MACROMOLECULES AND MEMBRANES IN HIGH MAGNETIC FIELDS

G. MARET and K. DRANSFELD
Hochfeld-Magnetlabor des Max-Planck-Instituts für Festkörperforschung, B.P. 166, 38042 Grenoble, France

(Invited paper)

In the past, magnetic fields have been frequently used to orient liquid crystals and large components of biological cells. The availability of high magnetic fields up to 20 Tesla makes possible the relatively strong orientation also of diamagnetically anisotropic macromolecules or small molecular clusters. From the degree of magnetic alignment – detected optically – the persistence length of flexible polymers and the size of various molecular clusters has been determined with high precision. In particular, this new method has been used recently in order to study the following structural problems:

1) The rigidity of nucleic acids in various solutions
2) The lateral structural correlation within a phospholipid membrane
3) The degree of local order in liquid polymers.

1. Introduction

Very important contributions to the present knowledge of structure and conformation of macromolecules in the liquid state – both in solution or in the melt – have been achieved by observing the molecular response to external fields such as gravitation, hydrodynamic velocity gradients or electric fields. In the case of polyelectrolytes, however, the application of such methods is frequently difficult because the effective forces on the molecules are not known exactly, since strong electric solvent–solute or solute–solute interactions may modify the effective hydrodynamic dimensions and charge densities of the molecule.

Using as examples various nucleic acids in solution, phospholipid membranes and liquid polymers, we show that a study of molecular orientation in *homogeneous* high magnetic fields yields valuable new information about macromolecular structure. The availability of highly *inhomogeneous* magnetic fields – discussed at the end – is equally of great importance for the study of macromolecules in solution.

2. Magnetic orientation of macromolecules

2.1. General principles

It is well known that diamagnetically anisotropic *small molecules* in the fluid state can be slightly oriented by an external magnetic field H (Cotton–Mouton-effect). Since partial orientation takes place in thermal equilibrium, the degree of orientation β is described by the Boltzmann statistics i.e. if – for instance – the molecules under consideration have diamagnetic susceptibility values $\chi_\parallel$ and $\chi_\perp$ parallel and perpendicular to a rotational symmetry axis respectively, $\beta = (\chi_\parallel - \chi_\perp)H^2/kT$ for $\beta \ll 1$, with k being the Boltzmann constant and T the absolute temperature [1]. At room temperature and conventionally available magnetic fields ($H \sim 1$ T) the magnetic orientability of single, even strongly anisotropic molecules like benzene ($\chi_\parallel - \chi_\perp = 60 \times 10^{-6}$ emu/mol) is very small ($\beta \sim 10^{-7}$).

However, β can dramatically be increased, when a great number N of such molecules are rigidly fixed together parallel to one other, since the effective diamagnetic anisotropy of such a *molecular cluster* – and hence β – is proportional to N.

$$\beta = N(\chi_\parallel - \chi_\perp)H^2/kT. \tag{1}$$

In fact, almost full magnetic alignment has been observed on very large biological systems like chloroplasts [2, 3], retinal rods [4, 5], muscle fibers [6] and on various liquid crystalline systems [7–10] even in magnetic fields around 1 T. It was demonstrated that measuring the magnetic orientation behaviour (β) yields information about molecular orientational correlation and about molecular arrangement within complex biological systems.

In order to obtain precise information about much smaller correlation lengths such as the persistence length $P = \frac{1}{2}l_0N$ of flexible polymers, when built up of much less magnetically anisotropic monomers (with monomeric length l_0) and even in dilute solutions, considerably higher

magnetic fields combined with an extremely sensitive detection of β are desirable. This extension has recently [1] been reported. By the measurement of the magnetically induced birefringence

$$\Delta n = n_{\parallel} - n_{\perp} = CM\ \lambda\ H^2 \sim \beta(\alpha_{\parallel} - \alpha_{\perp})C \qquad (2)$$

in fields up to 14 T (where $n_{\parallel}$, $n_{\perp}$ is the refractive index for light of wavelength λ when polarised parallel and perpendicular to H, respectively, $\alpha_{\parallel}$, $\alpha_{\perp}$ the molecular optical polarisabilities parallel and perpendicular to the molecular symmetry axis, C the monomer concentration and CM the Cotton–Mouton-constant) the persistence length of DNA was determined in dilute aqueous solutions under standard conditions.

