-EtOH condensates, and the DNA-polylysine aggregates.

5. A similar technique is shown in Fig. 1d where a front-
surface mirror recovers a large part of the back scattered light and
reflects it into the detector.

6. The beam goniometer mounted detector (Fig. 1a) provides
information on the CD scattering as a function of scattering angle.
The scattering acceptance angle is defined by the width of the slits
in front of the photomultiplier tube.

7. Vertical assembly and tilt angle device. The manufacture
of films that are extremely wet implies that the film has to lie parallel
to the horizontal until it is reasonably rigid. To measure the CD
of these wet films and the tilting angle studies we used the instrument
developed for CD film studies by Maestre (1970). This device allows
the rotation of the film assembly about the light beam (rotation of
ϕ, Fig. 1a) in order to study linear dichroism artifacts and the detection
of "null" scattering angles i by tilting.

PREPARATION OF THE FILMS

This is an art form and practice makes perfect. For example, calf thymus DNA is placed in a totally dehydrated lyophylized form on top of the bottom fused silica window. Distilled water or buffer is added drop by drop until all the DNA is submerged under a solution droplet. After the DNA fibers have swollen and gone into solution to form a very viscous liquid they are covered very gently by the top quartz plate and then pressed down with the proper twist (counterclockwise or clockwise). This twist is small – only about 45° to 90° rotation initially. Then the film is placed in the vertical assembly and its CD is measured. Usually the rotations are so large that the maxima of the CD peaks cannot be measured in the Cary 60 CD machine (2° maximum scale). The film is then squeezed some more to reduce the thickness enough to bring the measurement on scale. Keep trying! After measuring, the twist is then reversed until an opposite CD is obtained (usually about 90° to 180° twist) and so forth. The films usually have an optical density at 260 nm of between 0.3 to 0.7. Usually about four to five reversals can be done before the films become so disoriented that one observes the ordinary CD of DNA (conservative CD spectra). One must guard against introducting linear orientation components and this is checked by rotating the whole film assembly by 90°.

PREPARATION OF THE T7 PHAGE DNA-POLYLYSINE SCATTERING COMPLEXES $\psi(-)$ TYPE PARTICLES

0.85M and 1M salt. A solution of T7 phage DNA was obtained by phenol extraction from intact T7 bacteriophage and then dialyzed into 1.5M NaCl, 0.01 sodium cacodylate, pH 7.0. Poly-L-lysine hydrobromide (VIIB) was purchased from Sigma Biochemicals. This was reported to have a molecular weight of 32,000 and a DP of 150 by the supplier. A sample of the polylysine was submitted to the microanalytical laboratory of the chemistry department, University of California, Berkeley, for an elemental analysis in order that accurate solution concentrations of the polynucleotide could be obtained. Solutions were made up in 1.5M NaCl, 0.01 M sodium cacodylate, pH 7.0 and added to the above DNA solution so that a final 1:1 ratio of nucleotide to lysine residue ws obtained. In 1.5M salt, polylysine and DNA do not form particles while at lower salt concentrations, in which the two species do condense, a 1:1 ratio of lysine to DNA base is observed (Shapiro et al., 1969). The DNA, polylysine mixture was then dialyzed into 1M NaCl, 0.01 sodium cacodylate, pH 7.0, Shapiro et al. (1969) reported that at 0.85M to 1.0M NaCl, the salt concentration was at an optimum for the formation of reversible particles having $\psi(-)$ DNA character.

0.15M salt solutions. Two solutions of T7 DNA: polylysine in 0.15M NaCl, 0.01M sodium cacodylate, pH 7.0 were prepared using differing methods. Solution No. 1 was prepared by dialyzing the DNA, polylysine mixture into 0.85M NaCl and then into 0.15M NaCl, 0.01M sodium cacodylate, pH 7.0. Solution No. 2 was prepared by dialyzing the DNA, polylysine mixture into 1.0M, 0.7M, 0.4M and finally 0.15M NaCl, 0.01M sodium cacodylate, pH 7.0. Both types of particles showed the CD characteristics of $\psi(-)$ type particles.

E. coli DNA-polylysine complexes. E. coli DNA was obtained

from Worthington Biochemicals. A solution was made up in 1.5M NaCl,

0.01M sodium cacodylate, pH 7.0 and run through a 23 gauge needle 11 times

in order to reduce the size of the DNA molecules. The polylysine

was then added so that a final 1:1 ratio of lysine to nucleotide was

obtained. ψ(-) DNA particles were produced by dialysing the polylysine,

DNA mixture vs. 1.0M NaCl and 0.80M NaCl, 0.01M sodium cacodylate, pH 7.0.

In this case the salt concentrations were the same as those of Shapiro

et al. (1963).

E. coli DNA ethanolic condensates. E. coli DNA solution was placed

in 80% ethanol-buffered solutions as reported previously (Reich et

This particular preparation was highly concentrated, however, with

an optical density value of 10 at 260 nm, prior to the addition of the

ethanol. With this method most of the DNA is precipitated. The precipitated

material is concentrated in a high speed centrifuge after evaporating the

ethanol off under vacuum. The DNA is then redissolved in buffer and the

addition of ethanol to 80% w/w is repeated. In this manner DNA-ethanolic

ψ(+) particles with very large positive ellipticities are obtained.

(Fig. 14).

