ELSEVIER

Polymer 40 (1999) 4013–4023

polymer

Poly(ethylene glycol)-induced DNA condensation in aqueous/methanol containing low-molecular-weight electrolyte solutions Part I. Theoretical considerations

Gerd Kleideiter, Eckhard Nordmeier*

University of Osnabrück, Institute of Chemistry, Barbarastr. 7, 49069 Osnabrück, Germany

Received 3 February 1998; accepted 22 August 1998

Abstract

In a certain unfavourable environment, a single DNA molecule undergoes a conformational transition from an expanded coil state to a collapsed form. Here, this transition is induced by poly(ethylene glycol) (PEG) in a solvent mixture composed of an aqueous salt buffer and methanol. A theoretical description is presented in terms of the free energy of mixing DNA, PEG, and solvent, the elastic part of the free energy for DNA chains, and the translational entropy of the low molecular ions. Further effects taken into account are DNA–counterion binding and solvent quality. The theoretical predictions are: (1) The transition between the coil and the collapsed state is discontinuous. There exists a coexistence region where both states coexist side by side, but its width is very small. (2) The collapse takes place at a certain critical PEG concentration $c_{PEG,c}$. The value of this PEG-concentration depends on the degree of PEG polymerisation, $P_{w,PEG}$, the molar fraction, $x_{methanol}$, of methanol, and on the concentration, c_{salt}, of the added salt. For given values of $x_{methanol}$ and c_{salt}, $c_{PEG,c}$ decreases with increasing $P_{w,PEG}$. That is, it is easier to induce the DNA collapse with PEG of high molar mass than with PEG of low molar mass. If both $P_{w,PEG}$ and c_{salt} are constant, $c_{PEG,c}$ increases as the methanol concentration decreases. This means that addition of methanol promotes DNA condensation. If finally $P_{w,PEG}$ and $x_{methanol}$ are chosen constant, $c_{PEG,c}$ decreases as c_{salt} increases. Thus we can say, the collapsed DNA state is the more stabile the higher are $P_{w,PEG}$, $x_{methanol}$ as well as c_{salt}. That is, these three parameters act synergistically. (3) Theory gives some information about the DNA-molecule size. While in the coil state the expansion factor, α, is of the order of 1.8–2.4, it is of the order of 0.3 in the compact state. Results of measurements presented in the companion paper affirm these results.

Keywords: DNA condensation; Conformational transformation; Low molecular weight electrolyte solutions

1. Introduction

An individual DNA chain possesses the conformation of an elongated coil if it is freely dissolved in an aqueous salt solution. However, this is not the only conformation of a DNA-molecule. In biological cells DNA is packed into ordered structures with distinct morphology. For instance, in eukaryotes [1] DNA is folded 6–7-fold in comparison to the state where DNA is free, 60 μm of DNA must be compacted 600-fold to fit a virion of T2 or T4 bacteriophages [2] whose largest dimensions are 0.10 μm. Still more imposing is human diploid DNA. There 1.5 m of DNA must be compacted nearly 75 000-fold to fit into a nucleus.

This DNA compacting or condensation is a collective effect caused by mutual attraction of DNA-monomers. A major role play electrostatic forces. Binding of cationic compounds to DNA reduces the repulsion between the double-helical segments and produces a DNA-collapse in vitro. Model systems studied in several laboratories include synthetic polypeptides plus salt [3–5], polyamines [6], transition-metal ions plus polylysine [7], histones H1 [8], and organic solvents plus salt [9,10]. In most cases, well organised DNA toruses are produced, but the agents added must not necessary be charged. A DNA condenses also to a compact, relatively dense state in systems that contain neutral low molar mass polymers, such as poly(ethylene glycol) (PEG) [11,12] or poly(vinylpyrrolidone) (PVP) [13]. This suggests that DNA-condensation is governed in some measure by DNA–polymer excluded volume interactions. Further, it seems that DNA by itself can bend to form beaded structures with dimensions similar to nuclesome dimensions. Griffith [14] described such structures in DNA carefully isolated from prokaryotic cells. It is therefore proposed that the intrinsic properties of the DNA itself govern compacting in vivo.

* Corresponding author.

0032-3861/99/$ - see front matter
PII: S0032-3861(98)00643-0

A deeper understanding of this mature is obtained by studying the problem theoretically. First, it seems that the most unfavourable factor opposing DNA condensation is the electrostatic repulsion between DNA phosphates. Thus, there must be a free energy which counterbalances these forces. A possibility is a binding of cationic compounds such as polyamines to DNA. Unfortunately, this assumption is not strictly valid. Porschke [15,16] has shown that the binding of polyamines to DNA and the condensation of DNA–polyamine complexes are independent phenomena. That is, DNA-condensation cannot originate solely from the binding of multivalent cations. It is more likely, that cation binding decreases the repulsive forces and enhances simultaneously some attractive forces. A possible origin for these attractive forces are correlated fluctuations of bound cations as proposed by Oosawa [17]. It is also instructive to follow the ritual of Manning [18–20]. He postulates that the axis of the DNA double helix becomes spontaneously curved when the DNA reached a certain critical degree of neutralisation. The result can be a smooth bending, but also sharp bends spaced at regular intervals. That is, there are essentially three main energetic contributions to DNA condensation, namely charge neutralisation, DNA-bending, and correlated cation fluctuations.

