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Poly(ethylene glycol)-induced DNA condensation in aqueous/methanol containing low-molecular-weight electrolyte solutions Part II. Comparison between experiment and theory

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Abstract

Viscometry, UV/Vis–centrifugation and dynamic light scattering (DLS) were used to monitor the coil–globule transition of calf-thymus DNA by poly(ethylene glycol) (PEG) in aqueous/methanolic NaCl solutions. All three methods confirm that methanol and PEG promote the transition synergistically. The PEG concentration at which the DNA collapses decreases as the methanol fraction of the solvent is increased. The values found for the critical PEG and methanol concentrations agree quite well with those predicted by the modified Flory–Huggins theory. This is rather surprising, because effects such as selective solvent adsorption or intramolecular charge repulsion are neglected. The most informative experimental technique for the present investigations is DLS. The DNA molecules are not affected by outside forces and DLS allows the measurement of molecular size as well as the distribution of the conformational states. It was observed that there are no intermediate conformational states. A DNA molecule is either in the expanded coil state or in the collapsed state. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: DNA condensation; Poly(ethylene glycol); Coil–globule transition

1. Introduction

A DNA molecule packed into a virial capsid is hundreds of times more compact than when free in solution. An example is the DNA of bacteriophage T4. In solution it has a worm-like coil structure and a radius of gyration of 1 μm, while inside the T4 phage head, the DNA radius is only 50 nm. This compact, in vivo configuration is stabilized by multivalent cations and positively charged proteins, but can also be provoked in vitro by chemical agents [1–5].

Free DNA molecules having from about 400 to 10^5 [5] base pairs can be induced to collapse by the addition of cations such as spermine, spermidine, or hexamine cobalt(III) [1–5]. The conformational transition is called condensation or the coil–globule transition [6,7].

The agents required for condensation in aqueous solution are cations with valences of three or above or neutral polymers such as poly(ethylene glycol) (PEG) with a molar mass much smaller than the DNA molar mass. Divalent ions are much less effective. To make them effective, both the linear charge density of the DNA and the dielectric constant of the solvent must be lowered simultaneously. According to Bloomfield [8], a critical fraction of the DNA charge should be neutralized by the counterions, which is in the order of 90% in pure water as well as in a 50% (v/v) methanol/water mixture.

The aim of this paper is twofold, as follows.

1. To thoroughly test the importance of electrostatics for DNA condensation, a system was used which, besides DNA and monovalent cations (Na^+), contains PEG and methanol. The methanol content is varied to alter the dielectric constant of the solvent and the PEG concentration is varied to answer the question as to whether methanol acts synergistically with PEG. Since both additives can separately cause transitions in the DNA structure, it seems possible that the two work together cooperatively. This has indeed been found for other combinations of additives such as spermine with ethanol, which stabilizes the A family [9,10] of DNA structures.
2. The second issue is a comparison of the experimental results obtained here with the theoretical predictions of a theory presented by us in a previous paper [11]. Model calculations were presented on the widths that promote DNA condensation through intramolecular Flory–Huggins interactions, elastic Langevin forces, entropy effects, and electrostatic repulsions which must be

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overcome by altering the salt concentration inside a single DNA domain.

To investigate these issues, the DNA condensation of calf-thymus DNA ($M_w = 2.2 \times 10^3$ kg mol^{-1}) was studied in mixtures of water and methanol, where the salt concentration c_{NaCl} was kept constant at 0.5×10^3 mol m^{-3}. Viscometry and UV/Vis–centrifugation were used to detect the exact position of the PEG and methanol concentration that induced the onset of condensation and allowed the extent and course of the process to be followed. The third method applied was dynamic light scattering (DLS). This measures the diffusion coefficient distribution of DNA and shows whether condensation is a direct transition from a coil to a globule or whether there are any intermediate states. As an additional parameter, we varied the PEG degree of polymerization. This has also been done earlier [12], but to our knowledge not for calf-thymus DNA and not in the presence of methanol.

2. Experimental

2.1. Materials

Calf-thymus DNA was purchased from Sigma as a type I sodium salt. The residual protein content was determined according to Lowry [13] and was smaller than 0.2%. DNA stock solutions at high concentrations were centrifuged at 10 000 rev min^{-1} for 30 min and then diluted to their final concentrations in 10^{-3} mol l^{-1} HEPES buffer containing 0.5 M NaCl, 10^{-4} M EDTA and varying amounts of alcohol. The pH was adjusted to 7.2 with NaOH. The samples were then kept in a refrigerator at 5°C for no longer than 5 days, with occasional stirring.

DNA concentrations were determined photometrically with a Perkin-Elmer spectrometer using the standard extinction coefficient $\epsilon = 650$ m^2 mol^{-1} at $\lambda = 260$ nm and $T = 25$°C. At this wavelength, neither methanol nor PEG show any absorption.

Highly purified PEG was purchased from Fluka and used without further purification. The samples were dissolved in the same solvent mixtures as the DNA. For the removal of dust and other high-molecular-mass impurities they were passed through 0.22 μm Millipore filters. The PEG concentration was not affected, because the PEG molar masses used were rather small. They were 0.9, 2, 4, 6, 8, and 20 kg mol^{-1}, as given by the manufacturer. The uniformity coefficient is in the order of 1.7. More details and a comprehensive study on PEG were presented by Bailey and Koleske [14].

Other agents were water, methanol and NaCl. Water was doubly distilled and filtered through 0.1 μm filters before use. The methanol used was of high-performance chromatography grade and was passed through a 0.22 μm membrane (Schleicher and Schüll). NaCl (Merck) was used without further purification.

