DNA FILMS:
PREPARATION AND PHYSICAL CHEMICAL CHARACTERISATION

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SUMMARY

This thesis describes the preparation and properties of optically anisotropic deoxyribonucleic acid (DNA) films and of mixed films of DNA and poly(vinyl alcohol) (PVA).

The oriented pure DNA films were prepared by a wet spinning method and the preparation is discussed in terms of the homogeneity of the films and of the degree of orientation achieved in them. Isotropic PVA-DNA films were prepared by the casting of mixed solutions of PVA and DNA, and were oriented by mechanical stretching. The properties of the mixed films are considered in terms of an interaction between DNA and the PVA matrix.

The potential for the use of oriented DNA films in the study of the interactions between DNA and small molecules is explored briefly by the use of dyes.

Finally, an intensity method based on Mueller Calculus is described for the measurement of the birefringence of the films. The dispersion of the birefringence in absorption regions is interesting in that it is complementary to the dichroism.

To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, nor any material previously submitted for a degree or diploma at any University, except where due reference is made in the text.

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CHAPTER ONE

OPTICAL ANISOTROPY AND MOLECULAR STRUCTURE

When electromagnetic radiation interacts with matter, several observable effects may be produced, these being absorption, refraction, scattering and emission. The discussion will be limited to molecular structure analysis from absorption and refraction measurements. Although only large molecules will be considered, the principles involved are quite general in most cases.

1.1 Dichroism and Birefringence

The propagation of light in a medium can be described by a wave equation involving the complex refractive index, $\underline{n}'$, given by

$$\underline{n}' = n(1 - iK) \qquad (1.1)$$

where n is the refractive index and is the ratio of the velocity of light in vacuo to the velocity of light in the medium; K is the extinction coefficient and represents the attenuation of the light (Jenkins and White, 1957).

The nature of the interaction of electromagnetic radiation with matter gives rise to different intensities of absorption, depending on the direction which the electric vector of the radiation makes with the changing dipole moment responsible for the absorption; the absorption is greatest when the electric vector and the transition (dipole) moment are parallel (Sandorfy, 1964). In general, molecules are therefore characterised by three orthogonal absorption coefficients. However, it is usually practicable to measure only two of these, and so only uniaxial systems will be considered. The dichroism, $\Delta\epsilon$, is defined by

$$\Delta\epsilon = \epsilon_{11} - \epsilon_{\underline{1}} \qquad (1.2)$$

ϵ_{11} and $\epsilon_{\underline{1}}$ are the absorption coefficients for the electric vector parallel and perpendicular, respectively, to some well defined axis in the specimen under study. For example, the reference direction may be the fibre axis in a system consisting of parallel fibres. If ϵ_{11} is greater than $\epsilon_{\underline{1}}$ the system is said to exhibit positive dichroism and if ϵ_{11} is less than $\epsilon_{\underline{1}}$ it is said to exhibit negative dichroism. The dichroic ratio, R, is defined as the ratio of the two principal absorption coefficients (Shurcliff, 1966).

$$R = \frac{\epsilon_{11}}{\epsilon_{\underline{1}}} \qquad (1.3)$$

There is some confusion in the literature as to the definition of R, some authors using the reciprocal of the definition adopted here (Beaven et al., 1955; Bradbury et al., 1961; Gellert, 1961).

In addition to the anisotropy of absorption, molecules are also characterised by the anisotropy of the refractive index, n, and this is known as the birefringence, Δn (Shurcliff, 1966).

$$\Delta n = n_{11} - n_{\perp} \qquad (1.4)$$

n_{11} and $n_{\perp}$ are the refractive indices for the electric vector parallel and perpendicular, respectively, to a well defined axis in the specimen.

It is obvious that dichroism and birefringence will only be detected in oriented assemblages of molecules with polarised radiation. Neither randomly oriented systems, such as solutions, studied with polarised radiation, nor ordered systems studied with unpolarised radiation will show the anisotropy.

The type of molecular information which may be gained from the dichroism or birefringence depends on the conditions of the measurements. Single-wavenumber measurements of the dichroism allow the assignment of observed absorption bands to transitions predicted by theoretical calculations (Jakobi and Kuhn, 1962; Sandorfy, 1964). Conversely, if the direction of the transition moment in a chromophore is known, then the dichroic ratio allows the calculation of the angle between the chromophore and the molecular axis in long chain molecules. The usefulness of this type of calculation is restricted by the incompleteness of the molecular orientation (Bamford et al., 1956; Fraser, 1960). The dichroism can also give a measure of the flexibility of macromolecules in solution (Wada, 1964). The birefringence, at constant wavenumber, in a region of non-absorption can furnish information about the size, shape and flexibility of macromolecules and also about the polarisability of the molecules in various directions, enabling conclusions to be made about the arrangements of bonds in the molecules (Cerf and Scheraga, 1952).

The change in the dichroism, or the dichroic ratio, with the wavenumber of the incident radiation is useful to separate and assign absorption bands (Wada and Kozawa, 1964; Tanizaki et al., 1965). Although not much appears to have been done on the dispersion of birefringence (Oriel and Schellman, 1966), it has been shown that the dispersion of the electric birefringence near an absorption region allows the assignment of the polarisation of transitions from the shapes of the dispersion curves (Buckingham, 1962; Powers, 1966,1967; Powers and Peticolas, 1967). Sometimes a predicted shift in the absorption spectrum cannot be detected, but such a shift may be seen as a shift of the centre of the birefringence dispersion curve relative to the absorption curve (Powers, 1967).

1.2 Orientation of Molecules

Orientation of molecules may be achieved in several ways, both in solutions and in solids.

1.2.1 Solution Studies

The introduction of optical anisotropy into an isotropic solution of macromolecules may be achieved by the application of an external field. The anisotropy of the system is due to an equilibrium between the orienting field and the disorienting effect of Brownian motion which is characterised by the rotary diffusion coefficient which in turn is related to the shape of the molecules. The external orienting field may be hydrodynamic, magnetic or electric (Cerf and Scheraga, 1952; Dvorkin, 1961; Wada, 1964; Houssier and Fredericq, 1966; Oriel and Schellman, 1966; Powers, 1967). In the case of the hydrodynamic field, the macromolecules are aligned along the streamlines by shearing forces. In the case of the electric field, the molecules are aligned along the field, the orienting mechanism being due to permanent or induced dipoles; other mechanisms such as the distortion of the ionic atmosphere surrounding the molecules may also align them (O'Konski and Zimm, 1950).

Different methods are used to determine the rotary diffusion coefficient for the different methods of orientation. In the hydrodynamic case, it is usual to measure the angle of isocline as a function of the velocity gradient, or it is possible to measure the relaxation of the anisotropy after the external field has been removed (Cerf and Scheraga, 1952). In the electric case, square pulsed fields are used to promote the anisotropy and the time of decay of the anisotropy is a measure of the rotary diffusion coefficient (Tinoco, 1955). The rise of the electric birefringence upon the application of the square pulse in principle also allows the determination of the dipole moments of the oriented molecules (Tinoco, 1955). It must be noted that there is a greater danger of degradation of the macromolecules in the electric field owing to the high field strengths used (Houssier and Fredericq, 1966).

One main advantage of solution studies is that the concentration dependence of the anisotropy allows the observation of solute-solute interactions in the system (Dvorkin, 1961; O'Konski and Stellwagen, 1965).

1.2.2 Solid State Studies

Solid samples are usually produced by the evaporation of the solvent from a solution cast on a suitable flat plate (Fraser, 1960). Orientation may be induced at the time of casting by the undirectional stroking of the solution or by crystallisation. In the cases where the orientation of the molecules is lost before the solvent has evaporated it may be re-introduced into the detached or supported isotropic film by the mechanical stretching or rolling of the film.

Fibre spinning is also known to produce molecular orientation (Ziabicki and Kedzierska, 1959; Rupprecht, 1963, 1966a).

A study of anisotropic solids is useful from the point of view that the properties of the molecules in the solid state are not necessarily the same as in solution; this can arise partly from increased solute-solute interactions. Solvent effects can be studied directly in the case of solids (Falk et al., 1963a, 1963b). Solvent absorption can be largely eliminated in spectroscopic measurements (Falk, 1964). In addition, anisotropic solid films may lend themselves more readily than solutions to study by other techniques, such as nuclear magnetic resonance, electron spin resonance and semiconductivity, where anisotropic behaviour is not uncommon (Berendsen and Migchelsen, 1965; Elliott and Wyard, 1965; Hedén and Rupprecht, 1966; Rupprecht, 1966b; Ehrenberg et al., 1967).

1.3 Nucleic Acid Films

The aim of this work was to study the preparation of oriented deoxyribonucleic acid (DNA) films and their dichroic and birefringence properties in the visible and ultraviolet spectral regions. The reasons for this were:-

(a) In vivo, DNA probably occurs in a gel state and a study of DNA films would complement our knowledge of DNA behaviour in solutions and would improve our understanding of the nucleic acids in vivo.

(b) Very little work has been done on the birefringence dispersion of DNA. This information would complement the dichroism measurements.

(c) It was thought that the anisotropy would provide important geometrical information on small molecule-nucleic acid interactions, since small molecules such as dyes are known to interact with DNA in an ordered way (Lerman, 1961, 1963).

(d) Most of the methods for preparing oriented DNA for spectroscopic studies are not satisfactory.

Up to date, most of the methods for preparing oriented DNA films involved the shearing of a concentrated solution on a microscope slide, with a coverslip, or the stroking of a DNA gel to dryness with a spatula (Fraser and Fraser, 1951; Seeds, 1953; Sutherland and Tsuboi, 1957; Rich and Kasha, 1960; Bradbury et al., 1961; Gellert, 1961; Falk et al., 1963b; Houssier, 1964; Neville and Davies, 1966; Webb and Bhorjee, 1968). These methods are unsatisfactory because they produce inhomogeneous films with non-uniform orientation, making it necessary to use microspectrophotometry in their study. Microspectrophotometry would seem to be a difficult technique to use in the far ultraviolet region where there is a considerable interest at present (Falk, 1964). Another interesting way of orienting DNA has been to deposit oriented DNA fibres within sodium chloride crystals by evaporating solutions of the nucleic acid in sodium chloride; the sodium chloride crystals could be dissolved in methanol leaving the oriented fibres (White and Elmes, 1952). The degree of orientation by this method was not given by the authors of the work, and so this method was not used in this project. The objection of inhomogeneity also holds for the films prepared in this way. It was considered that the best method available for the preparation of oriented DNA films was the wet spinning method of Rupprecht (1963, 1966a). It was thought that the mechanical nature of the method would enable reproducible oriented DNA films to be made in large amounts. This method will not be dealt with here since it will be described in Chapter Two, Section 2.2, of this thesis. In addition to the Rupprecht method, a new method for preparing oriented nucleic acid films was tried. This involves the mechanical stretching of a film of poly(vinyl alcohol) containing the nucleic acid. The findings on this method will be described in Chapter Three, Sections 3.5 and 3.6.

CHAPTER TWO

THE WET SPINNING OF DNA

2.1 Fibre Spinning and Molecular Orientation

Fibre spinning involves the continuous extrusion of a fibre-forming polymer liquid through a circular orifice; the liquid stream, F, runs down on a spinning way and is converted to a solid fibre which is wound by a take-up device placed at a distance L from the spinneret (Fig. 2.1). On the spinning way, the polymer stream is drawn and its velocity increases from V_0, at the spinneret orifice, to V_E, at the take-up device; simultaneously the diameter of the fibre decreases from d_0 to d_E. The deformation ratio, S, for this process is given by

$$S = \frac{V_E}{V_0} \qquad (2.1)$$

The solidification of the fibre may be achieved by the precipitation of a polymer solution in "wet spinning", by the evaporation of a volatile solvent from a polymer solution in "dry spinning", or by the cooling of a polymer melt in "melt spinning". It has been found that fibre spinning produces molecular orientation (Ziabicki and Kedzierska, 1959, 1962a, 1962b; Rupprecht, 1963, 1966a).

In recent years, Ziabicki and his co-workers have been responsible for a number of experimental and theoretical publications about the nature of melt spinning in polymers

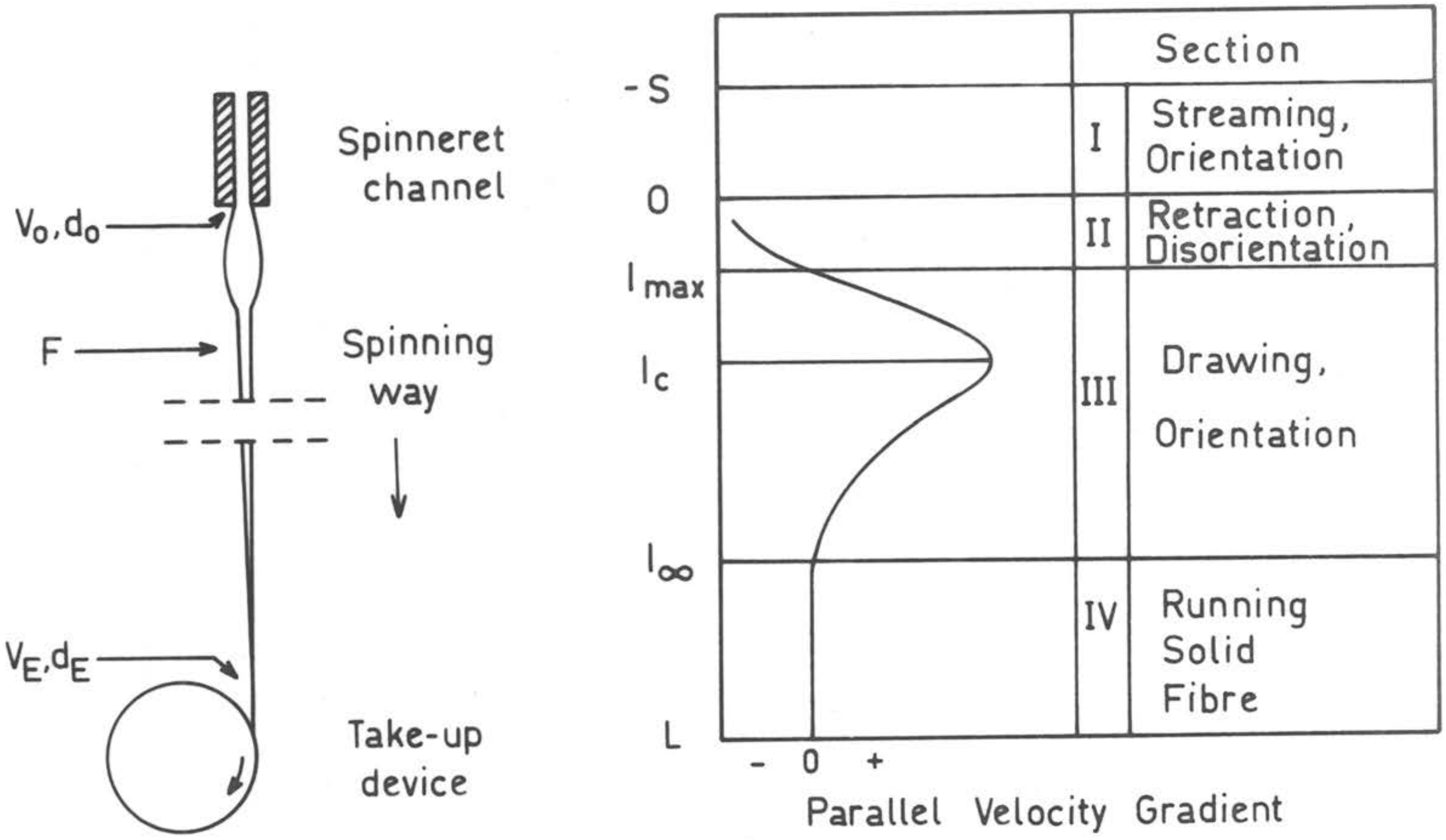


FIG. 2·1. Scheme of a Fibre Spinning Process . (Not drawn to scale) .

(Ziabicki and Kedzierska, 1959, 1960a, 1960b, 1962a, 1962b). Their findings may be generally applied to all three types of spinning. The molecular orientation, as judged from birefringence measurements, is the result of the action of the velocity gradient parallel to the spinning way and of relaxation factors. The deformation ratio affects the birefringence only if some network structures are present in the polymers under study. It must be noted that the orientation of macromolecules achieved in the spinneret channel by the action of a perpendicular velocity gradient is lost below the outlet and does not contribute to the molecular orientation in the spun fibre. Ziabicki has divided the spinning process into four parts which are realised in successive sections of the spinning way (Fig. 2.1), viz.,

Section I (-s to 0) (within the spinneret channel). The mean velocity along the channel is constant. However, a transverse velocity gradient exists and a streaming orientation is produced as a result of the action of the velocity field.

Section II (0 to l_{max}) (stream broadening). The velocity gradient along the spinning path is negative. There is a retraction of the polymer liquid. A disorientation of the macromolecules which were partially oriented within the spinneret channel takes place.

Section III (l_{max} to l_{∞}) (drawing).

The velocity gradient along the spinning way is positive and an orientation of macromolecules occurs. The velocity gradient reaches a maximum in the distance l_c from the spinneret; this is probably connected with a structural transformation in the flowing fibre-forming liquid, such as the formation of a gel during the set spinning process. After the maximum, the velocity gradient falls to zero at l_{∞}.

Section IV (l_{∞} to L) (running solid fibre).

The velocity is practically constant and no more orientation of macromolecules is produced.

Section III is very sensitive to external disturbances. That is, l_{∞} increases with the take-up velocity, V_E, and with the flow intensity; however, it is not affected by the spinneret orifice diameter.

2.2 The Wet Spinning of DNA

Various stages of the extractions of DNA from different tissues involve the precipitation of the nucleic acid in the form of a gelatinous mass which slowly loses water to form a fibrous precipitate (Steiner and Beers, 1961). The precipitation reaction was used by Rupprecht in the preparation of oriented DNA in large amounts by the wet spinning method (Rupprecht, 1963, 1966a).

This method seems promising for the preparation of reproducible films. Since it is still being investigated by Rupprecht and very little has been published about it, a report of our experiences in its use is in order.

2.2.1 The Method

The method of preparation of the oriented films involves first of all the precipitation of a DNA solution which is continuously fed into a precipitating liquid by means of a syringe. The gelatinous thread formed is wound onto silica slides attached to a rotating cylinder. The cylinder is capable of slow motion along its axis, within desired limits, thus ensuring that the thread is wound in parallel layers on the slides. Upon the achievement of a desired thickness of nucleic acid the cylinder is dried at a certain relative humidity (r.h.); this drying process causes the agglomeration of the parallel DNA threads into a film.

A notable difference between the apparatus used in this project and that of Rupprecht is the omission of a precipitation tower in our case. This meant that the length of the spinning way in our system was seriously limited.

The success of the spinning process for preparing oriented films can be judged by several criteria, amongst them being the optical quality of the films, the reproducibility of the preparations and the spinnability of the DNA solutions. Each of these factors will now be discussed.

2.2.2 The Appearance of the Wet Spun Films

In his preliminary note on the wet spinning method for preparing oriented DNA films Rupprecht (1963) claimed to have made an "apparently homogeneous film". However, no evidence for this was given even in his latest publication where this claim was renewed (Rupprecht, 1966a).

Visual inspection of our films showed fine striations parallel to the DNA fibres. However, it was difficult to distinguish individual fibres in most parts of the films with transmitted light under a microscope. Nevertheless, there were regions where fibres could just be made out in an undyed film. In the case of a film dyed with methylene blue it was much easier to see fibres (Plate 2.1). It seems that the fibres do fuse into one mass during the drying process and that the films do not correspond to a system of individual fibres.

In the case of Rupprecht's sodium nucleate films, a mention was made of "longitudinal stripes" which could be prevented by increasing the axial displacement per revolution of the cylinder during the preparation of the films. These were probably cracks in his films since the cracking tendency was also claimed to be influenced by the axial displacement per revolution of the cylinder. It is not certain whether the very finely ruled appearance of the

Plate 2.1

Wet Spun DNA Film Dyed with Methylene Blue

(Examined with transmitted light under a microscope)

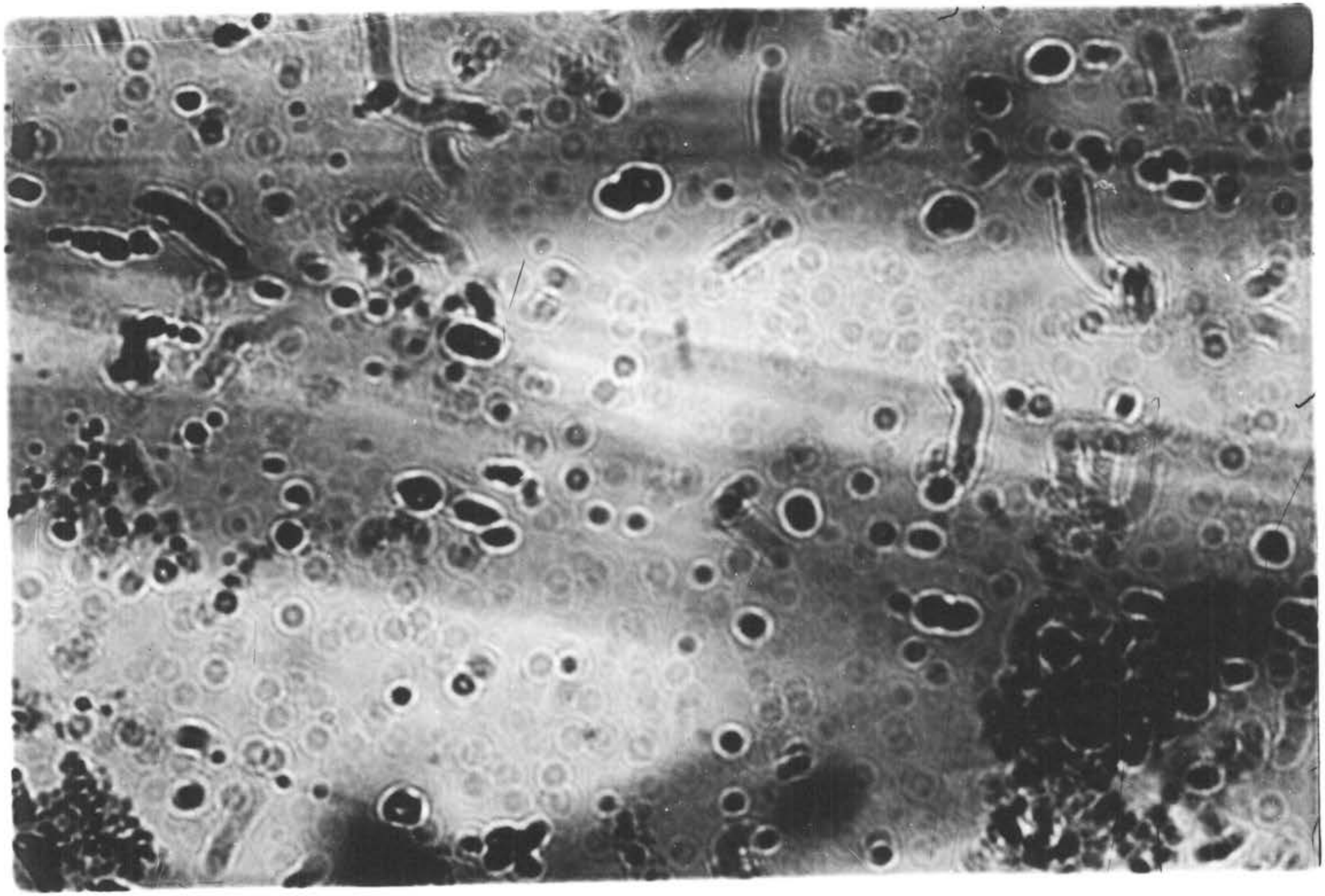
The film was prepared by the wet spinning of a solution of E. Coli. DNA III in an aqueous isopropanol solution (60% isopropanol) at a deformation ratio of 750. The precipitant was 0.2M with respect to sodium acetate. The DNA film was dyed by immersion in a solution of methylene blue in aqueous acetone (80% acetone) (Chapter Four, Section 4.2.1).

The dark streaks in the photograph are the DNA fibres.

It is not certain what the spots in this photograph are.

Microscope Variables

Magnification of objective:	45 x
Magnification of eye-piece:	6.3 x
Magnification of camera:	0.5 x



whole surface of our sodium nucleate films is in fact the "longitudinal stripe" appearance of Rupprecht's films, since the axial displacement per revolution of the cylinder in our preparations was greater than that used by Rupprecht (Chapter Six, Section 6.3.1).

