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Structure of DNA cholesteric spherulitic droplet dispersions

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Abstract

The aggregation of short (294-base-pair) linear double-stranded DNA molecules into cholesteric spherulitic droplets in a brine solution of polyethylene glycol has been studied using polarized light microscopy. The DNA concentration within the droplet is found to be constant and therefore the cholesteric pitch is independent of the total DNA concentration. The size of the droplets grows as a power law of the total concentration of DNA. The exponent of the power law relation is calculated using a progressive nucleation and growth model. The exponent is found to be equal to two-thirds and is in good agreement with the experimental data. By analysing fields of spherulitic droplets at low magnification using either hydrophobic or hydrophilic slides and cover-slips, we demonstrate that the positions of the droplets are correlated in solution. The long range correlation between the droplets indicates some kinetic arrest of the phase separating solution that may have been induced by the finite size of the system.

(Some figures in this article are in colour only in the electronic version)

DNA molecules can aggregate or condense without precipitating completely out of solution. The aggregation of DNA can be induced either by screening the electrostatic repulsion between the DNA molecules using multivalent cations, or by reducing the available volume (Chattoraj *et al* 1978, Gosule and Schellman 1976, 1978, Widom and Baldwin 1980, Bloomfield 1997, Raspaud *et al* 1998, Sikorav and Church 1991, Sikorav *et al* 1994, Pelta *et al* 1996). In the latter case, one can either directly dehydrate the DNA solution, or use an incompatible polymer which acts by an excluded volume effect (often called 'macromolecular crowding' (Zimmerman and Minton 1993, Zimmerman and Murphy 1996)). In 1971 Lerman (Lerman 1971) demonstrated that the condensation of DNA can be induced by the combined action of a crowding agent (he used polyethylene oxide to confine the DNA molecules) and monovalent cations (to screen the electrostatic repulsion between DNA chains). He denoted this state of DNA assembly as 'Ψ-DNA' (**p**olymer and **s**alt-**i**nduced condensation of DNA) (Lerman 1973). The final structure of the DNA aggregates obtained in water with monovalent cations (either with high DNA concentrations or in Ψ-DNA) is liquid crystalline (Rill *et al* 1991, Livolant and Leforestier 1996). The type and the structures of different liquid-crystalline phases depend on various parameters, such as DNA concentration, DNA length, counterion type and concentration, water concentration and concentration of the incompatible polymer in the case of the Ψ-condensation technique.

The discovery of Ψ-condensation of DNA generated many important experimental (Jordan *et al* 1972, Cheng and Mohr 1974, 1975, Evdokimov *et al* 1972, Laemmli 1975, Lerman 1971, Maniatis *et al* 1974, Grasso *et al* 1991, Minagawa *et al* 1994, Vasilevskaya *et al* 1995, Yoshikawa and Matsuzawa 1995) and theoretical papers (Grosberg and Zhestkov 1986, Grosberg *et al* 1982, de Vries 2001, Ubbink and Odijk 1995, Vasilevskaya *et al* 1995, Frisch and Fesciyan 1979, Kornyshev *et al* 2007), which have widened our insight into its mechanism. These papers were mainly concerned with understanding the important parameters which drive DNA condensation in the presence of neutral polymer and salt. Here we investigate both the structure and the spatial distribution of the DNA aggregates generated by the Ψ-DNA condensation technique.

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1. Materials and methods

To investigate this question we have used 118, 294 and 370 base pair long linear DNA (corresponding respectively to the sequence 4760–4879, 405–700 and 4760–5131 of the circular bacteriophage ϕX174 (Sanger *et al* 1978) (New England Biolabs)), which was obtained by PCR amplification using 96 ng of supercoiled bacteriophage ϕX174-RFI DNA (replicative form) as the template. The PCR product was separated from the Taq polymerase enzyme (New England Biolabs), unincorporated free nucleotides (New England Biolabs) and primers (30 bases each) (MWG) using a Sigma PCR Clean-Up Kit (Sigma). The recovered DNA samples were precipitated using ethanol (Sambrook *et al* 1989) and the pellet was suspended in 200 μl of ultra-pure water (HiPersolv for HPLC, BDH). The final concentration of DNA solution (1.5 g l^{-1}) was determined by UV absorbance at 260 nm. To assess the size distribution and the quality of the PCR products a fraction of the product, for the 294 base pair long DNA, was digested with alkaline phosphatase (New England Biolabs) prior to being 5′-end-labelled with T4 DNA kinase (New England Biolabs) using γ^{32} P-ATP (Amersham). The length distribution of the amplified fragments was quantified by electrophoresis on 7 wt% non-denaturing polyacrylamide gel (electrophoresis conditions: 3 V cm^{-1}, 6 mA for 15 h). To further check the quality of the PCR product (absence of single-stranded DNA breaks) a fraction of labelled fragment was denatured by heating (98 °C for 3 min) prior to being loaded onto the gel. Quantification of the radioactive bands was carried out on an InstantImager instrument (Packard).