To illustrate the power of this new method we present here a continuation of this study – especially dealing with the question of *why* nucleic acids are so rigid and *how* this rigidity is modified when interaction of the nucleic acid with the ionic solvent, with an intercalating dye or with the natural proteins in the chromosomes occurs. Furthermore, correlations of phospholipids in multilamellar planar and vesicular synthetic membranes and the conformation of high polymers in the amorphous state are investigated.

2.2. Experimental techniques

Several methods like microscopy [4], fluorescence polarization [2], linear dicroism [3], X-ray [5–7] and neutron scattering [11], and NMR [8] have been applied to detect high degrees of magnetic alignment. However, in order to obtain similar information on the structure of single, only partially orientable macromolecules, optical birefringence techniques have been used because of their much higher sensitivity for measurements of very small β-values [9, 10, 1].

Concerning the aspect of determining persistence lengths of flexible polymers, some advantages of the Cotton–Mouton-method with respect to other methods are indicated in [1] and in section 2.3. Now we briefly describe the experimental set up used for almost all studies reported here. Magnetic fields up to 14 T were produced with a Bitter type solenoid of 5 cm inner diameter. If not stated otherwise, samples were kept in standard – temperature stabilized (±0.05°C) – quartz containers with 1 cm optical path. The magnetic birefringence Δn was continuously measured using a photoelastic modulation technique and an automatically compensating Pockels-cell as shown in fig. 1. This method represents a refined modification of previously described techniques [13–15] and will be published elsewhere [16] in a more detailed form. We achieved a resolution $\Delta n/n < 10^{-9}$ for 1 cm sample length and $\lambda = 6328$ Å at a response time < 30 msec. A sample volume of only about 1 cm^3 is needed.

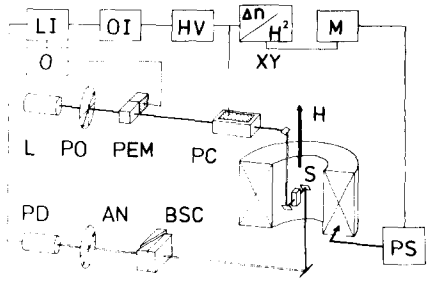

Fig. 1. Schematic representation of the experimental set up used to measure small magnetic birefringences. The photoeleastic modulator (PEM) [12] produces only a 100 kHz-modulation of laser (L) light intensity at the photodiode (PD), if no dc birefringence is present. Any steady magnetic birefringence gives rise to a superimposed 50 kHz-signal which – after dc conversion with a lock-in amplifier (LI) – serves as compensating voltage at the pockels cell (PC). Polarizer PO, Analyzer AN, Sample S, Babinet Soleil Compensator BSC, Oscillator 50 kHz O, Operational Integrator OI, High voltage amplifier HV, X–Y-recorder XY, Multiplier M, Magnet power supply PS.

2.3. The persistence length of nucleic acids

Because of its great rigidity and its enormous chain length (molecular weight usually $> 10^6$), DNA has often been used as a model substance for checking statistical theories on the coiling of semirigid – so-called wormlike – polymers which theoretically can neither be described by a random walk of the monomeric segments nor by an elongated rod. The important parameter P, the persistence length, connecting molecular weight and molecular extension – i.e. the radius of gyration – was not accessible to a direct measurement in thermal equilibrium and in dilute solution. From various hydrodynamic and light scattering experiments [17–19] even by employing theoretical corrections for "excluded volume" and assumptions about the effective

hydrodynamic chain diameter, P values still varying over one order of magnitude have been published [20] and only recently [19], they seem to converge to 450–600 Å. From magnetic birefringence data $P = 450 \pm 40$ Å for Calf-Thymus DNA in 1.5×10^{-1} M NaCl aqueous solution has been deduced [1], the experimental error mainly being due to the uncertainty of values for $\alpha_{\parallel} - \alpha_{\perp}$ and $\chi_{\parallel} - \chi_{\perp}$. However, small relative changes in P can be detected with high accuracy as demonstrated by the measurement of the temperature dependence of the Cotton–Mouton-constant (see fig. 3 in [1]). It has been shown that the partial alignment ($\beta = 0.88\%$ in $H = 12$ T) of the DNA filament perpendicular to H is caused by the strongly anisotropic aromatic bases.