2.2.3 Scattering Effects

The measured absorption of macromolecules is usually greater than the true (electronic) absorption as a result of scattering. In addition to Rayleigh scattering, scattering caused by an inhomogeneity of the sample may also be important (Fraser, 1960). The optical density due to Rayleigh scattering may be estimated usually from a double logarithmic plot of the optical density outside the absorption region against wavenumber and an extrapolation of the straight line obtained in this way to the absorption region (McLaren and Shugar, 1964). There is an uncertainty in this method in that the anomalous behaviour of the refractive index in the absorption region is not taken into account. The scattering caused by the inhomogeneity of the sample has no exact method of correction.

The films prepared during this project showed a slight absorption outside the DNA region (32,000 cm^{-1} to 30,000 cm^{-1}) (Gellert, 1961; Falk, 1965; Basu and Dasgupta, 1967). An accurate extrapolation was not possible in order to correct for scattering losses since the optical density between 32,000 cm^{-1} and

30,000 cm^{-1} varied only slightly with wavenumber. Furthermore, since it changed only very slightly for films of varying thickness and since there was good agreement between the spectra of films of varying thickness, scattering corrections were neglected (Falk, 1965).

2.2.4 The Degree of Orientation

The basis of the interpretation of dichroism in terms of molecular structure and orientation is the proportionality between the absorption coefficient and the square of the scalar product of the transition moment associated with a particular absorption band and the electric vector of the polarised radiation. Consider a simple model system consisting of a fraction, f, of molecules perfectly aligned to the optic axis and a fraction, (1 - f), randomly aligned. If all orientations of the chains of the perfectly aligned molecules are equally probable, then the transition moment vectors, $\underline{P}$, will be uniformly distributed on a right circular cone of semi-angle ϕ (Fig. 2.2). The dichroic ratio, R, for this system is given by (Fraser, 1960)

$$R = \frac{f\cos^2\phi + \frac{1}{3}(1 - f)}{\frac{1}{2}f\sin^2\phi + \frac{1}{3}(1 - f)} \qquad (2.2)$$

According to the Watson and Crick model, DNA consists of two interwoven helical chain molecules built up of phosphate + sugar +

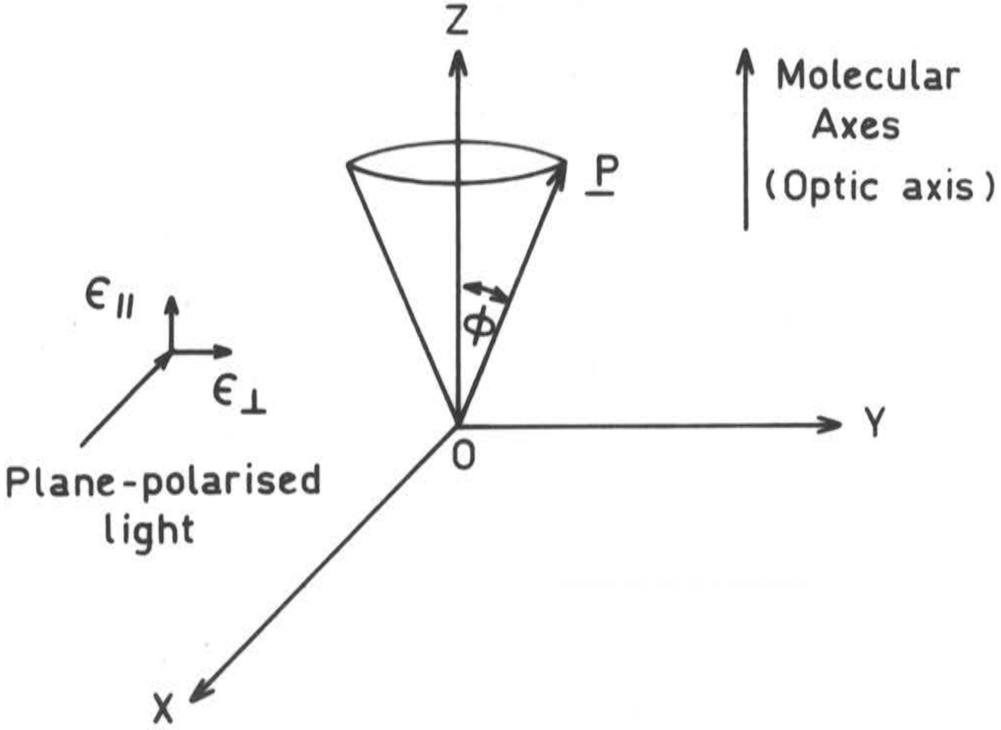


FIG. 2·2. Uniaxial Fibre Model for Calculation of Dichroic Ratio.

base repeating units (Steiner and Beers, 1961). The purine and pyrimidine bases point inwards to the axis of the helix and are hydrogen bonded to form the planar pairs adenine + thymine and guanine + cytosine. At relative humidities greater than 85%, the sodium salt of DNA exists in the B helical configuration where the base pairs are stacked perpendicular to the axis of the helix (Steiner and Beers, 1961). It is well known that the nucleic acids show selective absorption in the ultraviolet region centred at about 38,400 cm^{-1} as a result of the purine and pyrimidine bases. It is also known that the transition moments connected with the π-π^* transition responsible for this absorption lie in the planes of the purine and pyrimidine bases (Rich and Kasha, 1960). Consequently, it can be seen that $\phi = 90^\circ$ in equation (2.2), at high relative humidities, and that R will be a measure of the degree of orientation, f. Furthermore, R will decrease as f increases.

In our spinning system, the degree of orientation, as measured by the dichroic ratio at 38,400 cm^{-1} at 93% r.h., appears to depend on the deformation ratio. The reference direction in measuring the dichroic ratio was chosen as the fibre direction since the polymer chain axes were assumed to be oriented parallel to the fibre axis. The form of the dichroic ratio versus deformation ratio curve is qualitatively similar to the birefringence versus deformation ratio curve for polystyrene of Ziabicki and Kedzierska (1962b), except that in this case the dichroic ratio decreases with increasing orientation, whereas the birefringence increases (Fig. 2.3). It is difficult to estimate the effect of the parallel velocity gradient since the spinning path was small (of the order of a few millimetres) and was not reproducible. A comparison between the velocity difference ($V_E - V_O$), used by Ziabicki and Kedzierska (1962a) as a measure of the velocity gradient, and the dichroic ratio fails to show a regular interdependence between the two quantities (Table 2.1).

The deformation ratios used in this project to achieve a reasonable degree of orientation of the DNA were about fifteen times higher than used by Rupprecht (1963). This may have been the result of the short spinning way in our apparatus compared with the one metre spinning way in Rupprecht's apparatus (1966a).

An attempt was made to increase the spinning way from a few millimetres to about six centimetres at constant deformation ratio (S = 63) but this appeared to have no effect on the dichroic ratio (Table 2.2). Other experiments with an increased spinning way were unsatisfactory for a reason which will be discussed in Section 2.2.6 of this chapter.

As regards our best films, it is clear that the degree of orientation is not as good yet as in films prepared by other workers by different methods (Table 2.3). However, it is quite obvious that optimum conditions for orientation, such as optimum deformation

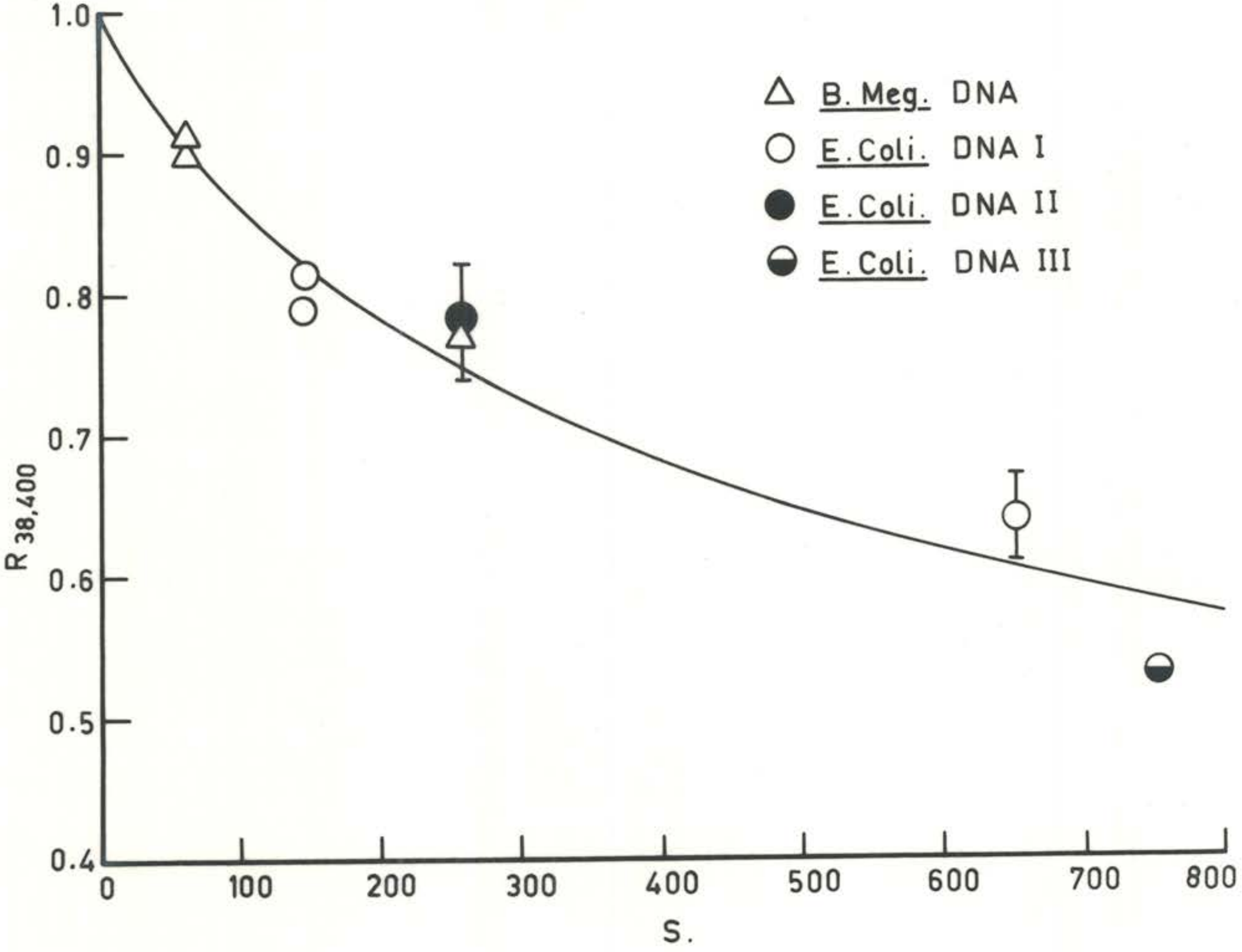


FIG. 2·3. Dependence of Dichroic Ratio on Deformation Ratio.

Table 2.1

Dichroic Ratio as a Function of Deformation Ratio at 93% r.h.

Sample of DNA	Number of Films per Run	$R_{38,400}$	$R_{38,460}$	$(V_E - V_O)$ cm.min.$^{-1}$	$S = \frac{V_E}{V_O}$
B. Meg.	1		0.91	314	63
B. Meg.	1		0.90	314	63
E. Coli. I	1		0.81	317	146
E. Coli. I	1		0.79	317	146
E. Coli. II	3		0.78 ± 0.04[1]	318	260
B. Meg.	1		0.77	318	260
E. Coli. I	6	0.64 ± 0.03[1]		319	650
E. Coli. III	1	0.53		554	750

[1] Mean deviation.

Table 2.2

Effect of Spinning Way on Dichroic Ratio

B. Meg. DNA

Precipitant: Aqueous acetone (20% H_2O)

Diameter of cylinder: 5 cm.

Rate of rotation of cylinder: 20 r.p.m.

Internal diameter of capillary: 0.030 cm.

Rate of feeding of DNA solution: 0.22 ml. per hr.

$V_E = 319$ cm.min.$^{-1}$ $V_O = 5.1$ cm.min.$^{-1}$

$S = V_E/V_O = 63$

	Film 1	Film 2
Spinning path length (cm.)	~0.2	6
Concentration of DNA Solution	0.1%	0.1%
Solvent for DNA	0.15M NaCl	0.20M NaCl
Spinning time (hrs.)	9.5	10
Drying conditions	9 days at 93% r.h.	6 days at 93% r.h.
$OD_{38,460}$ (unpolarised)	0.843	0.351
$R_{38,460}$	0.91	0.90

Table 2.3

Orientation in DNA Films

Method of Preparation	Method of Storage		R	Wavenumber	Reference
	% r.h.	time		cm.$^{-1}$	
Shearing gel between coverslip and slide	90		0.21	37,037	Seeds, 1953
Stroking gel			0.16	38,460	Rich and Kasha, 1960
Stroking gel	93		0.31	37,037	Falk et al., 1963b
Wet spinning	93	1 week	0.28	37,037	Rupprecht, 1963
Shearing gel between coverslip and slide	98		0.36		Houssier, 1964
Wet spinning	93	3 weeks	0.54	37,000	This work

ratio, optimum spinning way or optimum velocity gradient have not been achieved in our case.

2.2.5 Polarised Spectra of the Wet Spun Films

All the oriented DNA films showed negative dichroism in the ultraviolet region, 45,000 cm^{-1} to 32,000 cm^{-1}, which is in agreement with previous results (Seeds, 1953; Falk et al., 1963b). At 93% r.h., the dichroic ratio varied slightly with wavenumber (Fig. 2.4). In addition, there was a slight displacement of the absorption maximum to shorter wavenumbers for the parallel polarised spectrum compared with the perpendicular polarised spectrum. The magnitude of the shift is of the order of 200 to 300 cm^{-1}. The shift is more obvious on inspection of the dichroic ratio as a function of wavenumber since the minimum in the dichroic ratio curve does not coincide with the maxima in the polarised spectra (Fig. 2.4). This observation of the splitting of the two polarised components is in agreement with the work of Rich and Kasha (1960) and some of the measurements of Seeds (1953). It must be noted that Seeds' relative positions of the two polarised components are not always consistent.

A possible explanation for the slight displacement of the polarised spectra may be given as follows. The 38,400 cm^{-1} electronic band is thought to consist of two transitions from the purine and

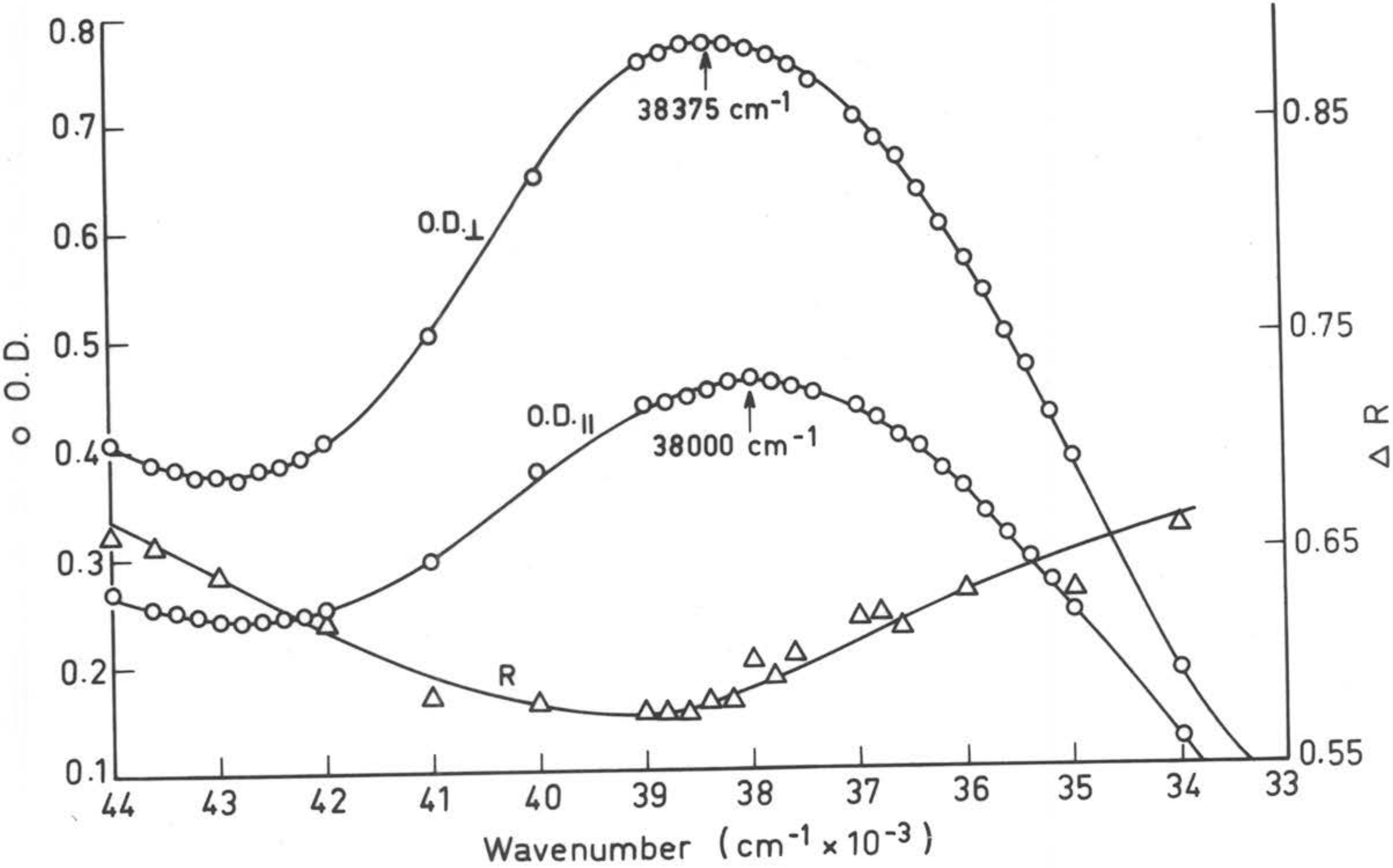


FIG. 2·4. Polarised Spectra of a Wet Spun DNA Film.

pyrimidine bases, namely a π-π^* transition, of high intensity, polarised in the plane of the bases, and an n-π^* transition, of low intensity, polarised perpendicular to the plane of the bases (Kasha, 1961). The n-π^* transition appears on the short wavenumber side of the nucleic acid spectrum in the form of a shoulder or inflection. This was indeed observed in the unpolarised spectra of our films at about 35,900 cm^{-1}. Owing to the way that the polarised spectra were measured this was not so obvious in the polarised spectra (Chapter Six, Section 6.5.3(b)). The fact that the n-π^* transition is situated on the shorter wavenumber side of the main band and that it is polarised parallel to the helix axis should cause it to contribute a greater optical density to the parallel polarised spectrum than to the perpendicular one at lower wavenumbers.

The transmittance of a partial polariser for incident unpolarised light is the arithmetic mean of the parallel and perpendicular transmittances for polarised light (West and Clark-Jones, 1951). This was confirmed for the wet spun films and may justify the disregard of scattering corrections. Table 2.4 shows the measured optical density (O.D.) of some films for unpolarised incident radiation and the optical density calculated for unpolarised incident radiation from the parallel and the perpendicular polarised optical densities.

Table 2.4

Calculated Optical Density for Wet Spun Films for Incident Unpolarised Light

Sample	Measured O.D. at		Calculated O.D. at	
	38,400 cm^{-1}	38,460 cm^{-1}	38,400 cm^{-1}	38,460 cm^{-1}
1	0.263		0.25	
2	0.698		0.72	
3	0.540		0.56	
4		0.653		0.66
5		0.822		0.82
6		0.621		0.63
7		0.843		0.84

2.2.6 The Reproducibility of the Wet Spun Films

The reproducibility of the wet spun films can be considered in two ways, namely as thickness reproducibility and as orientation reproducibility.

The optical density of films prepared individually was not reproducible even though the stock solution, the cylinder speed, the rate of feeding of the solution into the same precipitant and the spinning time were constant (Table 2.5).

Table 2.5

Thickness Reproducibility of Films Prepared Individually

E. Coli. DNA I

Concentration of DNA Solution: 0.1% in standard saline citrate

Diameter of cylinder: 5 cm.

Rate of feeding of DNA solution: 0.04 ml./hr.

Sample	Syringe Capillary Size (gauge)	Rate of Rotation of Cylinder (r.p.m.)	Precipitant	Storage of Film		$O.D._{38,400}$ (unpolarised)
				% r.h.	time (hrs.)	
1	19	2	Aqueous ethanol[1]	93	16	0.53
				93	53	0.48
2	19	2	Aqueous ethanol[1]	93	52	0.18
3	26	4	Aqueous acetone[2]	0	13	0.83
4	26	4	Aqueous acetone[2]	0	12	0.60
5	26	4	Aqueous acetone[2]	0	73	0.35

1. 33% H_2O

2. 20% H_2O

The differences in the optical densities of the different films are too large to be explained on the basis of a 44% hyperchromism in DNA films on dehydration (Falk, 1964).

The optical density reproducibility of films made during the same preparation was better than the reproducibility of films prepared individually. However, the films made at the one time were not identical (Table 2.6).

The reason for the lack of reproducibility in samples prepared individually is that different amounts of DNA were deposited during different preparations. A fibrous precipitate of DNA formed on the end of the delivery syringe and was not wound onto the cylinder. Different precipitants such as aqueous ethanol, aqueous acetone or aqueous isopropanol did not achieve the deposition of reproducible amounts of DNA in different runs. This is also the reason why the experiments involving an increased spinning way were unsatisfactory; not enough DNA was deposited on the cylinder; it was precipitated and adhered to the end of a glass tube which was immersed in the precipitant and was used to minimise the buffeting of the thread by turbulence over the increased spinning way. The buffeting of the threads by turbulence meant that they were not deposited as close as possible to each other on the cylinder and that the thickness of the films varied slightly from place to place. This could be a reason for the variation of the optical density of different films prepared

Table 2.6

Reproducibility of Films Prepared at the Same Time

E. Coli. DNA I

Concentration of DNA Solution: 0.1% in standard saline citrate

Precipitant: Aqueous acetone (20% H_2O)

Diameter of cylinder: 5 cm.

Rate of rotation of cylinder: 20 r.p.m.

Internal diameter of capillary: 0.097 cm.

Rate of feeding of DNA Solution: 0.22 ml./hr.

V_E = 319 cm.min.$^{-1}$ V_0 = 0.49 cm.min.$^{-1}$

$S = V_E/V_0 = 650$

Spinning Time: 8 hours

Drying conditions: 4 to 6 days at 93% r.h.

Sample	$O.D._{38,400}$ Unpolarised	$R_{38,400}$
1	0.698	0.65
2	0.617	0.64
3	0.512	0.61
4	0.629	0.62
5	0.559	0.58
6	0.540	0.73

in the same run (Jones, 1952). It would also have some bearing on the optical density of films prepared in different runs.

The dichroic ratio was quite reproducible both in films from different preparations and from the same preparation (Tables 2.2 and 2.6). The slight variation in R in films prepared at the one time may be assigned to the thickness variation along each sample.

2.2.7 Spinnability of DNA

The successful preparation of oriented DNA films by the wet spinning method depends on whether a DNA solution can be spun. Wet-spinnability may be expected to depend on the deformation ratio and the nature of the precipitant as already discussed. However, it has been found that the DNA sample and the solvent for the DNA also affect the spinnability.

The various nucleic acid samples which were used had been prepared from several sources. The concentration of each DNA solution was about 0.1%. The precipitating agents had been used for the precipitation of the DNA during its preparation as well (Marmur, 1961; Steiner and Beers, 1961). The results for the spinnability experiments are shown in Table 2.7. The spinnability was judged by the presence or absence of a film on the rotating cylinder; if a film was formed then the DNA was spinnable. It is not clear what factors about the sample affect the spinnability, since in some cases a change of the DNA-solvent system, in the

Table 2.7

The Spinnability of DNA

Source of DNA	Solvent	Precipitant	% H_2O in Precipitant	Electrolyte Content of Precipitant	S	Presence of film
E. Coli. I	Standard Saline Citrate	Aqueous Ethanol	0-33	0	48-160	yes
E. Coli. I	Standard Saline Citrate	Aqueous Ethanol	50	0	160	no
E. Coli. I	Standard Saline Citrate	Aqueous Acetone	20	0	4-650	yes
E. Coli. II	Standard Saline Citrate	Aqueous Acetone	20	0	260	yes
E. Coli. III	Standard Saline Citrate	Aqueous Acetone	20	0	880	no
E. Coli. III	0.3M Sodium Acetate	Aqueous Isopropanol	40	0.2M Sodium Acetate	750	yes
E. Coli. VI	H_2O	Ethanol	0	0	4	yes
B. Meg.	Standard Saline Citrate	Aqueous Acetone	20	0	63-260	yes
Calf thymus I	H_2O	Aqueous Ethanol	0-25	0	~160	no
Calf thymus II	H_2O	Aqueous Acetone	20	0	63	no
Calf thymus II	0.15M NaCl	Aqueous Acetone	20	0	63	no

same precipitant as for other samples, and in the same range of deformation ratios, changed the spinnability. It is possible that the presence of electrolyte was required in the precipitant in the case of the calf thymus samples (Herskovits et al., 1961), although it certainly was not necessary for most of the other samples. It is clear that an increase in the solvent power of the precipitant (e.g. 50% aqueous ethanol) decreases the spinnability of the DNA. It is also clear that the bacterial samples (E. Coli. and B. Meg. DNA) are better suited for the spinning process than the calf thymus samples. This could be an influence of the higher molecular weight of the bacterial samples.