2.2. Viscometry

The intrinsic viscosity, [η], of DNA was determined by applying the extrapolation method according to Huggins [15]

$$\frac{\eta_{sp}}{c_{DNA}} = [\eta] + k_H[\eta]^2 c_{DNA} \quad (1)$$

where

$$\eta_{sp} = (\eta_{solution}/\eta_{solvent}) - 1 = (t_{solution}/t_{solvent}) - 1$$

and c_{DNA} is the DNA concentration, k_H is the Huggins constant, $\eta_{solution}$ and $t_{solution}$ are the viscosity and the flow time of the DNA solution, respectively, and $\eta_{solvent}$ and $t_{solvent}$ are the solvent viscosity and the solvent flow time, respectively. That is, for a given data set of $x_{methanol}$, $M_{w,PEG}$, c_{salt} and c_{PEG}, η_{sp} was measured as a function of c_{DNA} and was used to calculate [η] with Eq. (1) above. It should be pointed out that by using Eq. (1), no densities are needed.

The apparatus was a Ubelohde capillary viscometer. It was thermostated at $T = 25 \pm 0.1$°C in a water bath. A correction according to Hagenbach [16] was necessary, but the error in [η] in all cases was no larger than 3%. This could have arisen because the reproducibility of the flow times was below 1%.

2.3. Light scattering

Static and dynamic light scattering [17,23,24] were performed simultaneously with the same spectrometer consisting of an AL-Sp 81 goniometer and an ALV-5000 multiple tau digital correlator. The light source was a He–Ne laser (Spectra Physics) operating at a wavelength of 632.8 nm and a power of 36 mW. Calibration was proved by the volume-correlated scattering intensities of toluene in the angular range from 30 to 150°. It was less than ±1%.

The measurements give the homodyne intensity–intensity correlation function, $g_2(q,t)$, where q is the amplitude of the wavevector and t the delay time. For a Gaussian distribution of the intensity profile, $g_2(q,t)$ can be recalculated into the electric field autocorrelation function [24], $g_1(q,t)$, by

$$g_2(q,t) = B(1 + \beta|g_1(q,t)|^2) \quad (2)$$

where B is the baseline and β a is constant that takes into account the number of coherence areas that generate the signal ($0 \le \beta \le 1$). When $q\langle R^2\rangle \ll 1$, and when the samples are monodisperse, $g_1(q,t)$ is a single exponential function

$$g_1(q,t) = \exp(-t/\tau) = \exp(-\Gamma t) = \exp(-q^2 D t) \quad (3)$$

where τ is the relaxation time, $\Gamma = 1/\tau$ is the relaxation rate, and $D = \lim_{q\to 0}(\Gamma/q^2)$ is the translational diffusion coefficient.

However, our samples are polydisperse. Therefore $g_1(q,t)$ must be expressed as an integral of the exponential decays

Table 1
Viscosity numbers, $[\eta]_{PEG}$ (10^2 m^3 kg^{-1}), of PEG as a function of $x_{methanol}$ and $M_{w,PEG}$

$M_{w,PEG}$ (kg mol^{-1})	$x_{methanol}$ (kg m^{-3})		
	0	122	244
2	0.78 ± 0.005	0.80 ± 0.05	0.83 ± 0.05
4	1.20 ± 0.05	1.15 ± 0.05	1.19 ± 0.05
6	1.53 ± 0.05	1.47 ± 0.05	1.51 ± 0.05
8	1.92 ± 0.05	1.90 ± 0.05	1.88 ± 0.05

weighted over the distribution, $A(\tau)$, of τ

$$g_1(t) = \int_0^\infty A(\tau) \exp(-t/\tau)\, d\tau \quad (4)$$

This Laplace integral, i.e. its inversion, constitutes an ill-posed problem. There exist a large number of possible solutions, all of which fit the data within the experimental error. To stabilize the optimal solution, regularization is the best possible method. Its principle consists of expanding the minimizing least-squares condition by an additional term called the regularizer, favoring a certain type of solution by implying any prior knowledge, e.g. the smoothness of the solution. Here, we used the program CONTIN 2DP [25,26]. This program is easy to handle and the regularization parameter is adjusted automatically. For the latter, the Fisher F test is performed or the unregularized solution is combined with the regularized one. As regularizer, the second derivative approximation of the distribution function is used, which tends to protect against artifacts and penalizes deviations from smoothness. In addition there is no need to adjust the sampling time. Blocks of eight channels with contact sampling time will be built and the sampling time is doubled from one block to the next. For instance, with 320 correlation channels, which we work with, delay times from 12.5 ns to many hours can be processed parallel with 39 different sampling times.

The final translational diffusion coefficient, D, was calculated by linear extrapolation of $D(q)$ to the zero wave vector q, i.e. we have $D = \lim_{q\to 0} D(q)$. An extrapolation to zero concentration was not necessary because the solutions were dilute enough such that no concentration dependence could be observed. Special tests also showed that there was no disturbance through a possible rotational diffusion coefficient.

Table 2
Viscosity number, $[\eta]_{DNA}$, of DNA as a function of the methanol content

$x_{methanol}$ (kg m^{-3})	$[\eta]_{DNA}$ (m^3 kg^{-1})
0	1.97 ± 0.04
61	1.94 ± 0.04
122	1.90 ± 0.04
183	1.86 ± 0.03
244	1.80 ± 0.03

Static light scattering was evaluated according to the method of Zimm. The weight-average DNA molar mass and the second virial coefficient obtained were $M_{w,DNA} = (2.2 \times 10^6 \pm 5 \times 10^5)$ g mol^{-1} and $A_2 = 3.2 \times 10^{-4}$ cm^3 g^{-2}. Both values are in accordance with the manufacture's specifications. Thus, our DNA molecules contained an average of 3160 base pairs.