Several theoretical attempts to describe these effects exist. Essentially, three lines can be distinguished: (1) the virial coefficient concept [21–25]; (2) the idea of Houssier [26]; and (3) the idea to connect the problem with the theory of the collapse of polyelectrolyte networks. The virial coefficient concept was first introduced by Zimm [21] and later extended by Grosberg [24]. The fundamental quantity is the free energy, ΔF, for the mixing of an isolated DNA chain with solvent and PEG molecules. According to Zimm the solvent and the PEG molecules build a unit or a continuum, respectively. The poorer the solubility force of this continuum the smaller is the DNA expansion coefficient, $\alpha = < R^2 >_z^{1/2} / < R^2 >_{z,\theta}^{1/2}$, where $< R^2 >_z^{1/2}$ and $< R^2 >_{z,\theta}^{1/2}$ are the z-average DNA radii of gyration in the continuum and under theta-conditions. The DNA equilibrium configuration is determined by the minimum of ΔF with respect to α at a given Flory–Huggins parameter, χ, describing the interaction between the DNA and the continuum. The collapse takes place when ΔF changes from a value of $\alpha \approx 1$ to a value of α much less than one. To find this point ΔF must be expanded into a virial series up to the third virial coefficient. Similarly, Grosberg works with the chemical potentials of PEG and DNA links, where at the equilibrium state the PEG chemical potential inside a DNA domain is equal to that outside the domain. The calculation procedure is a mixture of virial series developments and scaling theory. It explains among others the fact of DNA-condensation, the presence of a critical PEG-concentration, $c_{\mathrm{PEG,c}}$, and the dependence of the point of collapse on the degree of PEG-polymerisation. Unfortunately, the predictions are only qualitative. Effects such as chain flexibility or salt–polyion interactions are neglected or taken into account in a somewhat dubious way.

The second theoretical concept is that of Marquet and Houssier [26]. It is an estimation of the main free energies that contribute to DNA condensation. They are: (i) DNA bending; (ii) entropy of DNA segment mobility; (iii) repulsion between neighbourly charged DNA segments; and (iv) attraction forces between DNA segments due to correlated counterion fluctuations. If the sum of all these energies is negative a DNA-molecule can collapse, but if it is positive it can not. The results are that: (a) DNA condensation takes place spontaneously in the presence of tri- and tetra-valent cations; (b) condensation is possible for divalent cations, but only if the solvent is a mixture of water and an organic solvent such as methanol; and (c) condensation takes not place at all when the counterions are univalent.

The advantage of this method is that it takes into account nearly all relevant energy contributions and that one arrives relatively quickly to the answer of whether a polyion can collapse or not. The disadvantage is that it is very difficult, if not impossible, to determine exactly the point at which the transition should occur.

The third theoretical model was developed by Vasilevskaya et al. [27–29]. It may be the most interesting one, because while the virial coefficient concept was developed in analogy to the theory of the coil–globule transition for solutions of neutral polymers, this approach takes explicitly into account the fact that DNA coils are polyelectrolyte chains. Beside the difference in PEG concentration inside the DNA coils and that in the outside solution, additionally the theoretical analysis involves the Donnan equilibrium for the added salt between the interior and the exterior domain. Unfortunately, the mathematical formalism is rather complicated as a result of the fact that the physicochemical equations can be solved only numerically. However, the great advantage of this approach is that it is possible to compute absolute values for the critical PEG-concentration, $c_{\mathrm{PEG,c}}$, both as a function of the degree of PEG-polymerisation, $P_{\mathrm{w,PEG}}$, as well as a function of the salt concentration, c_{salt}. The predictions obtained are really good. They agree quite well with the experimental results.

In this paper, we study the collapse of a DNA-molecule that is dissolved in a PEG–low molecular salt solution, where the solvent is not pure water, but a mixture of water and methanol. We present the equations for the free energy describing the equilibrium state and we compute the physical conditions for the state at which the DNA collapses. There are several parameters that must be varied, this is a laborious work. They are, among others, the total PEG-concentration, c_{PEG}^{total}, the degree of PEG-polymerisation, $P_{\mathrm{w,PEG}}$, the total concentration, c_{salt}, of the added low molecular salt, and the molar fraction, x_{methanol}, of methanol in the solvent mixture. The theoretical concept used, follows with some minor alterations the line of Vasilevskaya et al.. However, (1) we use a slightly different notation. This is necessary in order to make the thematic didactically more