2.3 Conclusion

The wet spinning method can be used to prepare limited numbers of reasonably reproducible oriented films of DNA. The orientation of the molecules in the films made in this work has not been as good as that obtained by other workers. It would seem that optimum conditions for orientation have not been achieved. The search for the optimum orientation conditions has been hampered by the limited spinnability of the various nucleic acids which were used.

CHAPTER THREE

THE INTERACTION OF DNA WITH POLY(VINYL ALCOHOL)

3.1 Oriented Poly(vinyl alcohol) Films

The use of poly(vinyl alcohol) (PVA) is well known in the preparation of oriented films of small organic molecules (Tanizaki, 1959, 1960; Jakobi and Kuhn, 1962; Tanizaki et al., 1965; Tsunoda and Yamaoka, 1965; Tanizaki and Kubodera, 1967a, 1967b). A PVA sheet is usually immersed in an aqueous solution of the substance under study and the small molecules are taken up by the sheet when it swells. The mechanical stretching of the sheet induces the orientation of the small molecules.

Since certain DNA samples proved difficult to spin in order to produce oriented films, a different method for the preparation of the films was sought. The possibility of using the PVA sheets suggested itself as a new method. However, thick PVA films did not take up DNA molecules from solution and so mixed solutions of DNA and PVA had to be cast in order to make PVA-DNA films.

This work involves a comparison of the behaviour of pure DNA and mixed PVA-DNA films and solutions to determine whether the PVA in the mixed films has any influence on the DNA in them.

3.2 Solution Properties of DNA

The Watson and Crick double helical model of DNA has become widely accepted as a model for the DNA, both in solution and in the solid state (Steiner and Beers, 1961). The double helical structure can be disrupted under certain conditions (Marmur et al., 1963). This is known as denaturation and the conditions which can denature DNA include heat treatment, the dilution of a DNA solution, or the treatment of a DNA solution with various chemicals, such as the alcohols. The denaturing power of the alcohols, as measured by immunological and spectrophotometric methods, increases with increasing chain length in the series methyl, ethyl, n-propyl and n-butyl alcohols. However, masking of the hydrocarbon chain with hydroxyl substituents reduces the denaturing power; for example, inositol (cyclo-hexanehexol) is less effective than cyclo-hexanol (Geiduschek and Herskovits, 1961; Herskovits et al., 1961; Herskovits, 1962; Levine et al., 1963; Marmur et al., 1963). Some interaction between DNA and PVA, a polyhydric alcohol with a long hydrocarbon chain, might be expected in solution on the basis of the interaction between DNA and the simple alcohols.

Denaturation of DNA is accompanied by an increase in the ultraviolet absorption (hyperchromism) and by only small changes in the shape of the absorption spectrum. Thermal denaturation is characterised by the melting point, T_m, which is the temperature when the optical density has increased by half the total change. Those molecules which enhance denaturation decrease T_m relative to untreated DNA.

Thermal denaturation profiles (optical density versus temperature) were measured for a DNA solution in dilute saline citrate, a PVA-DNA solution in dilute saline citrate and a DNA solution in alcoholic dilute saline citrate. The PVA-DNA solution had been made by diluting one hundred times an alcoholic solution of PVA-DNA which was to have been cast as a film (Chapter Six, Section 6.2.5). The alcoholic DNA solution in dilute saline citrate contained an amount of ethanol equivalent to that in the PVA-DNA solution to be cast. It was not practicable to measure the thermal denaturation profiles of a redissolved PVA-DNA film since the films were not readily soluble without the application of heat. It can be seen that there is very little difference between the thermal denaturation profiles of the DNA solution and the PVA-DNA solution (Fig. 3.1); the melting points are almost identical; the 3% difference in the extent of hyperchromism is not significant and could have resulted from the degradation of the DNA since the concentrated PVA-DNA solutions had to be shaken vigorously before they were cast as films (Harrington and Zimm, 1965), and since a control experiment on both shaken and unshaken DNA solutions showed a similar difference in the absorption increases (Table 3.1). The T_m of the ethanolic DNA solution is lower than that of the DNA and the PVA-DNA solutions, in agreement with the results of Herskovits et al. (1961); hence, any measurement on an alcoholic PVA-DNA solution whose concentration was comparable to those which were cast would be difficult to interpret and was not attempted. The large hyperchromic change for

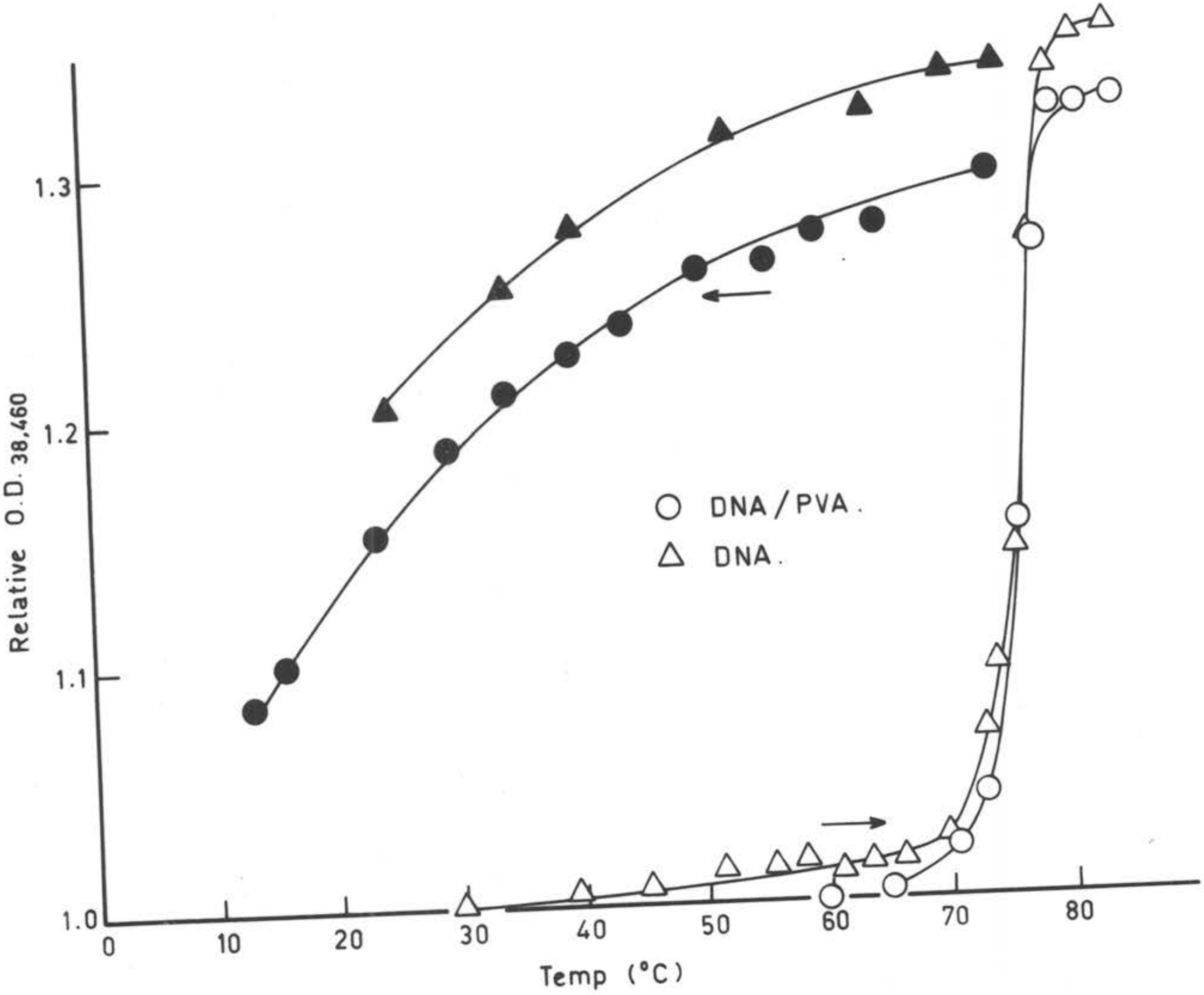


FIG. 3·1. Thermal Denaturation of DNA.

the alcoholic DNA solution may be explained by the evaporation of the solvent at higher temperatures.

It would seem that there is no interaction between the PVA and the DNA in dilute solution.

Table 3.1

Thermal Denaturation of DNA Solutions

Series	Solution	% Hyperchromic change at 38,460 cm^{-1} (corrected for solvent expansion)	T_m (^oC)
1	DNA (unshaken)	35	76.7
	PVA-DNA	32	76.4
	DNA (alcoholic)	49	70.0
2	DNA (unshaken)	37	
	DNA (shaken)	35	

3.3 The Behaviour of Pure DNA Films

Pure DNA films show an ultraviolet absorption band centred at about 38,400 cm^{-1}. The films show spectral changes which depend on their water content. The integrated intensity of the 38,400 cm^{-1} band increases upon dehydration and the absorption maximum shifts to shorter wavenumbers by about 250 cm^{-1} (Table 3.2) (Falk, 1964).

Table 3.2

Ultraviolet Spectra of Pure DNA and Mixed PVA-DNA Films

ν_{max} = wavenumber at the maximum absorption position

ν_{min} = wavenumber at the minimum absorption position

ν_{sh} = wavenumber at the point of inflection of the shoulder

Film	% r.h.	ν_{max} cm^{-1}	ν_{min} cm^{-1}	ν_{sh} cm^{-1}	Reference
Pure DNA	98	38,630 ± 30	43,290 ± 90	35,500 ± 300	Falk, 1964
PVA-DNA[1]	100	38,630 ± 60	43,400 ± 130	35,800 ± 100	This work
PVA-DNA[2]	93	38,610 ± 40	43,470 ± 70	35,800 ± 120	This work
Pure DNA	0	38,390 ± 30	43,290 ± 90	35,500 ± 300	Falk, 1964
PVA-DNA[3]	0	38,400 ± 50	43,370 ± 70	35,800 ± 140	This work

1. Average result of 31 measurements
2. Average result of 15 measurements
3. Average result of 40 measurements

The error quoted is the mean deviation.

The exposure of a dry film to an atmosphere of high relative humidity results in the complete reversibility of the absorption change. This is true even for a film which has also been heated after drying. The dichroic ratio of the 38,400 cm^{-1} band also changes from a low value to a higher value on dehydration and this change is also completely reversible (Falk et al., 1963b). Both the optical density and dichroic ratio changes occur, with hysteresis effects being observed, between 50% and 75% r.h. for the sodium nucleate films (Falk et al., 1963b; Webb and Bhorjee, 1968).

The explanation of the changes in the films is that there is a random tilting of the bases in the nucleic acid double helix on dehydration, but that the unwinding of the two strands of the helix cannot occur in the solid state; this restriction facilitates a rapid rematch of the bases upon the rehydration of the DNA. This explanation is analogous to the explanation of the loss of base stacking on denaturing DNA in solution except that the polynucleotide chains can uncoil in that case, making it difficult for the perfect rematch of the bases on renaturation and for the complete reversibility of the optical density change.

The presence of the absorption changes on hydration has been confirmed for films of the sodium salt of the DNA used in this work (Fig. 3.2). The optical density at 38,400 cm^{-1} was used to measure the extent of hyperchromism since the relative changes in this quantity were the same as the relative changes in the integrated absorption.

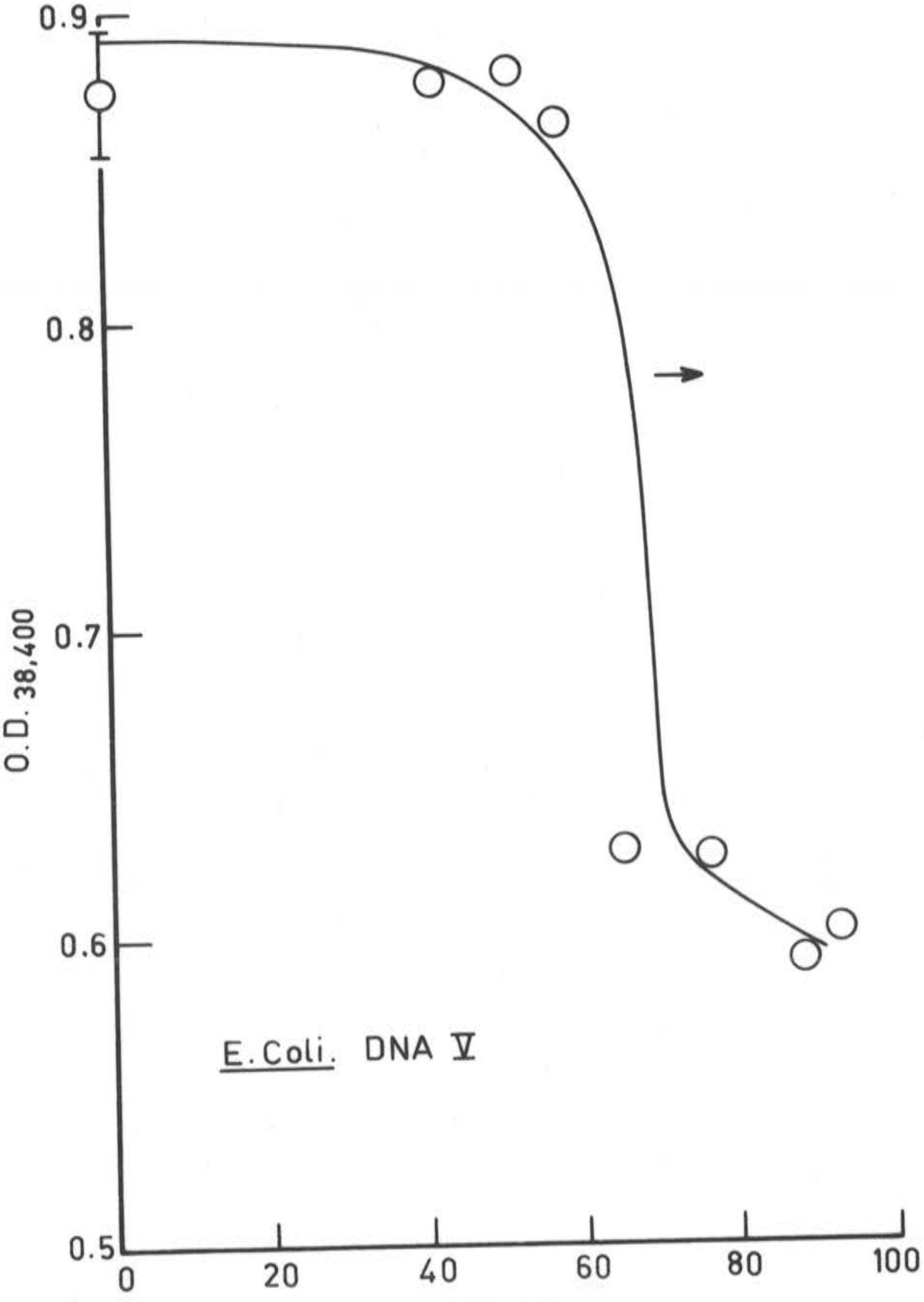


FIG. 3·2. Effect of Hydration on Pure DNA Film.

3.4 The Behaviour of Pure PVA Films

The ultraviolet spectrum of a pure PVA film, measured against air, arises from the absorption and the reflection of the film (Tanizaki and Kubodera, 1967a). There is no marked optical density change between 45,000 cm^{-1} and 30,000 cm^{-1}. Above 45,000 cm^{-1} the absorption increases (Kern and Dörr, 1961; Tanizaki and Kubodera, 1967a).

The films prepared in this project showed slightly different optical densities (Table 3.3). Each film also varied slightly in different parts owing to the relatively large area over which it was spread. However, the spectra of the films were independent of hydration.

In the infrared region, the PVA films showed a band at 1,650 cm^{-1} which may be attributed to water (Krimm et al., 1956). Although this band was not removed by allowing the films to stand at room relative humidity for a long time, it was removed by heating the films at 110°C for one hour.

3.5 The Behaviour of Mixed PVA-DNA Films

3.5.1 Appearance of the Films

In general, the mixed PVA-DNA films were clear and flat. The ultraviolet spectra of the mixed films were measured against pure PVA films and showed a slight optical density (of the order of 0.02)

Table 3.3

Reproducibility of PVA Films

Sample	Concentration of Solution Used for Casting Film (gm. PVA/ml. solvent)	O.D.$_{45,000}$	O.D.$_{38,400}$
1	0.093	0.120	0.092
2	0.095	0.108	0.072
3	0.094	0.093	0.082
3	0.094	0.102	0.095
4	0.093	0.103	0.084

between 32,000 cm^{-1} and 30,000 cm^{-1}. This was independent of the wavenumber and of the thickness of the mixed films. It was probably due to a slight mismatch of the sample and reference films (Table 3.3). When the films were subjected to an atmosphere of high relative humidity they swelled and became crinkly. However, perfect flatness was usually regained when they were re-exposed to room conditions. Upon the rehydration of a dry mixed film the optical density between 32,000 cm^{-1} and 30,000 cm^{-1} increased slightly over the first measurements for the thicker films but it was still independent of wavenumber, even up to 27,000 cm^{-1} in some cases. The optical density at 38,400 cm^{-1} was independent of time after the flatness had been regained. The reproducibility of the optical density for the same conditions was better than 1%; the mean deviation was usually 0.5%.

3.5.2 The DNA Absorption Region

The ultraviolet absorption spectra were not measured above 45,000 cm^{-1} because of the increasing absorption of the PVA and because a greater error was expected from the mismatch of the sample and reference films.

The unpolarised spectra of the unstretched mixed films were similar to those for pure DNA films in the positions of the maxima, minima and shoulders near 38,400 cm^{-1}, 43,300 cm^{-1} and 35,800 cm^{-1}, respectively. The wavenumber shifts on dehydration were also similar (Table 3.2, Fig. 3.3). However, there were differences in the absorption behaviour of the mixed films and the pure films on drying and hydration. The drying of a mixed film by decreasing the relative humidity of the atmosphere in which the film was equilibrated produced no change in the optical density unless the relative humidity was reduced to 0% r.h. (Fig. 3.4). The percentage change in the integrated absorption (measured by the increase in the optical density at 38,400 cm^{-1}) of a totally dry mixed film was much less

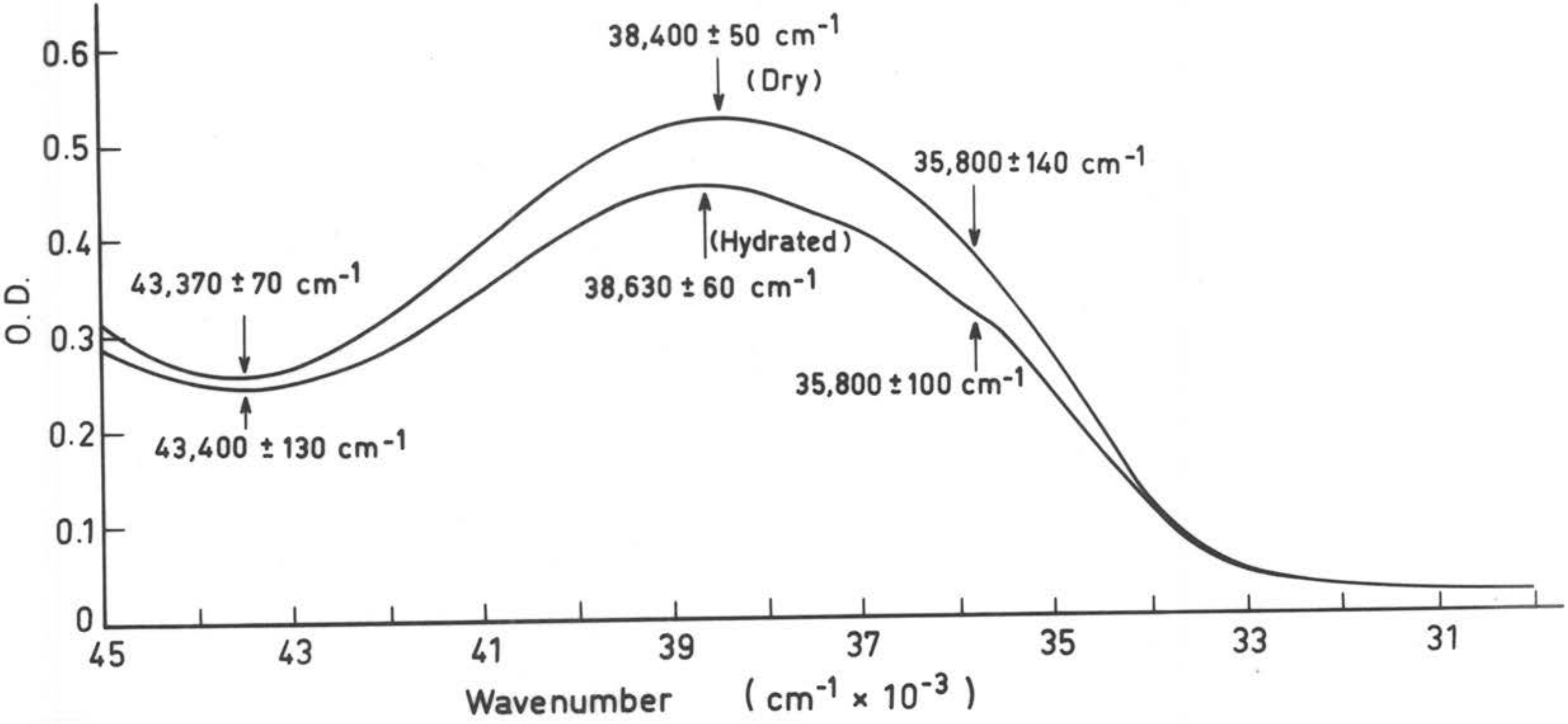


FIG. 3·3. Effect of Hydration on Spectrum of PVA-DNA Films.

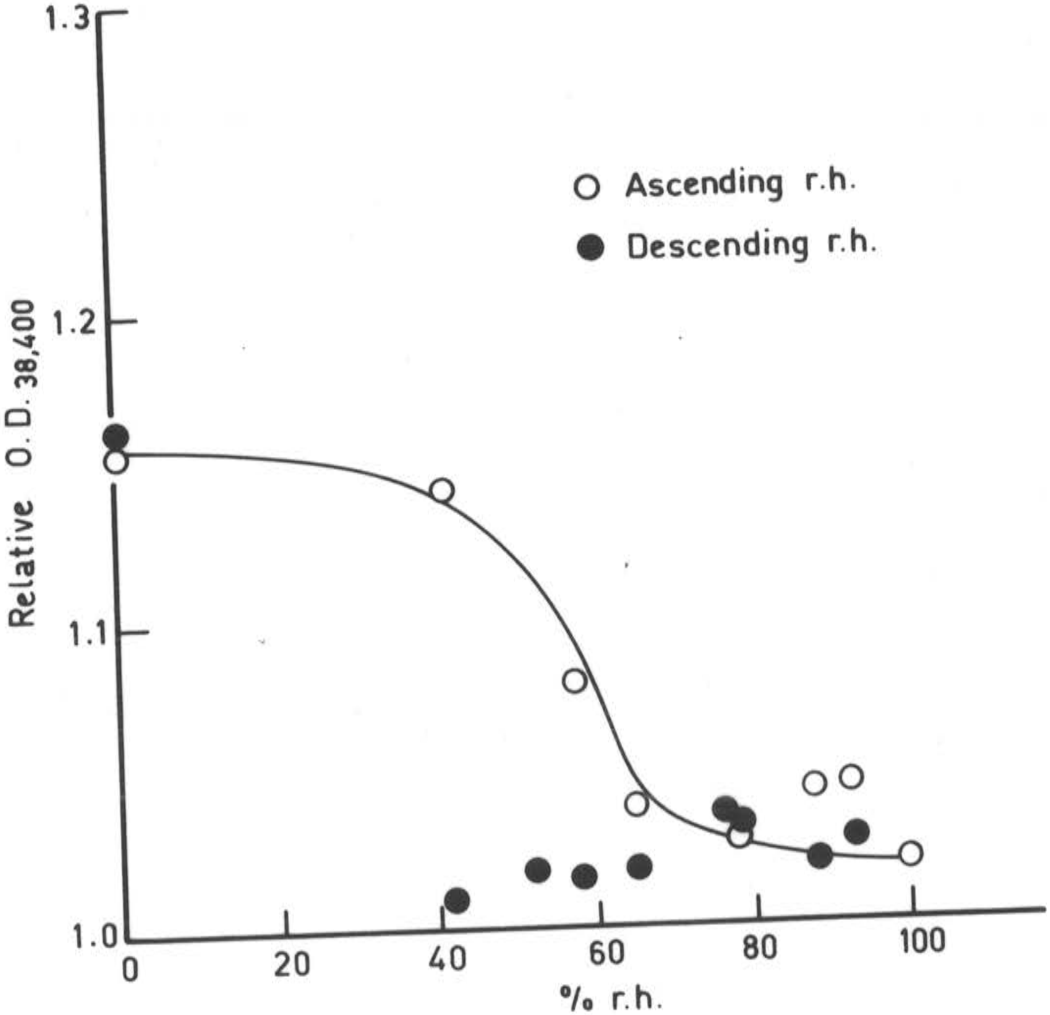


FIG. 3·4. Effect of Dehydration on PVA-DNA Films than for a pure film made from the same DNA, even when the mixed film had been heated at 107°C for one hour and then at 120°C for another hour (Table 3.4). Perfect reversibility of the absorption change on the rehydration of the mixed films was not achieved. Only in one case was there a perfect return to the original optical density. In other cases, differences up to 5% existed between the final optical densities on rehydration and the original optical densities of hydrated freshly prepared films, even when the optical densities at 32,000 cm^{-1} had been subtracted from those at 38,400 cm^{-1}, both for the original readings and the final readings.