We now present new information about the structural reasons for the high stiffness of DNA. At neutral pH, the DNA filament carries two negative charges per monomeric segment due to the phosphate groups. These surface charges are screened by solvent counter ions in a way depending on the Debye length $\lambda_D = 2.95(1/\sqrt{\mu})$, where λ_D is measured in Å and the ionic strength μ in moles NaCl per liter ($\triangleq M$). For $\lambda_D \ll P$, the persistence length of DNA should only be slightly influenced by electrostatic repulsion between monomer segments. Increasing λ_D by reducing μ, however, should lead to increasing P, since an increasing number of segments contribute to the mutual repulsion. This, in fact, is in good agreement with our observations (fig. 2). A saturation behaviour of CM/C – found by lowering μ above $\lambda_D > 100$ Å – might be explained in terms of conformational entropy: the gain in electrostatic energy from further increase of the mean distances between monomer segments is probably too small to compensate for the amount of energy needed to blow up the persistence length and hence the radius of gyration of the DNA filament (lowering of entropy). Fig. 2 shows that about half of the persistence length of DNA at $\mu = 10^{-4}$ M NaCl can be attributed to surface charges.

In order to find out why DNA is so stiff at $\mu > 10^{-1}$ M, let us first look more closely at the molecular structure of DNA. A DNA double helix is characterized by coplanar stacking of neighbouring basepairs. The base stacking tendency turns out to be sufficiently strong to cause a remarkable self-agglomeration even of the

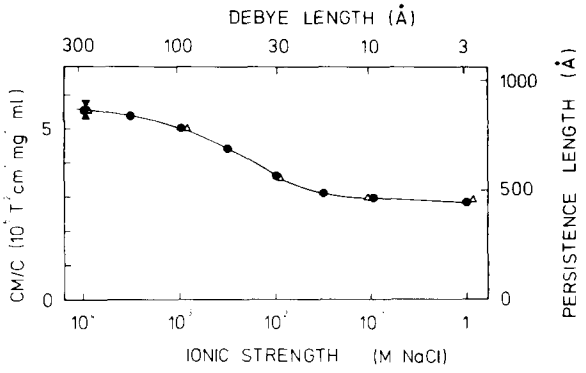

Fig. 2. Ionic strength dependence of the specific Cotton–Mouton-constant of Calf Thymus DNA. $T = 20.4$°C. Experimental parameters, sample preparation and characterization very similar to [1]. Starting from a first lot of DNA ($C = 3.1$ mg/ml in aqueous solution, pH = 6.8, $\mu = 10^{-4}$ M NaCl, 2×10^{-5} M EDTA), increasing μ was produced (●) either by addition of corresponding amounts of highly concentrated NaCl solution (final DNA concentration $C = 1.5$ mg/ml at 1 M), or by dialysis (△) $C = 3.1$ mg/ml, (▲) $c = 10$ mg/ml, (▼)$C = 5$ mg/ml.

monomeric bases in aqueous solution [21], its strength varying for different bases as demonstrated in fig. 3. In agreement with stacking affinity data [21] we observed greatest stacking probability for adenine. This result is confirmed by the temperature dependence of CM from aqueous solutions of Poly (rA) (fig. 3 in [1]) which, more clearly than data obtained by other methods, supports the following model [22] of