Condensation of DNA was induced in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.8) by addition of a mixture of polyethylene glycol 6000 (PEG, $M = 7000$–9000 g mol^{-1}) (BDH) and NaCl (Analar grade, BDH) to the DNA solution in order to reach a final NaCl concentration of 0.5 M for all performed experiments and a final PEG concentration of 5 wt/vol% for the experiments consisting of determination of the size distribution of the spherulitic droplets, the pitch of the cholesteric phase and the positional distribution of the droplets. In order to determine the variation of the cholesteric pitch with PEG concentration, the final NaCl concentration was fixed at 0.5 M and the PEG concentration was varied. In the experiments reported below the DNA concentration ranged from 0.2 to 1 g l^{-1}. After preparation and before mounting them between slide and cover-slip the solutions were left for a week to equilibrate. 15 μl of each solution was deposited between a glass slide and a glass cover-slip. The slide and cover-slip had been treated with a hot solution (90 °C) of 30% H_2O_2:70% H_2SO_4 (volume ratio) for 20 min in order to clean the oxide surface, followed by extensive rinsing with ultra-pure water. Prior to use, these surfaces were rinsed with chloroform then ultra-pure water. This surface treatment ensured a clean and hydrophilic surface for the slide and the cover-slip. In order to graft a polymer brush to the surface of the slide and the cover-slip we left some of the cleaned surfaces in a mixture of heptane (Aldrich) 1000:1 octadecyltrichlorosilane (Aldrich) (volume ratio) for 12 h. The resulting surface presents a hydrophobic character. The area of the cover-slip was 24 × 24 mm^2, with a sample thickness of 26 μm. After sealing with DPX epoxy resin (Fluka), samples were stored in a humid container to avoid any possibility of dehydration. The samples were observed using a Nikon Eclipse E600 polarizing microscope equipped with a 12 megapixel digital camera (Nikon DXM1200F). The pictures were acquired and analysed with a PC equipped with LUCIA image analysis software (Laboratory Imaging Ltd).

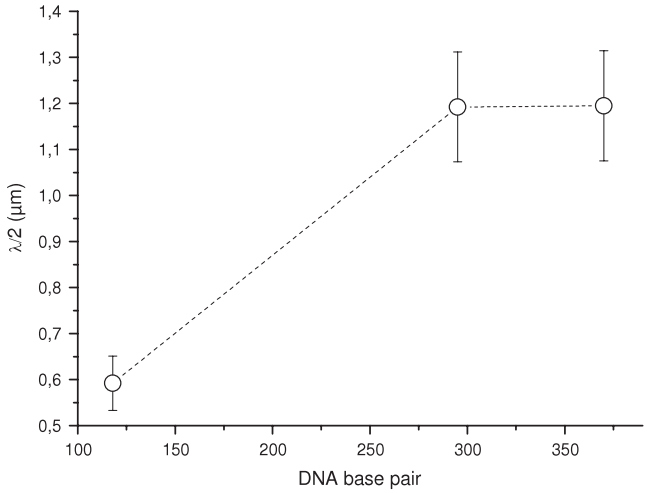

Figure 1. Variation of the half pitch of the cholesteric phase as a function of the DNA length.

2. Results and discussion

In order to be able to correlate the observed phenomena with the structure of the spherulitic droplets it is important to have a perfect control on the quality of the DNA samples. Figure 1 represents the variation of the cholesteric pitch as a function of DNA length for a PEG solution (5 wt/vol% and 0.5 M NaCl) containing the same amount of DNA chains (5×10^{-6} M of DNA molecules). As we can notice, the pitch of the cholesteric phase increases as the length of the DNA chain is increased. Therefore, to remove any kind of effect introduced by the polydispersity of the DNA chain size, we have used only the 294 base pair long DNA chain.

The DNA solutions contained a mixture of molecules of two different lengths (figure 2(a)). 50% of the DNA molecules were double stranded and 294 base pairs long, 11% of the DNA molecules were single stranded and 294 base long and about 34% of the DNA fragments had a much shorter length of about 30 bases, corresponding to the size of the primers used in the PCR reactions (figure 2(b)). These percentages are expressed in terms of numbers of molecules. The main (61%) part of the amplified DNA migrates as a single band in its native form (figure 2(a), lane (i)), indicating that the double-stranded molecules obtained have a very low polydispersity. To further assess the quality of the amplified DNA, an aliquot was submitted to heat denaturation prior to electrophoresis (figure 2(a), lane (ii)). The observation of a single band for the denatured form rules out the presence of single-stranded DNA breaks. We conclude that the amplified DNA consists