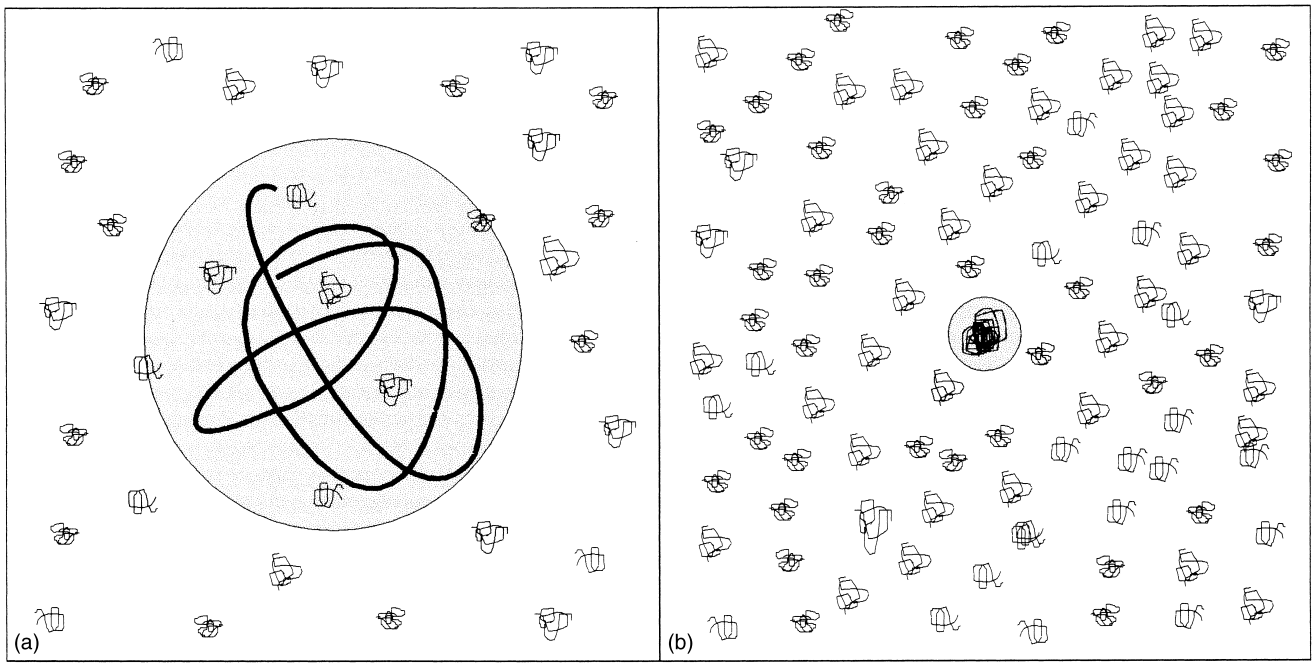

Fig. 1. Sketch of a DNA-domain surrounded by a number of PEG-molecules: (a) the DNA-molecule is in its expanded coil state; and (b) the DNA-molecule is collapsed.

easier understandable and to remain in line with the notation used in our other papers; (2) Vasilevskaya works with the Gaussian-distribution for the end to end chain distances. Instead of we use the more general Langevin-distribution, taking into account that a DNA-molecule cannot be stretched infinitely in length; (3) the effect of counterion binding is involved; and (4) we use a solvent mixture of water and methanol, while Vasilevskaya works with pure water.

We do not present any experiments as this is done in the companion paper where we also present a comparison between the theory and experiment.

2. Theory

We start our study with the model presented in Fig. 1. The solution contains DNA-macromolecules, linear flexible PEG-molecules, and a low-molecular salt which is dissolved in a mixture of water and methanol. A DNA-molecule is a polyion chain, possessing a relatively large persistence length. It occupies a volume domain, V_{dom}, which is so large that the much smaller PEG-molecules can penetrate inside. The solution contains n_{DNA} DNA-molecules. We call the region of domains occupied by the DNA-molecules the inner domains. The remaining volume of the solution is free of DNA and constitutes the outer domain. Additionally, we assume that the solution is dilute, i.e. there is no DNA-domain overlapping and there are no intermolecular DNA-interactions.

The notation for this model is as follows: We consider 10^{-3} m^3 of solution. In this volume are n_{DNA} DNA-molecules of molar mass $M_{\mathrm{w,DNA}}$. l_{DNA} is the distance between two base pairs, d_{DNA} the DNA-diameter, and $v_{\mathrm{sp,DNA}}$ the partial specific DNA volume. The volume of a DNA-repeat unit is then

$$V_{\mathrm{repeat,DNA}} = \pi(d_{\mathrm{DNA}}/2)^2 l_{\mathrm{DNA}} \tag{1}$$

With [30] $l_{\mathrm{DNA}} = 3.4{\cdot}10^{-10}$ m and $M_{\mathrm{w,DNA}} = 2.2{\cdot}10^3$ kg/mol we find $V_{\mathrm{repeat,DNA}} = 1.07{\cdot}10^{-27}$ m^3. The shape of a DNA-domain is very similar to that of a cylinder or an ellipsoid. However, to describe these geometric structures, two or three length parameters, respectively, are necessary. Unfortunately, these lengths are not known a priori. Therefore, we make a simplification. We describe the real DNA domains by spheres having the same volume as the real domains. This is an oversimplification, but it can be shown that the calculations that follow depend predominately on the DNA-domain volume and only marginally on the DNA domain shape.