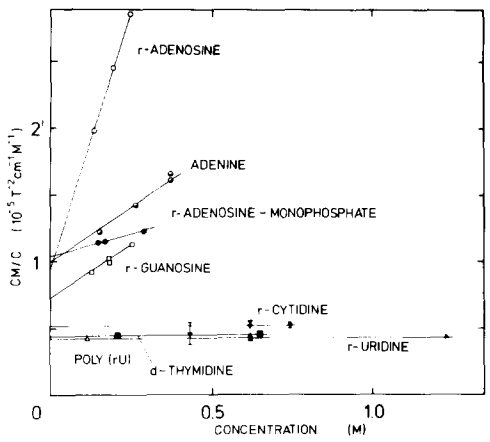

Fig. 3. Concentration dependence of the molar Cotton–Mouton-constant of aqueous solutions of different nucleosides, i.e. base + ribose and of the base Adenine (○) (in 0.5 M NaOH), Adenosine-Monophosphate (●) and Poly (rU) (Δ) (in 0.025 M Phosphate-buffer, 0.1 M NaCl). $T = 22.2$°C.

the poly (rA) structure in neutral pH: at 10°C the mean persistence length consists of helical arrays of about 15 rigidly and coplanarly stacked adenine bases which melt non-cooperatively with increasing temperature.

Base stacking should be an important contribution to the stability also of native DNA, since it occurs unspecifically between different bases, or even between bases and planar dye-molecules like ethidium having a molecular shape comparable to that of the bases. Such dyes are able to intercalate between two basepairs of DNA leading to a strong reduction of the local helicity, i.e. to a reduction of the torsional angle from 36° per monomer length l_0 (= 3.4 Å) to about 8° [23] (fig. 4). In this way the distance l_0 between two basepairs at the intercalation site is roughly doubled and, therefore, the phosphoribose backbones must be strongly deformed. Our data (fig. 4) show that the rigidity of DNA is not drastically changed by this intercalation process, even if more than 100 dye molecules are fixed within one persistence length of DNA. Since the hard core of stacked aromatics is the only common characteristic structural feature of both the unperturbed and the strongly perturbed DNA strands, we may conclude that predominantly basestacking is responsible for the inherent rigidity of nucleic acids.

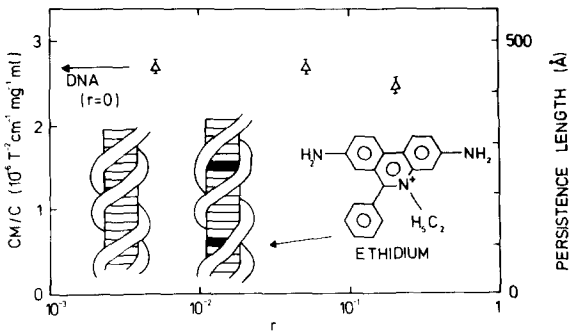

Fig. 4. Intercalation of Ethidium into Calf Thymus DNA. r denominates the mean number of intercalated dye molecules per phosphate group of DNA calculated from the intercalation equilibrium constant. T = 19.8°C, $\mu = 10^{-2}$ M NaCl, 2×10^{-5} M EDTA.

2.4. *The structure of chromatin*

In the nuclei of eukaryotic organisms complexes of DNA with some basic i.e. lysine and arginine rich proteins (= histones) are formed (weight ratio ~ 1 : 1) and usually called chromatin. Although many biological, chemical and physical techniques have been applied in order to clarify the structure of chromatin, the problem of how DNA in chromatin contracts considerably but in fairly regular manner, is by no means solved yet and a number of models are under discussion.

Since, following section 2.3, $\alpha_\parallel - \alpha_\perp$ and $\chi_\parallel - \chi_\perp$ as well as P are known for DNA, we are able to calculate the effective CM expected for some recently proposed structure models of chromatin. Although a detailed study of these calculations and comparative measurements will be published elsewhere [24], we shall briefly outline some of the results to illustrate the kind of structural information which can be obtained by the use of this technique.