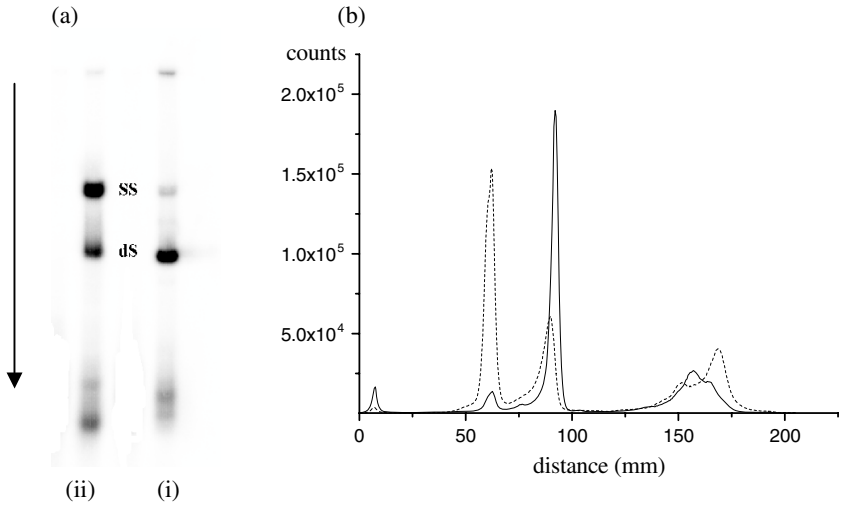

Figure 2. (a) Gel electrophoresis of the products of the PCR reaction. The arrow shows the direction of the migration of the DNA molecules in the gel. Lane (i) corresponds to the native DNA and lane (ii) to the heat denatured fragment. The bands labelled as **ss** correspond to single-stranded DNA and the bands labelled **ds** to double-stranded DNA. (b) Radiograph of the gel. The solid line is the radiograph of the native DNA (i) and the dashed line the radiograph of the denatured fragment (ii). By integrating the area under each peak of the radiograph we can infer that 50% of labelled fragment consists of 294-base-pair linear double-stranded DNA, 11% of labelled fragment consists of 294-base linear single-stranded DNA, 5% of the labelled fragment did not migrate and remained in the wells and 34% of the labelled fragment has a size smaller than 294 base pairs (mainly around 30 base pairs). The denaturation is only partial and shows only two bands for the 294-base-pair DNA and the smaller size DNA fragment: one corresponds to the double-stranded form (ds) (24%) and the other one to the single-stranded form (ss) (38%).

almost exclusively of intact double-stranded 294 base pair long fragments. Finally, we note that under the defined experimental conditions (5 wt/vol% PEG and 0.5 M NaCl) the 30-base single-stranded DNA fragments do not condense (Lis and Schleif 1975), and therefore they do not participate in the formation of the optical pattern that we observe from the DNA spherulitic droplets. However, it is not beyond the bounds of possibility that they have some stabilizing effect on the structure of the droplets. Indeed, because of their shorter length and their more flexible structure they could diffuse more easily into the droplet and congregate along the defects (disclination line), as do smaller atoms into solid crystals (Shewmon 1963).

3. Structure of the spherulitic droplet: theory and experiments

The structure of cholesteric spherulitic droplets was first analysed by Pryce and Frank in a personal communication to Conmar Robinson (Robinson *et al* 1958). They described the spherulitic texture using concepts based on stereographic projections and conformal transformation. Their construction implied a continuous structure except along a singular line (the disclination radius). The structure of the DNA cholesteric phase was first studied by Conmar Robinson (Robinson 1961), and the structure of DNA spherulitic droplets has been thoroughly characterized by Bouligand and Livolant (Livolant and Leforestier 1996, Bouligand and Livolant 1984). Spherulitic droplets have two types of characteristic optical textures, depending on the orientation of the disclination radius with respect to the plane of the sample (Livolant and Leforestier 1996). If the disclination radius lies in the preparation plane, a series of concentric circles is observed

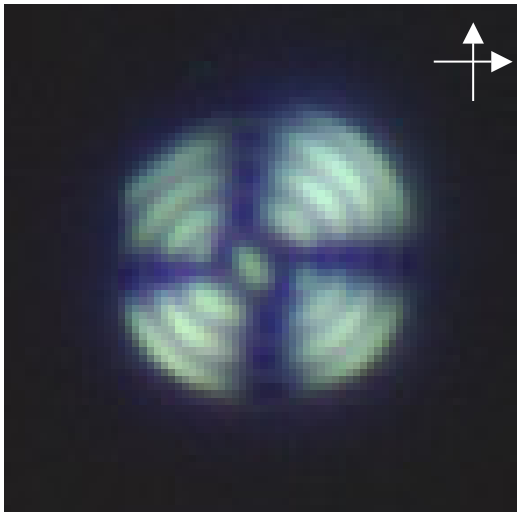

Figure 3. A DNA spherulitic droplet from a solution of 1 g l^{-1} concentration (under crossed polarizers, 40× magnification). The visible double spiral implies that the disclination radius is normal to the preparation plane. The black (Maltese) cross in the middle of the spherulites is due to the birefringent character of the cholesteric germs. The white arrows represent the direction of crossed linear polarizers.

under crossed polarizers. On the other hand, if the disclination radius is normal to the preparation plane, a double-spiral pattern is observed. In our preparations we observe mainly the double-spiral texture (figure 3). The existence, shape and size of the droplet result from a fragile balance between their bulk free energy and their surface free energy. The bulk free energy tends to increase the size of the droplet, whereas the surface