Computations of scattering Components

Several modifications have to be introduced in the FDCD equations

to allow computation of the back scattering or resonance reflection intensities

for each polarization. The appendix shows the equations for the following

cases:

i) Film with no scattering components gives with both cuvettes filled in Fig. 1(c)

$$\theta_F = (14.32)(2.303)\ A/(10^A+1)$$

$$\Delta A = \frac{\theta_F(10^A+1)}{32.98}$$

ii) Film scattering all in the back direction, both cuvettes filled in Fig. 1(c)

$$\Delta A = \frac{\theta_F(10^A+1)}{32.98}$$

iii) Film with scattering all in the back direction with the back cuvette full of fluorescer. Fig. 1(c)

$$\theta_F = -14.32\ \frac{\Delta A_s}{A_D}\ (10^{-A_F} - 10^{-A})$$

$$\Delta A_s = \frac{-\theta_F A_D}{14.32(10^{-A_F} - 10^{-A})}$$

where

ΔA_s = Differential back scattering component or resonance reflection (see Appendix)

A_D = Absorption of film

A_F = Absorption of fluorescer in back cuvette

A = Total absorption and scattering extinction of complete system

These are the equations used in computing the components obtained by the method described in Fig. 1(c).

RESULTS

Film experiments. Figures 2 to 6 show the results of a variety of

techniques employed in measuring the interaction with circularly polarized

light. Fig. 2 displays the circular dichroism as a function of twist.

The magnitudes at the maximum are $\Delta\varepsilon$ (right handed twist) = + 120

at 293 nm and + 100 at 233 nm. When the film is twisted clockwise

(i.e., a left handed twist) we observe $\Delta\varepsilon$ (left handed twist) = -210

at 280 nm and $\Delta\varepsilon$ = -169 at 222 nm. These are enormous values for

the CD of DNA. DNA in solution has values on the order of $\Delta\varepsilon$ = +2.2

at 275 nm and $\Delta\varepsilon$ = -3.3 at 245 nm. The CD is shown to be reversed

once more by again twisting counterclockwise. It should be noticed

that the positions of the maxima and minima shift toward lower wavelengths

as the films dehydrate with time or are squeezed tighter. This could

be interpreted as a change in some periodic parameter in the super

organization introduced by the twisting. It should be also noted

in Fig. 2 that rotating the whole assembly by 90°, a test for linear

dichroism artifacts (Eq. 7) that changes the signal shape little and only

affects the values by lower magnitude effects, are probably caused by nonuniform

thickness of the film.

Fig. 3 shows the CD of a film sandwiched between the two cuvettes

as shown in Fig. 1c. The direct CD measured showed values of $\Delta\varepsilon$ =

-260 at 287 nm, and -280 at 232 nm. 1. When the back scattering cone was

measured by the use of the FDCD technique by placing the α-napthylamine

fluorescer in the back cuvette we obtained a remarkable back scattering

CD signal with $\Delta\varepsilon$ = -120. The front scattering measured by FDCD

still gave a large value (-160). For the peak at around 230

the value either changed sign or was considerably reduced in magnitude.
Unfortunately in this region the α-napthylamine used in the FDCD
measurement absorbs strongly so the signal to noise ratio is poor
and consequently the measurement is not trustworthy. When a similar
measurement of the back scattering cone is made for a different DNA
film with counter-clockwise twist ("positive CD") by the mirror technique,
we again obtained similar behavior. This film had values of $\Delta\varepsilon$ =
+540 at 284 nm and +321 at 227 nm measured with regular CD (Fig. 4).

The back reflection mirror measurement shows a value of $\Delta\varepsilon$ = +742
at 297 nm and +414 in the 220-230 nm region. One must remember that
these magnitudes are only for true scattering components and can be
exaggerated by the denominator in Equation 6. It is obvious, however,
that in these cases, at least for the CD signals in the 270-290 nm
region, the back scattering components have the same polarization and
approximately the same order of magnitude as the signals measured
in the forward direction.

The behavior of the scattering components for the DNA film as
a function of scattering angle are shown in Fig. 5. The CD shows
a complicated behavior as the angle increases but in the region of
the large minimum the sign remains negative. Thus in that region
of the spectrum the scattered light shows essentially the same polarization
as is measured in the "ordinary" CD measurement. Of interest is
the CD scattering in the nonabsorbing regions, $\lambda > 300$ nm. These CD
"tails" into the visible have been interpreted as loss of light of
a particular polarization by deviation away from the detector (Dorman
and Maestre, 1973). For scattering angles $\theta = 0°$ to $\theta = 10°$ we see

that there is a change in sign and this indicates that scattered light of the opposite polarization is being measured. Thus in regions of the spectrum away from the absorbance bands, the CD scattering can be a complicated function of the scattering angle. Similar behavior will be seen later in the CD scattering of DNA-polylysine aggregates.

Fig. 6 is a study of the variation of "normal" CD measurement as the angle of incidence of the light beam impinging upon the films is altered. These measurements were done to see if the behavior of the twisted DNA films in any way mimicked that of twisted nematics or cholesteric liquid crystal structure. For this particular film we found that a minimum in the magnitudes of the CD peaks occurred at about a $\pm20°$ tilting angle. This implies that there are certain directions in which "nulls" occur (i.e., the interaction with circularly polarized light is a minimum or is <u>equal</u> for both polarizations).

The above results have shown that a) the CD of twisted DNA films has very large magnitudes (i.e. large interactions), b) is dependent on the sense of the twist of the film, c) can be reversed by reversing the sense of the twist, and d) the CD signal and CD scattering signal are very sensitive to the orientation of the film vs the light beam and/or scattering angle of the light after interaction. In analogy to the optical properties of liquid crystals (cholesteric or twisted nematics) we propose the interpretation that the CD signals are a result of the long range order and organization of DNA closely packed in films or aggregates.