The refractive index increment was determined at $T = 20 \pm 0.1$°C using a Brice–Phoenix differential refractometer. For $\lambda = 632.8$ nm, the result was $dn/dc = 0.164$, which is the same as that found by Borochov and Eisenberg [22].

2.4. UV/Vis–centrifugation

The idea of this method arose during a research journey at the BASF. Centrifugation experiments on DNA/PEG mixtures using the analytical ultracentrifuge XL-A from Beckmann showed that DNA exists in only two conformations. The one state can be described by the sedimentation coefficient $s_{1,app} \approx 17$ sved and the other by $s_{2,app} \leq 0.2$ sved. Intermediate sedimentation coefficients were not observed. Thus, DNA molecules can be separated by centrifugation.

In Osnabrück we have no analytical ultracentrifuge, but there is a laboratory centrifuge (UD 15 from Heraeus Christ). This centrifuge was used in connection with a UV/Vis spectrometer in the following manner. Two DNA solutions of equal composition were prepared. One sample was centrifuged with the laboratory centrifuge at 10 000 rev min^{-1} while the other sample was used as a reference. The UV/Vis absorption was measured at 260 nm and the results were compared. DNA molecules in the collapsed state become almost totally centrifuged so that the DNA absorption measured was close to zero. DNA molecules in the coil state do not deposit out and the absorption does not change. Consequently, the difference $A_{diff} \equiv A_{ref} - A_{res}$, where A_{res} is the residual absorption after centrifugation, gives the concentration of DNA molecules in the collapsed state. When all molecules are in the collapsed state, A_{res} should theoretically be negligible. However, in practice, A_{res} is never zero due to the presence of residual proteins and small DNA fractions that do not collapse, so that A_{res} is finite, but smaller than 6%.

3. Results and discussion

3.1. Viscometry

The viscometry measurements serve two purposes. Firstly, $[\eta]$ data are necessary to provide the Flory–Huggins interaction parameters χ_{PS} and χ_{DS}, described in the accompanying paper [11]. Secondly, $[\eta]$ is needed to determine the PEG concentration at which DNA condensation takes

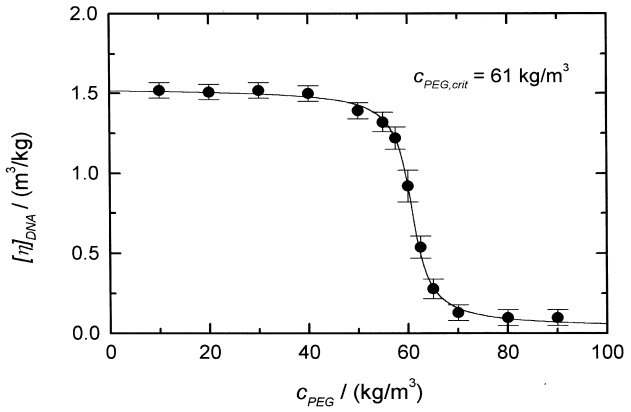

Fig. 1. Viscosity number, $[\eta]_{DNA}$, of DNA versus the PEG concentration, c_{PEG}, for $M_{w,PEG} = 4$ kg mol^{-1}, $c_{NaCl} = 0.5 \times 10^3$ mol m^{-3}, $x_{methanol} = 0$, and $T = 25$°C.

place. The intrinsic viscosity $[\eta]$ is proportional to the third power of the hydrodynamic radius, R_h, if it is assumed that the particles are spheres. Thus

$$[\eta]_{DNA} = \frac{10\pi N_A/3}{(M_{w,DNA}/R_{h,DNA})^3} \quad (5)$$

That is, $[\eta]_{DNA}$ is relatively large in the random coil conformation of DNA and comparatively small in the collapsed state.

Tables 1 and 2 show the viscosity of pure PEG and pure DNA for different methanol contents. The concentrations $c_{NaCl} = 0.5 \times 10^3$ mol m^{-3}, $c_{HEPES} = 1$ mol m^{-3} and $c_{EDTA} = 1$ mol m^{-3} were kept constant. It can be seen that $[\eta]$ increases as the PEG molar mass, $M_{w,PEG}$ is increased. However, within the experimental error of 5%, no dependence on $x_{methanol}$ was observed. For DNA, the situation was slightly different. The $[\eta]_{DNA}$ decreases as $x_{methanol}$ is increased, indicating that a DNA coil is less expanded the poorer the solvent quality. This behavior is normal and is found not only for polyions but also for neutral polymers [18].

Table 3
The critical PEG concentration and the width of the coexistence region as a function of the PEG molar mass

Parameter	$M_{w,PEG}$ (kg mol^{-1})				
	0.9	2	4	6	8
$c_{PEG,c}$ (kg m^{-3})	150	95	61	50	45
Δc_{PEG} (kg m^{-3})	8	6	4	4	4

Fig. 1 shows $[\eta]_{DNA}$ plotted against the total PEG concentration, where $M_{w,PEG} = 4$ kg mol^{-1}, $c_{NaCl} = 0.5 \times 10^3$ mol m^{-3}, $x_{methanol} = 0$, and $T = 25$°C. It can be seen that at low c_{PEG}, the viscosity remains constant, then suddenly drops, and finally converges at large c_{PEG} to a constant value which is about a factor 15 smaller than at the beginning. This sigmoidal curvature is typical for a conformational transition. At low c_{PEG}, the DNA is in its coil state, while it is in the collapsed state when c_{PEG} is large. The critical PEG concentration, $c_{PEG,c}$, is the value of c_{PEG} at which the DNA collapses. There the curvature of $[\eta]_{DNA}$ versus c_{PEG} has its turning point. Here, $c_{PEG,c}$ is 61 kg m^{-3}.