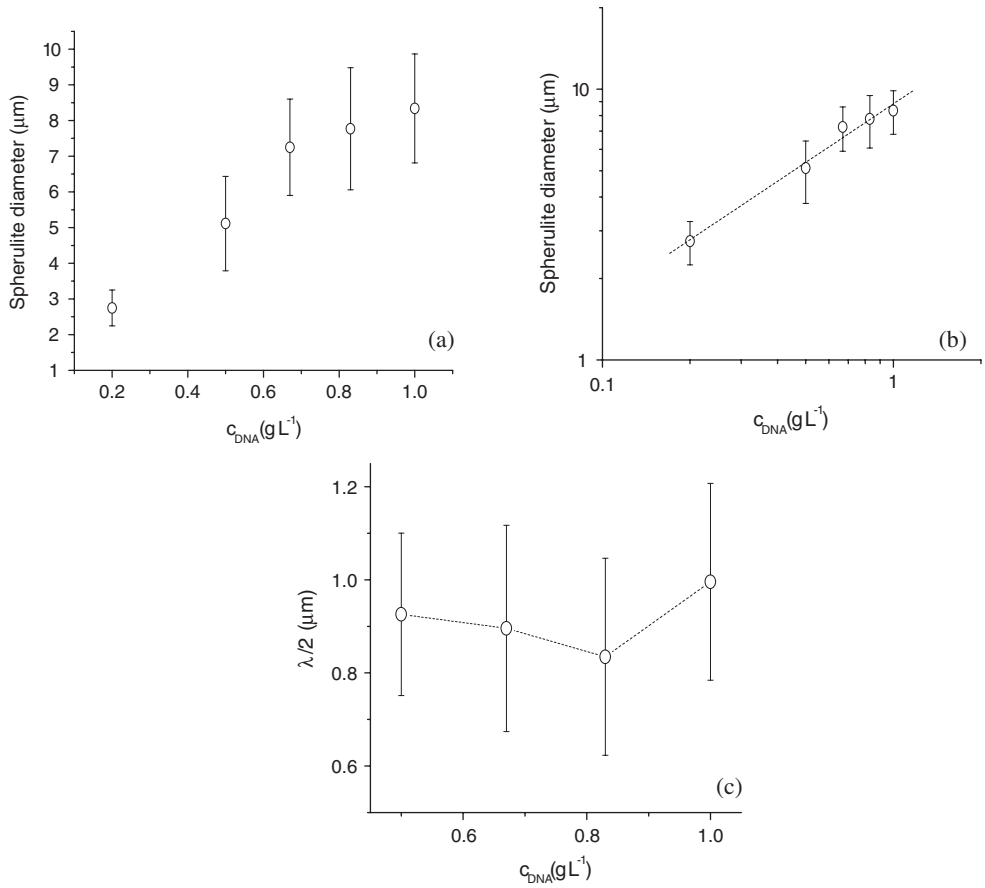

Figure 4. (a) Variation of the diameter of the spherulitic droplet as a function of total DNA concentration. (b) Variation of the diameter of the droplet as a function of total DNA concentration represented as a log–log plot. The dashed line represents a linear fit to the data points (open circles), from which a slope of 0.68 ± 0.05 is obtained. (c) Variation of the half pitch of the cholesteric phase as a function of total DNA concentration. Within the range of the error bars the pitch of the cholesteric phase is constant.

free energy, which depends strongly on the surface tension between the cholesteric phase and the isotropic phase, tends to reduce the size of the droplet (Ubbink and Odijk 1995, Livolant and Leforestier 1996). In the present study the DNA was condensed into the liquid crystalline phase by the cooperative action of the Na^+ cations and the osmotic pressure induced by addition of PEG to the solution. We measured the size of the DNA spherulitic droplets and the pitch of the cholesteric phase at different total DNA concentrations (figure 4) ten days after the samples were mounted between slide and cover-slip. The size (diameter $2R_s$) of the droplet increases with total DNA concentration (figure 4(a)), in a manner that we shall now explain.

The formation of DNA spherulitic droplets requires the cooperative action of the electrostatic screening induced by NaCl and the increase of osmotic pressure induced by PEG polymer. The synergic effect of the DNA charge screening by Na^+ cations and the increase of the osmotic pressure facilitate the aggregation of DNA molecules. However, the competition between the bulk free energy of the aggregate (which tends to expand the size of the aggregate) and its surface free energy (which tends to reduce the interface between the DNA rich phase and the surrounding isotropic solution) suggests that there exists a critical size below which the DNA aggregate is not stable. Therefore, there exists an energetic barrier that a DNA molecule must overcome in order to be incorporated into the spherulitic droplet. The presence of this energy barrier implies that DNA partitions between the isotropic phase outside the droplet and the cholesteric phase inside the droplet.

