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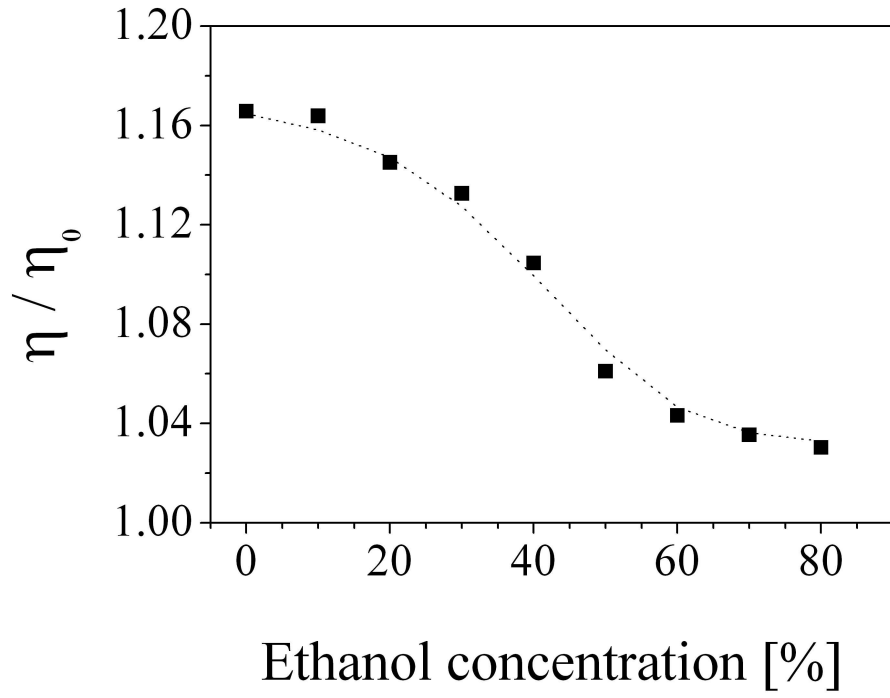


Ethanol-induced compactation of DNA: a viscosimetry and dynamic light scattering study