DNA-POLYLYSINE AGGREGATES EXPERIMENTS

Fig. 7 to Fig. 10 shows the CD scattering behavior of T7 phage DNA-polylysine and E. coli DNA-polylysine complexes at different stages of aggregation. Fig. 7 shows attempts at correction of the CD spectrum by FDCD methods. Presumably FDCD techniques as shown by Reich et al. (1979) will correct for all the scattering contribution that involve only deviation of the light away from the detector acceptance cone. It is shown that away from the negative CD band at 245 to 250 nm the FDCD correction does a fair job of eliminating the CD tails in the region between 270 nm to 290 nms. However, at the same time it increases the magnitude of the minimum from $\Delta\varepsilon(249 \text{ nm}) = -3.6$ to $\Delta\varepsilon = -5.0$. This is an analogous enhancement of the signal similar to the values reported in the EtOH-DNA particles study preceding this report. Fig. 8 shows two different preparations of T7 phage-DNA polylysine particles with the corrected FDCD spectra. The magnitude of the CD minima on those particles is about four times in value of that reported in Fig. 7. However, the CD tails are again corrected by the FDCD technique. Another type of particle, E. coli DNA-polylysine particle (Fig. 9), shows that its CD is essentially unchanged by the FDCD correction. It is evident from those particle studies that only a portion of the CD scattering pattern is "correctable" by large acceptance angle methods and that for those particles as well as the EtOH-DNA particles (Reich et al. 1979) a certain type of signal is not correctable and is even enhanced by increasing the collection efficiency of the detection system.

The CD scattering as a function of scattering angle θ (Fig. 10)

shows remarkably similar behavior to that of the DNA films (Fig. 5). The back scattering as measured by the mirror collection device (Fig. 1d) again shows the qualitative characteristics of the forward CD scattering or "normal" CD measurement.

Fig. 11 shows the CD scattering behavior of E-coli DNA-EtOH particles having extremely large CD values; i.e., $\Delta\epsilon$ (normal, 275 nm) = +80. For such a large "resonance" CD the FDCD methods enhanced the CD at the maximum up to a value of $\Delta\epsilon$ = +120.

One of the characteristics of the FDCD method is that the efficiency of correction increases as the total optical density of the solution reduced. In Fig. 11 a very low concentration of the DNA-EtOH particle solution is measured with a concomitant increase in noise in the signal. It can be seen that the magnitude of the signal does not change radically so that the CD spectrum at the resonance band is not a simple differential scattering signal but probably a manifestation of a liquid crystal (cholesteric or nematic) resonance reflection band having the associated anomalous transmission phenomenon.

In Fig. 11 it is also shown that the CD scattering at $\theta = 13°$ at the resonance band wavelength gives a scattering component having the same polarization as the forward scattering.

DISCUSSION AND CONCLUSIONS

We propose the thesis that the optical activity of the films, the DNA particles and the polylysine:DNA aggregates is a manifestation of a type of long range chiral asymmetry similar in nature to that found in cholesteric liquid crystals and twisted nematic liquid crystals.

The evidence for the above assertion is as follows:

a) Very large circular dichroism and ellipticities. In the films the circular dichroism ranges from $\varepsilon_\ell - \varepsilon_r$ = +800 to -220, two orders of magnitude larger than the intrinsic magnitude of DNA solution. For DNA ethanolic condensates, we have shown values as large as +150. For DNA histone complexes values of the order of $\Delta\varepsilon$ = -80 have been measured (Dorman; Maestre and Fasman, in preparation).

b) The sense of the measured optical activity can be directly correlated with the sense of the twist of the film. Moreover, for any given film this is a reversible phenomena; i.e., if the sense of the twist is reversed, so is the sign of the measured CD bands. Thus in Fig. 1 we show that a film with a counterclockwise twist of the top quartz plate, which produces a right handed helix, will produce large positive ellipticities at wavelengths, λ_1 = 298 nm and λ_2 = 233 nm. When this film is twisted in a reversed (clockwise) motion inducing a left handed helical twist or distortion we observe large negative values with minima at λ_3 = 280 nm and λ_4 = 210 nm.

This reversal of ellipticity can be produced several times until the orientation of the film is destroyed by the continual manipulation of the quartz plate.

c) The position of the CD maxima and minima of the films

seems to be related to their degree of hydration or thickness. As

the films dry out or are squeezed mechanically so that they become

thinner, the positions of the bands shifts towards the blue; i.e., smaller

wave length. Conversely, when the films are hydrated again the wavelength

of the bands increase in value (red shift). This particular property

mimics that found in cholesteric crystals in which a Bragg law behavior

is seen at those wavelengths of circularly polarized light which match

the periodicity and sense of helicity of the liquid crystal. For

cholesterics or twisted nematics (Maugin limit) we have

$$n\lambda = 2L \cos r' \qquad \text{Eq. 8}$$

where n = order, λ = wavelength at which the anomalous reflection or transmission

occurs, L = periodicity of helix, and r' = the internal refractive angle

(obtained from Snell's law). Thus for a beam at perpendicular incidence

and n=1 (first order reflection) we conclude that at wavelength λ = 2L

we should measure a very strong circular dichroism. However, in most

of the CD spectra reported here we measure two bands. Even in the

case where there is only a positive band, as in the DNA ethanolic particles,

we see evidence of another band in the shorter region of the spectrum.

It is probable that these two bands are a manifestation of two different

modes of interaction of a large order helix with light. Furthermore,

these bands show certain spatial distributions which also imply this

interpretation, as we shall discuss below.

    d) The DNA films also show a remarkable behavior as a function

of angle of incidence of the light beam. As shown in Fig. 6, as the

film is tilted with respect to the light beam incidence we go through

a minimum angle at $\pm 20°$ from the vertical in which the first negative

peak at $\lambda = 270$ nm disappears. As the angle is increased we get a recovery of the CD peak. This is the kind of optical behavior that is seen in cholesteric liquid crystals with a well defined structure and is interpreted as a shift in critical wave length outside of the absorbance region produced by the Bragg law behavior.