The range of relative humidities over which the spectral changes occurred in the mixed films lies between 40% and 65% r.h., compared with the values of 50% and 75% r.h. for the sodium salt of pure DNA (Fig. 3.5), (Falk et al., 1963b; Webb and Bhorjee, 1968). In Fig. 3.5, the results of Falk et al. (1963b) have been corrected to give the same extent of hyperchromicity as observed in one of the pure DNA films prepared in this project.

The absorption differences between the mixed and the pure films were the same whether the cast PVA-DNA solutions had been dried to films slightly above room relative humidity (55% r.h.) or slightly above 84% relative humidity.

3.5.3 Oriented PVA-DNA Films

Preliminary work has shown that the mechanical stretching

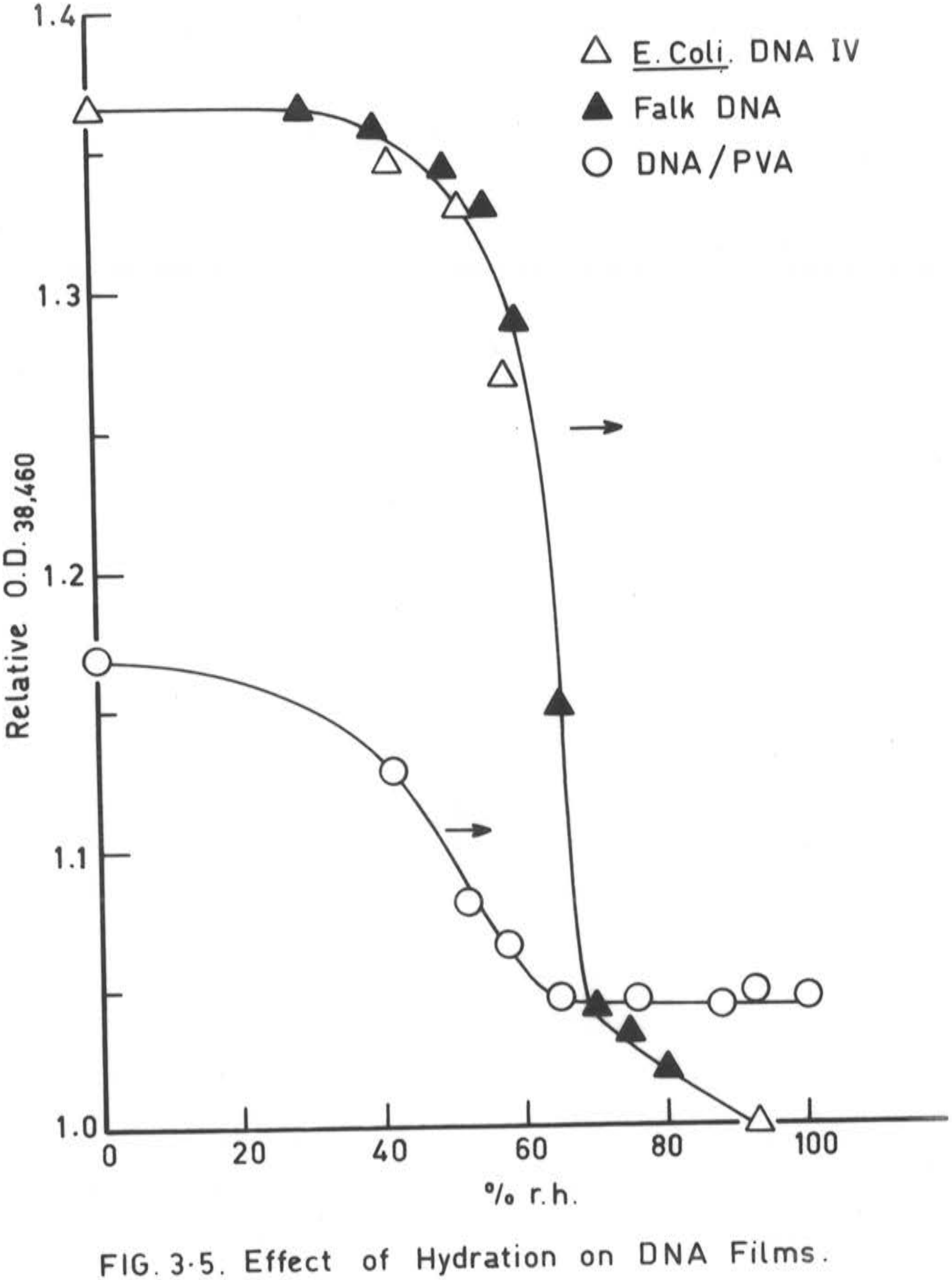


FIG. 3·5. Effect of Hydration on DNA Films.

Table 3.4

Hyperchromism in PVA-DNA Films on Drying

The percentage optical density change was calculated with respect to the minimum reproducible optical density of freshly prepared hydrated films.

Film	Treatment	Time (hrs.)	% Hyperchromism at 38,400 cm^{-1}
Pure DNA (*E. Coli.* IV)	P_2O_5	33	37
PVA-DNA (*E. Coli.* III, IV)	P_2O_5	110-310	16-17
PVA-DNA (*E. Coli.* IV)	P_2O_5	310	16
	and		
	107-120°C	2	18
Pure DNA (*E. Coli.* V)	P_2O_5	182	46
PVA-DNA (*E. Coli.* V)	P_2O_5	150-335	19

of the PVA-DNA foils induces the orientation of the nucleic acid. The reference direction for the measurement of the dichroism was the direction in which the films were stretched. The dichroism of the DNA absorption band was large and negative ($O.D._{\parallel} - O.D._{\perp} = -0.4$) in agreement with the results from the wet spun films. Although a silica cell was used as the reference in these measurements, the dichroic ratio at 38,400 cm^{-1} decreased with increased stretching of the hydrated films indicating that better orientation is achieved with an increased deformation (Fig. 3.6). The dichroic ratio also depended on the amount of moisture in the oriented film; for example, a film dried over silica gel for twenty hours gave a dichroic ratio of 0.88 while the same film gave a dichroic ratio of 0.61 at 38,400 cm^{-1} upon rehydration. A study of the variation of the dichroic ratio over a wide range of relative humidities has not been made.

In the polarised spectra of the mixed films the parallel component is again slightly displaced to shorter wavenumbers compared with the perpendicular component.

It is of great interest that the DNA used to prepare the oriented PVA-DNA films was calf thymus DNA II in Table 2.7 which would not readily lend itself to the wet spinning method.

3.6 Discussion

Evidence for the interaction of PVA and poly(methylmethacrylic acid) has been presented in the literature (Okhrimenko and D'Yakonova, 1964; D'Yakonova et al., 1965; Okhrimenko et al., 1966). The interaction, on the basis of infrared spectroscopy, is believed to occur by means of hydrogen bonding between the carboxyl groups of the poly-acid and the hydroxyl groups of the poly-alcohol

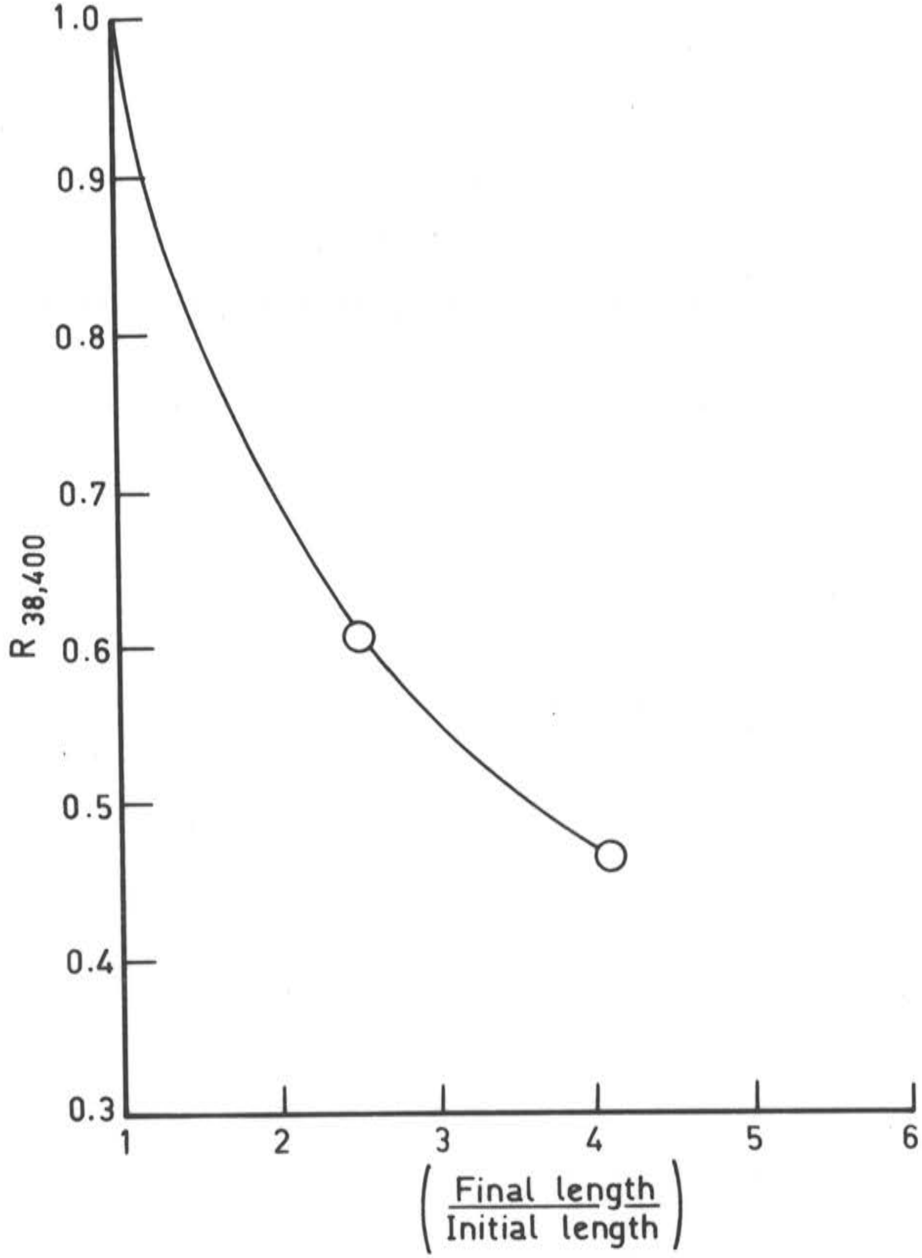


FIG. 3·6. Dichroic Ratio in PVA-DNA as a Function of Stretching.

(Distler et al., 1966). The results of the solution and solid state studies of the PVA-DNA system will be discussed in terms of an interaction between the PVA and the DNA. It is clear that the interaction takes place in the range of relative humidities between the solution state and the film state at 84% r.h.

Although the spectra of the films in the 38,400 cm^{-1} region failed to show any difference between the pure DNA and the mixed PVA-DNA films, it has recently been observed that this region is not very sensitive to changes in the DNA which may be caused by its interaction with inorganic salts, amino acids and other substances in solution. However, the spectral region near 52,630 cm^{-1} is claimed to be more sensitive to the interactions. The interactions result in a decrease of the absorption coefficient of the DNA absorption band at 52,083 cm^{-1} and in a shift of the band to shorter wavenumbers, relative to a salt free aqueous solution of the DNA (Tarnok and Röhrscheidt, 1968). It may be useful to compare the pure DNA and the mixed PVA-DNA film spectra in the far ultraviolet region, but this will require very carefully-matched preparations of the sample PVA-DNA and the reference PVA films.

The spectral changes of the pure DNA and mixed PVA-DNA films on complete dehydration and gradual rehydration are qualitatively similar. The slightly different range of relative humidities over which the spectral changes take place on rehydration of the mixed PVA-DNA films, compared with the pure DNA films, is not strong evidence for an interaction between the PVA and the DNA owing to the hysteresis effects noted by other workers in pure DNA films (Falk et al., 1963b). On the other hand, both polarised infrared spectroscopic studies and X-ray studies on the configurations of nucleic acid films and fibres have shown that different alkali metal salts of the acids undergo configurational changes at different relative humidities (Bradbury et al., 1961; Wilkins, 1963). In particular, even in the lithium salt, the X-ray patterns depend on both the lithium chloride content of the DNA fibres and on the relative humidity (Marvin et al., 1961).

The low hyperchromic change of the mixed PVA-DNA films on dehydration is the strongest evidence for an interaction between the PVA and the DNA. The small hyperchromic change in the mixed films cannot be explained by a mismatch of the sample PVA-DNA and the reference PVA films. The measured optical density of a hydrated mixed film, $(\mathrm{O.D.})_h$, is the sum of the optical density of the DNA in the film, $(\mathrm{O.D.})_h^{\mathrm{DNA}}$, and of the difference in the optical densities of the PVA in the sample film and in the reference film, $(\mathrm{O.D.}_S - \mathrm{O.D.}_R)_h^{\mathrm{PVA}}$ (equation (3.1)).

$$(\mathrm{O.D.})_h = (\mathrm{O.D.})_h^{\mathrm{DNA}} + (\mathrm{O.D.}_S - \mathrm{O.D.}_R)_h^{\mathrm{PVA}} \qquad (3.1)$$

Equation (3.2) gives the measured optical density of a dry film, $(\mathrm{O.D.})_d$, in terms of the components of the sample and the reference films.

$$(\text{O.D.})_d = (\text{O.D.})_d^{\text{DNA}} + (\text{O.D.}_S - \text{O.D.}_R)_d^{\text{PVA}} \qquad (3.2)$$

Since the spectrum of a PVA film is independent of hydration, the last terms on the right hand side of equations (3.1) and (3.2) are the same. If it is assumed that the DNA in the mixed PVA-DNA films is hyperchromic to the same extent as pure DNA, then $(\text{O.D.}_S - \text{O.D.}_R)^{\text{PVA}}$ may be calculated by the simultaneous solution of equations (3.1) and (3.2), where $(\text{O.D.})_h$, $(\text{O.D.})_d$ and the relation between $(\text{O.D.})_h^{\text{DNA}}$ and $(\text{O.D.})_d^{\text{DNA}}$ are known. The calculated mismatch of the PVA in the mixed PVA-DNA and the pure reference PVA films greatly exceeds not only the measured mismatch, but also the measured $(\text{O.D.})_R^{\text{PVA}}$ (Table 3.5).

Table 3.5

Calculated Mismatch of Sample PVA-DNA Films and Reference PVA Films at 38,400 cm^{-1}

$$(\text{O.D.})_h = (\text{O.D.})_h^{\text{DNA}} + (\text{O.D.}_S - \text{O.D.}_R)^{\text{PVA}}$$

$$(\text{O.D.})_d = (\text{O.D.})_d^{\text{DNA}} + (\text{O.D.}_S - \text{O.D.}_R)^{\text{PVA}}$$

$$\Delta(\text{O.D.}) = (\text{O.D.}_S - \text{O.D.}_R)^{\text{PVA}}$$

$$(\text{O.D.})_d^{\text{DNA}} = 1.37\ (\text{O.D.})_h^{\text{DNA}} \qquad \text{(Table 3.4)}$$

$(\text{O.D.})_h$ (measured)	$(\text{O.D.})_d$ (measured)	$(\text{O.D.})_h^{\text{DNA}}$ (calculated)	$\Delta(\text{O.D.})$ (calculated)	$\Delta(\text{O.D.})$ (measured) (Table 3.3)	$(\text{O.D.})_R^{\text{PVA}}$ (measured) (Table 3.3)
1.103	1.280	0.48	0.62	0.02	0.095
0.669	0.792	0.33	0.34	0.02	0.095
0.534	0.624	0.24	0.29	0.02	0.095

The smaller hyperchromic change in the mixed PVA-DNA films compared with the pure DNA films means that there is a smaller loss of base pairing and stacking in the DNA in the mixed films upon complete dehydration. The changes in the dichroic ratio upon hydration verify that there is less base pairing and stacking in the dry mixed films than in the hydrated mixed films (Falk et al., 1963b). There are two interpretations for the small hyperchromic change in the mixed PVA-DNA films. Both interpretations are based on the assumption that the removal of all the water molecules from pure DNA films leads to full hyperchromism. The water molecules stabilise the B form of DNA in the solid state (Falk et al., 1963b).

The first interpretation for the low hyperchromism in the mixed PVA-DNA films supposes that the PVA in the mixed films has taken the place of some of the water molecules which stabilised the DNA and that the DNA is still fully native. The removal of the stabilising molecules of PVA cannot be achieved on drying the films with phosphorus pentoxide or heating; the removal of all the water molecules, indicated by the disappearance of the 1,650 cm^{-1} band in the pure PVA films, does not lead to full hyperchromism in the mixed films. The lack of the spectral changes upon subjecting the PVA-DNA films to atmospheres of decreasing relative humidity may be explained by this model for the interaction, but it would depend at what sites the PVA has replaced the water molecules in the DNA (Falk et al., 1963a). On the other hand, the lack of spectral changes may also be explained by the strong association of water with the PVA matrix itself (Krimm et al., 1956).

This model for the interaction of PVA with DNA is consistent with the study of Webb and Bhorjee (1968) on the interaction of inositol, a polyhydric alcohol, with DNA in oriented films. Infrared spectroscopy in the regions 4,000 cm^{-1} to 2,000 cm^{-1} and 1,800 cm^{-1} to 1,400 cm^{-1} proved that the inositol replaces the water molecules in the DNA double helix and completely prevents the occurrence of ultraviolet hyperchromic effects.

The mode of interaction between the PVA and the DNA may be hydrogen bonding between the hydroxyl groups of the PVA and available groups such as the phosphate groups of the DNA. Infrared spectroscopy may reveal hydrogen bonding in the PVA-DNA system (Distler et al., 1966). Up to date, the mixed PVA-DNA films have contained too much PVA for a successful study of the shapes of the absorption bands in the O-H stretching region in the infrared. Much thinner films with less PVA in them are therefore recommended.

The second interpretation for the small hyperchromic change in the mixed PVA-DNA films is that the DNA has interacted with the PVA in the hydrated film and that the optical density of the PVA-DNA complex is greater than the optical density of a native DNA film at the same concentration of the nucleic acid. It is assumed that the total removal of the water molecules will lead to the same final optical density as for a pure DNA film, but the relative change will be less for the mixed PVA-DNA film.

The reason for the lack of a perfect reversibility of the absorption change on rehydration of the mixed films is not clear.

The fact that the T_m measurements failed to show any interaction between the PVA and the DNA in solution may be the result of concentration differences in the solid films and in the solutions used in the T_m measurements. It must be remembered that the solutions on which the thermal denaturation measurements were made were one hundred times more dilute than the solutions which were cast, although the PVA:DNA ratio was the same as in the concentrated solutions.

3.7 Other Attempted Studies

Substances such as poly-L-lysine and various diamines stabilise the DNA double helix to denaturation in solution and increase T_m relative to native DNA (Mahler and Mehrotra, 1963; Tsuboi et al., 1966). It was desirable to interact the DNA with some of these substances before introducing the PVA and to cast interacted PVA-Substance-DNA films and to test the extent of hyperchromicity in such films. An increased hyperchromicity over a plain PVA-DNA film might have validated the second interpretation for the interaction between PVA and DNA in the films.

Two substances were used - poly-L-lysine and 1,5-diaminopentane. However, the precipitation of the DNA with poly-L-lysine, before the introduction of the PVA, and the precipitation of the DNA with 1,5-diaminopentane, after the introduction of the PVA, made these two substances unsuitable for this type of study. It should be noted that there was also precipitation of the poly-L-lysine-DNA complex even when it was prepared in dilute solution and the resulting solution was concentrated by freeze-drying. It should also be noted that no precipitation was observed when the PVA was introduced into a solution containing 1,5-diaminopentane and no DNA.

3.8 Conclusion

The poly(vinyl alcohol) films are probably not suitable for quantitative studies of interactions between DNA and small molecules because of the interaction between the PVA and the DNA. However, they may be suitable for qualitative spectroscopic studies because the changes of the DNA in the mixed films are qualitatively similar to those which occur in the pure DNA films in the region 45,000 cm^{-1} to 32,000 cm^{-1}. The mixed films have the advantage that the DNA in them may be oriented when they are stretched, whereas it may not be easy to orient the DNA by other methods.

This method of orientation may be applicable to other polyelectrolytes. One example could be atactic and isotactic sodium polystyrene sulphonate, where ultraviolet dichroism measurements on oriented films may show configurational differences between the two types of compound. Configurational differences between the atactic and isotactic sodium polystyrene sulphonates have not been detectable in the solid state from the infrared spectra of potassium bromide discs or from X-ray diffraction patterns of powders (Martin, 1966). Another example could be ribonucleic acid (RNA) where the spatula-stroking technique has been unsuccessful in the preparation of oriented films to examine the possibility of configurational changes on hydration by means of polarised spectroscopy (Falk, 1965). The possibility of using PVA-RNA films in polarised spectroscopic studies would seem remotely possible because the hydration effects of RNA are slightly different from those of DNA (Falk, 1965).

CHAPTER FOUR

THE INTERACTION OF DNA WITH DYES

The interaction of DNA with small molecules such as dyes has been an important subject in connection with mutagenesis and with the histological staining of tissues (Orgel and Brenner, 1961; Steiner and Beers, 1961). A lot of studies have been made in solution in an attempt to elucidate the mode of the interaction. Various techniques have been used, including ordinary and difference spectrophotometry (Morthland et al., 1954; Peacocke and Skerrett, 1956; Bradley and Wolf, 1959; Yamabe, 1967), flow dichroism (Lerman, 1963; Nagata et al., 1966), circular dichroism (Gardner and Mason, 1967), electro-optical techniques (Powers and Peticolas, 1967) and nuclear magnetic resonance (Blears and Danyluk, 1967). Relatively few solid state studies have been made on dyed DNA, especially optical measurements (Lerman, 1961; Neville and Davies, 1966). This chapter briefly describes some optical studies of the interaction of methylene blue, and two triphenylmethane dyes, with DNA in the oriented wet spun DNA films.

4.1 Metachromasia in Dyes

4.1.1 Polymer-free Solutions

A large number of flat aromatic dyes aggregate in aqueous solution. The aggregation is accompanied by changes in the dye spectrum in the visible region. In particular, Beer's law is not obeyed at high concentrations and there is a blue shift of the absorption peak. This phenomenon is known as metachromasia.

The thiazine dye methylene blue (MB) is one example of a metachromatic dye (Fig. 4.1). In dilute aqueous solution, the visible spectrum of the monomer shows a strong band at 15,000 cm^{-1} which has a vibrational shoulder at 16,250 cm^{-1} (Fig. 4.2). As the concentration of the MB is increased, the peak at 15,000 cm^{-1} decreases in intensity and the shoulder increases in intensity owing to the formation of dimers (Bergmann and O'Konski, 1963). Above concentrations of 10^{-3}M, trimers are formed and the 16,300 cm^{-1} peak shifts further to the blue with a reduction in the overall absorption coefficient (Braswell, 1968).