Fig. 2 presents the DNA viscosity, $[\eta]_{DNA}$, as a function of c_{PEG} for various PEG molar masses. The other parameters were fixed at $x_{methanol} = 0$ kg m^{-3}, $c_{NaCl} = 0.5 \times 10^3$ mol m^{-3}, and $T = 25$°C. Three effects were observed, as follows.

1. In the coil state the value of $[\eta]_{DNA}$ is larger the smaller is $M_{w,PEG}$. That is, in the presence of small PEG molecules, a DNA molecule is more expanded than when the PEG molecules are large.

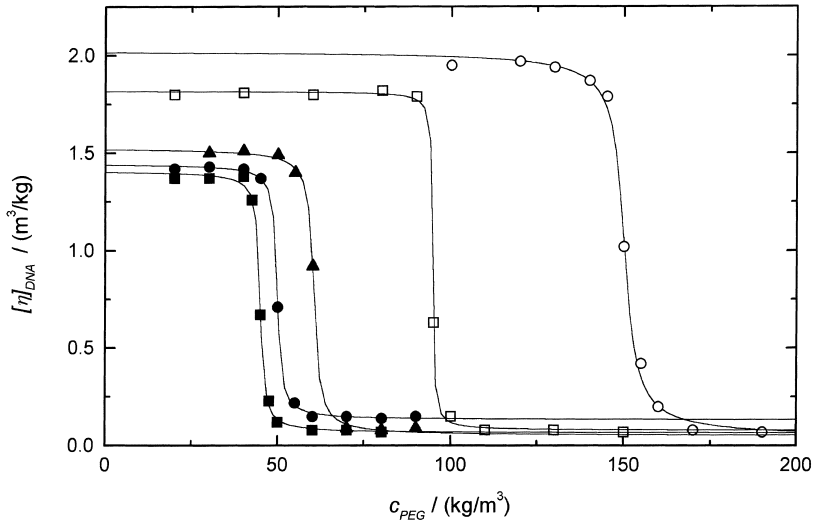

Fig. 2. Viscosity number, $[\eta]_{DNA}$, of DNA versus the PEG concentration, c_{PEG}, at various PEG molar masses (kg mol^{-1}): (■) $M_{w,PEG} = 8$; (●) $M_{w,PEG} = 6$; (▲) $M_{w,PEG} = 4$; (□) $M_{w,PEG} = 2$; (○) $M_{w,PEG} = 0.9$ ($x_{methanol} = 0$; $c_{salt} = 0.5 \times 10^3$ mol m^{-3}; $T = 25$°C).

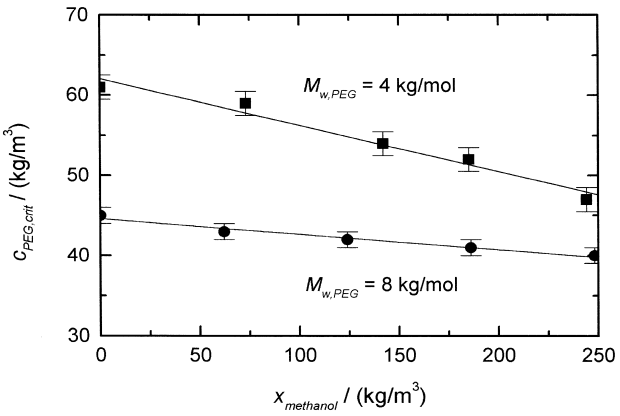

Fig. 3. Critical PEG concentration as a function of x_{methanol} for two molar masses of PEG.

2. The critical PEG concentration decreases as $M_{\text{w,PEG}}$ increases.
3. The conformational transition is sharp. There is a coexistence region within which both the coil and the collapsed DNAs coexist, but the width, Δc_{PEG}, of this region is very small.

Table 3 shows that Δc_{PEG} does not exceed 10 kg m^{-3}, and decreases with increasing $M_{\text{w,PEG}}$. All these effects are in line with the theoretical predictions given in the previous paper [11]. They are also in accordance with comparable experimental results of other groups [5,19].

A representative graph on the influence of the methanol content, x_{methanol}, on the critical PEG concentration is shown in Fig. 3. There, $c_{\text{PEG,c}}$ is plotted versus x_{methanol} for $M_{\text{w,PEG}} = 4$ and 8 kg mol^{-1}. The c_{salt} and T were fixed as before, c_{NaCl} is 0.5×10^3 mol m^{-3} and T is 25°C. The result is that $c_{\text{PEG,c}}$ decreases as x_{methanol} increases. The decreasing increment is larger the lower is $M_{\text{w,PEG}}$.

3.2. UV/Vis–spectroscopy combined with centrifugation

To confirm the viscometry results, an independent second method is required. Ultracentrifugation experiments [20] showed that condensed DNA molecules sediment to the bottom of a centrifuge cell where they aggregate. The DNA molecules that are in the coil state do not sediment out. Therefore, DNA molecules can be separated by centrifugation with respect to their conformational state.