The scattering behavior of the films, the DNA-EtOH particles and the nucleohistone-DNA and polylysine-DNA particles show that the measured CD is mainly a scattering resonance phenomenon that occurs at certain critical wave lengths. In particular the measurement of the back scattering regions of the film show magnitudes of the same order as those measured in the forward direction. At the critical wavelengths the circular polarization of the scattering seems to remain constant as a function of scattering angle. However, in the region of the spectrum away from the critical wavelength we observe a reversal of the sense of circular polarization. Thus, different regions of the CD spectrum of these structures behave with a different spatial distribution which indicates that they are produced by different scattering mechanisms.

e) What is most remarkable is the large increase in the magnitude of some of the CD curves at the critical wavelength when attempts are made to recover all of the scattered light by large angle integrating methods such as FDCD or fluorescent cells. It is as if more light is coming out of the system at a given critical wavelength than is being put in. Thus, when the scattered light is measured over a sphere of 4π steradians there is a sudden increase in the measured radiation at the critical wavelength. A similar mechanism exists in cholesteric liquid crystals analogous to the Bormann effect (1941) for x-rays,

and investigated by Nitayananda (1973) and Luresh (1976). This
can be viewed as an anomalous transmission phenomenon.

In our particular case we propose the following mechanism for
the critical wavelengths: The light is absorbed by the normal electronic
transition in the nucleic acid bases of the DNA. However, this energy
which is usually lost by radiationless transition processes in the
normal DNA structure is now coupled to the helical super organization
matching the polarization and repeat periodicity of the incoming light
beam. In analogy to a cholesteric liquid crystal this will cause
a resonance phenomenon such that the absorbed energy is re-radiated
in particular directions wih the same polarization and wavelength
as the incoming light beam. This mechanism is well known in the interaction
of helical antennae with circularly polarized radiowaves. A model
system of optical activity was studied by Freeman and Tinoco (1957)
using helical coils of copper wire and a microwave generator. These
workers found that the coils showed strong cotton effects at wavelengths
determined by the periodicity of the wire helix and given by the equation
$n\lambda \approx 2L$ (where L is a linear function of the repeat period of the
wire coil). There were two circular dichroic bands, one at the axial
mode of interaction of the coil ("end fire" mode in antenna theory,
see Krauss, 1950) and the other at shorter wavelength at the normal
mode of oscillation of the coil.

Fig. 3 shows the back scattering cone as measured by FDCD and
Fig. 4 shows it as measured by the mirror back collection device. In
both cases the higher wavelength CD peaks have the same polarization
as the CD peak measured in the forward direction. The short wavelength

peak is either absent or suppressed in magnitude. We are led to the
assumption that this mode is probably oscillating in a perpendicular
direction to the light beam and thus is weakly detected by back scattering
detection devices.

Therefore, because of reasons enumerated above we have concluded that
the extremely large CD values reported for films, nucleohistones and
certain types of condensed DNA at specific wavelengths reflect a certain
long range order of a helical nature. The sense of the helicity is
reflected in the sign of the so-called psi spectra (i.e., a positive
CD reflects a right handed helical arrangement and a negative CD reflects
a left handed coil). Furthermore, the positions of these large peaks
reflect the repeat periodicity and are probably given by a Bragg reflection
law analogous to the law for cholesteric liquid crystals, Eq. 8, or
to that obtained for helical antenna arrays (Krauss, 1950).

$$n \lambda = 2L \quad \text{axial mode}$$
$$\text{or} \quad n \lambda = 2d \quad \text{normal mode}$$

Eq. (9)

L = helical periodicity, d = diameter of helix.

It is important to realize that it is the helical ordering that
provides the mechanism for the large CD values. Any random packing
of nucleic acids will not produce any change in CD except that produced
by changes in secondary structure. Gosule and Schellman (1976) have
reported DNA-putrescine and DNA-spermine aggregates of very defined
morphological sizes in which no changes in CD were detected. Similarly
Tunis-Schneider and Maestre (1970) have shown that films prepared
in the proper way show little difference from the CD of DNA and RNA. We

have found that it is easy to change one of the psi type films to
obtain the regular conservative CD spectra of DNA by the proper manipu-
lation of the quartz plates.

It is also important to note that the secondary structure of the
DNA has little or nothing to do with the psi type spectra. It may
be possible to correct for the psi spectra by tilting the films until
a null angle is obtained and then measure the remaining spectra which
are presumably caused by the secondary structure, but this is a difficult
experimental problem. In particles in solution this will be possible
only if the psi type bands are fortuitously far away from some of
the significant CD spectrum regions of the DNA which reflect secondary
structures (for example, the 270 - 290 nm region).