It is known that less aggregation takes place in organic solvents (McKay and Hillson, 1967). This has been confirmed for solutions of methylene blue in an acetone-water mixture (80% acetone) for the concentration range 0 to 5 x 10^{-4}M. The spectrum of the methylene blue in the acetone-water mixture resembles the monomer spectrum of aqueous methylene blue, except that the main peak is shifted to 15,200 cm^{-1}. Beer's law is obeyed over the range of concentrations measured and the ratio of the optical densities of the main band to the point of inflection of the shoulder is constant (Fig. 4.3).

4.1.2 Solutions Containing Polymers

In the case of an interaction between cationic dyes and

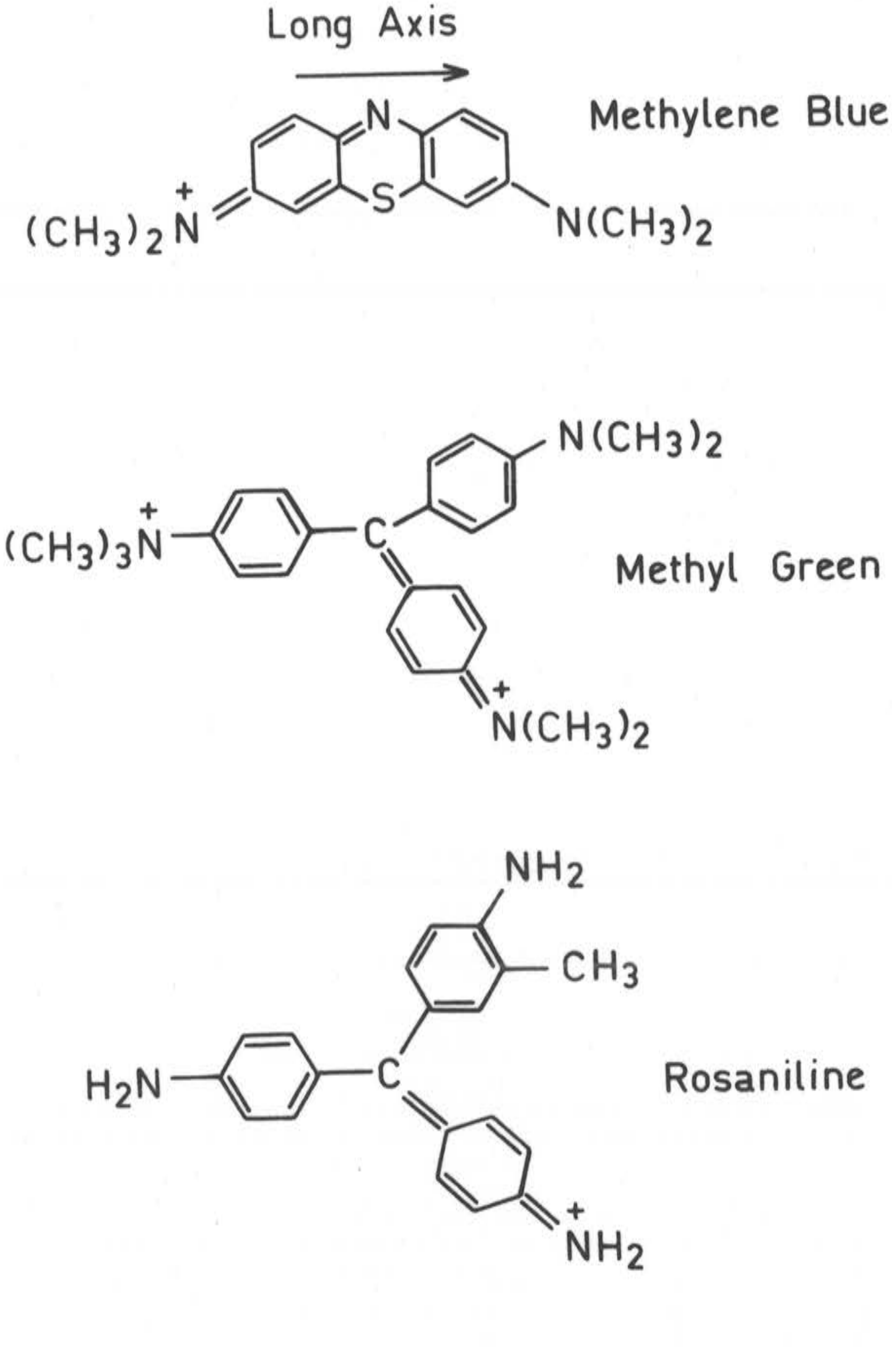


FIG. 4·1. Dye Structures.

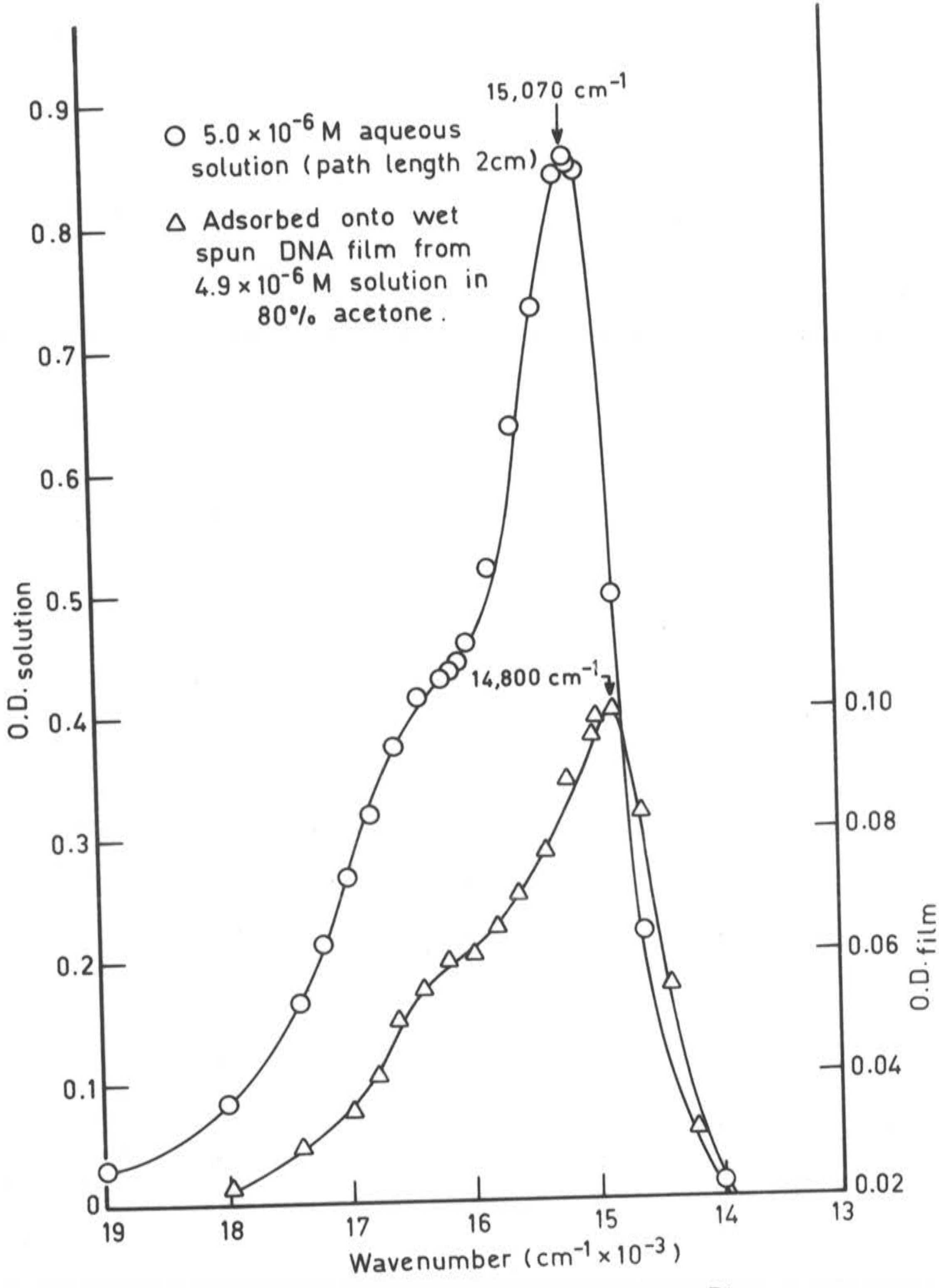


FIG. 4·2. Unpolarised Spectra of Methylene Blue.

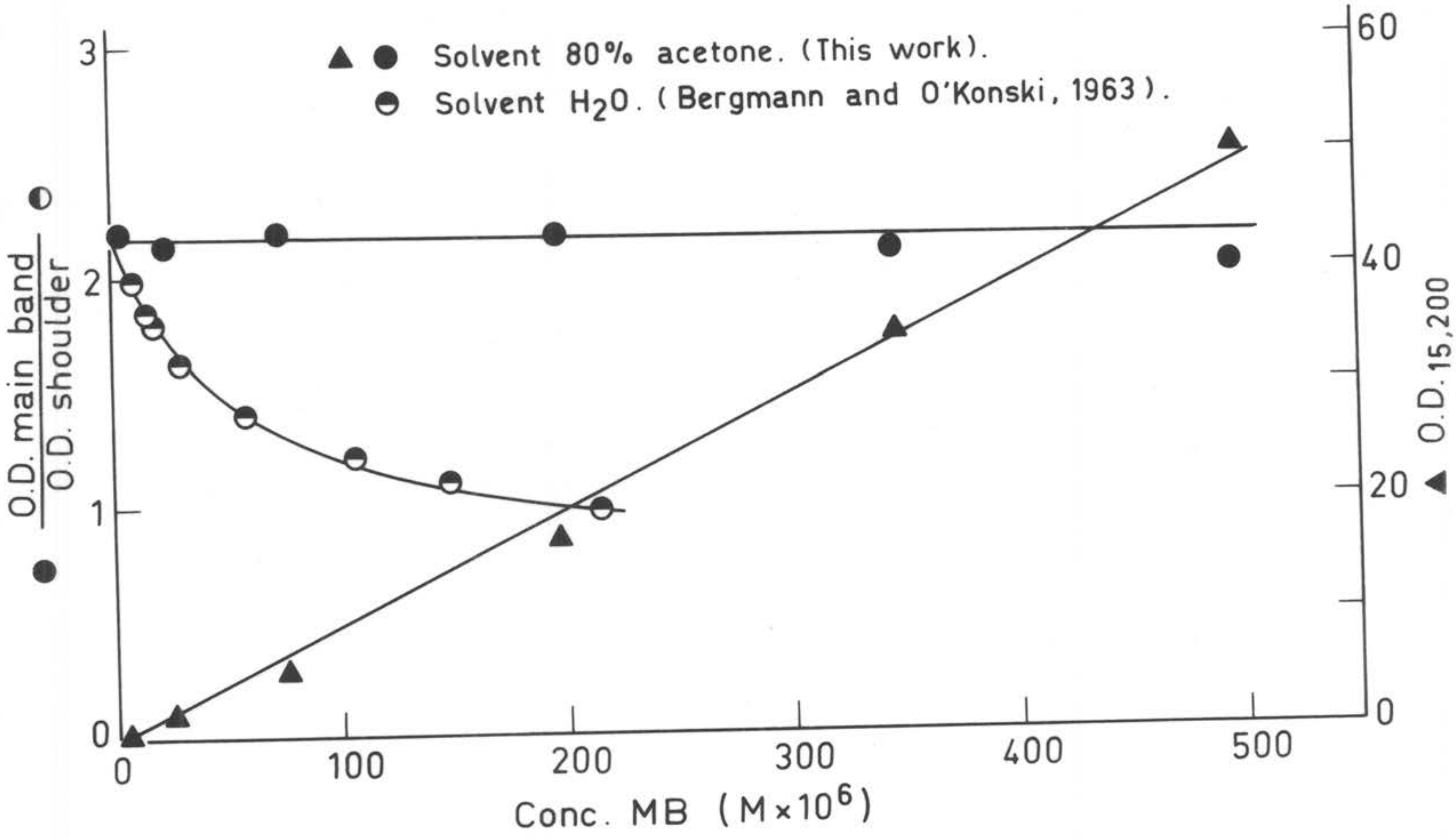


FIG. 4·3. The Behaviour of Methylene Blue in 80% Acetone.

polyanions in dilute aqueous solution metachromasia is evident, even though the concentration of the dye is such that only monomers are present in an equivalent polyanion-free solution (Bradley and Wolf, 1959). The extent of the metachromasia depends on the relative amounts of the polyanion and the dye which are present in the solution. This behaviour has been attributed to the formation of dye aggregates on the polyanion. It should be noted that at high ratios of polyanion to dye the shape of the visible spectrum of the dye is similar to the spectrum of unbound monomer, except that the main absorption peak lies at lower wavenumbers (Morthland et al., 1954; Bradley and Wolf, 1959).

The interaction of DNA with the acridine dyes has been interpreted in terms of two basic models. In the first model, at high polymer to dye ratios, the dye is thought to be intercalated or sandwiched between adjacent base pairs within the double helix (Lerman 1961, 1963). At lower polymer to dye ratios, micelles of the dye are thought to be taken up by the phosphate groups on the outside of the helix (Peacocke and Skerrett, 1956). In the second model, the dye molecules are only bound externally to the phosphate groups and are oriented with their long axes perpendicular to the helix axis (Bradley and Wolf, 1959). Other models, which are modifications of the intercalation model, have also been proposed for the interaction (Pritchard et al., 1966; Gardner and Mason, 1967).

4.2 The Interaction of Methylene Blue with DNA in Oriented Films

4.2.1 Preparation of the Dyed Films

It was not possible to prepare oriented, dyed DNA films by the direct wet spinning of a mixed solution of methylene blue and DNA, since the dye was washed out of the resulting film by the precipitant. However, pure wet spun films of DNA adsorbed methylene blue from solutions of the dye in aqueous acetone (80% acetone) in most cases. The films which had been spun in aqueous isopropanol containing sodium acetate adsorbed the dye when they were first immersed in the dye bath, but the adsorbed dye was released as the time of immersion increased. These films did not adsorb methylene blue if they were first kept in 80% acetone. The explanation of this effect is not certain, since the films which had been wet spun in 80% acetone adsorbed the methylene blue from the dye bath and did not appear to release appreciable amounts of it on standing in 80% acetone. It has been shown that the binding of methylene blue by DNA in solution is reduced by increasing the acetone concentration of the solution or by increasing the salt concentration (Simons, 1968). It seems that the dyeing properties of the wet spun DNA films are connected with the electrolyte content of the precipitant and hence with the electrolyte content of the films.

The PVA-DNA films were unsuitable for studying the interactions of dyes with DNA because of the binding capacity of PVA for methylene blue and other dyes (Dörr, 1966).

4.2.2 Unpolarised Visible Spectra of the Dyed Films

The spectra of the dyed wet spun DNA films are subject to the inhomogeneity effects discussed in Chapter Two, Sections 2.2.2 and 2.2.6 (Jones, 1952).

The dyed films whose properties will be discussed were prepared from the DNA films described in Table 2.6. Each film contained a similar amount of DNA, and the polymer to dye ratio was changed by immersing different films in solutions of methylene blue in aqueous acetone (80% acetone), but of different concentrations of the dye, for an equal time. The unpolarised spectra of the methylene blue adsorbed onto the films are shown in Figs. 4.2 and 4.4, after the films had been stored at 93% r.h. for five days. Two peaks may be seen in the spectra, one at 14,800 cm^{-1} and the other between 16,000 cm^{-1} and 16,500 cm^{-1}, the relative intensity of the two peaks depending on the polymer to dye ratios in the different films (Table 4.1). The position of the long wavenumber peak also depends on the polymer to dye ratio. For the film immersed in the most dilute methylene blue solution, the spectrum of the adsorbed dye resembles the monomer spectrum of the free dye in aqueous solution, except that the position of the main band has shifted to the red (Fig. 4.2). This is indicative of the binding of methylene blue to DNA (Morthland et al., 1954).

The dyed film prepared using 198 x 10^{-6}M methylene blue as the dye bath seems anomalous in the series in that the optical density

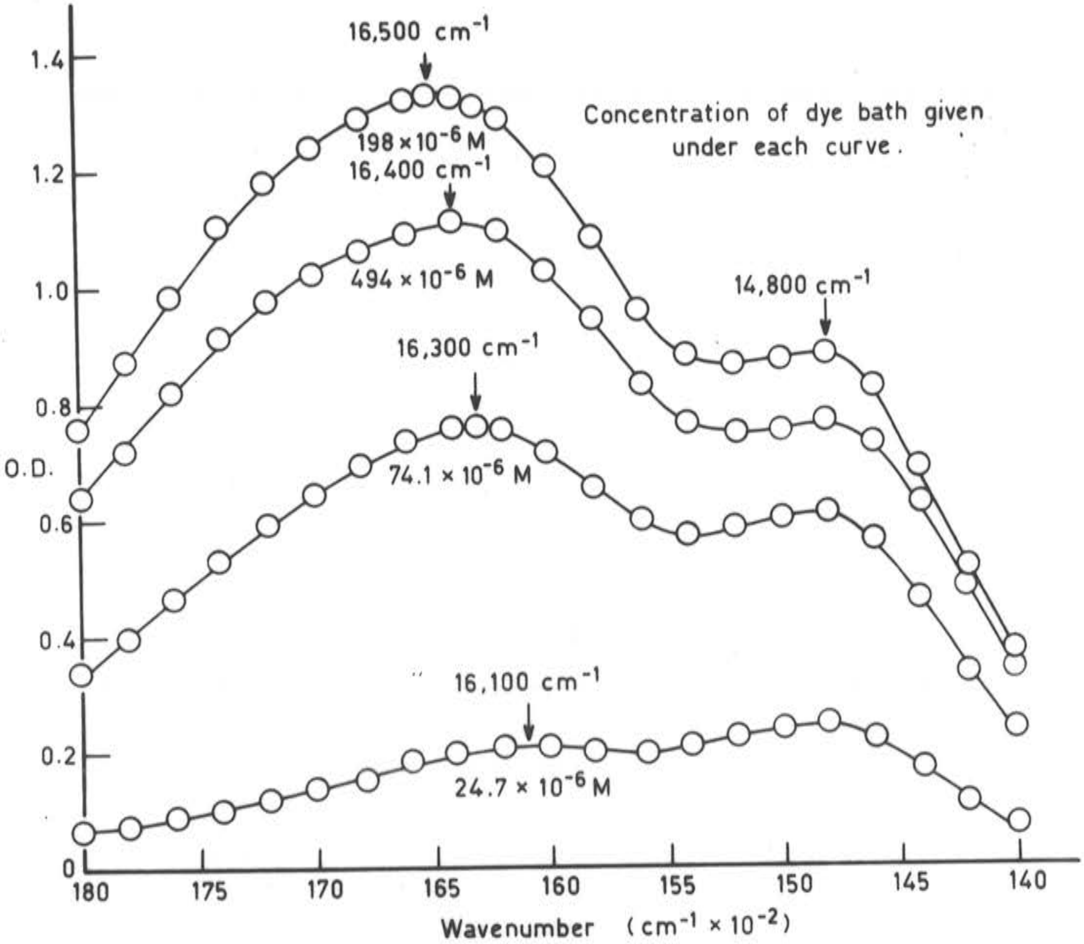


FIG. 4·4. Unpolarised Spectra of Methylene Blue Adsorbed onto Wet Spun DNA Films.

Table 4.1

Adsorption of Methylene Blue onto DNA Films

Concentration of MB in dye bath ($M \times 10^6$)	Properties of Undyed Films		Properties of Dyed Films			
	Unpolarised	Polarised	Unpolarised		Polarised	
	$O.D._{38,400}$	$R_{38,400}$	$O.D._{16,400}$	$O.D._{14,800}$	$R_{16,400}$	$R_{14,800}$
4.94	0.698	0.65	0.055	0.100	-	-
24.7	0.617	0.64	0.197	0.243	0.88	0.87
74.1	0.512	0.61	0.759	0.605	0.89	0.90
198	0.629	0.62	1.327	0.881	0.77	0.80
494	0.559	0.58	1.113	0.766	0.71	0.75

of the adsorbed dye is higher than for a film dyed in a more concentrated MB solution. In fact, the amount of DNA in the particular film was greater than in the film immersed in the more concentrated dye solution, and the data fit quite well on the adsorption isotherm in Fig. 4.5.

Preliminary experiments involving the wetting and the drying of the dyed films showed changes in the spectra of the adsorbed dye. These changes are interesting from the points of view that the state of aggregation of various dyes in sublimated layers depends on the water content of the layers (Lysina and Vartanian, 1959), and that the DNA configuration in the films also depends on the water content of the films (Falk et al., 1963b).

The results for the methylene blue interaction with the DNA in the wet spun films are similar to the results for solution studies of acridine dyes bound to DNA (Bradley and Wolf, 1959). They are also similar to the results for the adsorption of methylene blue to montmorillonite where it has been interpreted that the adsorbed dye exists as bound monomer and bound aggregate (Bergmann and O'Konski, 1963). It seems that in the case of the DNA films single dye molecules are adsorbed from a monomeric dye solution and that the aggregation of the dye molecules takes place on the DNA. This also agrees with the findings of Bergmann and O'Konski (1963) on the binding of methylene blue by montmorillonite.

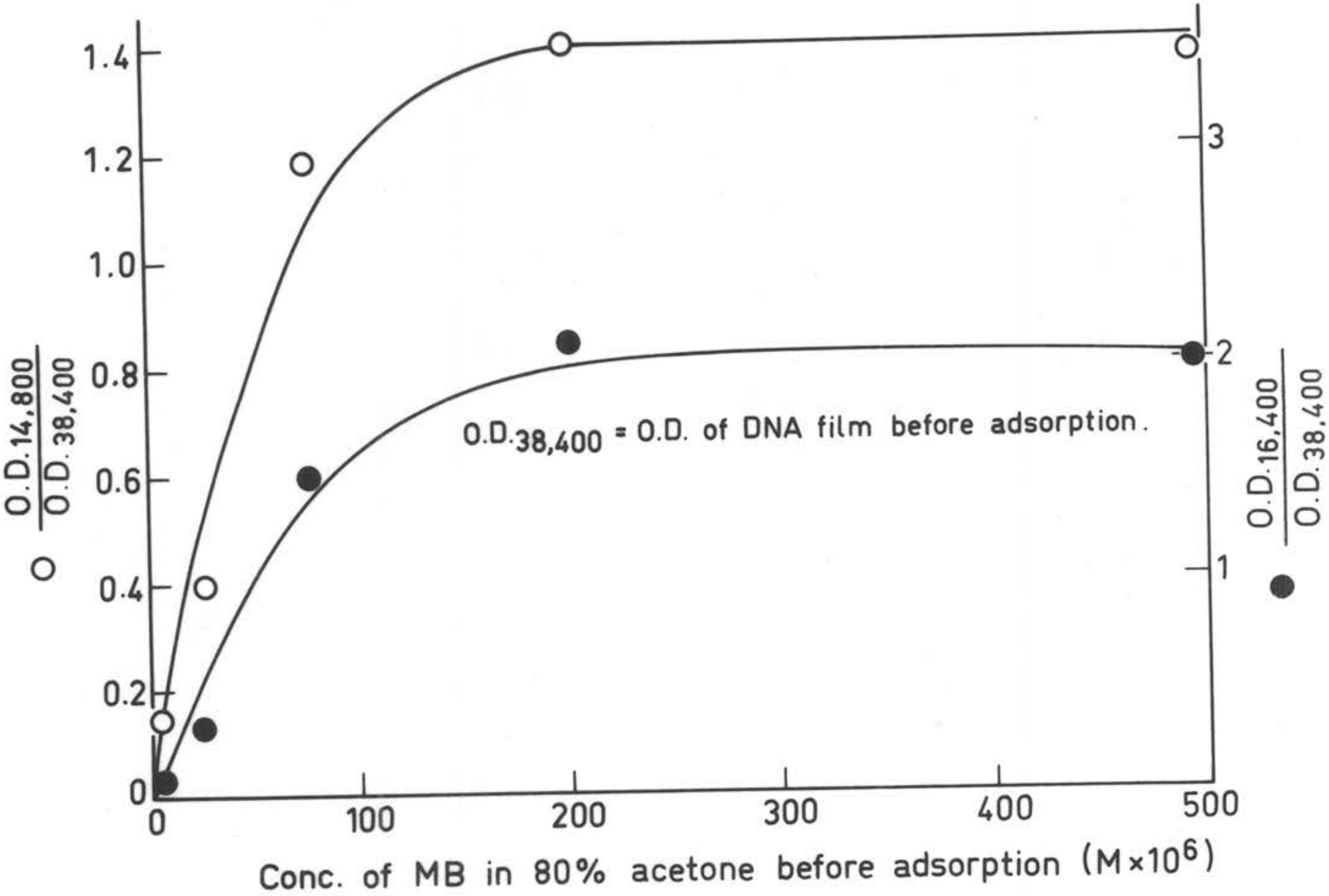


FIG. 4·5. Adsorption of Methylene Blue onto Wet Spun DNA Films at Room Temperature.