A typical UV/Vis centrifugation result is shown in Fig. 4. The residual absorption A_{res} is plotted versus c_{PEG}, where $x_{\text{methanol}} = 183$ kg m^{-3}, $M_{\text{w,PEG}} = 4$ kg mol^{-1}, $c_{\text{salt}} = 0.5$ mol m^{-3}, and $T = 25$°C. The curve can be described by the empirical equation

$$A_{\text{res}} = k_1 \arctan[(c_{\text{PEG}} - k_2)/k_3] + k_4 \quad (6)$$

where $k_1 = -0.5016$, $k_2 = 56.7698$ kg m^{-3}, $k_3 = 0.72119$ kg m^{-3}, and $k_4 = 55.8436$. The critical PEG concentration is almost (57 ± 1) kg m^{-3} which is somewhat larger than $c_{\text{PEG,c}} = (51 \pm 3)$ kg m^{-3} as determined by viscometry, but both are in the same order of magnitude. The results found for the other PEG molar masses are summarized in Table 4. As with the viscometry measurements, two effects were observed: $c_{\text{PEG,c}}$ decreases as both $M_{\text{w,PEG}}$ and x_{methanol} increase. That suggests that both methods yield comparable results.

The data in Table 4 can be well described by multiple regression. An appropriate fit formula is

$$c_{\text{PEG,c}} = k_1(x_{\text{methanol}}) \times \left(\frac{1 \text{ g mol}^{-1}}{M_{\text{w,PEG}}} \right) k_2 \quad (7)$$

where $k_1 = 7754$ (kg m^{-3}) $- 3.44 \times x_{\text{methanol}} - 0.0245$ (m^3 kg^{-1}) $\times$ $(x_{\text{methanol}})^2 + 1.81 \times 10^{-5}$ (m^3 kg^{-1})^2 $\times$ $(x_{\text{methanol}})^3$, $x_{\text{methanol}} \in [0, 260$ kg m$^{-3}]$, $k_2 = 0.576$, and $M_{\text{w,PEG}} \in [0.9, 20$ kg mol$^{-1}]$. This relationship holds only for $c_{\text{NaCl}} = 0.5 \times 10^3$ mol m^{-3}, but similar relationships can

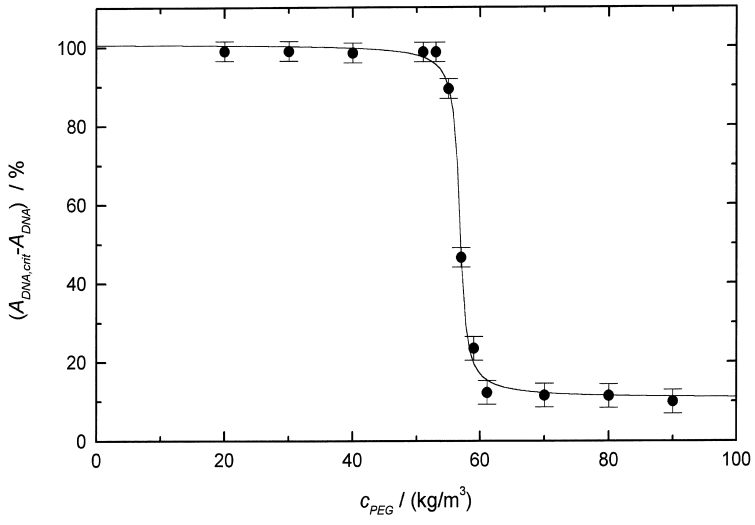

Fig. 4. Residual absorbency, $A_{\text{res}} = A_{\text{DNA,crit}} - A_{\text{DNA}}$, versus the critical PEG concentration for $x_{\text{methanol}} = 183$ kg m^{-3}, $M_{\text{w,PEG}} = 4$ kg mol^{-1}, $c_{\text{salt}} = 0.5 \times 10^3$ mol m^{-3}, and $T = 25$°C.

Table 4
The critical PEG concentration, $c_{PEG,c}$ (kg m^{-3}) as a function of the PEG molar mass, $M_{w,PEG}$ (kg mol^{-1}) and the methanol content, $x_{methanol}$ (kg m^{-3}) for $c_{NaCl} = 0.5 \times 10^3$ mol m^{-3} and $T = 25$°C, determined by UV/Vis–centrifugation

$M_{w,PEG}$											
0.9		2		4		6		8		20	
$x_{methanol}$	$c_{PEG,c}$	$x_{methanol}$	$c_{PEG,c}$	$x_{methanol}$	$c_{PEG,c}$	$x_{methanol}$	$c_{PEG,c}$	$x_{methanol}$	$c_{PEG,c}$	$x_{methanol}$	$c_{PEG,c}$
0	166 ± 2	0	100.5 ± 2	0	70 ± 1.5	0	57 ± 1.5	0	47 ± 1.5	0	31.5 ± 1
62	158.5 ± 2	63.2	97 ± 2	62	66.5 ± 1.5	63	55 ± 1.5	62	45 ± 1.5	62	30 ± 1
123.9	149 ± 2	126.3	91 ± 1.5	123.9	63 ± 1.5	125.9	51 ± 1.5	123.9	42 ± 1	123.9	29 ± 1
185.9	136 ± 1.5	189.5	82 ± 1.5	183	57 ± 1.5	188.9	46 ± 1.5	185.9	37.5 ± 1	185.9	27 ± 1
247.9	121 ± 1.5	252.6	72 ± 1.5	247.9	51.5 ± 1.5	252.8	41 ± 1	252.3	34 ± 1	247.8	24 ± 1

be derived for other salt concentrations, and it is also possible to formulate a general expression which contains all four relevant parameters, $c_{PEG,c}$, $x_{methanol}$, $M_{w,PEG}$ and c_{salt}, but this is beyond the scope of this paper.