The volume of such an equivalent sphere is

$$V_{\mathrm{dom}} = \frac{4\pi}{3} < R^2 >^{3/2}_{\mathrm{z,DNA}} = \frac{4\pi}{3}\alpha^3 < R^2 >^{3/2}_{\mathrm{z},\theta,\mathrm{DNA}} \tag{2}$$

where $< R^2 >^{1/2}_{\mathrm{z,DNA}}$ and $< R^2 >^{1/2}_{\mathrm{z},\theta,\mathrm{DNA}}$ are the z-average DNA radii of gyration at the actual state and under theta-conditions. $\alpha \equiv < R^2 >^{1/2}_{\mathrm{z,DNA}} / < R^2 >^{1/2}_{\mathrm{z},\theta,\mathrm{DNA}}$ is the so-called expansion coefficient. Its value depends among other parameters on the solvent composition, the salt concentration, and the PEG-concentration. The most important parameter is the volume fraction, $\phi^{\mathrm{in}}_{\mathrm{DNA}}$, of the DNA in

the inner domains. We have:

$$\phi_{\mathrm{DNA}}^{\mathrm{in}} \equiv \frac{\text{eigenvolume of a DNA-molecule}}{V_{\mathrm{dom}}} = \frac{P_{\mathrm{w,DNA}} V_{\mathrm{mono,DNA}}}{V_{\mathrm{dom}}} = \frac{1}{\alpha^3} \frac{3 L_{\mathrm{DNA}} d_{\mathrm{DNA}}^2}{16 < R^2 >_{z,\theta,\mathrm{DNA}}^{3/2}} \quad (3)$$

where $P_{\mathrm{w,DNA}}$ is the degree of DNA-polymerisation.

Eq. (3) can be recalculated into the DNA-concentration, c_{DNA}, by

$$c_{\mathrm{DNA}}^{\mathrm{in}} = \frac{\phi_{\mathrm{DNA}}^{\mathrm{in}} 4 M_{\mathrm{w,DNA}}}{\pi (d_{\mathrm{DNA}})^2 L_{\mathrm{DNA}} N_{\mathrm{A}}} \quad (4)$$

with L_{DNA} the DNA contour length and N_{A} the Avogadro-number.

There are $n_{\mathrm{PEG}}^{\mathrm{in}}$ PEG-molecules in the inner DNA domains. $M_{\mathrm{w,PEG}}$ is their molar mass, $P_{\mathrm{w,PEG}}$ their degree of polymerisation, l_{PEG} their repeat unit length, and d_{PEG} the PEG repeat unit diameter. Thus, the PEG volume fraction, $\phi_{\mathrm{PEG}}^{\mathrm{in}}$, in the inner DNA domains is

$$\phi_{\mathrm{PEG}}^{\mathrm{in}} = \frac{(n_{\mathrm{PEG}}^{\mathrm{in}} \cdot 10^{-3} m^3) P_{\mathrm{w,PEG}} \pi (d_{\mathrm{PEG}}/2)^2 l_{\mathrm{PEG}}}{(4\pi/3) \alpha^3 < R^2 >_{z,\theta,\mathrm{DNA}}} \quad (5)$$

Recalculation into the concentration gives

$$c_{\mathrm{PEG}}^{\mathrm{in}} = \frac{\phi_{\mathrm{PEG}}^{\mathrm{in}} 4 M_{\mathrm{mono,PEG}}}{\pi (d_{\mathrm{PEG}})^2 l_{\mathrm{PEG}} N_{\mathrm{A}}} \quad (6)$$

where $M_{\mathrm{repeat,PEG}}$ is the PEG repeat unit molar mass [31] ($M_{\mathrm{repeat,PEG}} = 44.05 \cdot 10^{-3}$ kg/mol).

Finally, we need to establish the solvent volume fraction, $\phi_{\mathrm{solvent}}^{\mathrm{in}}$, in the inner domains. It is simply obtained by the law of conservation for the number of particles. We find

$$\phi_{\mathrm{solvent}}^{\mathrm{in}} = 1 - \phi_{\mathrm{DNA}}^{\mathrm{in}} - \phi_{\mathrm{PEG}}^{\mathrm{in}} \quad (7)$$

Similarly, we calculate the volume fraction for the outer domain. Obviously, we have $1 - n_{\mathrm{DNA}} V_{\mathrm{dom}}$. Since n_{DNA} is the number of DNA-molecules per 10^{-3} m^3 and V_{dom} a volume, this quantity is dimensionless. Totally, there are n_{PEG} PEG-molecules in 10^{-3} m^3 solution. $n_{\mathrm{PEG}}^{\mathrm{out}}$ of these molecules are in the outer domain and $n_{\mathrm{PEG}}^{\mathrm{in}}$ molecules are in the inner DNA domains. Thus, we find

$$n_{\mathrm{PEG}}^{\mathrm{out}} = n_{\mathrm{PEG}} - n_{\mathrm{DNA}} \cdot 10^{-3}\ m^3 \cdot n_{\mathrm{PEG}}^{\mathrm{in}} \quad (8)$$

The corresponding volume fraction is

$$\phi_{\mathrm{PEG}}^{\mathrm{out}} = \frac{n_{\mathrm{PEG}}^{\mathrm{out}} P_{\mathrm{w,PEG}} \pi (d_{\mathrm{PEG}}/2)^2 l_{\mathrm{PEG}}}{1 - n_{\mathrm{DNA}} V_{\mathrm{dom}}} \quad (9)$$

so that the concentration becomes

$$c_{\mathrm{PEG}}^{\mathrm{out}} = n_{\mathrm{PEG}}^{\mathrm{out}} \left(\frac{M_{\mathrm{w,PEG}}}{N_{\mathrm{A}}} \right) \frac{1}{1 - n_{\mathrm{DNA}} V_{\mathrm{dom}}} \quad (10)$$