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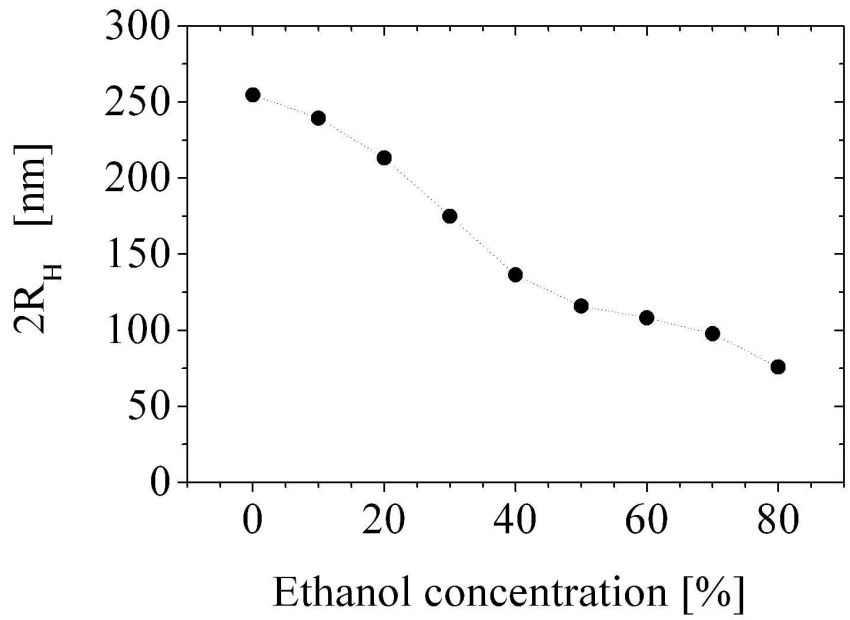
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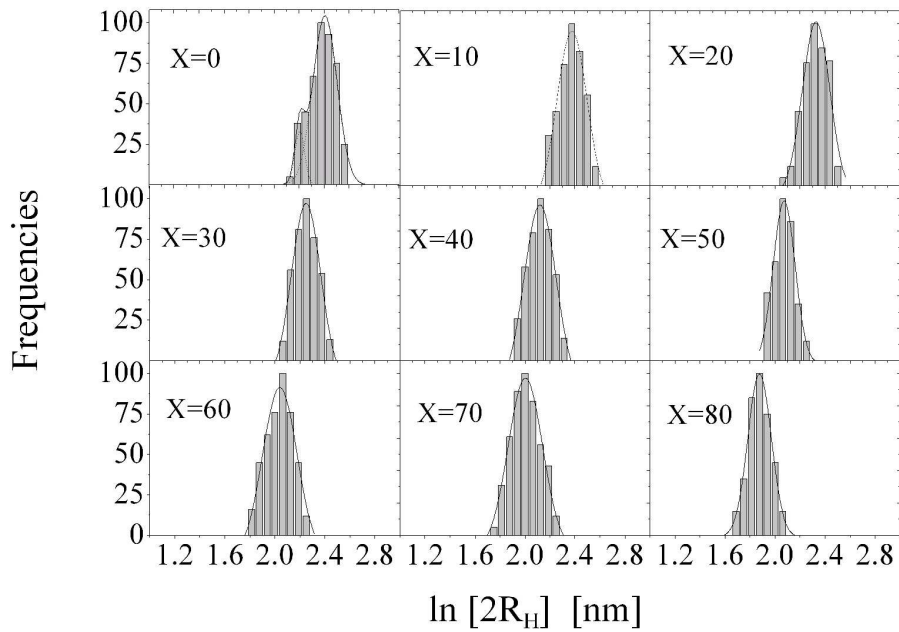
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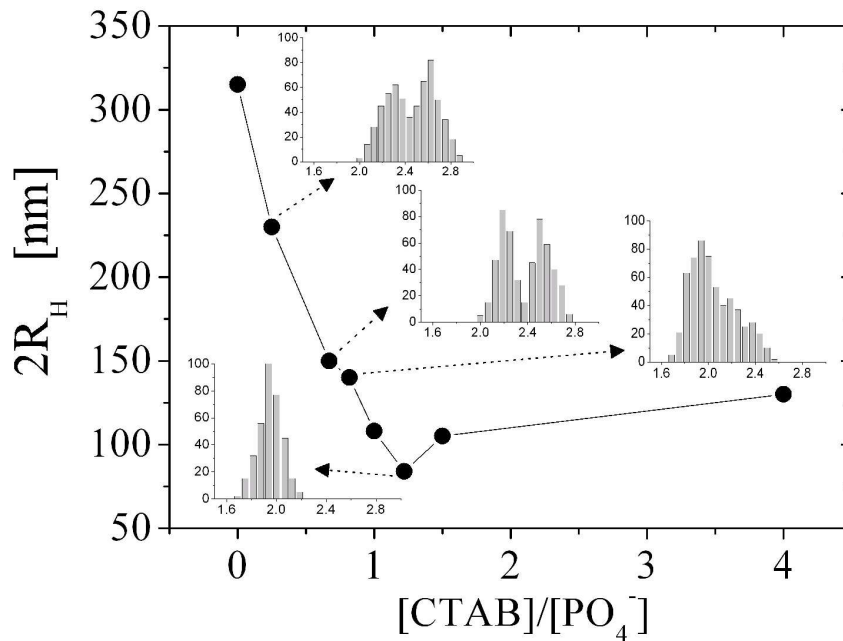