Let $N_A(t)$ be the number of DNA molecules incorporated into a droplet at a time t, and N_T the total number of DNA molecules present in the solution. We assume that in order for a DNA molecule to be incorporated in an aggregate it should first encounter the aggregate and then overcome the energetic barrier that we have described above. Therefore, the frequency of incorporation of DNA into the aggregate is proportional to the rate of encounter between DNA molecules and therefore should be linearly proportional to the remaining number of DNA molecules in the isotropic phase ($N_T - N_A(t)$). Let us represent by K_p the rate of aggregation of DNA molecules per unit time. This parameter takes into account the probability of encounter between DNA molecules and the probability of incorporation of a DNA molecule into an aggregate. However, to analyse our data set it is not necessary to know exactly the form of the aggregation rate K_p (which in a general manner can be time dependent) and this is beyond the scope of this paper. Using the arguments developed above, we write the variation of the number of DNA molecules incorporated into a droplet

as

$$\frac{\mathrm{d}N_A(t)}{\mathrm{d}t} = K_\mathrm{p}(t)\,[N_\mathrm{T} - N_A(t)]\,. \tag{1}$$

In order to solve this ordinary differential equation we need to determine the boundary conditions. At the start of the aggregation process ($t = 0$) we do not observe any spherulites; this observation corresponds to the initial condition $N_\mathrm{A}(0) = 0$. After one to two days of equilibration we could clearly distinguish in the solution small DNA aggregates. The size of these droplets increases as a function of time (figure 5). Finally after a few weeks of observation we observed a few coalescence events. Therefore, at very long time one expects that all the DNA molecules present in the solution will be incorporated into a single cholesteric DNA rich macroscopic phase. This observation implies that at very long times the system tends towards an equilibrium state where all the DNA molecules in the solution have phase separated from the PEG solution. This corresponds to the boundary condition $t \to \infty N_\mathrm{A}(t) = N_\mathrm{T}$. Using these boundary conditions we solve equation (1) and find the following expression for the kinetics of incorporation of DNA molecules into a droplet:

$$N_A(t) = N_\mathrm{T}\left(1 - \mathrm{e}^{-\int_0^t K_\mathrm{p}(t)\,\mathrm{d}t}\right)$$
$$\text{with } \lim_{t\to\infty} \int_0^t K_\mathrm{p}(t)\,\mathrm{d}t \to \infty. \tag{2}$$

Now that we have calculated the number of DNA molecules incorporated into a spherulitic droplet at a given time we calculate the average volume of a droplet using the Pryce–Frank construction. The basic idea in the treatment of cholesteric droplets by Pryce and Frank is to build up the droplet by progressive addition of spherical shells of different DNA orientations. Therefore, along every radius of the droplet (except the disclination line) there is a constant cholesteric twist. This means that the twist angle of each shell is related to the radius of that shell. Therefore the volume of a shell is equal to the number of molecules present inside the shell times the average volume (v_DNA) occupied by each DNA molecule in the cholesteric phase. The shell structure of the spherulitic droplet implies that to calculate the average volume of a spherulitic droplet at a time t one should take into account how the successive shells are added to the droplet up to time t. This means that the average volume of the droplet at time t is given by

$$V_\mathrm{spherulite}(t) = \frac{1}{tM}\int_0^t \frac{\mathrm{d}N_A(\tau)}{\mathrm{d}\tau} N_A\,(t-\tau)\,v_\mathrm{DNA}\,\mathrm{d}\tau$$
$$\text{with } 0 \leqslant \tau \leqslant t \tag{3}$$

where M is the number of droplets. By inserting equations (1) and (2) into equation (3) and considering a spherulitic droplet as a sphere of average radius R_s we obtain that

$$R_\mathrm{s}(t) = \left[\frac{3V_\mathrm{T}^2 v_\mathrm{DNA} f\,(t)}{M4\pi}\right]^{\frac{1}{3}} C_\mathrm{T}^{\frac{2}{3}} \qquad \text{where}$$
$$f(t) = \frac{1}{t}\int_0^t [K_\mathrm{p}(\tau)\mathrm{e}^{-\int_0^\tau K_\mathrm{p}(t')\mathrm{d}t'}(1-\mathrm{e}^{-\int_0^{t-\tau} K_\mathrm{p}(t')\mathrm{d}t'})]\mathrm{d}\tau. \tag{4}$$

These considerations predict that the average radius of a spherulitic droplet at time t increases as the total concentration of added DNA in the solution (C_T) to the power of 2/3. Figure 4(b) shows that the size of the spherulite does indeed grow with the total DNA concentration as $\sim c_\mathrm{T}^{0.68}$.

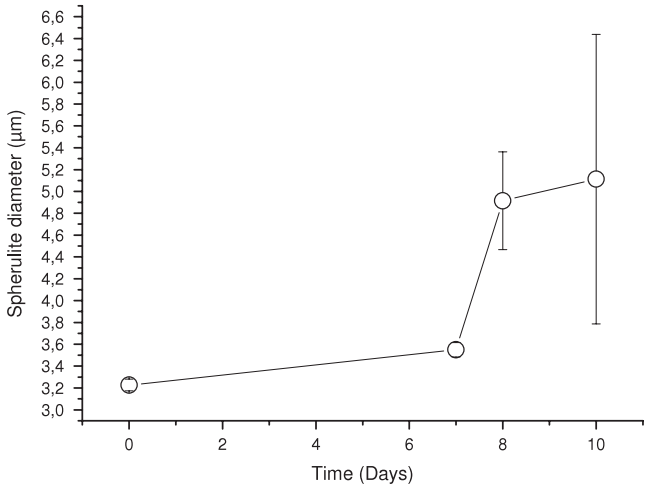

Figure 5. Variation of the diameter of the spherulitic droplet as a function of time. This measurement was performed on a preparation of DNA concentration of 0.5 g l^{-1} in 5% PEG6000 deposited between slide and cover-slip. The solution has been equilibrated for a week before being deposited between slide and cover-slip.