4.2.3 Polarised Visible Spectra of the Dyed Films

All the films measured showed negative dichroism in the visible region. The dichroic ratio was fairly constant over the range 18,000 cm^{-1} to 14,000 cm^{-1}, possibly increasing with decreasing wavenumber (Fig. 4.6). The dichroic ratio was slightly lower at the higher concentrations of the adsorbed dye (Table 4.1), but this could be due to the higher accuracy in the measured polarised spectra for the higher optical densities, since the polarised spectra were measured by subtracting the background spectrum of the polariser from the spectrum of the polariser and the sample (Chapter Six, Section 6.5.3(b)).

It has been shown that the particular transition which was studied in the dyed films is polarised along the long axis of the methylene blue molecule (Kern and Dörr, 1961). Hence, the dichroism of the dyed DNA films implies that the long axis of the methylene blue molecules makes an angle of between 45° and 90° with the helix axis for all polymer to dye ratios studied. This is consistent with both the intercalation model and the external binding model for the interaction of the acridine dyes with DNA, and also with flow dichroism measurements on the methylene blue-DNA system itself (Nagata et al., 1966).

4.3 The Interaction of Triphenylmethane Dyes with DNA in Oriented Films

The triphenylmethane dyes, methyl green and rosaniline, were also

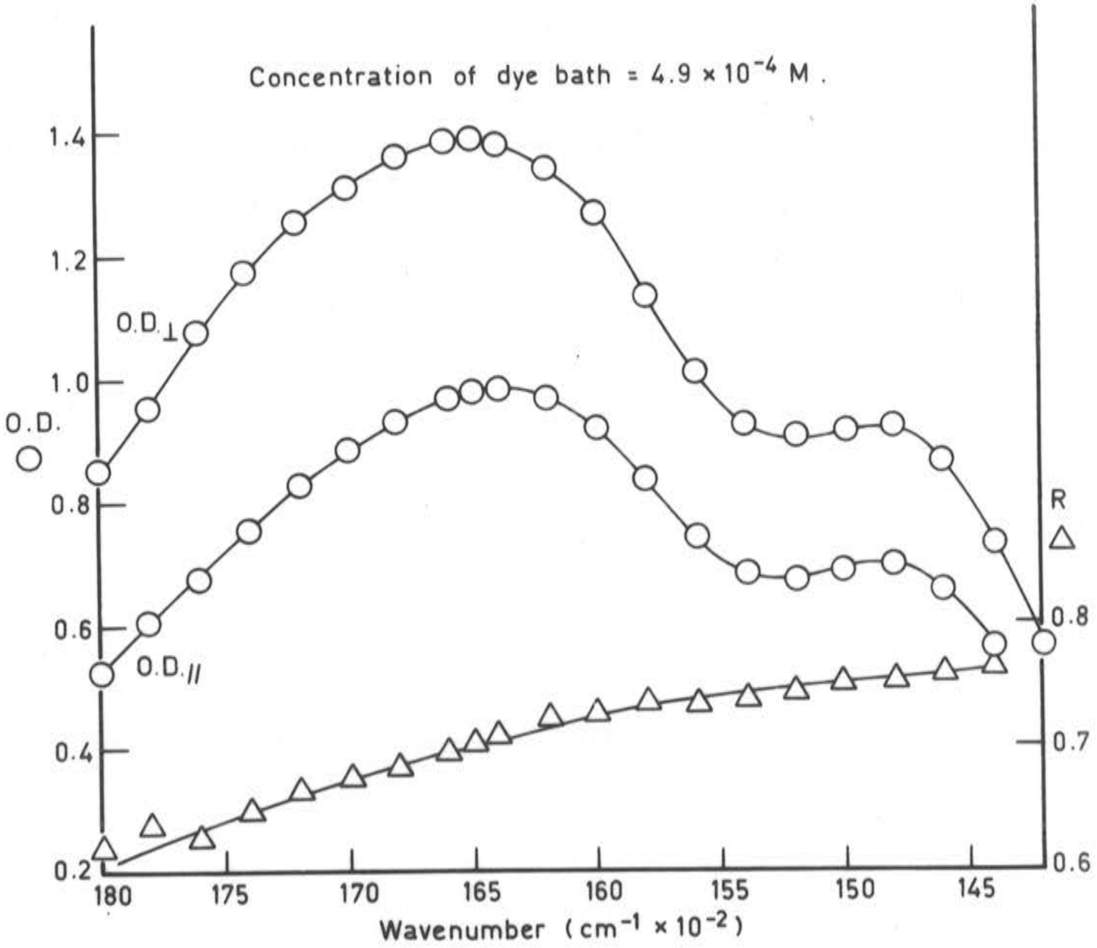


FIG. 4·6. Polarised Spectra of Methylene Blue Adsorbed onto Wet Spun DNA Film.

used to prepare dyed oriented films of DNA (Fig. 4.1). The reason for this is that X-ray measurements show that rosaniline is only bound externally to the DNA (Neville and Davies, 1966). No visible dichroism was noted in the dyed films, however. This is in contrast to the positive dichroism of nucleic acid fibres stained with methyl green (White and Elmes, 1952), but in agreement with the lack of dichroism in films of DNA stained with rosaniline (Neville and Davies, 1966). These results do not disprove the lack of a definite geometric arrangement of the dyes when they are bound to DNA. Perhaps the degree of orientation in the DNA films used was not high enough to detect dichroism in the spectrum of the bound dyes; the degree of orientation in the DNA films used with methyl green and rosaniline was less than in the films used with methylene blue. On the other hand, the orientation of the dye transition moment may be such as to show no dichroism.

4.4 Conclusion

The adsorption of methylene blue onto the wet spun, oriented DNA films is similar to the interaction of other planar aromatic dyes with DNA in solution. Dichroism measurements are consistent with both the intercalation and external binding models for the interaction of the dyes with DNA. The need for better oriented specimens in order to determine transition moment directions is shown by the lack of dichroism in DNA films dyed with the triphenylmethane dyes, methyl green and rosaniline.

CHAPTER FIVE

BIREFRINGENCE

The potential of the measurement of the birefringence dispersion near absorption bands has already been discussed in Chapter One, Section 1.1. Most of the birefringence measurements to date have been monochromatic measurements and have been used either to study the shape and size of macromolecules in solution or, from the sign of the birefringence, merely to corroborate structural details determined by other methods (Singer et al., 1938; Seeds and Wilkins, 1950; Oriel and Schellman, 1966). White and Elmes (1952) measured the dispersion of the visible birefringence in pyronin-dyed nucleic acid fibres, but further details of their measurements have not been found. This work was intended to extend the conventional microscopic measurements of the birefringence of DNA films in the ultraviolet spectral region, in order to establish the use of the birefringence dispersion for molecular structure determinations. The method of measurement of the birefringence with the apparatus which has been built will be described.

5.1 The Measurement of Birefringence by Compensators

Consider linearly polarised light incident normally on a doubly refracting plane-parallel plate and vibrating in a direction which does not coincide with the optic axis. Two mutually perpendicular polarised waves are formed in the interior of the plate and are propagated with different velocities. On emerging from the plate, the two waves combine to form, in general, an elliptically polarised wave. If OX($\underline{1}$) and OY(11) are the main vibration directions in the plate, and OP is the vibration direction of the incident wave, then the incident light vector may be represented by

$$D_P = a \cos (\omega t - \psi_P) \qquad (5.1)$$

and the emergent components may be represented by

$$D_{\underline{1}} = a \cos \Upsilon \cos (\omega t - \psi_{\underline{1}}) \qquad (5.2)$$

$$D_{11} = a \sin \Upsilon \cos (\omega t - \psi_{11}) \qquad (5.3)$$

where a is the amplitude, Υ is the angle between OP and OX, ω is the angular frequency, t is the time and ψ is the phase constant (Fig. 5.1) (Jenkins and White, 1957).

If the thickness of the plate is d_r and the wavenumber of the light is ν, the phase difference, δ, on emergence is given by

$$\delta = \psi_{11} - \psi_{\underline{1}} = 2\pi\, \nu\, d_r(n_{11} - n_{\underline{1}}) \qquad (5.4)$$

$n_{11} - n_{\underline{1}}$ is the birefringence.

In general, the phase difference or retardation, δ, is measured by means of "compensators", namely instruments which are themselves doubly refracting plates and, if suitably placed in the path of the waves to be examined, can compensate the phase difference and reform linearly polarised light on emergence. Compensators are used in

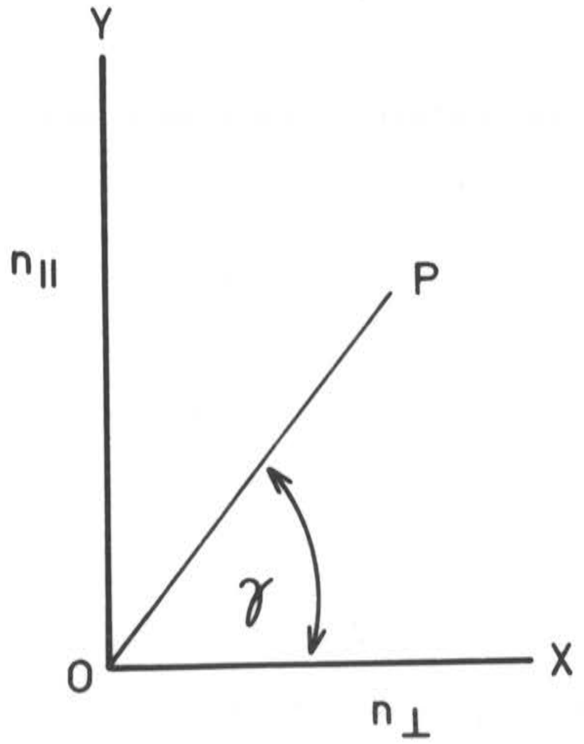


FIG. 5·1. The Propagation of Polarised Light in a Birefringent Plate.

conjunction with an analyser and a polariser and enable a direct measurement of δ (Jerrard, 1948).

Although compensators may in principle be used in the ultraviolet region, they are not readily available for this region (Goerner, 1965); research is being carried out in order to produce large aperture retardation plates for this region (McDermott and Novick, 1961). A different method was sought to measure δ in the ultraviolet region. Such a method is an intensity method based on Mueller Calculus.

5.2 Mueller Calculus

The Mueller Calculus is a phenomenological matrix-algebraic method of specifying a beam of light and the optical devices encountered by the beam, and computing the outcome (Shurcliff, 1966). The advantage of the Mueller Calculus over conventional algebraic and trigonometric methods is its relative simplicity.

Completely or partially polarised light can be specified by a set of four time-averaged quantities which are known as the Stokes parameters and have the dimensions of intensity. The four parameters are usually represented by a column vector (the Stokes vector) as

$$\begin{bmatrix} I \\ M \\ C \\ S_1 \end{bmatrix}$$

The first parameter, I, is the total intensity of the light. The other parameters, M, C and S_1, describe the state of polarisation of the light. The Stokes vector for incident unpolarised light, [U], is given by

$$[U] = c_i \begin{bmatrix} 1 \\ 0 \\ 0 \\ 0 \end{bmatrix} \qquad (5.5)$$

c_i is a normalisation constant used to keep the first element of the Stokes vector unity.

In Mueller Calculus, an optical device is represented by a 4 x 4 matrix which operates on the Stokes vector of the incident light to give the Stokes vector of the transmitted light.

5.3 Mueller Matrices of Optical Devices

5.3.1 Polarisers

A linear polariser is characterised by two principal transmittances - the major principal intensity transmittance, k_1, and the minor principal intensity transmittance, k_2. k_1 is the ratio of the transmitted intensity to the incident intensity when the incident beam is linearly polarised in that vibration direction which maximises the transmittance. k_2 is the ratio obtained when the transmittance is minimised. For the convenience of notation, the major and minor amplitude transmittances are also used and these can be converted to the intensity transmittances by squaring them. The intensity transmittance ratio of a polariser is r_p^2 where r_p is the ratio of the minor amplitude transmittance to the major amplitude transmittance. In the case of an ideal polariser, $r_p = 0$.

The Mueller matrix, $[P_\alpha]$, for a polariser whose major transmittance axis is displaced by an angle α from the horizontal is given in Table 5.1. In the case of an ideal polariser this becomes

$$[P_\alpha] = c_\alpha \begin{bmatrix} 1 & \cos 2\alpha & \sin 2\alpha & 0 \\ \cos 2\alpha & \cos^2 2\alpha & \sin 2\alpha \cos 2\alpha & 0 \\ \sin 2\alpha & \sin 2\alpha \cos 2\alpha & \sin^2 2\alpha & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix} \qquad (5.6)$$

5.3.2 Retardation Plates

The Mueller matrix, $[R_\beta]$, for an absorbing, non-ideal birefringent plate is given in Table 5.2, where δ is the phase difference of the plate, β is the angle by which the fast axis is displaced from the horizontal, and r_r^2 is the intensity transmittance ratio of the slow axis transmittance to the fast axis transmittance. In the case of an ideal retarder, $r_r = 1$.

5.4 The Spectrophotoellipsometer

The schematic representation of the spectrophotoellipsometer

Table 5.1

Mueller Matrix for Polariser (Shurcliff, 1966)

α = angular displacement of the major transmittance axis from the horizontal

$r_p^{\,2}$ = intensity transmittance ratio

c_α = normalisation constant

$$[P_\alpha] = c_\alpha \begin{bmatrix} (1 + r_p^{\,2}) & (1 - r_p^{\,2})\cos 2\alpha & (1 - r_p^{\,2})\sin 2\alpha & 0 \\ (1 - r_p^{\,2})\cos 2\alpha & (1 + r_p^{\,2})\cos^2 2\alpha + 2r_p\sin^2 2\alpha & (1 + r_p^{\,2})\sin 2\alpha\cos 2\alpha - 2r_p\sin 2\alpha\cos 2\alpha & 0 \\ (1 - r_p^{\,2})\sin 2\alpha & (1 + r_p^{\,2})\sin 2\alpha\cos 2\alpha - 2r_p\sin 2\alpha\cos 2\alpha & (1 + r_p^{\,2})\sin^2 2\alpha + 2r_p\cos^2 2\alpha & 0 \\ 0 & 0 & 0 & 2r_p \end{bmatrix}$$

Table 5.2

Mueller Matrix for an Imperfect Retardation Plate (Hodgdon, 1965)

β = angular displacement of the fast axis from the horizontal

${r_r}^2$ = intensity ratio of slow axis transmittance to fast axis transmittance

δ = retardation

c_β = normalisation constant

$$[R_\beta] = c_\beta \begin{bmatrix} (1 + {r_r}^2) & (1 - {r_r}^2)\cos 2\beta & (1 - {r_r}^2)\sin 2\beta & 0 \\ (1 - {r_r}^2)\cos 2\beta & \begin{array}{l} \tfrac{1}{2}(1 + {r_r}^2 + 2r_r\cos\delta) + \\ \quad \tfrac{1}{2}(1 + {r_r}^2 - 2r_r\cos\delta)\cos 4\beta \end{array} & \tfrac{1}{2}(1 + {r_r}^2 - 2r_r\cos\delta)\sin 4\beta & 2r_r\sin\delta\sin 2\beta \\ (1 - {r_r}^2)\sin 2\beta & \tfrac{1}{2}(1 + {r_r}^2 + 2r_r\cos\delta)\sin 4\beta & \begin{array}{l} \tfrac{1}{2}(1 + {r_r}^2 + 2r_r\cos\delta) - \\ \quad \tfrac{1}{2}(1 + {r_r}^2 - 2r_r\cos\delta)\cos 4\beta \end{array} & 2r_r\sin\delta\cos 2\beta \\ 0 & -2r_r\sin\delta\sin 2\beta & 2r_r\sin\delta\cos 2\beta & 2r_r\cos\delta \end{bmatrix}$$

which was built for the measurement of δ is given in Fig. 5.2.

The matrix representation of incident unpolarised light passing through an ideal polariser, a non-ideal birefringent plate and a non-ideal analyser is given by

$$[E] = [A_\theta][R_\beta][P_\alpha][U] \qquad (5.7)$$

$[E]$ = Stokes vector of the emerging light.

$[A_\theta]$ = Mueller matrix for a non-ideal analyser (represented by the subscript "a") whose major transmittance axis is displaced by an angle θ from the horizontal.

Since only the intensity, I, of the emerging light is to be measured, only the first element of $[E]$ needs to be considered. The intensity of the emerging light, $I_{\alpha,\beta,\theta}$, is given by

$$I_{\alpha,\beta,\theta} = c_*(B_1 + B_2\sin2\beta + B_3\cos2\beta + B_4\sin4\beta + B_5\cos4\beta) \quad (5.8)$$

where $c_* = c_i \cdot c_\alpha \cdot c_\beta \cdot c_\theta$.

$$B_1 = (1 + r_a^2)(1 + r_r^2) + \tfrac{1}{2}(1 - r_a^2)(1 + r_r^2 + 2r_r\cos\delta)\cos2(\alpha - \theta)$$

$$B_2 = (1 + r_a^2)(1 - r_r^2)\sin2\alpha + (1 - r_a^2)(1 - r_r^2)\sin2\theta$$

$$B_3 = (1 + r_a^2)(1 - r_r^2)\cos2\alpha + (1 - r_a^2)(1 - r_r^2)\cos2\theta$$

$$B_4 = \tfrac{1}{2}(1 - r_a^2)(1 + r_r^2 + 2r_r\cos\delta)\sin2\theta\cos2\alpha + \tfrac{1}{2}(1 - r_a^2)(1 + r_r^2 - 2r_r\cos\delta)\sin2\alpha\cos2\theta$$

$$B_5 = \tfrac{1}{2}(1 - r_a^2)(1 + r_r^2 - 2r_r\cos\delta)\cos2(\alpha + \theta)$$

Fig. 5.2

Scheme of Spectrophotoellipsometer

Key to Figure

OPTICAL SYSTEM

1. Light source (Xenon arc lamp)
2. Focussing lens
3. Monochromator
4. Monochromator exit slit
6. Collimating lens
7. Polariser (rotatable)
10. Sample film (rotatable)
11. Iris
12. Analyser (fixed)
13. Chopper
14. Photomultiplier

ANGLE-MEASURING SYSTEM

5. Mirror
8. Perspex disc with angular scale (rotatable)
9. Projection system which illuminates the angular scale via a cross hair

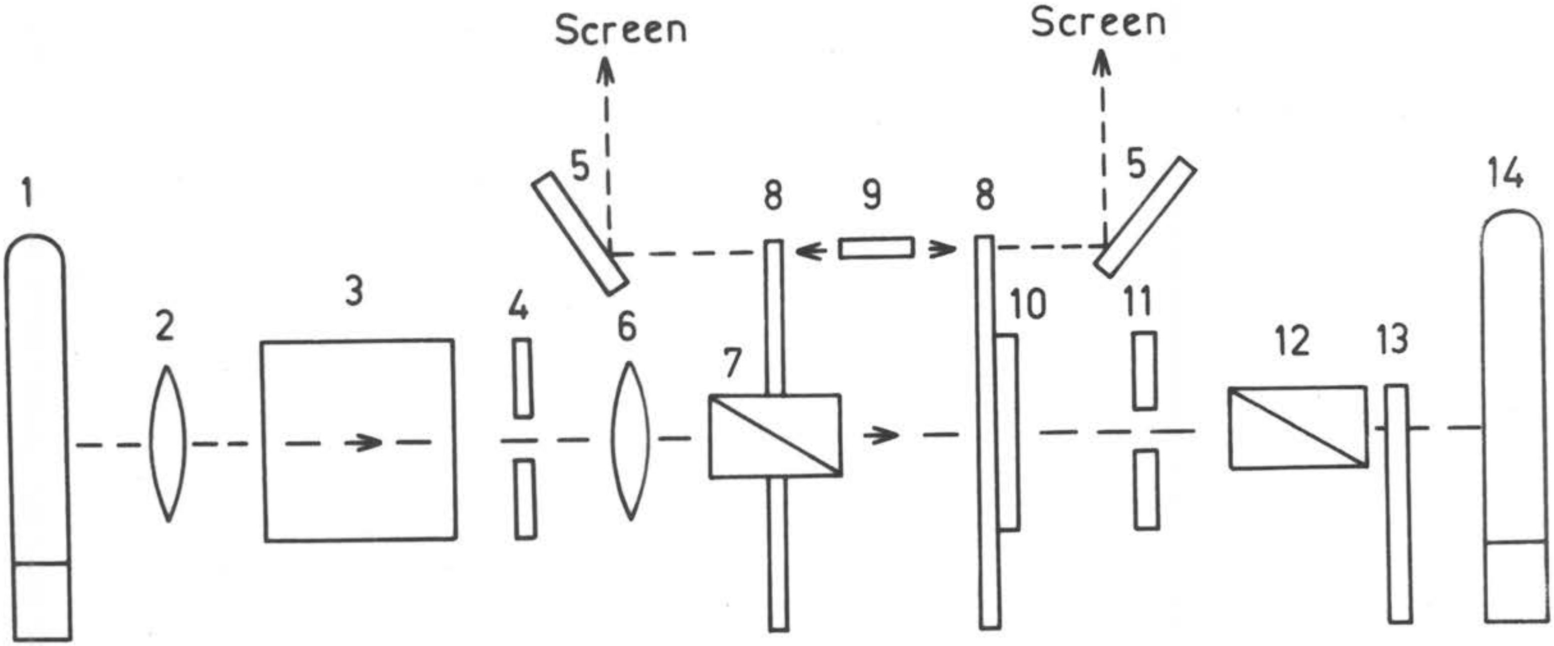


FIG. 5·2. Scheme of Spectrophotoellipsometer.

From equation (5.8), it follows that

$$I_{0,0,0} = 4c_* \tag{5.9}$$

$$I_{0,\frac{\pi}{2},0} = 4c_* r_r^2 \tag{5.10}$$

$$I_{0,0,\frac{\pi}{2}} = 4c_* r_a^2 \tag{5.11}$$

$$I_{0,\frac{\pi}{2},\frac{\pi}{2}} = 4c_* r_a^2 r_r^2 \tag{5.12}$$

$$r_r^2 = \frac{I_{0,\frac{\pi}{2},0}}{I_{0,0,0}} = \frac{I_{0,\frac{\pi}{2},\frac{\pi}{2}}}{I_{0,0,\frac{\pi}{2}}} \tag{5.13}$$

$$I_{0,\frac{\pi}{4},\frac{\pi}{2}} = c_*[(1 + r_a^2)(1 + r_r^2) - 2r_r(1 - r_a^2)\cos\delta] \tag{5.14}$$

$$I_{\frac{\pi}{2},\frac{\pi}{4},\frac{\pi}{2}} = c_*[(1 + r_a^2)(1 + r_r^2) + 2r_r(1 - r_a^2)\cos\delta] \tag{5.15}$$

$$I_{\frac{\pi}{4},\frac{\pi}{4},\frac{\pi}{2}} = 2c_*(1 + r_a^2) \tag{5.16}$$

$$2r_r\cdot\frac{(1 - r_a^2)}{(1 + r_a^2)}\cdot\cos\delta = \frac{(I_{\frac{\pi}{2},\frac{\pi}{4},\frac{\pi}{2}} - I_{0,\frac{\pi}{4},\frac{\pi}{2}})}{I_{\frac{\pi}{4},\frac{\pi}{4},\frac{\pi}{2}}} \tag{5.17}$$

It can be seen that measurements of the intensities in equations (5.9) to (5.12) and (5.14) to (5.16) allow the calculation of δ, provided that r_a^2 is known. In fact, r_a^2 may be determined in a separate measurement in the absence of the birefringent plate. The position

$\beta = \frac{\pi}{4}$ may be found by rotating the birefringent plate between crossed polariser and analyser and should correspond to the position of maximum intensity. The determination of the phase difference by the intensity measurements requires the use of a homogeneous retardation plate. That is, the anisotropic films whose birefringence is to be measured must be homogeneous. Otherwise errors will arise when different parts of the films are exposed to the light beam by the rotation of the films in order to change the value of β.