Inside the outer domain are no DNA-molecules. Therefore, $\phi_{\mathrm{DNA}}^{\mathrm{out}}$ is zero and the solvent volume fraction is

$$\phi_{\mathrm{solvent}}^{\mathrm{out}} = 1 - \phi_{\mathrm{PEG}}^{\mathrm{out}} \quad (11)$$

Next, we divide the solution volume into a number, N_{total}, of lattice cells of equal size V_{cell}. Since our reference volume is 10^{-3} m^3 it follows

$$N_{\mathrm{total}} = 10^{-3}\ m^3 / V_{\mathrm{cell}} = N_{\mathrm{total}}^{\mathrm{out}} + n_{\mathrm{DNA}} \cdot 10^{-3}\ m^3 \cdot N_{\mathrm{total}}^{\mathrm{in}} \quad (12)$$

where $N_{\mathrm{total}}^{\mathrm{in}}$ and $N_{\mathrm{total}}^{\mathrm{out}}$ are the number of cells per DNA-domain inside the inner DNA domains and the outer domain, respectively. In detail we have

$$N_{\mathrm{total}}^{\mathrm{in}} = V_{\mathrm{dom}} / V_{\mathrm{cell}} \quad (13)$$

$$N_{\mathrm{total}}^{\mathrm{out}} = \left(1 - n_{\mathrm{DNA}} \cdot 10^{-3}\ m^3 \cdot V_{\mathrm{dom}}\right) / V_{\mathrm{cell}} \quad (14)$$

$$N_{\mathrm{DNA}} = n_{\mathrm{DNA}} \cdot 10^{-3}\ m^3 \cdot N_{\mathrm{total}}^{\mathrm{in}} \cdot \phi_{\mathrm{DNA}}^{\mathrm{in}} \quad (15)$$

$$N_{\mathrm{PEG}} = n_{\mathrm{DNA}} \cdot 10^{-3}\ m^3 \cdot N_{\mathrm{total}}^{\mathrm{in}} \cdot \phi_{\mathrm{PEG}}^{\mathrm{in}} + N_{\mathrm{total}}^{\mathrm{out}} \cdot \phi_{\mathrm{PEG}}^{\mathrm{out}} \quad (16)$$

$$N_{\mathrm{solvent}} = N_{\mathrm{total}}^{\mathrm{in}} \cdot n_{\mathrm{DNA}} \cdot \phi_{\mathrm{solvent}}^{\mathrm{in}} + N_{\mathrm{total}}^{\mathrm{out}} \cdot \phi_{\mathrm{solvent}}^{\mathrm{out}} \quad (17)$$

Here, N_{DNA}, N_{PEG}, and N_{solvent} are the number of cells that are occupied with DNA-segments, PEG-segments, and solvent-molecule segments, respectively.

The central part of the theory are the free energies. We have three terms: (1) the free energy of mixing the polymers together with the solvent; (2) the elastic part of the energy for the DNA chains; and (3) the translational free energy of the salt ions. Of course there are further energies which could be taken into account. For instance, there are electrostatic interactions between the charged species, conformational contributions of the PEG-molecules, or polymer–solvent selective adsorption effects to name only a few. We neglect these contributions because: (1) the solution is assumed to be diluted (thus intermolecular DNA interactions can be neglected); and (2) theoretical calculations [43] and experimental results [44,45] show that these energies are comparatively small with respect to the main contributions.

The expression for the free energy of mixing the solution is easily obtained by the Flory–Huggins approach [32]. This is a very simplified model, but it is the most familiar concept in polymer physics. Here, we have two energies of mixing, $F_{\mathrm{mix}}^{\mathrm{in}}$ and $F_{\mathrm{mix}}^{\mathrm{out}}$, one for the inner domains and one for the outer domain. The results can be written down as

$$F_{\mathrm{mix}}^{\mathrm{in}} = \mathrm{k_B T} \Big[n_{\mathrm{PEG}}^{\mathrm{in}} ln\left(\phi_{\mathrm{PEG}}^{\mathrm{in}}\right) + n_{\mathrm{solvent}}^{\mathrm{in}} ln\left(\phi_{\mathrm{solvent}}^{\mathrm{in}}\right) + \chi_{\mathrm{DS}}^{\mathrm{in}} \phi_{\mathrm{DNA}}^{\mathrm{in}} n_{\mathrm{solvent}}^{\mathrm{in}} P_{\mathrm{s}} + \chi_{\mathrm{DP}}^{\mathrm{in}} \phi_{\mathrm{DNA}}^{\mathrm{in}} n_{\mathrm{PEG}}^{\mathrm{in}} P_{\mathrm{w,PEG}} + \chi_{\mathrm{PS}}^{\mathrm{in}} \phi_{\mathrm{PEG}}^{\mathrm{in}} n_{\mathrm{solvent}}^{\mathrm{in}} P_{\mathrm{s}} \Big] \quad (18)$$

and

$$F_{\mathrm{mix}}^{\mathrm{out}} = \mathrm{k_B T} \left[\chi_{\mathrm{PS}}^{\mathrm{out}} \phi_{\mathrm{PEG}}^{\mathrm{out}} n_{\mathrm{solvent}}^{\mathrm{out}} P_{\mathrm{s}} + n_{\mathrm{PEG}}^{\mathrm{out}} ln\left(\phi_{\mathrm{PEG}}^{\mathrm{out}}\right) + n_{\mathrm{solvent}}^{\mathrm{out}} ln\left(\phi_{\mathrm{solvent}}^{\mathrm{out}}\right) \right] \quad (19)$$

where $\chi_{\mathrm{PS}}^{\mathrm{i}}$, $\chi_{\mathrm{DP}}^{\mathrm{i}}$, and $\chi_{\mathrm{DS}}^{\mathrm{i}}$ (with i = inner, outer) are the