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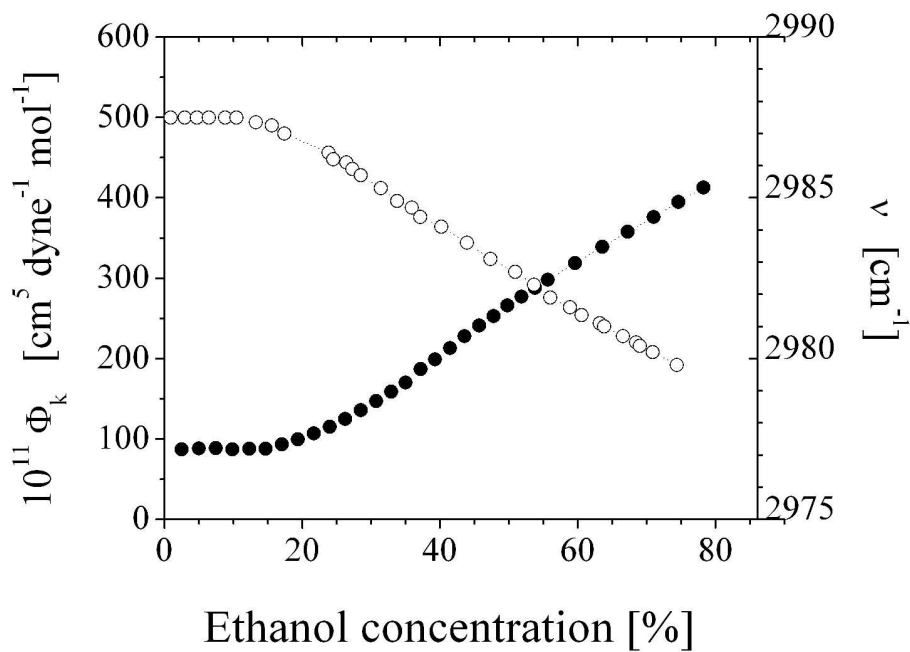
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Ethanol-induced compactation of DNA: a viscosimetry and dynamic light scattering study

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The condensation effect of ethanol on the DNA conformation in ethanol-water mixtures has been investigated by means of viscosimetry and dynamic light scattering measurements. **A conformational transition** was observed at a volume fraction close to 40%, where a sort of structural transition occurs from normal B-DNA to a more compact form. This behaviour differs from the one experienced by the same system when the condensation effects are induced by charged surfactants. In this latter case, the analysis of the scattered light auto-correlation functions evidences the presence of bimodal chain size distribution, characterized by two different average values and indicating the simultaneous presence of a coil and compact globule state. In the present case, a monomodal distribution is found and the mechanisms of DNA compaction are preferably governed by solvent interactions both of electrostatic nature, through changes in the solvent permittivity, **as well as interactions** associated to some kind of "hydrophobic clustering" of alcohol molecules, above a threshold concentration.

Keywords: DNA compaction, ethanol-water mixtures, light scattering, molar compressibility, hydrophobic clustering

1 Introduction

Addition of certain solutes or solvents to DNA in aqueous solutions converts its chain from an extended conformation to compact toroids, rods or spheres [1]. In vitro compaction of DNA is of interest for both biological and physical implications. Compact structures of DNA are of biological interest because, within viruses and living cells, DNA molecules generally exist in a highly compact conformation which is characterized by an eminently regular structure. From the physical point of view, DNA compaction is an example of a polymer coil-globule transition (well known to polymer physicists) in which the polymer chain may pass from a state to another one, by changing the quality of the solvent [2].

Many different types of substances, including multivalent cations such as polyamines, basic proteins, cationic surfactants and also neutral polymers, such as polyethylene glycol [PEG] and monohydric alcohols, can induce compaction of DNA in vitro [1]. In particular, compaction of DNA induced by cationic surfactants has been extensively studied in recent years. This is due to the large and increasing interest for using cationic surfactants as a possible way for in vivo gene transfer [3, 4].