3.3. Dynamic light scattering

This method gives the distribution of the DNA diffusion coefficients. A good example is the sample $c_{DNA} = 0.3$ kg m^{-3}, $c_{NaCl} = 0.5 \times 10^3$ mol m^{-3}, $M_{w,PEG} = 4$ kg mol^{-1}, $x_{methanol} = 0$ and $T = 25$°C, where the PEG concentration was changed step by step. The results are shown in Fig. 5, where the probability, $w(D)$, of finding a DNA molecule having a diffusion coefficient D is plotted versus D. At low PEG concentrations, such as $c_{PEG} = 10$ kg m^{-3}, there is only one distribution, $w(D)$, and the corresponding D values are low, i.e. in the order of 10^{-2} s m^{-2} or lower. According to the Einstein–Stokes relationship, $R_h = k_B T/6\pi\eta_0 D$, the hydrodynamic radius, R_h, is inversely proportional to D. Thus, R_h is large and all DNA molecules are in the coil state. As c_{PEG} increases, a second $w(D)$ distribution is seen to occur, whose D values are much larger than those of the primary distribution. They are in the order of 0.1 up to 1 s m^{-2} and the corresponding DNA molecules are in the collapsed state. This second distribution is first observed at the concentration $c_{PEG} \approx 50$ kg m^{-3}. This concentration can thus be defined as the critical PEG concentration. Other values are given in Table 5.

When the PEG concentration is further increased, the primary distribution vanishes and the second distribution becomes dominant. This is achieved at $c_{PEG} = 90$ kg m^{-3}. At this final state we have only the collapsed DNA conformation.

It can thus be concluded that DLS can be used efficiently to detect the transition of a DNA coil into its collapsed form. The dependences found for the critical PEG concentration are qualitatively the same as those detected from viscometry and UV/Vis–centrifugation. There are some quantitative discrepancies, which may be due to the different definitions of $c_{PEG,c}$. For DLS, it is unclear whether it is realistic to define $c_{PEG,c}$ so that the areas under the two $w(D)$ distributions are equally large. This can be done, but a DNA coil and a compact DNA globule are different particles. They behave differently hydrodynamically, so such a definition seems incorrect.

The most important result of DLS is that there are only two DNA states. There is unequivocally no intermediate state between the coil and the condensed DNA form. If such a conformation existed, there would be three or more peaks, but only two are observed.

3.4. Comparison between experiment and theory

In the accompanying paper [11] a theoretical model was presented using which the critical PEG concentration can be calculated. The model parameters such as the cell volume, the DNA persistence length, the Flory–Huggins interaction parameters, and the molar volume of the solvent were chosen so that they describe the DNA/PEG system studied here as realistically as possible. A comparison between experiment and theory is therefore possible.

We start with the dependence of the PEG molar mass on the critical PEG concentration. The results are of equal quality. Thus, we discuss only one system, namely the situation where $x_{methanol} = 0$, $c_{salt} = 0.5 \times 10^3$ mol m^{-3}, and $T = 25$°C. A summary of the results is given in Fig. 6. The solid line is computed according to the theoretical model. The other lines represent the experimental results obtained by UV/Vis–centrifugation and viscometry, respectively. All three curves can be well fitted by hyperbolic functions

$$c_{PEG,c} = k_1 + k_2 \left(\frac{10^{-3} \text{ kg mol}^{-1}}{M_{w,PEG}} \right)^{k_3} \tag{8}$$

where the values of the constants k_1, k_2, and k_3 are given in the figure caption. The experimental curves are in good agreement with the theoretical one, but there are discrepancies depending on the PEG molar mass. At $M_{w,PEG} = 1$ kg mol^{-1}, the difference between $c_{PEG,c,theory}$ and $c_{PEG,c,UV/Vis}$ is 25 kg m^{-3}, which is in the order of 10%, and for $M_{w,PEG} = 20$ kg mol^{-1}, the deviation is in the order of 30%. The reason for this is that the reference values found for the DNA persistence length, the Flory–Huggins