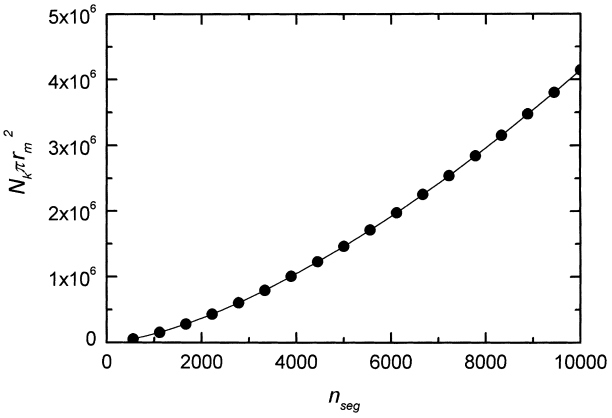

Fig. 2. The normalisation constant, N_k, as a function of the number of segments per chain.

Flory–Huggins interaction parameters between PEG and solvent, DNA and PEG, and DNA and solvent, respectively. k_B is the Boltzmann constant, T the absolute temperature, and P_s the number of solvent molecule segments in a lattice cell. It should be pointed out that a solvent segment is defined so that it contains as much solvent molecules that it is as large as a DNA-segment.

The absolute values of χ^i_{PS} and χ^i_{DS} depend on the solvent composition and the temperature. We can calculate them by the empirical relation of Kok and Rudin [33]. According to these authors we have

$$\chi^i_{jS} = \frac{1}{2} - \frac{3.24\left([\eta]^i_{jS} - [\eta]^i_{j,\theta}\right)}{M_{w,j}\left[1 + 0.81 c_j\left([\eta]^i_{jS} - [\eta]^i_{j,\theta}\right)\right]} \delta_j^2 V_{mol,solvent} \tag{20}$$

where $[\eta]^i_{jS}$ is the viscosity number of the polymer j dissolved in the solvent S, $[\eta]^i_{j,\theta}$ the viscosity number under theta-conditions, $M_{w,j}$ the weight average molar mass of polymer j, δ_j the polymer density, c_j the polymer concentration (unit: 10^3 kg/cm^3), and $V_{mol,solvent}$ the molar volume of the solvent (unit: 10^{-6} m^3). For a mixture of

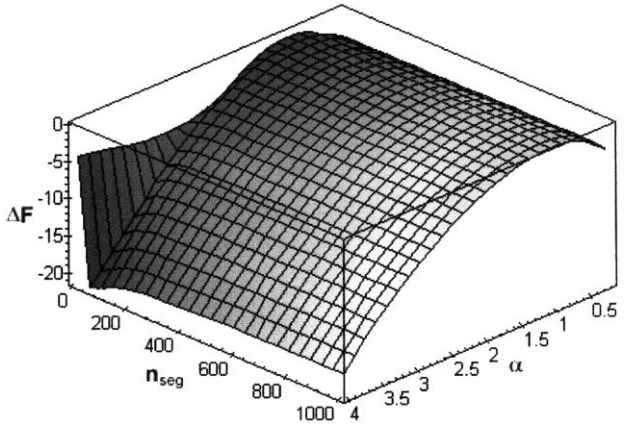

Fig. 3. The reduced elastic free energy, F_{elas}/k_BT, as a function of the expansion coefficient, α, and the number of segments per chain n_{seg}.

water and methanol [34] at $T = 25$°C we find

$$V_{mol,water/methanol} = \left[18.1 + 0.172 w_{meth} + 7.6\cdot10^{-5} w_{meth}^2 + 4.7\cdot10^{-6} w_{meth}^3\right] \cdot 10^{-6}\,\mathrm{m}^3 \tag{21}$$

with

$$w_{meth} = 0.10953 x_{meth} - 2.66431\cdot10^{-5} x_{meth}^2 + 6.15045\cdot10^{-8} x_{meth}^3 \tag{22}$$

where w_{meth} and x_{meth} are the weight percent and the concentration of methanol in kg/m^3.

A problem is the Flory–Huggins parameter x_{DP}. According to literature [29], χ_{DP} is 0.54 and constant, but it is more likely to assume that it depends on the degree of PEG-polymerisation.