DNA compaction has been observed by means of a variety of techniques that detect changes in polymer size or chirality, including various forms of electron microscopies, light scattering, sedimentation, viscosimetry, circular dichroism, etc. With these conventional techniques, it is still uncertain whether the coil-globule transition is a continuous or discrete process in individual DNA molecules, since the values of the pertinent physical quantities are in general obtained as an average over an ensemble of different molecules. Recently, a fluorescence microscopy (FM) method was proposed to study the conformational behaviour of a single large DNA chain in aqueous solution, in the presence of various condensing agents [5, 6, 7, 8, 9]. These studies have revealed that, although the transition of the ensemble of DNA molecules follows a typical sigmoidal curve and does not correspond to any kind of phase transition, the transition of individual DNA chain is largely discrete and can be considered a kind of first-order phase transition between elongated coil and collapsed globule. This means that coil and globule forms coexist in the solution, at a concentration interval in which the continuous transition is observed in a macroscopic ensemble of DNA chains. The existence of a bimodal distribution between elongated coil and compact globule state has been also evidenced, in a direct way, in our recent work, where the compaction of calf thymus DNA [ctDNA] by the cationic surfactant cetyltrimethylammonium bromide [CTAB] has been investigated by means of combined viscosity and dynamic light scattering [DLS] measurements [10].

DLS technique, which probes the changes in the translational diffusion coefficient from the elongated coil state to the compact globule state induced by the addition of the surfactant, provides a direct observation of the surfactant-induced conformational changes in a single DNA chain. The use of the DLS technique allowed us to observe the coil-globule coexistence in bulk, in a direct way, clearly supporting the suggestion of the fluorescence microscopy studies that the transition between the two states, elongated coil and compact globule,

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8 induced by cationic surfactants, is continuous when observed in the macroscopic
9 ensemble of DNA chains, but occurs in discrete steps for each DNA chain.

10 The condensation of a single molecule of bacteriophage T4-DNA, a virus
11 **which infects *E. Coli* and has been used extensively for molecular**
12 **biology research**, in the presence of primary alcohols has been recently stud-
13 ied by fluorescence microscopy technique [11, 12, 13]. Among other results, there
14 are a number of different aspects that match with the behaviour of DNA com-
15 paction in the presence of cationic surfactants. In the globular phase near the
16 transition point, a metastable state is observed in which the globular and coil
17 states coexist within a single DNA chain, i.e., phase separation is induced along
18 a single chain. Therefore, in the presence of alcohols, coiled **and** globular states
19 could coexist within a single DNA chain. The possibility of the formation of an
20 intermediate conformational state of single DNA molecule has been also noticed
21 in the presence of other condensation agents [14, 15, 16] as well as seen in recent
22 computer simulations [17]. This result reveals that, although the conformational
23 behaviour of large DNA chains in aqueous solution in the presence of various
24 condensation agents shows analogous properties at the level of ensemble of DNA
25 molecules, however the compaction of DNA occurs by different ways and mech-
26 anisms, at the single-molecule level. Taking into account these observations, it
27 needs a **careful study** of the general features of compaction of single DNA
28 chains in aqueous solution, upon the addition of certain condensing solutes or
29 solvents, not only flexible polycations, multivalent ions and cationic amphiphiles
30 but also primary alcohols and organic solvents.

31 In the present work, viscosity and DLS techniques have been used to study
32 the compaction of ctDNA induced by ethanol and the collected data has been
33 compared to our recent results on the compaction of ctDNA by CTAB, where
34 we applied the same experimental approach [10]. The results are discussed in
35 connection with previous adiabatic compressibility and infrared absorption data
36 of water/ethanol mixtures in the same concentration range [18]. The main aim
37 of the present work is to extend information about the influence of the solvent
38 on the coil-globule transition of DNA molecule.