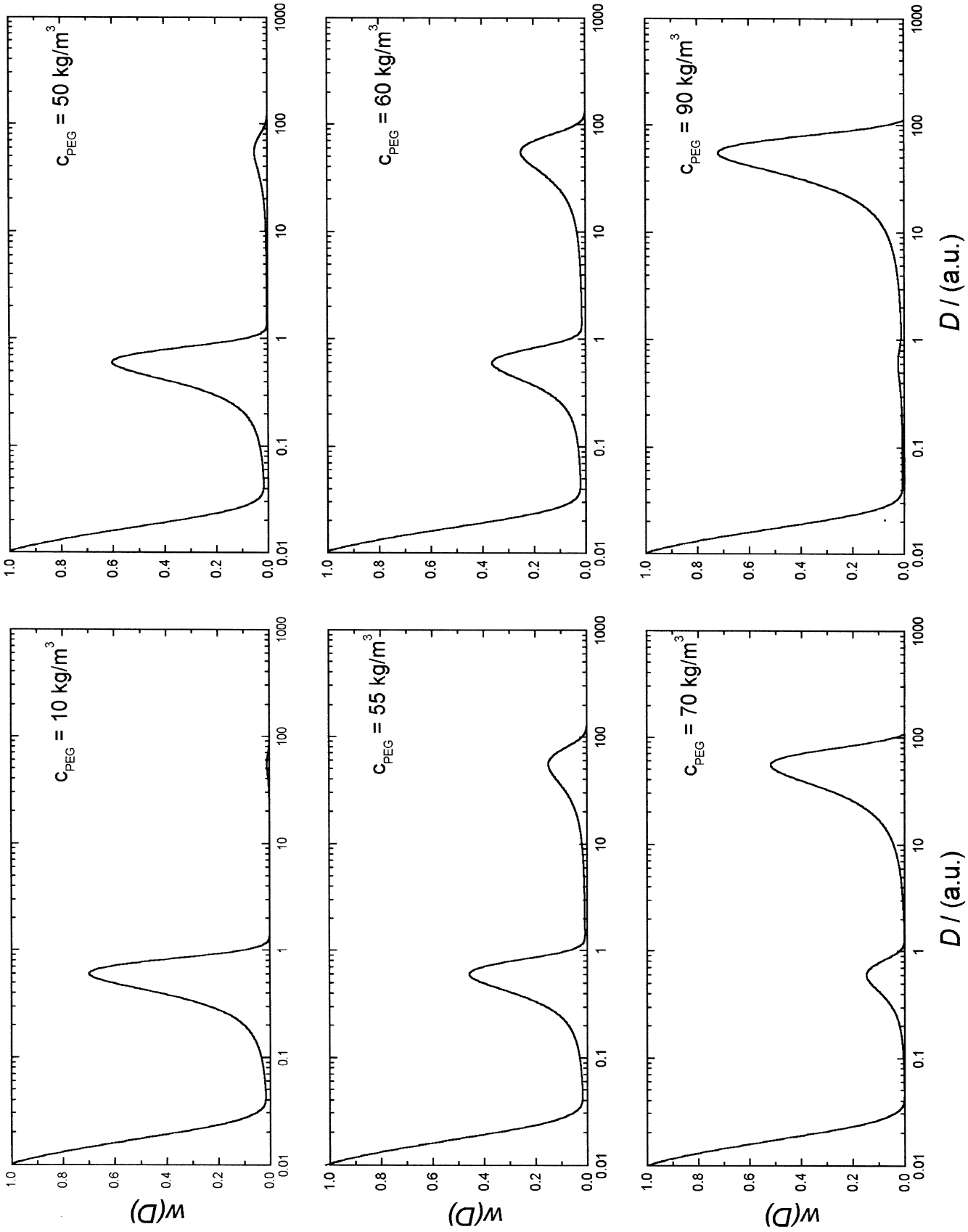

Fig. 5. Dynamic light scattering experiments; plot of the translational diffusion coefficient distribution $w(D)$.

Table 5
The $c_{PEG,c}$ values determined by dynamic light scattering as a function of $x_{methanol}$ for $M_{w,PEG} = 4$ kg mol^{-1}

$x_{methanol}$ (kg m^{-3})	$c_{PEG,c}$ (kg m^{-3})
0	64
62	60
125	55
183	50

parameters and the other parameters differ from one literature source to the next and show different functional dependencies.

We have chosen the system parameters in such a way that the agreement between theory and UV/Vis–centrifugation is exact for $M_{w,PEG} = 6$ kg mol^{-1}. For the other PEG molar masses the system parameters were kept constant. However, it is seen that this assumption is not correct. Probably, the DNA persistence length and also the other parameters depend on $M_{w,PEG}$. Exact agreement can be obtained if l_p, χ_{DS}, χ_{PS}, and χ_{DP} are varied appropriately. The only problem is the correct choice of the experimental reference method. Since this is as yet unclear, we have omitted such an analysis.

Fig. 7 illustrates the comparison between theory and experiment for the dependence of $c_{PEG,c}$ on the methanol concentration. Theory predicts a linear decrease of $c_{PEG,c}$ as $x_{methanol}$ increases. This linearity is indeed found but only as long as $x_{methanol}$ is small. Qualitatively, the theoretical and experimental curves correspond well with each other, suggesting that the model applied is realistic. However, there are some quantitative differences. The quantitative agreement is best at high $M_{w,PEG}$.

However, the discrepancy between theory and experiment increases with increasing MeOH concentration. This could be explained if the solvent quality decreases at high $x_{methanol}$. A DNA molecule could then not only alter its

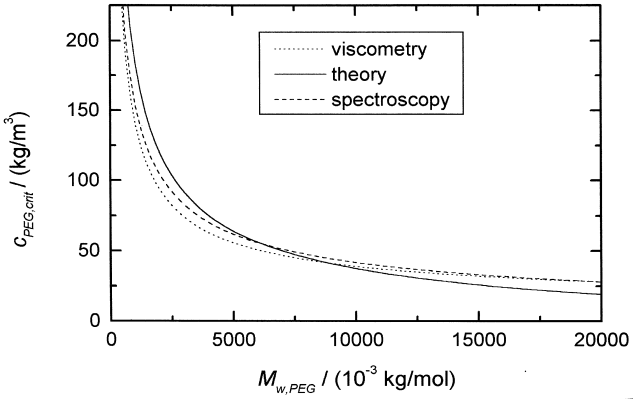

Fig. 6. Plot of the critical PEG concentration versus the PEG molar mass, determined experimentally by viscometry ($k_1 = 9.36$ kg m^{-3}; $k_2 = 11\,543.2$ kg m^{-3}; $k_3 = 0.567$), UV/Vis–centrifugation ($k_1 \approx 0$ kg m^{-3}; $k_2 = 7754.2$ kg m^{-3}; $k_3 = 0.567$), and computed according to our model.