The pitch λ (figure 4(c)) of the cholesteric phase inside the spherulitic droplet appears to be independent of the total DNA concentration added to the solution ($\lambda = 1.8 \pm 0.4\ \mu$m). The independence of the cholesteric pitch on varying DNA concentration (in the DNA concentration range where the cholesteric phase can exist) has also been observed for liquid-crystalline DNA obtained from concentrated DNA solutions (Van Winkle *et al* 1990). In the case of the DNA cholesteric phase obtained by DNA aggregation using PEG, it has been shown that the cholesteric phase unwinds (the pitch increases) as the total amount of PEG is increased (the osmotic pressure is increased) in the solution (Leonard *et al* 2001). In our experiments the total concentration of PEG is kept constant and only the total concentration of DNA added to the solution is changed. As demonstrated by Stanley *et al* (2005), the pitch of the cholesteric phase inside the spherulitic droplets depends strongly on the concentration of DNA molecules inside the droplets. Changes in the DNA cholesteric pitch imply a reorganization of the structure of the spherulitic droplet: for example, the DNA cholesteric pitch increases (figure 6) as the cholesteric–hexagonal columnar phase transition is approached (Stanley *et al* 2005). In the case of Ψ-DNA aggregation, the DNA concentration inside the spherulitic droplets is controlled solely by the amount of PEG added to the solution (Stanley *et al* 2005, Rau *et al* 1984). Thus, invariance of the pitch with total DNA concentration added to the solution should be expected, as the concentration of DNA inside the spherulitic droplets is controlled by the amount of PEG in the solution. This is not too surprising, as under the described experimental conditions the DNA and the rest of the solution undergo a phase separation process. The formation of DNA droplets involves, as described above, a nucleation and growth process which is not unlike traversing a

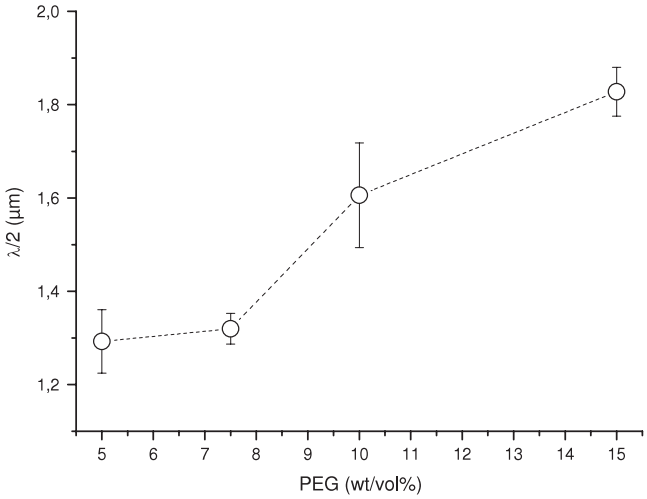

Figure 6. Variation of the half pitch of the cholesteric phase as a function of the PEG concentration for 294 base pair long DNA chain.

horizontal tie lie within a two-phase region on a phase diagram. Moreover, the pitch invariance demonstrates that the variation of the size of the spherulitic droplets does not involve a change in the bulk structure of the droplet, and thus that the increase in size of the droplet can be understood as the addition of a new spherical shell to the surface of the DNA droplet.

4. Analysis of the spatial distribution of the spherulitic droplets

We have also measured the spatial distribution of the spherulitic droplets in the plane of the preparation as a function of DNA concentration after 10 days. Figure 7(a) shows a typical picture of a sample at low magnification (10×) between crossed polarizer and analyser. We arbitrarily chose one of the DNA spherulites as a reference point, and measured the distance between the reference spherulite and all other spherulites present in the field of view (figure 7(b)). Figure 7(c) shows a typical distribution of the number of spherulites as a function of distance. By measuring the area under the distribution function we calculated the total number $N(r)$ of spherulites that lie within a disc of radius r. This number is related to the correlation function $C(r)$ through the equation

$$N(r) = \int_0^r N\left(r'\right) C\left(r - r'\right) \mathrm{d}r' \qquad \text{with } r' < r. \tag{5}$$

In order to extract the correlation function we differentiated the logarithm of the number of droplets (normalized to the total number of droplets N_0) with respect to the distance r

$$C(r) = \frac{\mathrm{d}\ln\left(\frac{N(r)}{N_0}\right)}{\mathrm{d}r}. \tag{6}$$

The correlation function (figure 8(a)) does not monotonically drop to zero, but exhibits a number of discrete peaks.