If the N molecules defined above in eq. (1) are not parallel to each other, but arranged within a regular superstructure, N in eq. (1) has to be reduced by a factor f which is determined by the superstructure alone. f has been calculated [24] for a helix of such molecules and is plotted for illustration in fig. 5a as a function of pitch h at constant radius R. Since the effective

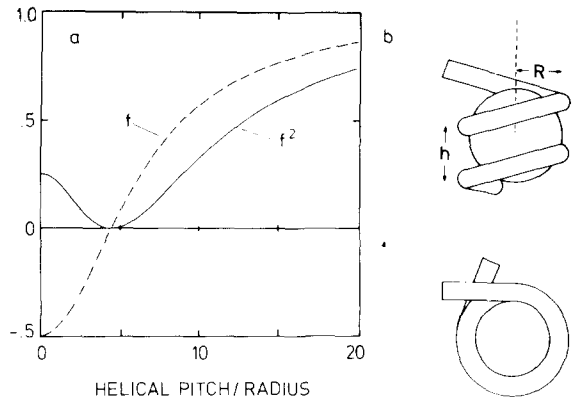

Fig. 5. (a) Calculated relative anisotropy factor f (----) and relative Cotton–Mouton-constant f^2 (——) of a superhelical arrangement of anisotropic segments as a function of h/R. Negative, positive f-values indicate that helices built up of segments with $\chi_\parallel > \chi_\perp$ orient with the helical axis parallel, perpendicular to H respectively. (b) Schematic representation of a so-called nucleosome viewed from the side and from the top similar to the recent proposal by Baldwin et al. [25] showing the histone ball with $R \sim 50$ Å surrounded by ~ 160 basepairs of DNA fixed on the histones with a helical pitch $h = 55$ Å. Free pieces of ~ 40 basepairs are believed [25] to connect these nucleosomes to form a statistical chain.

optical anisotropy per segment of the helically twisted filament is $f\ (\alpha_{\parallel} - \alpha_{\perp})$, CM is proportional to f^2 [see eq. (2)]. As shown in fig. 5a CM depends sensitively on the helicity and its determination therefore may yield precise information about h/R. However, in recent chromatin models [25] the DNA is no longer thought to be twisted in a monotonic superhelix around a core of proteins, but seems to be folded around a ball of histones possibly as indicated in fig. 5b. These nucleosomes are connected by free pieces of DNA ("beads-on-a-string-model"). For both the nucleosomes shown in fig. 5b and the statistical chain of nucleosomes, f^2 has been calculated in a way very similar to that used by Champion et al. [26] for conformation studies of sodium chromolyn. It was found to be 0.39 ± 0.07 and 0.30 ± 0.10 respectively for the two cases. Comparison of these values with the Cotton–Mouton-measurements (fig. 6) which give 0.42 and 0.35 respectively for CM (at $\mu = 0$)/CM (at $\mu = 3$ M) $= f^2$ shows good agreement between experiment and calculation and therefore confirms the "bead-on-a-string" model of chromatin.

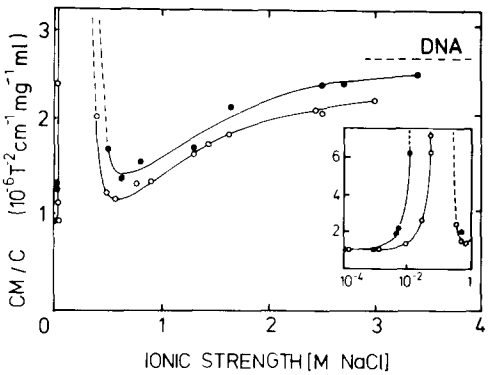

Fig. 6. Ionic strength dependence of the specific Cotton–Mouton-constant of Calf Thymus chromatin (●) and its nucleosomes (○). Preparation of samples as described in [25]. $T = 20.5$°C. Various concentrations C between 3.0 and 5.0 mg/ml. At $\mu > 3$ M, the CM-value of free DNA is almost reached as expected, since the histones are removed from the complex with DNA by increasing μ. Strong magnetic orientability between $\mu \sim 10^{-2}$ M and 0.6 M is observed and probably due to cross linking between different chromatin fibers by the histone H1 [27].

2.5. *Phospholipid membranes*

Recently the effect of a magnetic field on artificial phospholipid membranes (lamellar multilayers of hydrated egg lecithin) has been reported [28]. However, with the technique used, the proposed interpretation of this effect – magnetic orientation of diamagnetically anisotropic domains within the membrane bilayer – was not properly proved.