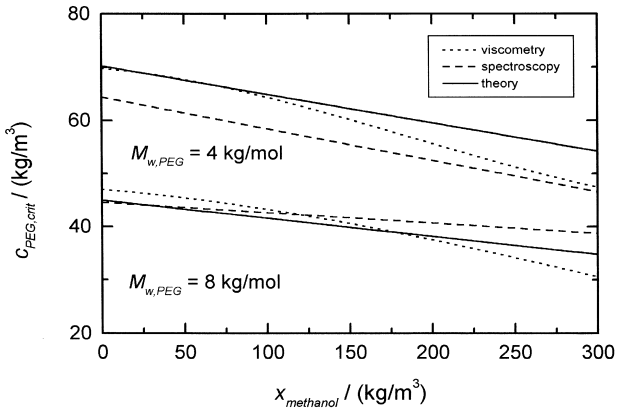

Fig. 7. Critical PEG concentration versus $x_{methanol}$ determined experimentally and theoretically for $M_{w,PEG} = 4$ kg mol^{-1} and $M_{w,PEG} = 8$ kg mol^{-1} at $c_{NaCl} = 0.5 \times 10^3$ mol m^{-3} and $T = 25$°C.

secondary structure from a double helix to a partially denaturated form, but aggregation could take place too. Theory does not take the possibility of aggregation into account. For $x_{methanol} \leq 200$ kg m^{-3} these effects are not present but they may become important as the MeOH concentration increases.

No measurements were performed to study the influence of the salt concentration on $c_{PEG,c}$. Theory predicts that in the absence of MeOH for given values of $M_{w,PEG}$, $c_{PEG,c}$ behaves as $c_{PEG,c} = k_1 + (k_2/c_{salt})$. This behavior agrees with the theoretical predictions of Yevdokinow and Skundin [18] and agrees with the experimental results of Vasilevskaya et al. [19]. To date, there appear to be no measurements in mixed solvents with varying salt content. This will be the subject of a future study.

3.5. The coexistence zone

Experiments have shown that the width of the coexistence zone is rather small and more or less independent of $M_{w,PEG}$, $x_{methanol}$ and c_{salt}. These results are consistent with both our theoretical computations and with the results of other groups. Vasilevskaya et al. [19] report a slight increase in the width of the zone as c_{salt} is increased. They find also that a collapsed DNA returns to the expanded coil state if the PEG concentration becomes very high, i.e. when it is in the order of 100 kg m^{-3} or higher. This was not observed in the present study. The reason may be that Vasilevskaya et al. used T4 DNA which has a molar mass M_w of 1.08×10^5 kg mol^{-1}, while calf-thymus DNA of a much lower molar mass (2.2×10^3 kg mol^{-1}) was used in this study.

4. Conclusions

Viscometry, UV/Vis–centrifugation and DLS experiments were carried out to obtain a deeper insight into the features of DNA coil–globule transitions in PEG water/methanol solutions. In the present work both the methanol

concentration and the PEG molar mass were changed while the salt concentration of NaCl was kept constant. The results obtained verify the predictions of the theoretical model calculations presented in the companion paper [11]. All three methods yield a critical PEG concentration at which the transition takes place. The absolute values measured for $c_{PEG,c}$ agree quite well with those computed theoretically and they show the same dependences on $x_{methanol}$ and $M_{w,PEG}$. This could thus be taken as a confirmation of the theoretical model applied.

UV/Vis–centrifugation probably yields the most reliable values for $c_{PEG,c}$. This method works relatively fast and the DNA concentration employed is so low that intermolecular DNA/DNA interactions can be neglected. That is, the experimental conditions are very similar to those considered in the theoretical model. The disadvantage of UV/Vis–centrifugation is, however, that no reliable information about the DNA size can be obtained. Viscometry thus appears to be a more appropriate method. By measuring the viscosity, a value for the DNA hydrodynamic radius can be derived. According to Fig. 5, the ratio $R_{h,DNA,coil}/R_{h,DNA,globule}$ is in the order of 15. Unfortunately, the DNA concentration necessary for the viscometry experiments is a factor of 10 larger than for UV/Vis–centrifugation. Thus, intermolecular DNA/DNA interactions are possible. According to Post and Zimm [21], the collapsed DNA state is a metastable state for which aggregation can occur if the DNA concentration is high enough.

The most interesting experimental technique for the present purpose is DLS. It yields directly the DNA state, the DNA size, and the distribution of the DNA molecules over both states (coil and globule). At small PEG concentrations, only the coil state is observed, while at $c_{PEG,c}$ both the coil and the globule state, and at high c_{PEG}, only the collapsed state are observed. There appear to be no further intermediate states. The main advantage of DLS over the other methods is that the DNA molecules are not affected by outside disturbances. The samples do not have to be centrifuged, as in UV/Vis–centrifugation, and there are no flow rates, as in viscometry. A similarly good method would be fluorescence spectroscopy; however, this involves labeling the DNA molecules with a dye, which could influence the DNA conformation.

The most interesting result of this study is that the addition of methanol promotes DNA condensation. This is in accordance with the results of Arscott et al. [8]. That is, there are synergistic effects between the solvent, the salt, and the alcohol content, where the latter has both an electrostatic as well as a structural influence on the DNA.

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