The last remaining topic of discussion concerns those CD scattering
perturbations that can be corrected by integrating methods; e.g.,
the long tails outside of the absorbance regions of the spectrum.
These tails are also measured in the CD spectrum of cholesteric crystals
(Holzwarth and Holzwarth, 1973). Invariably the tails can be corrected
by FDCD or Fluorscat cell methods indicating that the light is lost
by differential scattering (i.e., one polarization is deviated more
from the optical axis that the other). The explanation for this behavior
is reasonably straightforward. It is a manifestation of the entire
size and shape of the particle (i.e., Mie or "form" type of scattering)
but in this case differential scattering occurs for different polarizations.
This is also true for bacteriophage T2 and T4 (Dorman and Maestre
1975, Holzwarth et al. 1976). These tails were shown to be proportional
to the Kronig-Kramers transforms of the Fluorscat corrected signal

of the psi type spectrum. Therefore, in this sense it is a true Mie type

optically active or "form" dichroic scattering phenomenon. The DNA-

EtOH particles show a more complicated behavior since for this type of

particle the corrected scattering is at right angles to the beam. This

is probably a reflection of the smaller size of the particle obtained

for this particular set of measurements. We did not measure a large back

scattering reflection with FDCD and the mirror device for these particles;

thus most of the scattering components are probably perpendicular to the

light beam. Therefore this type of "correctable" CD scattering is a

reflection of index of refraction dispersion and the bulk size, shape

and sense of helicity of the particle.

The nature of this type of scattering is analogous to that of

form birefringence manifested by arrays of rods or plates when studied

with linearly polarized light (Wiener, 1926). In this case the form effect

is a circular birefringence form effect which differentiates between

right handed and left handed helical structure.

It should be noted that in the case of the polylysine T-7 DNA

condensates (Fig. 10) there is a strong back scattering component;

therefore the resonance mode of liquid crystal type scattering points

to a different organization than that of DNA-EtOH condensates.

CONCLUSIONS

From the studies of the CD and the scattering differential CD
of films of DNA with twisted helical structure, polylysine-DNA
psi type particles (negative) and DNA-EtOH salt aggregates (positive
ψ spectra) we have arrived at the following conclusions:

a) Psi type CD spectra are mainly a manifestation of resonance
scattering phenomena with an associated anomalous transmission in the
absorbing bands.

b) The CD bands outside of the absorbing region can be corrected
by the use of devices that increase the collection geometry such as
Fluorscat cells and FDCD methods. This is the birefringent dispersion
component and is not only a function of the bulk index of refraction
but reflects the size and shape of the particle as pointed out by
Holzwarth and Holzwarth (1973), and Holzwarth et al. (1974).

c) We have shown that the CD scattering of these particles can
be divided into two types which reflect the different properties of
the scattering structure. Information can be obtained on the long
range organization by demonstrating that some of the scattering exhibits
optically active behavior such as is found in cholesteric crystals
or twisted nematic crystals. In such cases the sense of twist or super helix
can be determined from the sign of the CD bands. A right handed helix
gives positive CD signals and a left handed helix gives negative CD.
signals. The periodicity is given by the Bragg law: $n\lambda = 2L \cos r$.
Thus for a typical psi (-) nucleohistone aggregate which exhibits a
critical $\lambda = 270-280$ nm, the helical periodicities would be on the
order of 1350 to 1400 Å. As indicated in the experimental results

it is probable that DNA molecules stacked side by side and dehydrated

will twist from a parallel arrangement to a left twisted arrangement

as they dehydrate and come closer together.

e) In the few cases in which correction can be obtained at low

salt concentration for DNA-polylysine complexes we find that the corrected

CD spectrum has a depressed band in the 270-290 nm region indicating

that this salt concentration the secondary structure of the DNA is changing

towards the C form geometry. However, since the resonance bands increase

in magnitude it is impossible to obtain any information concerning the

secondary structure of these molecules. It would be possible to measure

such information if some type of quasi-liquid crystal bulk organization

(such as a film) could be obtained and then measurement could be performed

at certain null angles given by the Bragg reflection law for liquid

crystals.

f) A model of the geometry of DNA in these aggregates is shown

in Fig. 12. By a helical array we mean a parallel organization of

DNA molecules which are then twisted slightly so that each layer of

DNA molecules is at a slight angle of twist with respect to the two

neighboring ones. Each layer of DNA would be then twisted with respect

to the two neighboring layers in a manner similar to that found in cholesteric

liquid crystals or twisted nematic liquid crystals. The long range

order of these layers would then provide the necessary helical periodicity

to produce the strong resonance bands measured by circular dichroism.

The planes in the figure are placed far apart for clarity but in the

DNA film we assume that these planes are in proximal contact. Indeed

from our experiment this contact seems to be so close as to bias the

twist in only one direction since DNA films tend to give negative psi type spectra if not forced into a given twist mechanically or forcibly disoriented. Therefore it is probable that some type of minimum energy configuration exists between quasi-parallel arrangements of DNA molecules that imposes a long range left-handed organization as in Figure 12(b).

g) The above would explain the behavior of condensed DNA in bacterio-phage T2, T4, T6 in which an apparent liquid crystalline component negative in value and centered at 260 nm is obtained (Holzwarth et al. 1974). The periodicity for the condensed T2 DNA package then would be of the order of 1300 A if we assume that it obeys the Bragg scattering law, Eq. (8), for a first order reflection. The CD tails would also be a property of the size of the particle since they are caused by birefringence dispersion. Smaller bacteriophage such as T5 and T7 (Maestre et al. 1971) show a much smaller contribution to the CD tails.

REFERENCES

Borrmann, G., Physick. 2 42 157 (1941).

Brunner, W.C. and Maestre, M.F. (1974) Biopolymers 13, 345.

Carroll, D. (1972) Biochemistry 11 426 and Thesis, University of California, Berkeley, CA.

Chandrasekhar, S. (1977) Liquid Crystals, Cambridge University Press, London.

De Gennes, P.G. "The Physics of Liquid Crystals" (1974) Clarendon Press, Oxford.

Dorman, B.P. and Maestre, M.F. (1973) Proc. Nat. Acad. Sci. U.S. 20 255.