5.5 Dispersion of the Birefringence of a Stretched PVA Film Dyed with Methylene Blue

A stretched PVA film dyed with methylene blue was used to test the spectrophotoellipsometer. Such a film is birefringent and absorbs visible and ultraviolet light owing to the presence of the dye. The results of the measurements are shown in Figs. 5.3 and 5.4. The transmittance ratio curve appears to agree with the dichroism results of Kern and Dörr (1961) in that the smallest r_r^2 occurs where the methylene blue absorbs strongly, viz. near 34,000 cm^{-1} and 15,000 cm^{-1}. The phase difference dispersion plot shows that δ varies linearly with the wavenumber over the range 40,000 cm^{-1} to 16,000 cm^{-1}. It follows from equation (5.4) that the birefringence is independent of wavenumber for the dyed film. This

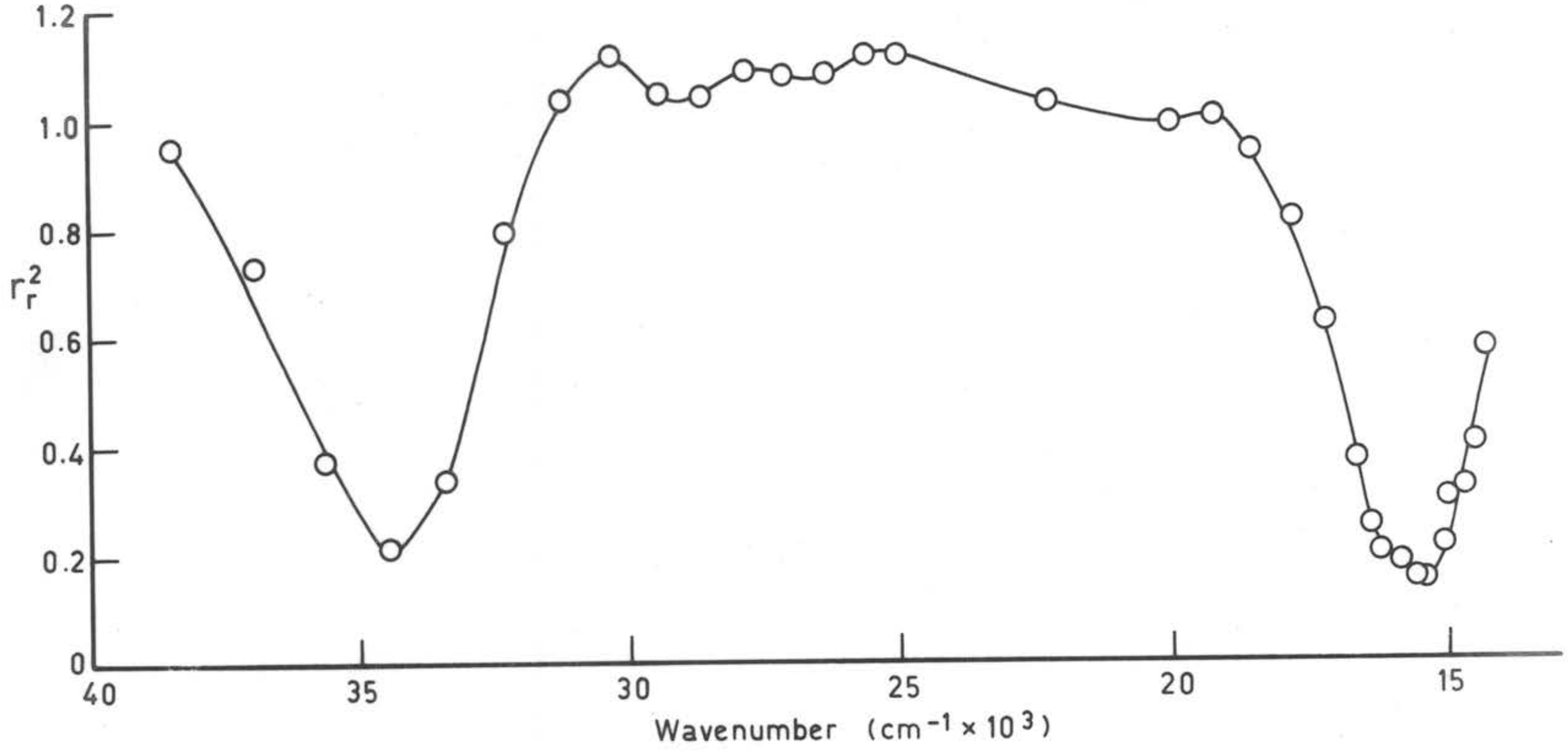


FIG. 5·3. Variation of Transmittance Ratio of PVA-MB Film with Wavenumber .

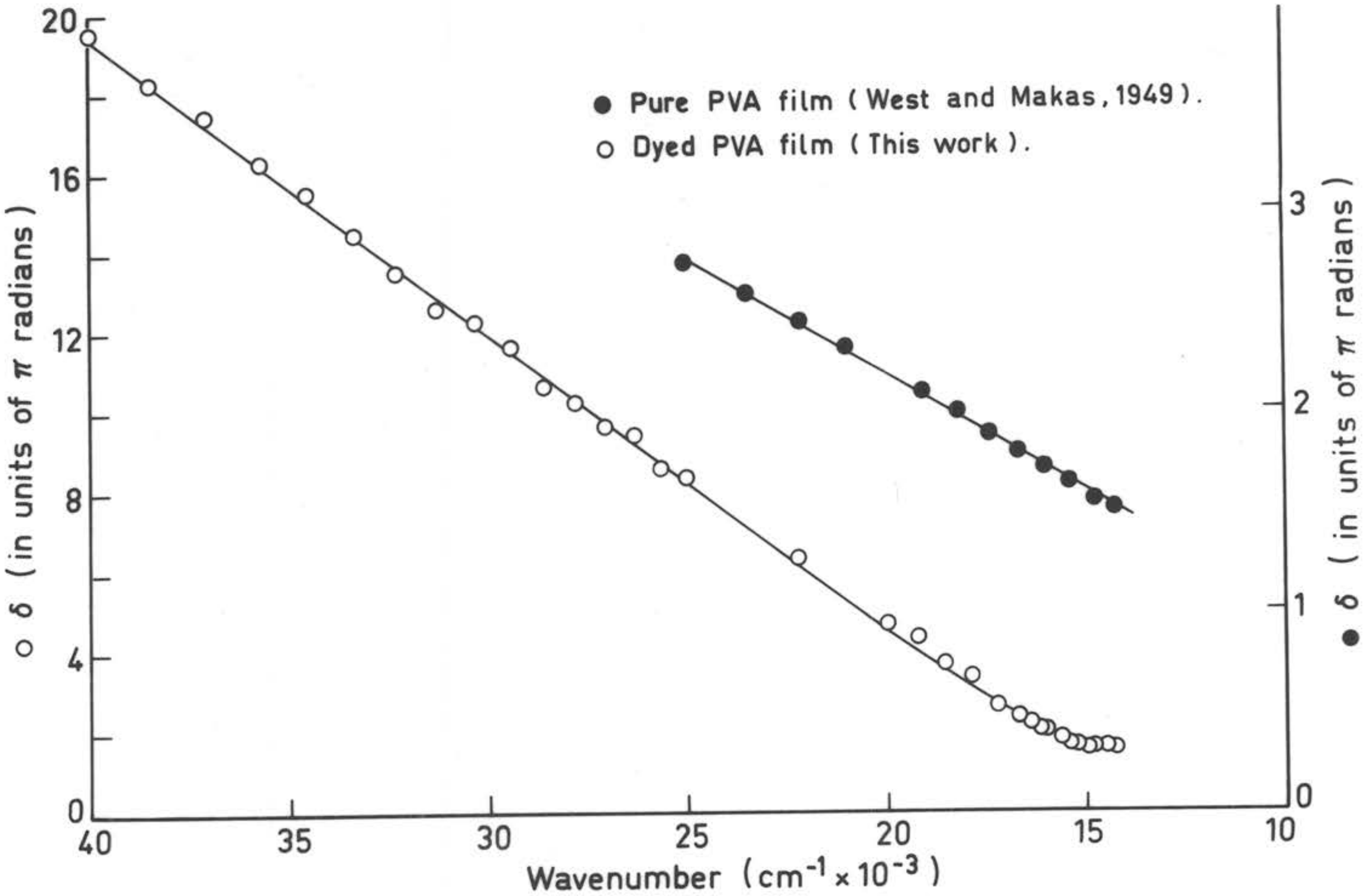


FIG. 5·4. Dispersion of the Phase Difference for Stretched PVA Films.

agrees with the results of West and Makas (1949) for a stretched pure PVA film when their values of δ are plotted against the wavenumber (Fig. 5.4). The magnitudes of δ and the slopes of the straight lines for the dyed and the pure PVA films are different owing to differences in both the thicknesses and degrees of stretching in the films. It seems that the birefringence of the PVA in the dyed film predominates over most of the spectral range measured. However, the birefringence behaviour of the dyed film is slightly modified at the red end of the spectrum by the methylene blue where the dye shows the strongest absorption.

5.6 Dispersion of the Birefringence of Wet Spun DNA Films

The development of the spectrophotoellipsometer and the method of the preparation of the oriented DNA films took place simultaneously. The birefringence measurements on the wet spun DNA films have not been satisfactory up to date, although changes in the birefringence have been observed in the region of the DNA absorption (45,000 cm^{-1} to 35,000 cm^{-1}) as expected. The reasons for the lack of success in the birefringence dispersion measurements are connected with slight thickness inhomogeneities in the films and with the low degree of orientation and hence with the low birefringence of the films.

CHAPTER SIX

EXPERIMENTAL

6.1 Reagents

6.1.1 Deoxyribonucleic Acid

The sodium salts of the DNA from the *E. Coli.* and *B. Meg.* bacteria and from the calf thymus gland were used. The bacterial DNA had been prepared by the method of Marmur (1961). Table 6.1 gives some properties of the various nucleic acids used and shows that the preparations were probably native. It was not possible to characterise the *E. Coli.* DNA VI, the *B. Meg.* DNA and the calf thymus DNA I at the time of the spinning experiments. Calf thymus DNA I could be precipitated by ethanol, even though a solution of it did not give a film during the wet spinning process. The physical state in which the nucleic acids were supplied is important in that solid DNA films show considerable hyperchromicity on drying and this change is reversible even over long storage periods (Falk et al., 1963b); this means that an originally native DNA is unlikely to denature when it is stored as a solid for some time.

6.1.2 Poly(vinyl alcohol)

"Baker grade" PVA (99-100% hydrolysed; viscosity of a 4% aqueous solution at 20°C = 55-65 cp) was used for the preparation of the unoriented PVA-DNA films and for the oriented PVA-MB film (J.T. Baker Chemical Company, New Jersey). A weak band in the

Table 6.1

Deoxyribonucleic Acids

Source	State Supplied	T_m ($^\circ$C)	% Hyperchromicity at 38,460 cm^{-1}	Solvent
E. Coli. I	Solution (0.1%)	94	40	Standard Saline Citrate
E. Coli. II	Solution (0.1%)	-	35	0.1M NaCl
E. Coli. III	Solid	91	36	Standard Saline Citrate
E. Coli. IV	Solid	92	38	Standard Saline Citrate
E. Coli. V	Solid	-	45	Solid State
E. Coli. VI	Solution (0.1%)	-	-	H_2O
B. Meg.	Solid	-	-	-
Calf thymus I	Solid	-	-	-
Calf thymus II	Solid	-	37	Dilute Saline Citrate

infrared at 1,710 cm^{-1} showed the presence of some acetate groups, although these did not affect the ultraviolet spectrum.

Elvanol 50-42 (86-89% hydrolysed PVA) was used for the preparation of the stretched PVA-DNA films (Monsanto Chemicals (Aust.) Ltd.).

6.1.3 Dyes

Methylene blue (B.D.H., Ltd.) was used without further purification. The visible spectrum of a dilute aqueous solution of the dye compared very well with the spectra of the purified methylene blue used by other workers (Bergmann and O'Konski, 1963; Braswell, 1968) (Table 6.2).

Table 6.2

Visible Spectrum of Methylene Blue

ν_{max} = wavenumber at the maximum absorption position

ν_{sh} = wavenumber at the point of inflection of the shoulder

ϵ_{max} = absorption coefficient at ν_{max} (uncorrected for dye adsorption to the walls of the glass vessels and cells)

$\frac{O.D._{peak}}{O.D._{sh}}$ = ratio of optical densities at the maximum and at the point of inflection of the shoulder

Reference	Concentration of MB ($M \times 10^6$)	ν_{max} cm^{-1}	ν_{sh} cm^{-1}	$\epsilon_{max} \times 10^{-4}$ $l.mole^{-1}cm^{-1}$	$\frac{O.D._{peak}}{O.D._{sh}}$
Bergmann and O'Konski, 1963	9.8	15,060	16,327	8.45	1.99
Braswell, 1968		15,038		7.8	
This work	5.0	15,070	16,250	8.5	2.01

Methyl green (George T. Gurr Ltd., London) and Rosaniline Hydrochloride (B.D.H. Ltd.) were used as supplied.

6.1.4 Other Reagents

"A" grade 1,5-diaminopentane dihydrochloride (Calbiochem., Los Angeles) was used as supplied.

Poly-L-lysine hydrobromide (L. Light and Co., Ltd., Colnbrook, England) was used as supplied.

6.2 Reagent Solutions

6.2.1 Dilute Saline Citrate

0.015M sodium chloride plus 0.0015M trisodium citrate.

6.2.2 Standard Saline Citrate

0.15M sodium chloride plus 0.015M trisodium citrate

6.2.3 DNA for Wet Spinning

Salt-free aqueous DNA solutions were prepared by dissolving DNA directly in water in the cold over a period of several days. Occasional shaking was needed. The concentration of the salt-free solutions was greater or equal to 0.1%, the DNA still retaining its native configuration at these concentrations (Kurucsev, 1963).

When a DNA solution in standard saline citrate, or in 0.1M to

0.3M sodium chloride or sodium acetate was required, the salt-free aqueous DNA solution was diluted with an appropriate amount of a concentrated salt solution. All the DNA solutions were stored in a refrigerator.

Before use, the DNA solutions were degassed by freezing them in liquid nitrogen, evacuating the container, and then thawing the frozen solutions in vacuo. This was repeated several times. It has been shown that the double helical structure of the DNA is not broken down by freezing solutions to -192°C and thawing them (Shikama, 1965).

6.2.4 DNA for PVA-DNA Interaction Studies

DNA solutions for preparing the unoriented pure DNA films or the mixed PVA-DNA films were made by dissolving DNA in water or 10^{-3}M sodium chloride at a concentration of 1% in the cold over a period of several days. The solvent had been degassed before use. The resulting viscous solutions were stored in the frozen state.

6.2.5 PVA-DNA Solutions

The following recipe is the final one used. Degassed ethanol (1.0 ml.) and degassed dilute saline citrate (5.0 ml.) were added to the PVA (0.7130 g.) and the resulting slurry was heated on a water bath for five minutes. The clear solution formed was shaken vigorously for thirty minutes in order to homogenise it.

It was then allowed to equilibrate to room temperature overnight. Each PVA solution was prepared in duplicate.

The stock DNA solution (1.5000 g.) was added to one of the PVA solutions and degassed dilute saline citrate (1.5000 g.) was added to the other PVA solution at room temperature. The PVA solutions were then shaken vigorously for one hour at room temperature. It is unlikely that the DNA was denatured during this process (Chapter Three, Section 3.2). The resulting homogeneous PVA-DNA solutions were free of bubbles.

All the solutions were protected as much as possible from the room lights by means of aluminium foil.

The omission of the ethanol from the PVA-DNA solutions caused them to froth considerably during the shaking. The froth was very difficult to remove, even when the solutions were evacuated and left to stand. Films cast from the frothed solutions were unsuitable for spectrophotometry because of the presence of very many bubbles in them.

The presence of ethanol in the mixed solutions did not affect the properties of the PVA-DNA films cast. A solution which did not contain ethanol but which had been allowed to stand at room temperature for one week, to become sufficiently free of froth, gave a film which behaved similarly to the films cast from the ethanolic solutions. Furthermore, the denaturation of DNA by ethanol in solution is reversible when the ethanol-denatured DNA is dialysed back into 0.2M aqueous sodium chloride (Herskovits et al., 1961). Neville and Davies (1966) have shown that sodium-DNA fibres made from an ethanolic gel of DNA behave in the same way as fibres made from an ethanol-free gel.

6.3 Film Preparations

6.3.1 Wet Spinning

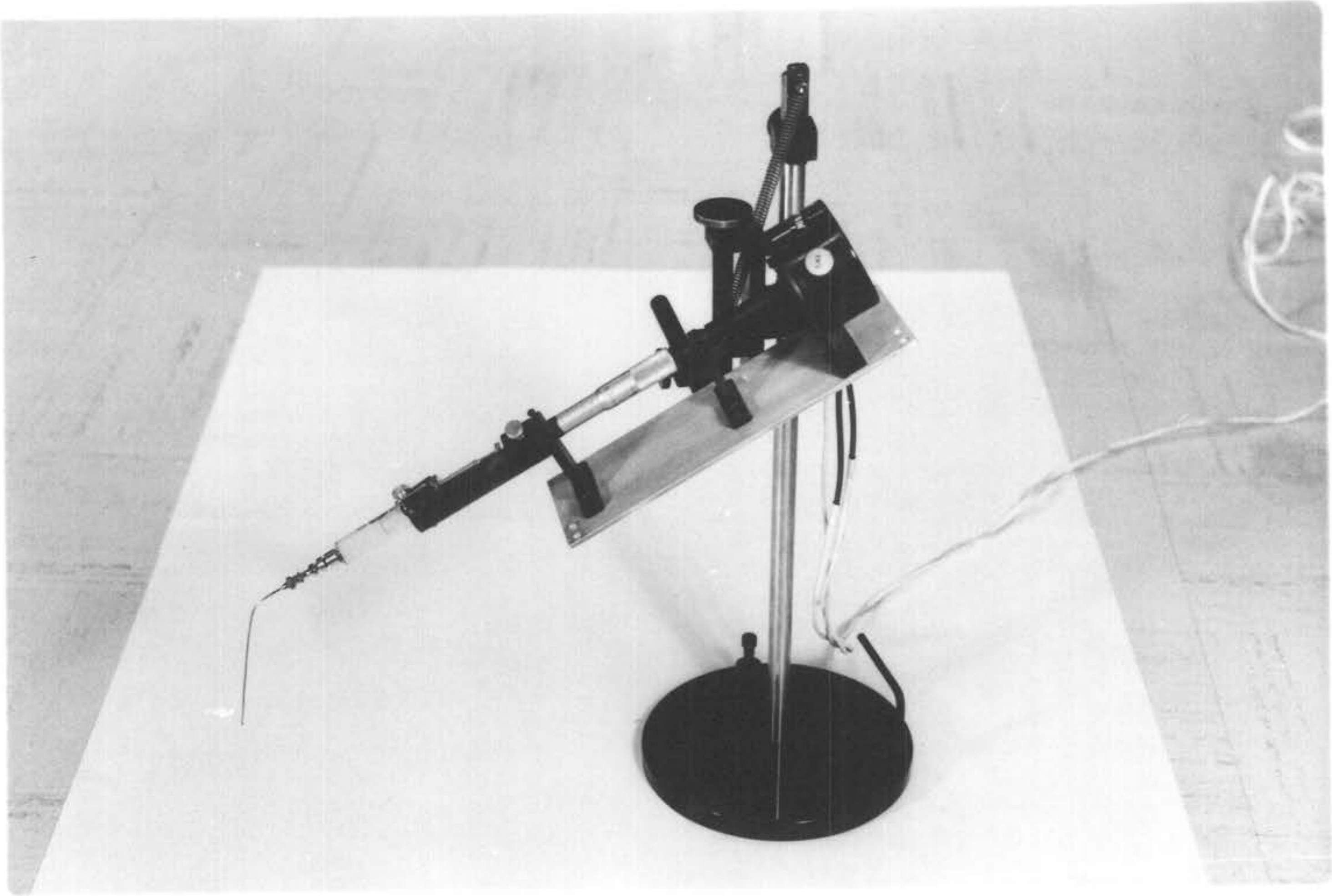
A motor-driven syringe was used to inject the DNA solutions into the precipitating liquid. It consisted of a Venner synchronous motor (1/15 r.p.m.) connected to the micrometer head of an "Agla" micrometer syringe (Burroughs Wellcome Ltd., London); the syringe itself was clipped into a metal holder which was attached to the micrometer head (Plate 6.1). The syringe system could be lowered and raised by means of a screw.

Both an "Agla" glass syringe (capacity, 0.5 ml.) and a "Van" syringe (capacity, 5 ml.) were used. The rates of feeding of the solutions were 0.04 ml./hr. and 0.22 ml./hr., respectively. Stainless steel capillaries of different internal diameters were used to change the speed of extrusion, V_0, of the DNA solutions. The internal diameters of the capillaries varied up to 0.097 cm. Thicker-bored capillaries (internal diameter ~ 0.16 cm.) were not suitable because they allowed the syringes to empty by gravity.

It is unlikely that any degradation of the DNA occurred in

Plate 6.1

The Motor-driven Syringe for Feeding DNA Solutions into the Precipitant



the extrusion process, since the rate of shear at the walls of the capillaries was small. The rate of shear, calculated from equation (6.1), amounted to 23 $\sec^{-1}$ for a capillary of internal diameter 0.030 cm., and for a feed rate of 0.22 ml./hr. (Harrington and Zimm, 1965).

$$G = \frac{4}{\pi b^3}\frac{dv}{dt} \qquad (6.1)$$

G = maximum (wall) shear rate.

b = radius of capillary.

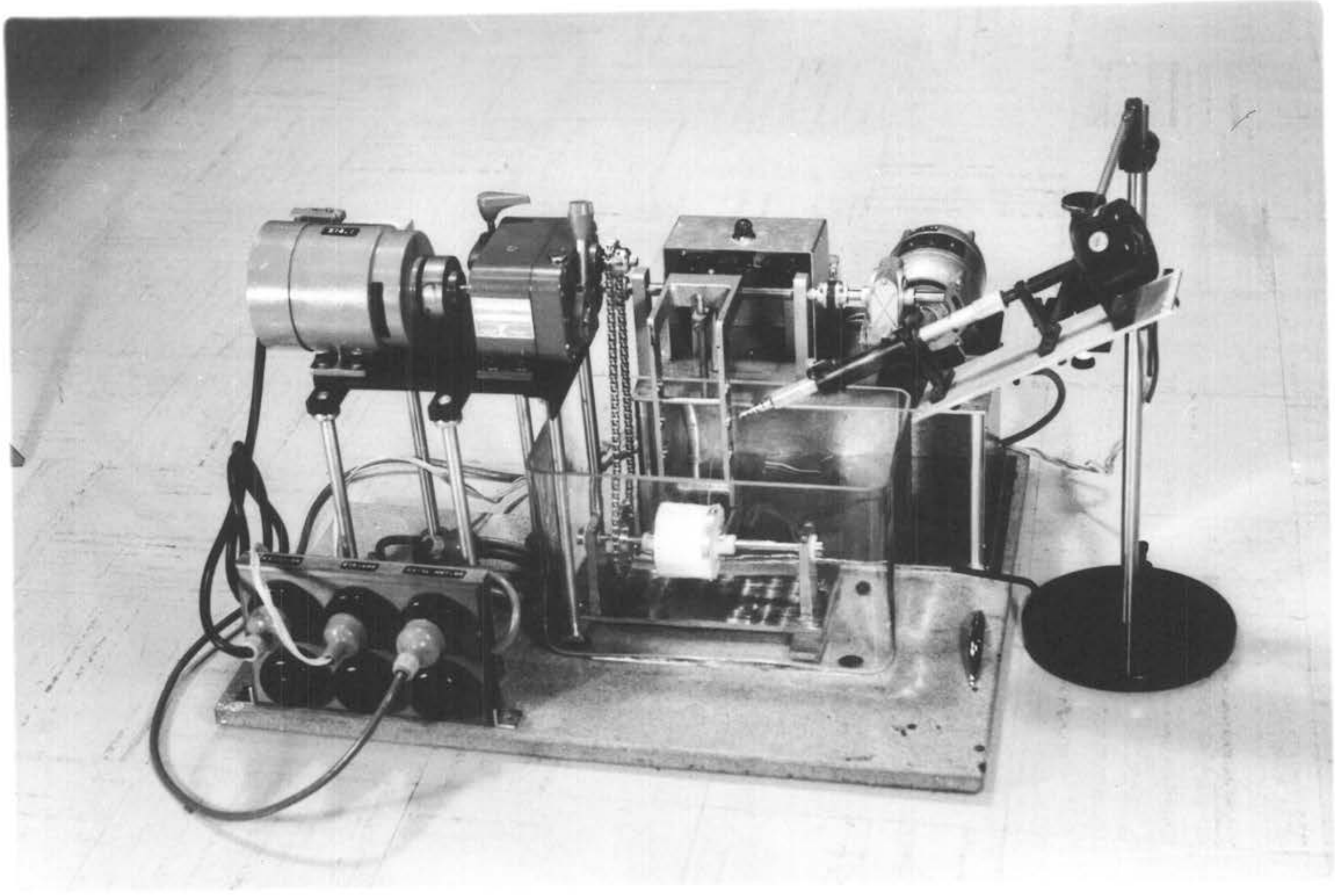
$\frac{dv}{dt}$ = observed volume flow rate

The final device built for the winding of the precipitated thread is shown in Plate 6.2. All the metal parts which came into contact with the precipitant were constructed from stainless steel. A teflon cylinder (length, 5 cm.; diameter, 6.3 cm.) was rotated about a keyed shaft mounted in two teflon end bearings. The cylinder was immersed in the precipitating liquid contained in a rectangular glass tank. The shaft was rotated by means of an electric motor with an infinitely variable gearbox (Model 10-E-400R MC1; Zeromax Company, Minnesota) mounted on aluminium legs above the precipitant. The rotation from the motor to the shaft was transmitted, with a 2:1 reduction, by means of a chain and two sprockets. The speed of the cylinder was usually about 30 r.p.m.