40 2 Experimental section

41 2.1 Materials

42 Calf thymus Na-DNA [ctDNA], purchased from Sigma Chem. Co., was diluted
43 in deionised water and fragmented by sonic vibration with a Vibra Cell sonifier
44 by Sonic and Materials Inc. (5 mg/ml concentration, 1 min sonication time).
45 **The reason for the fragmentation by sonic vibration was to obtain**
46 **DNA fragments shorter and more regular than those of commercial**
47 **products. The molecular weight distribution of DNA was determined**
48 **by agarose gel electrophoresis and was widely spread from 0.5 to 1**
49 **kbp: in order, the molecular weight of our samples after fragmenta-**
50 **tion is (3.3 ÷ 6.6) · 10⁻⁵ g/mole.**
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8 **Furthermore, it has to be noticed the condensation of DNA has been**
9 **observed over a wide range of DNA lengths. Only when DNA becomes**
10 **very short, about 150 base pairs, it can not be condensed from dilute**
11 **solutions into discrete particle of orderly morphology [19].** Alcohol em-
12 ployed was ethanol (absolute, >99,5%, Carlo Erba) and was used without further
13 purification. The solutions were prepared by weighing, mixing a fixed amount
14 of the DNA solution (5 mg/ml) with water/alcohol mixtures at the desired al-
15 cohol volume fraction. DNA concentration in all sample solutions (0.1 mM in
16 terms of phosphates for both viscosity and light scattering measurements) was
17 determined spectrophotometrically by using the molar extinction coefficient for
18 **native DNA** at 260 nm of $6600 \text{ M}^{-1} \text{ cm}^{-1}$ [20]. The pH of the solutions
19 was measured by means of a Crison micropH mod. 2000. **At the different**
20 **%vol/vol compositions, the pH values recorderd is about 7.**
21

22 2.2 Viscosity measurements

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24 Viscosity measurements were carried out in a themostated bath at a temperature
25 of $25.0 \pm 0.1 \text{ }^\circ\text{C}$ by means of a viscosity measuring system Schott Gerate mod.
26 AVS 400, equipped with an Ubbelohde viscometer. A reproducibility of 0.1%
27 was obtained. Viscosity depends on a variety of parameters, such as the shape
28 of dissolved particles, their rigidity and the state of aggregation, the amount
29 of hydration, and the forces acting between the particles. We characterize the
30 viscosity behaviour of the DNA-surfactant solutions through the relative visco-
31 sity, η_r , defined as the ratio $\eta_r = \eta/\eta_0$ between the viscosity η of the suspension
32 and the viscosity η_0 of the pure solvent.
33

34 2.3 Dynamic light scattering measurements

35
36 The size and size distribution of DNA in water-ethanol mixtures have been cha-
37 racterized by means of dynamic light scattering measurements, using a spectro-
38 meter equipped with a Brookhaven BI9000AT logarithmic correlator and a 10
39 mW He-Ne laser source of wavelength $\lambda=632.8 \text{ nm}$.

40 Light scattered by the sample, placed in a thermostated bath maintained at the
41 temperature of $25.0 \pm 0.2 \text{ }^\circ\text{C}$, was detected at an angle of $\theta=90^\circ$. In dynamic
42 light scattering technique, the normalized time autocorrelation function of the
43 intensity of the scattered light $g^{(2)}(\tau)$ can be expressed in terms of the electric
44 field autocorrelation function $g^{(1)}(\tau)$ through the Siegert relation:
45

$$46 \quad g^{(2)}(\tau) = A[1 + B(g^{(1)}(\tau))^2] \quad (1)$$

47
48 where A is the baseline and B the coherence factor. For a dilute suspension of
49 monodisperse particles, $g_1(\tau)$ decays exponentially with a decay rate $\Gamma = Dq^2$,
50 where $q = (4\pi n/\lambda)\sin(\theta/2)$ is the magnitude of the scattering wave vector and
51 D the translational diffusion coefficient which is related to the hydrodynamic
52 diameter $2R_H$ through the Stokes-Einstein relationship $D = K_B T / (6\pi\eta R_H)$,
53 where $K_B T$ is the thermal energy and η the viscosity of the solvent phase. For a
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polydisperse sample, $g_1(\tau)$ has no longer a single exponential decay and can be written as the Laplace transform of the spectrum of the relaxation decay rates

$$g^{(1)}(\tau) = \int_0^{\infty} G(\Gamma) \exp(-\Gamma\tau) d\Gamma \quad (2)$$