Dorman, B.P., Heart, J.E., and Maestre, M.F. (1973) Metheod Enzymol. 27D, 767.

Duysens, L.M.N., Biochem. Biophys. Alta 19, 1 (1956).

Fasman, G.D., Biochemistry 9, 2815 (1970).

Gitter-Amir, A., Schneider, A.S., and Rosheneck (1976) Biochemistry 15, 3131 and 3138.

Gordon, D.J. (1972) Biochemistry 11, 413.

Gordon, D.J. and Holzwarth, G. (1971) Proc. Nat. Acad. Sci. U.S. 68, 2365.

Gosule, L.C. and Schellman, I.A. (1976) Nature 259 333.

Grosjean, M. and Legrand, C.N. Head Seances Acad. Sci. 251, 2150 1960).

Haynes, Garret, and Gratzer, Biochemistry 9, 4410 (1970).

Holzwarth, G. and Holzwarth, N.A. Journal Opt. Soc. Am. (1973) 63, 325.

Holzwarth, G., Gordon, D.G., McGiness, Dormon, B.P., and Maestre, M.T. Biochemistry (1974) 13 126.

Jordan, C.F., Lerman, L.S. and Venable, J.H. (1972) Nature New Biol. 236, 76-70.

Krauss, T.D. Antennae (1950) McGraw-Hill, New York.

Levy, M. and Felsenfeld, G., Proc. Nat'l. Acad. Sci. U.S.A. 56, 1324 (1966).

Luresh, K.A., Mol. Cryst. Liquid Cryst. 21, 315 (1973).

Maestre, M.F. (1970) J. Mol. Biol. 52, 543.

Maestre, M.F., Gray, D.M. and Cook, R.B. (1971) Biopolymers 10, 2537.

Nitayananda, R., Kini, U.D., Chandrasekhar, S., and Luresh, K.A., Proceedings of the International Liquid Crystals Conference, Bangalore, December 1973, p. 325.

Philipson, K.D. and Sauer, K. (1973) Biochemistry 12, 3454.

Reich, C. Maestre, M.F., Edmondson, S. and Gray, D. Biochemistry.

Schneider, A.S. and Harmatz, D. (1976) Biochemistry 15, 4158.

Schneider, A. (1973) Methods Enzymol. 270, 751.

Shapiro, J.T., Leng, M. and Felsenfeld, G., Biochemistry 8, 3219 (1969).

Shurcliff, W.A. (1962) Polarized Sight, Harvard U. Press Cambridge, Mass.

Tinoco, Jr. and Freeman (1957) J. Phys. Chem. 61, 1196.

Turner, D.H., Tinoco, Jr., I., and Maestre, M.F. (1974) J. Am. Chem. Soc. 96, 4340.

Tunis-Schneider, M.J. and Maestre, M.F. (1970) J. Mol. Biol. 52, 521. deVries, He. (1951) Acta Cryst. 4, 219.

Wiener, O. (1926), Kolloidchem. Beih, 23, 189.

FlGURE LEGEND

Fig. 1.   Schematics of the differing techniques and devices used
in the measurement of the CD scattering components of films,
DNA-polylysine particles and DNA-ETOH condensates. All
these devices were used in a modified Cary 60 dichrograph.

Fig. 2.   CD of DNA films measured in a vertical CD assembly as a
function of twist of film and orientation of film about
the optical axis of the light beam. Twisting occurred in the
sequential order described in curve labels.

Fig. 3.   DNA film with clockwise twist producing a negative ψ (-)
CD spectrum. This film was produced between two cuvettes
as described in Figure 1c to enable measurement of "normal"
CD. Forward cone (forward cell full of fluorescer in FDCD
mode) and back cone (back cell full of fluorescer in FDCD
mode). Note that scattered light is equivalent to transmitted
light, and since the CD instrument measures (T_r-T_l), the
curve labeled FDCD back scatter is $(A_{sr}-A_{sl}) = - A_s$ as deter-
mined by the equations in the appendix.

Fig. 4.   DNA film with counterclockwise twist measured by normal
CD and the back mirror device as in Fig. 1d. Notice the polari-
zation remains essentially the same throughout the spectrum.

Fig. 5.   DNA film with counterclockwise twist as a function of scatter-
ing angle. The goniometer device is sketched in Fig. 1a.

Fig. 6.   DNA film normal CD as a function of changing incidence angle
of light beam. This is measured in the vertical mode device.

Fig. 7.   T7 phage DNA-polylysine complexes produced in different

salt concentrations. The FDCD correction is achieved by the device shown in Fig. 1b.

Fig. 8. T7 DNA polylysine particles with large CD negative bands. Note that the two different preparations have different minima in the uncorrected CD spectra. Upon correction the CD is very similar.

Fig. 9. E. coli DNA-polylysine complex particles. For these particles the correctable scattering contribution using the FDCD method is minimal.

Fig. 10. THe E. coli-DNA polylysine particles of Fig. 9 were measured by the goniometer device (Fig. 1a) to determine the characteristics of the CD spectrum scattering. A comparison with film scattering (Fig. 5) shows very similar behavior qualitatively although the magnitudes are smaller in the particle case. The curve with crosses (xxxx) shows the back scattering obtained with the mirror device shown in Fig. 1(d).

Fig. 11. FDCD corrections of DNA-EtOH particles as a function of optical density of the sample. Also shown is normal CD and CD scattering at $\theta = 13^{\circ}$ scattering angle.

Fig. 12. Proposed model of DNA packing in films and aggregates. Figs. 12(a) and 12(b) show the sense imposed upon the film by the mechanical twisting. Fig. 12(c) represents the actual interpretation given to twisted nematic and cholesteric liquid crystals applied to the DNA fibers in a twisted DNA film.