The second energy contribution is the elastic free energy, F_{elas}, of a DNA chain. We have two possibilities to calculate it. First we can assume the DNA-chains are infinitely long and second we can state they are finitely long. In the first case we have to work with the Gaussian-segment distribution. The result is the well-known Flory expression [35]

$$F_{elas} = k_B T\left[ln\left(\alpha^3\right) + 1.5\left(1 - \alpha^2\right)\right] \tag{23}$$

However, the second case is the more realistic one. The real distribution is a Langevin-distribution [36]:

$$w(\vec{r})_{Langevin}\mathrm{d}r = N_k \exp\left\{\int_0^{r_m} L^{-1}(r/r_m)\mathrm{d}r/l\right\} 4\pi r^2 \mathrm{d}r \tag{24}$$

where L^{-1} is the inverse Langevin-function, r the actual length of the polymer chain, r_m its maximal length, i.e. its contour length, and N_k the normalisation constant. To obtain N_k the integral

$$\int_V w(\vec{r})_{Langevin}\mathrm{d}V = 1 \tag{25}$$

must be solved. This can be done only numerically where $w(\vec{r})_{Langevin}$ has to be expanded into a series. As a consequence, the absolute value of N_k depends on the number of terms used in this series. We restrain ourselves to the first three terms, the result then obtained is

$$N_k\cdot4\pi r_m^3 \int_0^1 x^2 \exp\left(-\frac{3}{2} n_{seg}\left[x^2 + \frac{3}{10}x^4 + \frac{33}{175}x^6\right]\right)\mathrm{d}x = 1 \tag{26}$$

with $x \equiv r/r_m$ and n_{seg} the number of segments per chain.

This integral can be computed by Maple [46], a program whose name can be derived from some combination of the letters in the phrase 'Mathematical manipulation language', but in fact it is simply chosen as a name with a Canadian identity.

A typical result is shown in Fig. 2, where $N_k \pi r_m^2$ is plotted

versus n_{seg}. It can be quite well described by the function

$$N_k\left(n_{seg}\right) = \frac{1}{4\pi r_m^3}\left(111.682 + 4.14616 n_{seg}^{3/2}\right) \tag{27}$$

with $n_{seg} \in [10,\ 10000]$. Next, we transform $w(\vec{r})_{Langevin}$ into spherical coordinates and after that we follow the procedure described by Treloar [37] to compute F_{elas}. Thereby we assume that $\alpha = \alpha_x = \alpha_y = \alpha_z$, i.e. that the expansion of the DNA chain is uniformly in all directions. Then the final result is

$$F_{elas} = k_B T\left(N_k 4\pi r_m^3\right)\frac{1}{\alpha^3}\int_0^1 x^2 \exp\left\{-\frac{3}{2}n_{seg}\left[x^2\frac{1}{\alpha^2} + x^4\frac{3}{10\alpha^4} + x^6\frac{33}{175\alpha^6}\right]\right\}\cdot\left[ln\left(\alpha^3\right) + \left(\frac{3}{2}n_{seg}\left[x^2\left(\frac{1}{\alpha^2}-1\right) + x^4\left(\frac{3}{10}\right)\left(\frac{1}{\alpha^4}-1\right) + x^6\left(\frac{33}{175}\right)\left(\frac{1}{\alpha^6}-1\right)\right]\right)\right]dx \tag{28}$$

A view on this formula shows that the system parameters $x_{methanol}$, $P_{w,PEG}$, and c_{salt} are not explicitly present. They influence the DNA-persistence length and thus they are embodied in the number of segments n_{seg}.

Fig. 3 shows a three-dimensional plot of F_{elas}/k_BT as a function of n_{seg} and α. The most striking appearance is a furrow. This furrow separates the region that can be sufficiently well described by the Gaussian approximation from the region where the Langevin-distribution should be applied.

Finally, we come to the expressions for the translational entropies of the small ions. First, we consider the inner domains. There are two counterions per DNA base-pair. That is, each DNA-molecule can set free $Q = 2\cdot P_{w,DNA}$ counterions, but not all of these counterions are free. Some counterions are territorially bound. According to Manning [38] the average degree of counterion binding is

$$\theta_z = \frac{1}{z}\left[1 - (z/\xi)\right] \tag{29}$$

with z the counterion valence and $\xi = e^2/(4\pi\varepsilon_0\varepsilon k_B T l_0)$the charge density parameter. Here, z is 1, $l_0 = 1.7\cdot10^{-10}$m, and for the dielectric constant of the solvent it holds [34].

$$\varepsilon = 80.6 - 48.5\cdot\frac{w_{methanol}}{100} \tag{30}$$

The salt added is of the stöchiometry $\nu_c : \nu_b$, and its concentration inside the inner domains is c_{salt}^{in}. That is, totally we have $N_{c,DNA}^{in} = (1-\theta_z)Q$ counterions that come from the DNA, $N_{c,salt}^{in} = \nu_c c_{salt}^{in} N_A V_{dom}$ counterions that are from the added salt, and $N_{b,salt}^{in} = \nu_b c_{s,salt}^{in} N_A V_{dom}$ byions. The entropy of mixing is defined as

$$\Delta S_{mix} = -k_B\left[ln\left(\frac{n_+}{n_{total}}\right) + ln\left(\frac{n_-}{n_{total}}\right)\right] \tag{31}$$

with n_+, n_-, and n_{total} the number of positive, negative, and ions totally present. Thus, we have