We report here first magnetic birefringence data of planar multilayers of egg lecithin and of dipalmitoyl lecithin (DPL) vesicles as further application of the Cotton-Mouton-method on very small samples. A detailed study will be given elsewhere [29]. Planar multilayers of egg lecithin show a linear dependence of Δn on H^2 (fig. 7a) indicating that up to $H = 12$ T, the magnetic orientation of the lipids is far from saturation. However, from the great value $CM \sim 2.5 \times 10^{-3}\,T^{-2}\,cm^{-1}$, which is about 10^3 times higher than the Cotton–Mouton-effect of free molten alcane chains, we conclude that large intermolecular correlations within some kind of domains must be present. The great apparent effective diamagnetic anisotropy of such a lipid multilayer, observed when the light beam is parallel and H perpendicular to the normal r of the glass surface (i.e. membrane plane (fig. 7)), can be explained [29], if domains

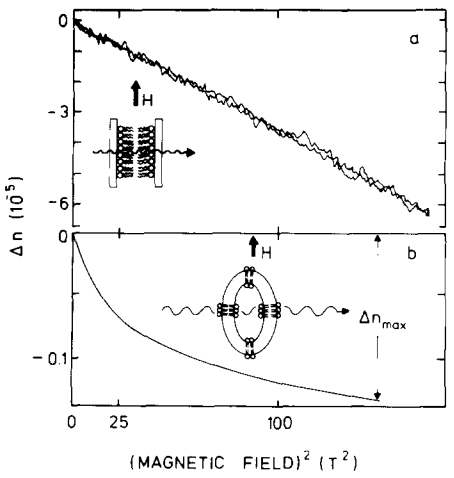

Fig. 7. Cotton–Mouton-effect of (a) planar egg lecithin multilayers (samples oriented by shear between two glass plates of 8 μm distance, as reported by [28], H_2O content = 50% w/w, $T = 21.4$°C) and of (b) 15 min sonicated vesicles of Dipalmitoyl lecithin (DPL, $C = 0.9$ mg P/ml H_2O, 5×10^{-3} M $La(NO_3)_3$, $T = 36.8$°C, mean vesicle size ~ 600 Å evaluated by electron microscopy on negatively stained, dried samples). Open circles represent polar head groups and straight lines hydrocarbon chains of the phospholipids. ~~~~ Laserbeam.

are postulated which include a sufficiently large number of correlated lipids with non-vanishing tilt angle θ between r and the long axis of at least parts of the lipid chain.

Although using only the data presented here other contributions to the observed great magnetic birefringence seem to be possible, it should be kept in mind that θ is known to be $\sim 30°C$ for the unsaturated chains of egg lecithin [28] and has very recently been proposed by Rand et al. [30] to be different from zero for DPL at temperatures below 35°C. At 35°C, where hydrated not too small DPL vesicles show the so-called pretransition [31], θ is supposed to decrease to zero [30] (fig. 8), and at 41°C DPL undergoes the well-known gel-liquid-crystalline phase transition where high lipid mobility and rapidly moving kinks appear. Therefore a temperature dependent magnetic birefringence measurement on DPL should be an independent check of whether the picture of magnetic orientation of domains of tilted lipids within the bilayer is correct.

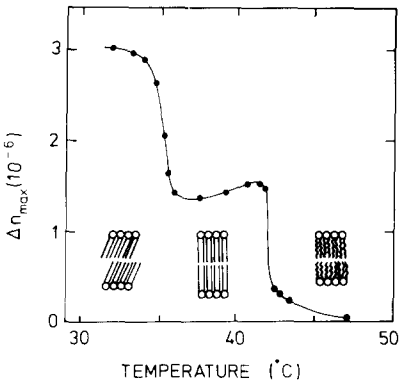

Fig. 8. Temperature dependence of Δn_{max} for DPL vesicles. Time between consecutive points ~ 20 min. Sample as defined in fig. 7. For comparison a schematic representation of different phases of lipid arrangement for DPL is shown as proposed recently [30].