Linear motion of the cylinder along the keyed shaft was achieved

Plate 6.2

The Spinning Machine for the Preparation of Oriented DNA Films



by means of a leadscrew operated by a reversible induction motor with a double reduction gearbox (Parvalux, Model SD-10), giving a final speed of rotation of 3 r.p.m. The leadscrew was also mounted above the precipitant. The axial motion was transmitted from the leadscrew to the cylinder by means of two doubly-cranked arms. The rate of axial movement was 0.63 cm./min. The axial displacement per revolution was 0.019 cm., and was greater than the value, 0.0115 cm., used by Rupprecht (1966a) during the spinning of the sodium salt of DNA.

The change of direction of the axial motion was governed by two microswitches mounted near the leadscrew. When an end point was reached, a microswitch gave an impulse to a relay which changed the rotational direction of the motor driving the leadscrew, and hence changed the direction of the axial motion of the cylinder (Fig. 6.1).

Another apparatus was also used for the winding of the precipitated thread where the axial motion of an anodised aluminium cylinder (length, 5 cm.; diameter, 5 cm.) was achieved by means of a cam coupled to the cylinder. The cam was operated by a worm wheel and a worm on the keyed shaft. The axial motion was not independent of the rate of rotation of the cylinder. The axial displacement per revolution in this apparatus was 0.022 cm. Constant speed motors (Parvalux SD-22 at 20 r.p.m. and Venner at 2 r.p.m. and 4 r.p.m.) were used to rotate the keyed shaft.

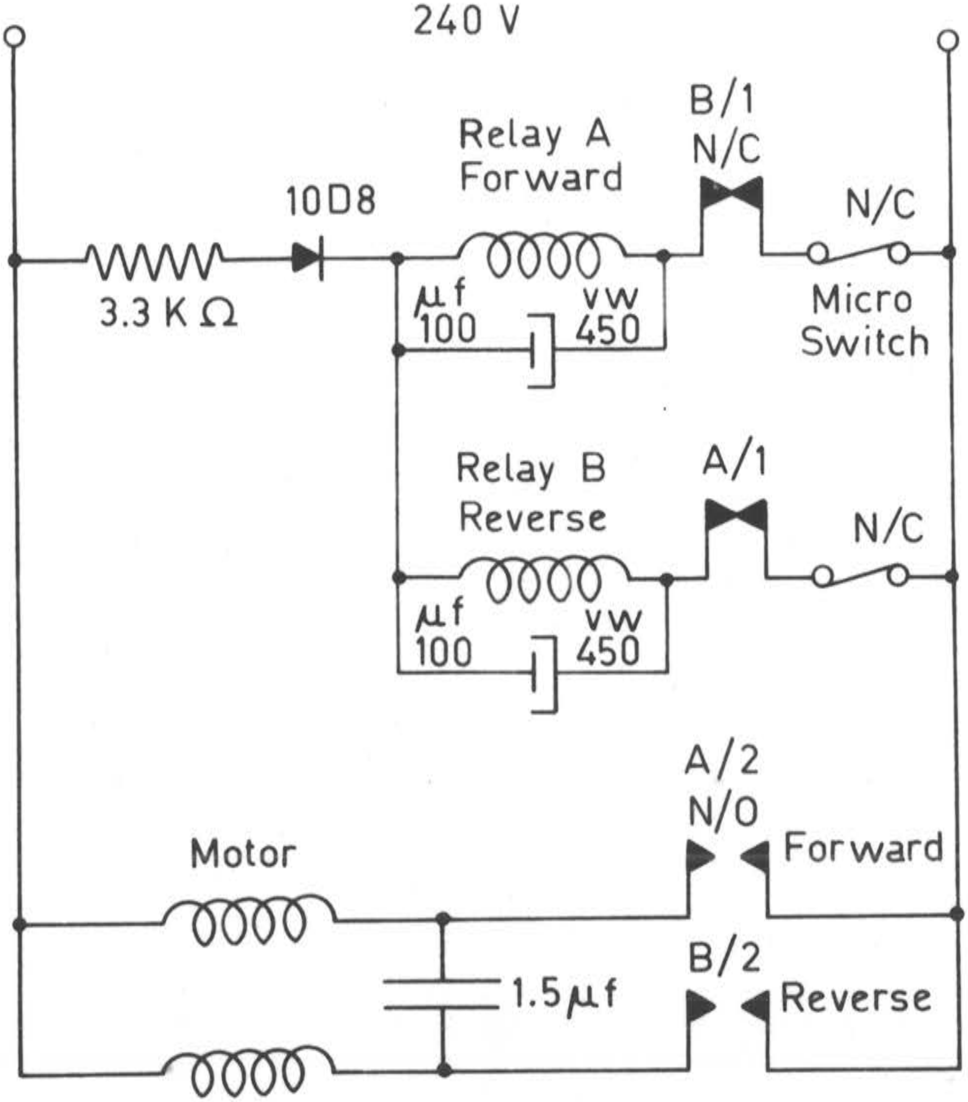


FIG. 6·1. Circuit Diagram for Reversing Motor.

Silica slides (3.5 cm. x 1.2 cm. x 0.1 cm.) were clamped in grooves machined in the cylinders. The maximum number of slides which could be attached was 10 and 8 for the teflon and aluminium cylinders, respectively. The area of the film obtained on each silica slide was 3.0 cm. x 1.2 cm.

Before the start of a preparation, the syringe containing the degassed DNA solution was switched on and allowed to empty for thirty minutes to get rid of any air bubbles which had formed when the solution had been sucked into the syringe. When a continuous stream of DNA flowed down through the precipitant, the cylinder and the axial driving apparatus were also started to begin the preparation of the films.

The capillary from the syringe was normally positioned only a few millimetres from the cylinder. When an increased spinning way was required, the end of the capillary was positioned in a glass tube (internal diameter, 0.6 cm.) above the cylinder. The end of the capillary was kept just below the surface of the precipitant in this case. The glass tube was mounted in a perspex plate which rested on top of the tank containing the precipitant. It prevented the buffeting of the DNA thread which flowed down onto the cylinder from a height of about 6 cm.

6.3.2 Casting of Isotropic PVA-DNA Films

The PVA-DNA and the corresponding PVA solutions were poured onto separate glass plates (15 cm. x 15 cm. x 0.63 cm.) and were spread over an area 15 cm. x 7.5 cm. with a brass roller. The spread solutions were allowed to dry in the air, under a light-proof box, at room temperature and room relative humidity. The drying time was less than a day. The thickness of the films was approximately 0.002 cm.

The brass roller consisted of a brass rod which had been machined to take two brass rings of slightly larger diameter than the rod on either end of it. A clearance of 0.033 cm. between the rod and the glass plates was achieved in this way.

The glass plates had previously been cleaned in chromic acid, alcoholic potassium hydroxide and distilled water and had been rendered non-polar with Releasil 7 (Midlands Silicones Ltd., U.K.), to facilitate the detachment of the films. A control experiment using untreated perspex plates (15 cm. x 15 cm. x 1.2 cm.) showed that the silicone did not affect the dehydration behaviour of the PVA-DNA films.

6.3.3 Stretching of the PVA Films

Small strips of the PVA films (2.5 cm. x 2.5 cm.) were stretched by means of a stretching device operated by a screw (Tsunoda and Yamaoka, 1965). Adhesive tape was not sufficient to mount the films on the stretching device. It was necessary to clamp the films as well, by means of small brass plates screwed onto the stretching device through the tape and the films. A brass strip mounted between the films and the screw protected the films from contamination by the oil from the screw (Fig. 6.2).

The stretching of the films at room temperature was easiest after the films had been subjected to an atmosphere of water vapour in a desiccator. It was necessary to stretch the thin films slowly, over a period of several days, lest breakage occurred. In this respect much thicker films are stronger and may be extended further. It was necessary to dry the films after they had been stretched in order to overcome relaxation effects, and hence to prevent the contraction of the films after they were removed from the stretching device.

6.3.4 Casting of Isotropic Pure DNA Films

Isotropic pure DNA films were prepared by the evaporation of several drops of a DNA solution (concentration, 1%) spread on the inside surface of a silica cell (path length, 1.0 cm.). The drying time was several days at room temperature and room relative humidity in the dark.

6.3.5 Dyed Films

Oriented dyed films of DNA were prepared by immersing the wet spun DNA films into solutions of the dyes in aqueous acetone

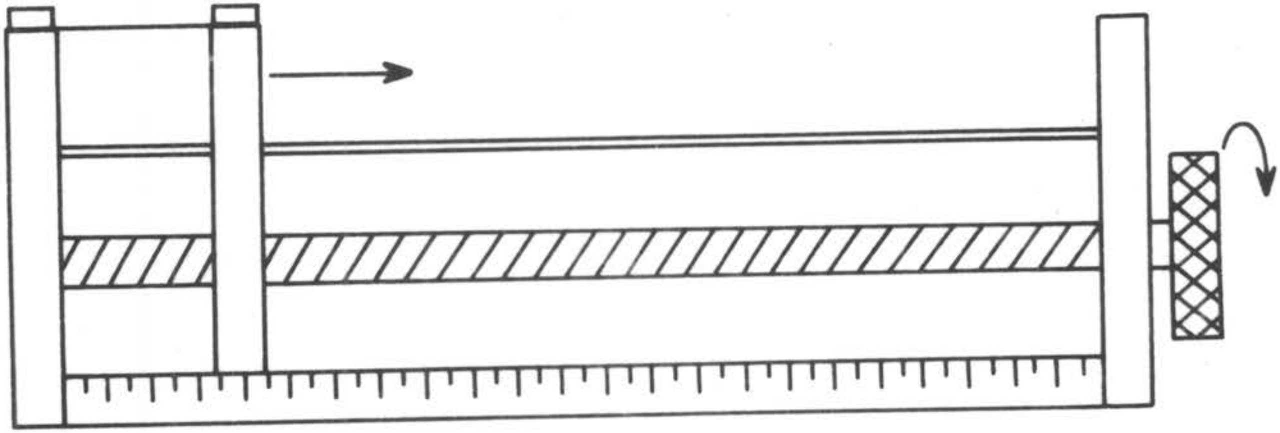


FIG. 6·2. Stretching Device.

(80% acetone) (Table 6.3). After a sufficient amount of dye had been adsorbed by the films, they were first rinsed in aqueous acetone (80% acetone) and were then stored in a fresh solution of aqueous acetone (80% acetone) for about forty minutes in order to remove any unbound dye. Methylene blue was adsorbed faster than either methyl green or rosaniline.

Table 6.3

Adsorption of Dyes onto Wet Spun DNA Films

Dye	Concentrations of Dye Solutions	Time of Immersion of Films (hrs.)
Methylene blue	4.94×10^{-6} to 494×10^{-6}M	1.5
Methyl green	0.0094%	96
Rosaniline	0.015%	96

The uncertain purity of the methyl green and the rosaniline is the reason for the percent concentration units for these dyes in Table 6.3.

The PVA-MB film for the birefringence measurements was prepared by immersing a relatively thick PVA film (thickness, 0.017 cm.) in an aqueous solution of methylene blue (concentration, 8×10^{-4}M) for several hours. The dyed PVA film was then rinsed in water and was stretched in order to orient the molecules.

6.4 Storage of the Films

6.4.1 Wet Spun Films

After the deposition of the DNA on the cylinder, the cylinder was dried overnight with silica gel (0.5 g.). The cylinder was then subjected to an atmosphere of 93% r.h. in a desiccator at room temperature. In the case where the precipitant contained sodium acetate, the cylinder was first immersed in ethanol for 16 hours and was then kept in aqueous ethanol (90% ethanol) for 3 days in the cold, the aqueous ethanol being changed every day.

6.4.2 PVA-DNA Films

The PVA-DNA films were stored in the dark in blackened desiccators since a dry film which had been stored unprotected from room lights did not show the optical density changes upon hydration which a film stored out of the light showed (Fig. 6.3).

6.4.3 Humidity Control

The relative humidity inside the desiccators containing the films was controlled by using saturated aqueous solutions of various salts (Table 6.4). When the films were stored over water itself, the relative humidity was taken as 100%. Phosphorus pentoxide was used to maintain 0% r.h. Storage times up to several weeks at a particular relative humidity were used for the equilibration of

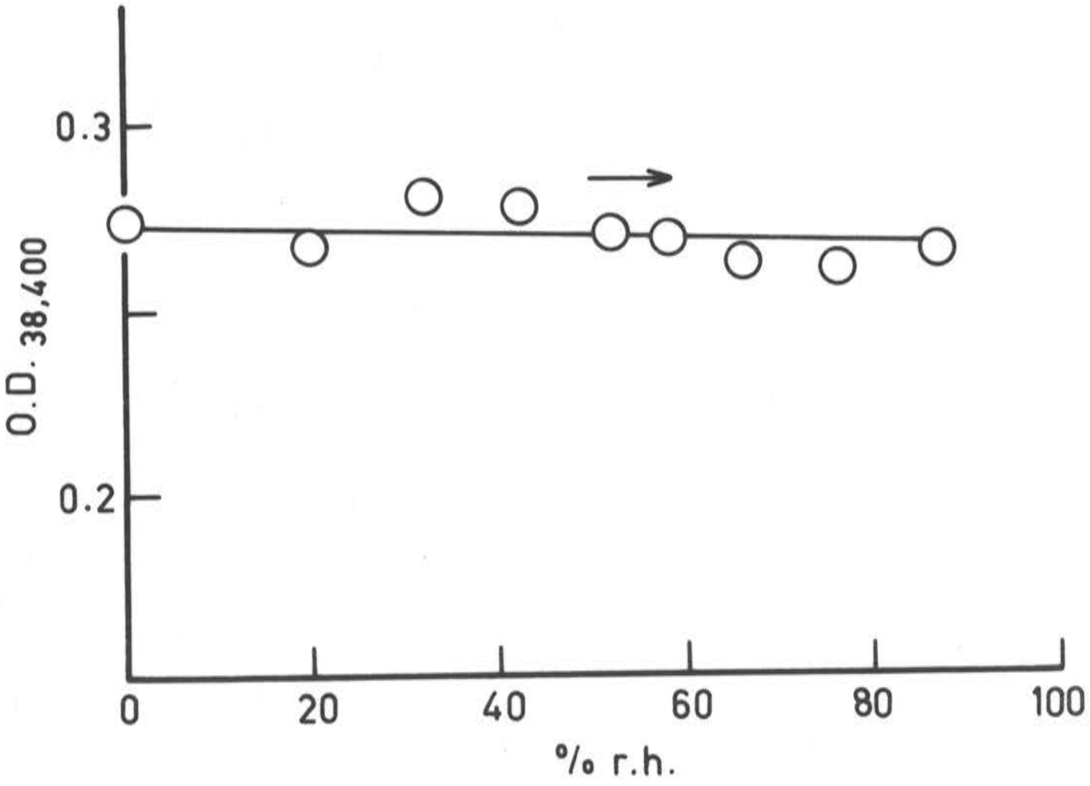


FIG. 6·3. Effect of Light on Hydration of PVA-DNA Films.

some films to constant optical density.

A saturated solution of sodium nitrite was unsuitable for the use in providing an atmosphere of 66% r.h., since both pure PVA and mixed PVA-DNA films which had been stored over such a solution showed an unexpected increased absorption virtually throughout the region 45,000 cm^{-1} to 30,000 cm^{-1}, the increased absorption being particularly noticeable in the region 45,000 cm^{-1} to 40,000 cm^{-1}.

Table 6.4

Relative Humidity Control

Room Temperature = $(21.2 \pm 0.8)^{\circ}C$

Salt	% r.h.	(Lange, 1961)
Potassium acetate	20	
Calcium chloride	32	
Zinc nitrate	42	
Sodium bisulphate	52	
Sodium bromide	58	
Magnesium acetate	65	
Sodium chloride	76	
Sodium thiosulphate	78	
Potassium bromide	84	
Potassium chromate	88	
Sodium sulphate	93	

Room relative humidity was measured over a period of twelve days by means of a wet and dry bulb hygrometer (Weast and Selby, 1965-1966).

6.5 Spectroscopy

6.5.1 Instruments

Polarised and unpolarised measurements at 38,460 cm^{-1} (260 mμ) were made by means of a Unicam SP500 spectrophotometer, whereas the spectra were recorded by means of a Unicam SP700 spectrophotometer. Unpolarised measurements at 38,460 cm^{-1} were also made by means of a Beckman DU spectrophotometer. The optical density scale of the spectrophotometers was calibrated using an alkaline solution of potassium chromate as the transmittancy standard (Haupt, 1952), whereas the wavenumber scale was checked by the position of the hydrogen line. Unpolarised infrared measurements were made using a Perkin Elmer Model 21 spectrophotometer.

6.5.2 Mounting of the Films

The sample cell for the wet spun films consisted of a blackened brass case (4.4 cm. x 1.2 cm. x 1.2 cm.) to which two silica windows were cemented by means of Apiezon grease. Small frames, fitting over the windows to protect them from dislocation, were screwed onto the case by means of countersunk screws. A small glass tube, which was connected to the cell by means of quickfit joints (size, B5) via a glass bridge, contained a saturated solution of a salt in order to maintain a constant relative humidity inside the cell (Fig. 6.4).

In the case of the pure isotropic DNA films, constant relative humidity was ensured by sealing the silica cell with a stopper and teflon tape (Permacel - Johnson and Johnson Pty. Ltd., Sydney) after the cell had been taken out of a desiccator containing a saturated salt solution.

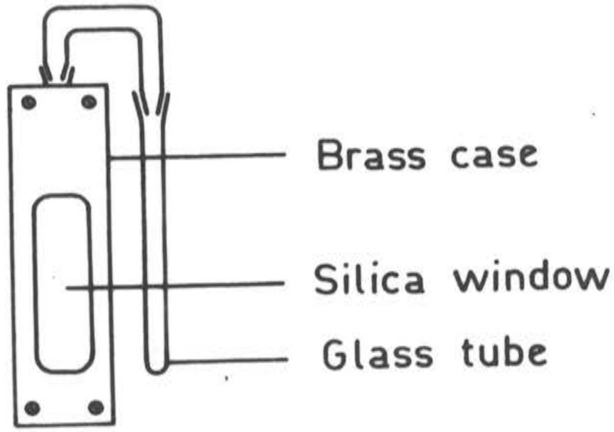
For the ultraviolet measurements, the PVA films were fastened to a blackened machined block (4.3 cm. x 1.2 cm. x 0.9 cm.) by means of adhesive tape. The films formed a window in the block. A small frame with a "Viton-A" gasket was screwed onto the brass block and over the films to prevent them from contracting when they were exposed to atmospheres of high relative humidity.

For the infrared measurements, the PVA films were fastened in stainless steel frames which could be slid into the measuring position in the spectrophotometer.

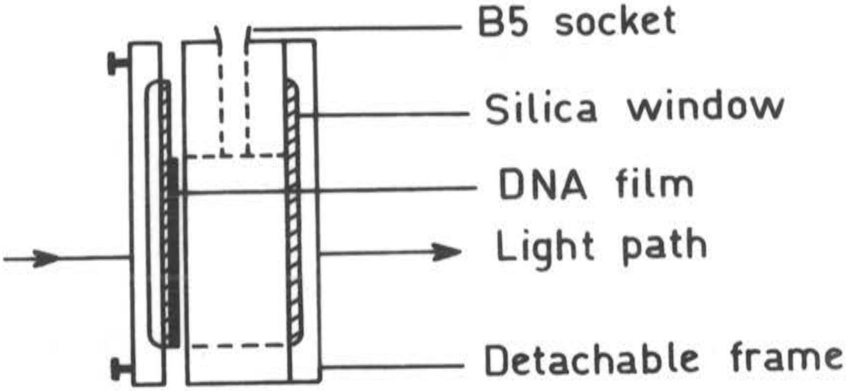
6.5.3 Polarised Spectroscopy

(a) Unicam SP500 Spectrophotometer

In the SP500, the polarised light was produced by means of dichroic ultraviolet polarising filters (formula PL40, Polacoat Inc., Ohio) mounted in the filter holder of the instrument (McDermott and Novick, 1961). Two filters were used, one to produce



Front View



Side View

FIG. 6·4. Sample Cell for Wet Spun Films.

horizontally polarised light and the other to produce vertically polarised light.

The transmittance ratio, r_p^2, for the filters was determined by measuring the transmittance for incident unpolarised light of the two polarisers superposed parallel and then crossed. Since the transmittances for the single polarisers were the same within experimental error, it was assumed that the two filters were identical, and r_p^2 was calculated from the expression

$$r_p^2 = \frac{(\sqrt{H_{11} + H_{\underline{1}}} - \sqrt{H_{11} - H_{\underline{1}}})}{(\sqrt{H_{11} + H_{\underline{1}}} + \sqrt{H_{11} - H_{\underline{1}}})} \qquad (6.2)$$

H_{11} and $H_{\underline{1}}$ are the intensity transmittances for incident light for the two filters superposed with their major transmittance axes parallel and crossed, respectively (Land and West, 1946).

The principal intensity transmittances, k_{11} and $k_{\underline{1}}$, of the oriented DNA films were calculated using the formulae

$$k_{11} = \frac{(H'_{11} - r_p^2 H'_{\underline{1}})}{(1 - r_p^2)} \qquad (6.3)$$

$$k_{\underline{1}} = \frac{(H'_{\underline{1}} - r_p^2 H'_{11})}{(1 - r_p^2)} \qquad (6.4)$$

H'_{11} was measured directly on the spectrophotometer and is the ratio of the intensity transmittance of the DNA film and the polariser parallel relative to the intensity transmittance of the polariser alone. $H'_{\perp}$ is defined similarly except that the DNA film and the polariser were crossed. The dichroic ratio, R, of the DNA films is given by

$$R = \frac{-\log_{10} k_{\parallel}}{-\log_{10} k_{\perp}} \qquad (6.5)$$

In each measurement an empty silica cell (path length, 1.0 cm.) was used as the reference.

(b) Unicam SP700 Spectrophotometer

In the SP700, the principal optical densities of the oriented DNA films were measured using a calcite polarising prism (Archard-Taylor modification of a Glan-Foucalt polariser) mounted after the sample in the sample beam (Shurcliff, 1966). It was assumed that the prism was a perfect polariser. Provision was made for the rotation of the prism and not the sample. A background spectrum of the prism against the reference beam was recorded in each measurement. The reference cell was an empty silica cell (path length, 1.0 cm.).

6.5.4 Solution Measurements

Solution spectra were measured at room temperature using silica cells of different path lengths (0.03 cm. to 1.0 cm.) and a glass cell (path length, 2.0 cm.).

Thermal denaturation profiles of the DNA were measured with the Beckman DU spectrophotometer. The sample and the reference cells (path length, 1.0 cm.) were placed in an electrically heated block which could be moved so as to bring the cells alternatively into the light path. The temperature of the solutions was measured by means of a thermistor immersed in the reference solution. The surface of both the sample and the reference solutions was covered with paraffin oil to minimise the evaporation of the solvent.

In the heating experiments, the optical densities were corrected to 25°C owing to the thermal expansion of the solvent. This was done by multiplying the measured optical densities by the ratio of the density of water at 25°C to the density of water at the measured temperature. It was assumed that the density of the solvent behaved in the same way as the density of water behaves with the temperature.

6.6 Microscopy

A Reichert Zetopan Research microscope was used to examine the wet spun DNA films in transmitted light. Photographs were taken by means of a Praktica IV reflex camera mounted on the microscope.

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