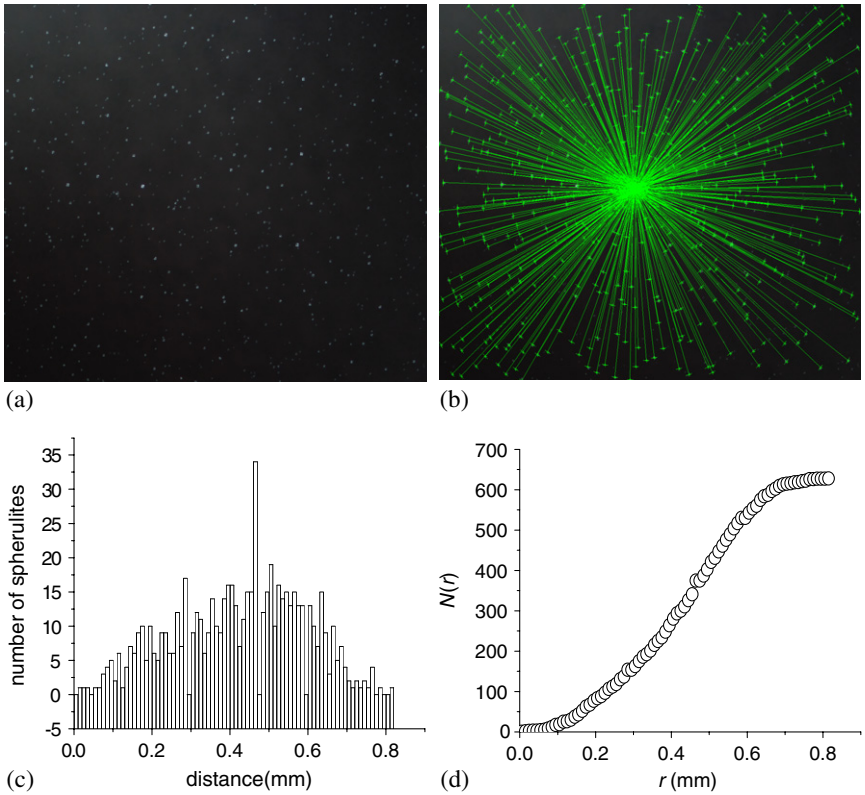

Figure 7. (a) A typical field of view of the DNA spherulitic droplets (white dots). This image is from a preparation of DNA at a concentration of 0.83 g l^{-1} (crossed polarizers, 10× magnification). (b) A droplet is chosen as a reference point and each line corresponds to the distance between the reference droplet and its neighbours. (c) A typical measured distribution of the number of droplets as a function of distance ($c_{\mathrm{DNA}} = 1$ g l^{-1}). (d) Number of droplets within a disc of radius r ($c_{\mathrm{DNA}} = 1$ g l^{-1}).