APPENDIX

DNA FILM SCATTERING

The assumption is made here that the film is thin compared to the wavelength of light so that it can be considered to be two-dimensional with no thickness. The experimental set-up is as in Fig. 1(d). The front cuvette is on the left before the DNA film. The back cuvette is on the right after the DNA film. The light beam travels from left to right in Fig. 1(d).

A_F = absorption of the fluorescent solution in one of the cuvettes; i.e., $0.2 \, \varepsilon_F C_F$.

$A_{DL,R}$ = absorption of the DNA film for left (L) and right (R) circularly polarized light.

$$A = A_F + A_D; \quad A_L = A_F + A_{DL}; \quad A_R = A_F + A_{DR};$$

$$\theta_F = \text{FDCD ellipticity}$$

I. Suppose film does not scatter and both cuvettes are filled:

Intensity of light absorbed in first cuvette by fluorescer

$$(1) \quad I_1 = I_o(1 - e^{-2.303A_F})$$

Intensity of light reaching DNA film is:

$$(2) \quad I' = I_o \, e^{-2.303A_F}$$

Intensity of light after going through film is:

$$(3) \quad I'_o = I'e^{-2.303A_D} = I_o e^{-2.303A}$$

Intensity of light absorbed in second cuvette is:

$$(4) \quad I_2 = I'_o(1 - e^{-2.303A_F}) = I_o e^{-2.303A}(1 - e^{2.303A_F})$$

Total intensity of light absorbed by the fluorescer solution for left circularly polarized light is:

$$(5) \quad I_L = I_{IL} + I_{2L} = [I_{o_L} + I_{o_L}e^{-2.303A_L}][1 - e^{2.303A_F}]$$

$$(6) \quad I_R = I_{1R} + I_{2R} = [I_{o_R} + I_{o_R}e^{-2.303A_R}][1 - e^{-2.303A_F}]$$

$$(7) \quad \theta_F = k \frac{(e^{-2.303A_L} - e^{-2.303A_R})[1 - e^{-2.303A_F}]}{(2 + e^{-2.303A_L} + e^{-2.303A_R})[1 - e^{2.303A_F}]}$$

$$A = (A_L + A_L)/2$$

$$(8) \quad \theta_F = k \frac{10^{-A_L} - 10^{-A_R}}{2 + 10^{-A_L} + 10^{-A_R}} \quad \text{(multiply num. and denom. by } 10^A\text{)}$$

$$\text{where } \Delta A = A_L - A_R$$

$$(9) \quad \theta_F = k \frac{10^{-\Delta A/2} - 10^{+\Delta A/2}}{2 \cdot 10^A + 10^{-\Delta A/2} + 10^{+\Delta A/2}} \quad \text{(expand around } \Delta A\text{)}$$

$$(10) \quad \theta_F = \frac{k(-2.303\Delta A)}{2 \cdot 10^A + 2} \quad \text{where } k = -28.65$$

$$(11) \quad \theta_F = \frac{(14.32)(2.303)\Delta A}{10^A + 1}$$

$$(12) \quad \Delta A = \frac{\theta_F(10^A + 1)}{(32.98)}$$

II. Suppose film back scatters $180°$ totally (i.e., beam reverses upon hitting DNA film). It can easily be seen that since the film is

between two cuvettes of equal dimensions, the absorption of the back
scattered light is exactly the same as the forward unscattered light
and, thus, Eq. (12) holds for this case also.

III. Suppose only the first or front cuvette is filled and the DNA film
has a $180°$ component of back scattering: The second cuvette or back
cuvette is empty. A_s = apparent absorption due to back scattering.
Intensity of light scattered by DNA film is:

$$(13) \quad I_{os} = \frac{A_s}{A_D} I'(1 - e^{-2.303A_D})$$

$$= \frac{A_s}{A_D} I_o(1 - e^{-2.303A_D})e^{-2.303A_F}$$

Intensity of scattered light reaching L is:

$$(14) \quad I_s = I_{os} \, e^{-2.303\varepsilon_F C_F(d-\ell)}$$

Intensity of scattered light absorbed by L is:

$$(15) \quad dI_s = 2.303\varepsilon_F C_F I_s d\ell$$

$$= e^{-2.303A_F} I_o \frac{2.303\varepsilon_F C_F A_s}{A_D} (1 - e^{-2.303A_D})$$

$$x[e^{-2.303\varepsilon_F C_F(d-\ell)}]d\ell$$

Total intensity of scattered light absorbed is:

$$I'_s = [\frac{2.303\varepsilon_F C_F A_s}{A_D} I_o \, e^{-2.303A_F} (1 - e^{-2.303A_D})e^{-2.303\varepsilon_F C_F d}]$$

$$x \int_0^d e^{2.303\varepsilon_F C_F \ell} d\ell$$

$$= I_{os} \, e^{-2.303A_F} (e^{2.303A_F} - 1)$$

(16) $\quad I_s' = I_{os}(1 - e^{-2.303A_F}) \quad$ which is expected.