A fit routine CONTIN [21], which employs the constrained regularization method, was used to obtain $G(\Gamma)$ and hence the size particle distribution, when the correlation function markedly deviates from a single exponential decay.

Since it is well known that, in light scattering experiments, the inversion of autocorrelation data is an "ill-conditioned" problem, we have compared the $G(\Gamma)$ distributions calculated using CONTIN with ones obtained by different method, the Lawsons's NNLS algorithm, and we have only considered those peaks in the particle distributions that are independent of the algorithm employed. The control parameters of both algorithms were set in order to obtain, in terms of the particle size, the intensity-weighted size distribution. The values of the refractive index n and the viscosity η of the solvent at different alcohol concentrations employed to calculate the average hydrodynamic diameter $2R_H$ through the Stokes-Einstein relation were taken from literature data [22].

3 Results and Discussion

Fig.1 shows the behaviour of the relative viscosity η_r of ctDNA in ethanol/water mixtures as a function of alcohol volume fraction. Measurements were performed up to 80% of alcohol concentration because ethanol, whose influence on the DNA structure is well known [23], cooperatively transforms natural DNA chain from the B to the A conformation at about 75% concentration [24, 25, 26, 27]. Fig.1 shows that the relative viscosity, η_r , of the aqueous solution of ctDNA chains in elongated coil state ($X = \text{Ethanol concentration } [\%] = 0$) is around 1.16, which is considerably higher than the expected value for the same ctDNA molecule in the globular state. Hence, it must be expected that the coil-globule transition of DNA, induced by ethanol, may be accompanied by a significant decrease of the relative viscosity value. It was found that, at low values of alcohol concentration ($X < 20\%$), the values of η_r are similar to those of an aqueous solution of DNA. As can be seen, the relative viscosity decreases rather steeply above $X \simeq 20\%$, reaching a saturation value at about $X=60\%$. The observed decrease of the viscosity is comparable to the same effect observed for DNA compaction by cetyltrimethylammonium bromide [CTAB] [10], in agreement with the formation of a globular structure in DNA chain. The average hydrodynamic diameter $2R_H$ of the DNA chains in the ethanol/water mixtures as a function of the alcohol volume fraction is shown in Fig. 2. As can be seen, with the increase of alcohol volume fraction in the mixture, a progressive decrease of the hydrodynamic diameter $2R_H$ appears, from the value of about 260 nm for DNA in pure water to about 80 nm for DNA at $X=80\%$. The overall behaviour of the

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8 hydrodynamic diameter recalls **the one** observed by means of viscosity mea-
9 surements (Fig. 1) and the percentage size reduction is in agreement with the
10 ethanol-induced compaction of individual single duplex T4-DNA molecules ob-
11 served by FM [13]. Typical size distributions of DNA in water/ethanol mixtures
12 are shown in Fig. 3, up to an ethanol concentration of $X=80\%$. The dependence
13 of the average hydrodynamic diameter $2R_H$ and the typical size distribution of
14 DNA as a function of the ethanol concentration reflects the change of the hel-
15 ical structure that DNA may adopt. As can be seen, three distinct regimes of
16 aggregation can be individuated. At low volume fraction of ethanol ($X < 20\%$),
17 the size distributions of DNA in the mixture is only little changed with respect
18 to the one in the pure aqueous solution. Further increase in the alcohol volume
19 fraction in the range $X=20\% \div 60\%$ leads to a compaction of DNA molecules.
20 At higher ethanol concentration, close to $X=80\%$, a further transition takes
21 place, according to the literature [24], attributed to the transition of DNA from
22 the B-form to a highly condensed and highly stabilized A-form. It is worth not-
23 ing that only a single population is present in the solution, whose average size
24 progressively decreases as the ethanol volume fraction increases.