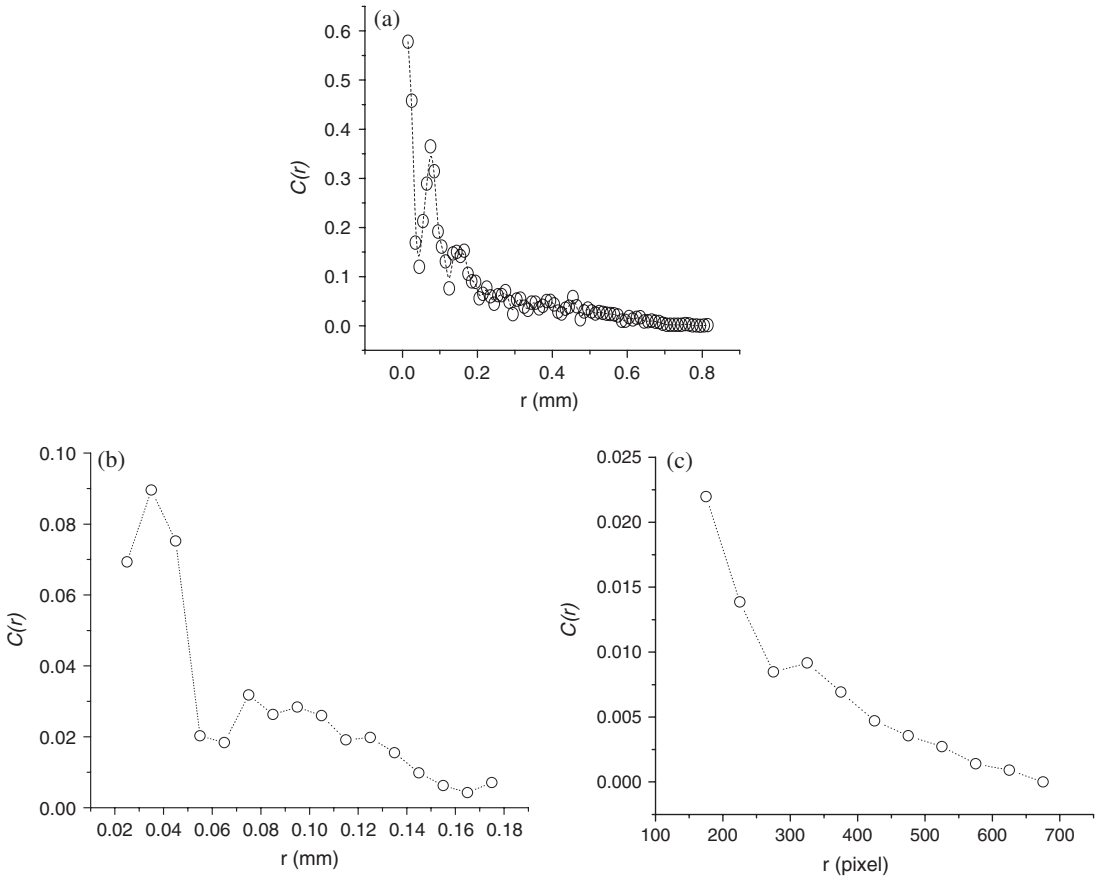

Figure 8. (a) A typical profile of the correlation function $C(r)$ as a function of the distance r ($c_{\mathrm{DNA}} = 1$ g l^{-1}) in a hydrophilic sample. (b) A typical profile of the correlation function $C(r)$ as a function of the distance r ($c_{\mathrm{DNA}} = 1$ g l^{-1}) in a hydrophobic sample. (c) The profile of the correlation function $C(r)$ as a function of the pixel distance ($c_{\mathrm{DNA}} = 10$ g l^{-1}) obtained by analysing figure 2(c) of Rill *et al* (1991).

The presence of these peaks implies that the droplets are correlated in position. It is surprising to observe that these correlation peaks are located at such a large distance (from 10 to 100 μm for the first correlation peak). The large value of the correlation distance between the droplets rules out interaction mechanisms involving PEG molecules (depletion mechanism (de Vries 2001)), or any geometrical interaction between droplets (scaled particle theory (Reiss *et al* 1959)). Moreover, as the correlation length between the droplets is 10^4–10^6 times larger than the Debye screening length in the solution it seems very unlikely that the interaction between the droplets is due to electrostatic interactions. In order to verify that the observed correlation function is not an experimental artefact we changed the properties of the slide and cover-slip surfaces by coating them with a thin hydrocarbon brush layer, and measured the correlation function in these samples. Figure 8(b) shows the correlation function obtained from a sample with hydrophobic surfaces. The shape of the function is the same as observed with hydrophilic surfaces, although the positions of the peaks have changed and their amplitude decreased. Moreover, by analysing figure 2(c) of Rill *et al* (1991), where DNA spherulitic droplets were obtained using a very concentrated DNA solution (10 mg ml^{-1}), we find that the positions of these droplets (figure 8(c)) were also correlated in the same manner as our presented data. To exclude all types of optical artefacts (which have previously led to the erroneous conclusion that there exists an attraction between like-charged colloids in confined environments) we follow the methodology defined by Bechinger and co-workers (Baumgartl *et al* 2006). These authors measured accurately the pair potential between charged colloidal particles in thin samples, and proposed that the observed distribution of particles by optical microscopy, $P(r)$, corresponds in fact to the true distribution of particle, $P_{\mathrm{t}}(r_{\mathrm{t}})$, multiplied by the variation of the true distance between the particles, r_{t}, relative to the observed one, r ($P(r) = \frac{\mathrm{d}r_{\mathrm{t}}}{\mathrm{d}r} P_{\mathrm{t}}(r_{\mathrm{t}})$). r_{t} corresponds to the observed distance between the particles, r, minus the relative effect of other particles on the position of the reference point, $2\Delta r$. In our case, the positions of the spherulitic droplets do not change as a function of time; it seems that the droplets are frozen in their given positions. Therefore, the observed configuration is not an equilibrium configuration (we do not observe thermal motion). Using these observations, in our case, there is an exact equality between r_{t} and r. Therefore, the observed distribution should correspond to the true distribution of the particles. However, we do not consider that there exist any intrinsic attractive interactions between the spherulite particles, as was previously suggested in the case of like-charged colloidal particles.

As described in the previous section we are dealing with a phase separating binary mixture (DNA/PEG). Therefore, the

final structure of the system is determined kinetically but not thermodynamically. Moreover, this phase separating system is sandwiched between two glass plates, where each component of the mixture could interact with the surfaces of the glass slides. One possible explanation for the strong positional correlations observed in our system is that the dynamics of the phase separation is coupled to that of a surface wetting phenomenon. Thus, the difference of the wetting ability of each phase could induce a hydrodynamic instability in the binary mixture, which in turn could induce an indirect long-range interaction between the droplets. The strength and the spatial range of this interaction depends strongly on the thickness of the sample and the ability of different phases to interact with the surface of glass plates (Tanaka 1993).

Our work has a bearing on the understanding of previous work on Ψ-DNA. Studies of Ψ-DNA by circular dichroism (CD) and calorimetric measurements have mainly focused on the structure of the DNA aggregates, considering them as being independent of one another (Keller and Bustamante 1986, Jordan *et al* 1972, Cheng and Mohr 1974, 1975, Grasso *et al* 1991). As the quantities measured by these techniques are macroscopic, one has to consider the correlation function, shown in figure 8, in order to be able to extract potential information relevant to the behaviour of a single DNA molecule. Also, the existence of positional correlations between the spherulites implies that at higher DNA concentrations (or PEG concentrations) the structure and the shape of the resulting liquid crystalline domains should be related to the correlation length.

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