(17) $\quad I_s' = \dfrac{A_s}{A_D} (1 - e^{-2.303A_D})(1 - e^{-2.303A_F}) e^{-2.303A_F}$

I_L = total intensity of left cpl absorbed by fluorescer

I_R = total intensity of right cpl absorbed by fluorescer

(18) $\quad I_L = (I_o + I_{OSL})(1 - e^{-2.303A_F})$

$$= [I_o + I_o \dfrac{A_{SL}}{A_{DL}} (1 - e^{2.303A_{DL}}) e^{-2.303A_F}](1 - e^{-2.303A_F})$$

$\therefore$

(20)

$$\theta_F = k \cdot \dfrac{[\dfrac{A_{SL}}{A_{DL}} (1 - e^{-2.303A_{DL}}) e^{-2.303A_F}] - [\dfrac{A_{SR}}{A_{DR}} e^{-2.303A_F}(1 - e^{-2.303A_{DR}})]}{2 + [\dfrac{A_{SL}}{A_{DL}} e^{-2.303A_F}(1 - e^{-2.303A_{DL}}) + \dfrac{A_{SR}}{A_{DR}}(- e^{-2.303A_{DR}}) e^{-2.303A_F}]}$$

for: $A_D \equiv (A_{DL} + A_{DR})/2$ = DNA absorption

$\Delta A_S \equiv A_{SL} - A_{SR}$ = differential back scattering

$\Delta A \equiv A_{DL} - A_{DR}$ = differential DNA absorption

$A_S \equiv (A_{SL} + A_{SR})/2 \quad$ total scattering in back direction.

then

$$\theta_F = k \cdot \frac{(\frac{A_{SL}A_{DR}-A_{SR}A_{DL}}{A_{DL}A_{DR}})(10^{A_D}-1) + \frac{2.303\Delta A}{2}(\frac{A_{SL}A_{DR}+A_{SR}A_{DL}}{A_{DL}A_{DR}})}{2\cdot10^A + (\frac{A_{SL}A_{DR}+A_{SR}A_{DL}}{A_{DL}A_{DR}})(10^{A_D}-1) + \frac{2.303\ A}{2}(\frac{A_{SL}A_{DR}-A_{SR}A_{DL}}{A_{DL}A_{DR}})}$$

Note: $A_{DL} \cong A_{DR} \cong A_D$.   Then we get approximation

$$\theta_F = k \cdot (\frac{(\frac{\Delta A_S}{A_{DL}} - \frac{A_{SR}\Delta A}{A_{DL}A_{DR}})(10^{A_D}-1) + \frac{2.303\ A}{2}(\frac{2A_S}{A_{DL}} + \frac{A_{SR}\Delta A}{A_{DL}A_{DR}})}{2\cdot10^A})$$

Assume A_S is small compared to total absorption A_D

(Note A_S is not <u>total</u> scattering but back scattering, i.e.

measured by the front cuvette).

(23) $\therefore \qquad \theta_F = k \cdot \frac{\Delta A_S}{A_{DL}}(10^{A_D}-1)/2\cdot10^A$

$$= k\frac{\Delta A_S}{A_{DL}}(10^{-A_F} - 10^{-A})$$

$$\cong -14.32\frac{\Delta A_S}{A_D}(10^{-A_F} - 10^{-A})$$

(24) $\quad \Delta A_S = \frac{-\theta_F A_D}{14.32(10^{-A_F}-10^{-A})}$

FIG. 1a

X

INCIDENT LIGHT BEAM

DNA FILM

φ

ι

Y

θ

Z

ι = ANGLE OF TILT OR INCIDENCE ANGLE

θ = SCATTERING ANGLE

φ = ORIENTATION ANGLE ABOUT OPTICAL AXIS OF LIGHT BEAM

SLITS

PHOTOMULTIPLIER (1-1/8" DIAMETER HAMAMATSU #376)

BEAM GONIOMETER MOUNT

FIG. 1a

FIG. 1b

CUVETTE

LIGHT BEAM

RAYLEIGH HORN

UV FILTER

2" HAMAMATSU #375 PM TUBE

FDCD DEVICE

FIG. 1b

FIG. 1c

BACK CUVETTE — FRONT CUVETTE

LIGHT BEAM

DNA FILM

UV FILTER

#375 PM TUBE

FDCD BACK REFLECTION DEVICE

FIG. 1c

FIG. 1d

FRONT SURFACE MIRROR

SLIT

LIGHT BEAM

DNA FILM OR CUVETTES

FRONT SURFACE MIRROR

1-1/8" HAMAMATSU #376 PM TUBE

BACK REFLECTION MIRROR DEVICE

FIG. 1d

XBL796-3548

XBL 796-3550

XBL796-3551

XBL 796 - 3547

XBL796-3549

T7 DNA-POLYLYSINE COMPLEXES

CD   .85M SALT
FDCD CORR.   .85M SALT
CD   1M SALT
FDCD CORR.   1M SALT

EPSILON LEFT - EPSILON RIGHT

WAVELENGTH (NANOMETERS)

T7 DNA-POLYLYSINE COMPLEXES, .15M SALT

EPSILON LEFT - EPSILON RIGHT

————— CONVENTIONAL CD
— — — FDCD CORR.
– – – – CONVENTIONAL CD
·········· FDCD CORR.

WAVELENGTH (NANOMETERS)

E. COLI DNA-POLYLYSINE COMPLEX

WAVELENGTH (NANOMETERS)

EPSILON LEFT - EPSILON RIGHT

——— NORMAL CD
— — — FDCD CORRECTED

E. COLI DNA-POLYLYSINE GONIOMETER CD

EPSILON LEFT – EPSILON RIGHT

WAVELENGTH (NANOMETERS)

0 DEGREES
13 DEGREES
23 DEGREES
33 DEGREES
xxxxxx BACK SCATT. MIRROR X .5

XBL796-3546

Counterclockwise twist in films

DNA molecules

Repeat periodicity

Right Handed Array Organization
(a)

Axis of light propagation

Clockwise twist

DNA axis

Left Handed Array Organization
(b)

2nd Layer + twist

1st Layer of DNA molecules

DNA molecule

Optical axis

(c)

XBL7811-3693