25 This behaviour is very different from the conformational changes occurring in
26 DNA compaction induced by charged surfactants, such as for example cetyltri-
27 methylammonium bromide [CTAB]. In relation to this, a direct observation
28 comes by comparing Figs. 2 and 3 with Fig. 4, where the average hydrodynamic
29 diameter $2R_H$ of DNA-CTAB complexes as a function of the surfactant to DNA-
30 phosphate molar ratio $[CTAB]/[PO_4^-]$ is shown. As the DNA charge neutraliza-
31 tion proceeds, the chain undergoes a coil-globule transition. **The insets of Fig.**
32 **4 show** the size distribution of the complexes at some selected charge ratios.
33 In this case, in agreement with FM evidence, a bimodal distribution with the
34 simultaneous presence of coil and compact globule state, whose relative concen-
35 tration changes with surfactant concentration, is clearly evidenced. The mole-
36 cular mechanism leading to this peculiar behaviour has been attributed to an
37 initial electrostatic interaction of the cationic surfactant with a DNA molecule,
38 followed by a binding of surfactant ligands to the same DNA molecule, driven
39 by hydrophobic forces [6]. The mechanism of DNA condensation by ethanol
40 and other primary alcohols is basically different from the one occurring in the
41 presence of cationic surfactants.

42 It is well known that the dielectric permittivity of the solvent phase is a
43 key factor that determines the conformational behaviour of DNA in solution.
44 Wilson and Bloomfield [28] showed that DNA condenses under a variety of ionic
45 conditions, when approximately 90% of its charge is neutralized by the counter-
46 ion condensation. Theoretical estimates of the various contributions to the free
47 energy of condensation also involve electrostatics as the major factor [29, 30]. It
48 is thought that, in the presence of alcohol, DNA compaction is brought about
49 by its own counterions through the change of the dielectric properties of the
50 solution [11, 28, 31]. However, other studies have suggested that factors other
51 than electrostatics, such as hydration and hydrophobic interactions, are relevant
52 in the condensation of DNA molecules [32, 33]. On this regard, it is worth noting
53 that the alcohol-induced compaction of DNA occurs in the same concentration
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8 range where anomalous behaviours in several properties of water/alcohol mixtures themselves are also observed. In this region of composition, a maximum is found in sound absorption [34], X-ray scattering [35] and light scattering [36, 37].

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11 Our recent studies on the properties of water/alcohol mixtures show that this anomalous behaviour can be associated to some kinds of *hydrophobic clustering* of alcohol molecules in the water rich region of composition, beyond a threshold value of alcohol concentration [18, 38].