Since by using vesicles as characterized in fig. 7 and an optical path of 3 cm the effective number of bilayers obtained with r perpendicular to H is about 100 times greater than for planar multilayers, we measured the temperature dependence on DPL vesicles in order to obtain larger signals. In agreement with our expectation that the non-ideally spherical vesicles and the few Bangham-type larger aggregates [31], which we observed with electron microscopy, can be very strongly oriented in high magnetic fields, Δn versus H^2 shows a saturation behaviour (fig. 7b). In fig. 8 Δn_{max} is plotted as a function of temperature and both phase transitions are clearly resolved by this method, indicating independently that Bangham-type structures determine the magnetic birefringence signal since small vesicles do not show a remarkable pretransition [31]. Above 42°C, only small lateral lipid correlation exists and therefore Δn_{max} is small, whereas between 35°C and 42°C Δn_{max} is strongly increased by an increase of the effective diamagnetic surface anisotropy because of increased lateral lipid correlation, leading to stronger orientability of the nonspherical particles. Below 35°C a contribution to the magnetic birefringence might arise from orientation of domains within the membrane built up of tilted lipids.

2.6. Molecular organization in high polymers

Furthermore high magnetic fields have been applied to some synthetic polymers and – here again – orientation effects became observable: the polymerisation velocity of liquid crystalline monomers (p-methacryloxy-benzylidene-p-ethyloxyaniline) increased by as much as 40% in $H = 7$ T because of the almost full magnetic alignment of the monomer matrix [32]. On the other hand the magnetic birefringence technique described above has been used [33, 34] to study whether in amorphous molten polymers a long range orientational order of the chains exists. Following eq. (1) such an order should lead to a linear increase of CM with the degree of polymerisation. From the observation, that a few degrees above the glass temperature CM of polystyrene is only about 1.7 times that of the corresponding monomers it has been concluded [33] that the amorphous state of polystyrene is better characterized by a randomly coiled chain conformation rather than by long range molecular order. Other polymers like polycarbonate, polyethyleneoxide and hydrocarbons show similar results [34]. A deviation from the strictly linear relation between Δn and H^2, i.e. a saturation of Δn at $H < 1$ T reported earlier [35] on polystyrene, was only observed on impurified commercial samples.

3. Magnetic separation of macromolecules

In inhomogeneous magnetic fields concentration gradients of dissolved macromolecules can be produced, if macromolecule and solvent have different magnetic susceptibilities [36, 37]. Since the effective force on the macromolecule is proportional to H grad H, high fields combined with locally high field gradients are useful for obtaining measurable separation effects and hence information about magnetic states and molecular weight even of small macromolecules.

Red blood cells have been almost completely separated in $H = 1.7$ T by the use of the high field gradient ($\sim 80\ \mathrm{T\ cm^{-1}}$) near a ferromagnetic wire [38], and a paramagnetic aqueous solution (holmium EDTA chloride) of the macromolecule γ-globulin showed a relative concentration change of 10^{-3} in $\sim 10^2\ \mathrm{T^2\ cm^{-1}}$ [36]. A new optical absorbance technique sensitive to concentration differences between points of highest and lowest H in the sample cell has recently been tested on the paramagnetic macromolecule ferritin in our laboratory [39]. More than 10% separation was observed in $H =$ 10 T and grad H = $10\ \mathrm{T\ cm^{-1}}$. The apparatus will be used to study enzymatic reactions in high fields and gradients in order to understand the observed [37] magnetically induced change in the enzymatic activity of catalase.

The experiments were carried out in the Service National des Champs Intenses, Grenoble and we acknowledge the support of its technical staff. Furthermore we are very grateful to Prof. A. Mayer, Prof. P.M. Vignais, Prof. E.M. Bradbury and Dr. J.P. Baldwin for many helpful discussions and we thank Dr. J.J. Lawrence for preparation of most of the DNA samples, Dr. B. Carpenter and Dr. K. Simpson for preparation and characterization of the chromatin samples and F. Cuault and J. Chabert for preparation and control of phospholipid vesicles.

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