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15 **In particular the compressibility behaviour of water/ethanol system was studied in detail in a previous paper [39] by means measurements of sound velocity, v , and density ρ of mixture at the different ethanol-water composition.** A plot of ethanol apparent molar compressibility, Φ_k as a function of alcohol volume fraction is reported in Fig. 5. This quantity is nearly constant at low ethanol concentration, but increase steeply as more alcohol is added to the solution. In the same Fig. 5, the frequency, ν , of the C-H stretching vibrations is also reported. **In fact, infrared radiation has been used to spectroscopically investigate the formation of micellar microaggregates in aqueous solutions and a large shift to a lower frequency was observed for the C-H stretching modes of some ionic surfactants upon micelles formation [40]. Similar effects accompany the association of ethanol and of other monohydric alcohols in water [38].** As it can be seen in Fig. 5, a shift of the frequency is observed as the alcohol volume fraction is increased. The dependence on alcohol concentration of ν matches the one of Φ_k . The two sets of data, adequately normalized, superimpose within the experimental errors. The compressibility and IR data have been discussed in Ref. [38] by considering three concentration ranges defined by two "signpost" alcohol volume fractions X_a and X_b , respectively. For water/ethanol mixtures at 25 °C, $X_a \simeq 17\%$ and $X_b \simeq 57\%$. The data suggest that in the $0 \div X_a$ range, where Φ_k and ν do not change with X , the alcohol molecules are essentially dispersed and surrounded by "water cages" of fairly regular and longer-lived H bonds. In the $X_a \div X_b$ region, a transition attributed to a progressive clustering of alcohol molecules with strong modification in the solvation of hydrophobic groups is observed. A correlation between the effects of ethanol on the compaction of DNA (Fig. 1) and changes in properties of the solvent (Fig. 5) clearly appears from the data. These data suggest that the DNA coil-globule transition in water/ethanol mixtures is driven by the concomitant transition in the bulk solvent due to the clusterization of alcohol molecules. In the $X_a \div X_b$ region, a qualitative change in the nature of the interactions between solvent components in the mixture occurs. Interactions between hydrophobic groups become increasingly favourable and progressively replace the interactions of these groups with water molecules. In the water/DNA/ethanol system, a modification in the water-DNA and alcohol-DNA interactions is also expected. Present results on condensation of DNA induced by ethanol suggest that the total mixing scheme of the binary water/alcohol mixtures, including the clustering of alcohol molecules, can have a dramatic influence on DNA compaction. **Obviously, some new experiments and simulations of the behaviour of ethanol molecules could give some light on the subject. The complex self-association behaviour presented**

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8 by ethanol in the water-rich region is not limited to ethanol alone,
9 but is generally exhibited in dilute aqueous solutions of monohydric
10 alcohols [18, 38]. So, we think that more measurements in aqueous
11 solution of other alcohols in the low-concentration region may be
12 desirable to confirm the results obtained in the present work.
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8 Fig.1 The relative viscosity $\eta_r = \eta/\eta_0$ of DNA in water-ethanol mixtures,
9 as a function of the ethanol concentration. **The errors are comparable with**
10 **the symbols dimension.**

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14 Fig.2 The average hydrodynamic diameter $2R_H$ of the DNA chains in ethanol-
15 water mixtures as a function of the ethanol concentration. **The errors are**
16 **comparable with the symbols dimension.**

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21 Fig.3 Size distribution of DNA chains **at different** ethanol-water mixture
22 composition, derived from the CONTIN analysis of the correlation function.
23 The concentration of ethanol was varied from $X=0\%$ to $X=80\%$.

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28 Fig.4 Average hydrodynamic diameter $2R_H$ of DNA-CTAB complexes as a
29 function of the surfactant to DNA-phosphate molar charge ratio $[\text{CTAB}]/[\text{PO}_4^-]$.
30 The errors are comparable with the symbols dimension.
31 The insets show the size distribution, **i.e. the frequencies versus $\ln [2R_H]$ (in**
32 **nm)**, at four different values of $[\text{CTAB}]/[\text{PO}_4^-]$, before the neutralization con-
33 dition, $[\text{CTAB}]/[\text{PO}_4^-]=0.25$, $[\text{CTAB}]/[\text{PO}_4^-]=0.67$, where there is a bimodal
34 distribution and close to the neutralization condition, $[\text{CTAB}]/[\text{PO}_4^-]=0.75$,
35 $[\text{CTAB}]/[\text{PO}_4^-]=1.22$, where only a monomodal distribution appears.

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40 Fig.5 Apparent ethanol molar compressibility Φ_k (left side, full circles) and
41 frequency ν of the C-H stretching vibrations (right side, open circles) in water-
42 ethanol mixtures as a function of the ethanol concentration, at the temperature
43 of 25 °C. **The errors are comparable with the symbols dimension.**Data
44 from Ref. [34]