$$\Delta S_{mix}^{in} = -k_B\frac{V_{dom}}{V_{cell}}\left\{\left[\left(1-\theta_z\right)Q\frac{\phi_{DNA}}{P_{w,DNA}} + \nu_b c_{salt}^{in} N_A\right]\times ln\left[\left(1-\theta_z\right)Q\frac{\phi_{DNA}}{P_{w,DNA}}\right] + \nu_b c_{salt}^{in} N_A V_{cell} + \nu_c c_{salt}^{in} N_A ln\left[\nu_c c_{salt}^{in} N_A V_{cell}\right]\right\} \tag{32}$$

so that the free energy becomes

$$F_{trans}^{in} = k_B T\frac{V_{dom}}{V_{cell}}\left\{\left[\left(1-\theta_z\right)Q\frac{\phi_{DNA}}{P_{w,DNA}} + \nu_b c_{salt}^{in} N_A\right]\times ln\left[\left(1-\theta_z\right)Q\frac{\phi_{DNA}}{P_{w,DNA}} + \nu_b c_{salt}^{in} N_A V_{cell}\right] + \nu_c c_{salt}^{in} N_A ln\left[\nu_c c_{salt}^{in} N_A V_{cell}\right]\right\} \tag{33}$$

At this point it should be noted that $\phi_{DNA}V_{dom}/(V_{cell}P_{w,DNA})$ is unity.

Analogously, we can describe the outer domain. If c_{salt}^{out} is the salt concentration in the outer domain it holds

$$N_c^{out} = \nu_c c_{salt}^{out} N_A\left(1 - n_{DNA}V_{dom}\right)\cdot10^{-3}m^3 \tag{34}$$

$$N_b^{out} = \nu_b c_{salt}^{out} N_A\left(1 - n_{DNA}V_{dom}\right)\cdot10^{-3}m^3 \tag{35}$$

where N_c^{out} and N_b^{out} are the number of counterions and byions in the outer domain, respectively. The total salt concentration is

$$c_{salt} = \left(1 - n_{DNA}V_{dom}\right)c_{salt}^{out} + n_{DNA}V_{dom}c_{salt}^{in} \tag{36}$$

Thus, by combination of these equations we can recalculate c_{salt}^{in} into c_{salt}^{out} or vice versa.

For the translational free energy of the outer domain we have

$$F_{trans}^{out} = \frac{k_B T}{V_{cell}}\left(1 - n_{DNA}V_{dom}\right)\cdot10^{-3}m^3\cdot\left\{2\nu_c c_{salt}^{out} N_A V_{cell} \times ln\left[\nu_c c_{salt}^{out} N_A V_{cell}\right]\right\} \tag{37}$$

where the parameter $\nu_c$is lacking because for NaCl it holds $\nu_c = \nu_b = 1$.

Eqs. (33) and (37) depend directly on the salt concentration employed, c_{salt}, and indirectly on the dielectric constant, ε, of the solvent, but there is no influence of the degree of PEG-polymerisation $P_{w,PEG}$.

It is important to comment the quantities and parameters

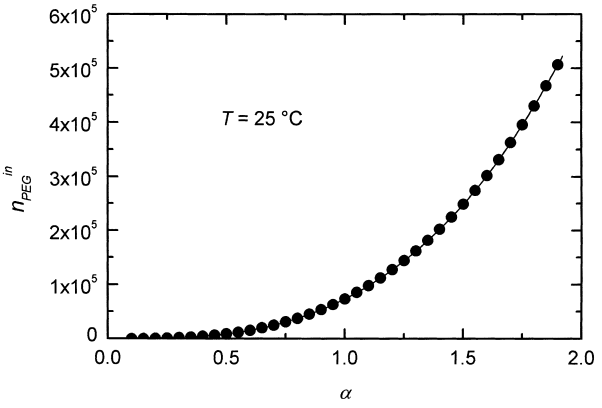

Fig. 4. The number of PEG-molecules, $n_{\mathrm{PEG}}^{\mathrm{in}}$, inside the inner domains versus the expansion coefficient α.

involved in the above equations. c_{salt}, c_{PEG}, $P_{\mathrm{w,PEG}}$, and x_{methanol} are presented by the experimenter. They become systematically varied. Also no problem is the DNA-concentration, c_{DNA}, determining the number n_{DNA} of DNA-domains per $10^{-3}\,\mathrm{m}^3$. As long as the solution is dilute, $n_{\mathrm{DNA}}V_{\mathrm{dom}}\langle\langle 1$, so that in principle we consider a single DNA-molecule.

In conclusion, there remain only three quantities that are unknown a priori. They are the salt concentration, $c_{\mathrm{salt}}^{\mathrm{in}}$, inside the inner domains, the number, $n_{\mathrm{PEG}}^{\mathrm{in}}$, of PEG-molecules in the inner domains, and the expansion coefficient, α, of a DNA-coil. All other quantities are constants or parameters that can be calculated or derived from literature data.

Our task is now to compute the critical PEG-concentration at which the DNA collapses. For this purpose we need three equations. They are the following equilibrium conditions:

- (1) We have the free energies

$$F_{\mathrm{in}} = F_{\mathrm{mix}}^{\mathrm{in}} + F_{\mathrm{elas}} + F_{\mathrm{trans}}^{\mathrm{in}} \tag{38}$$

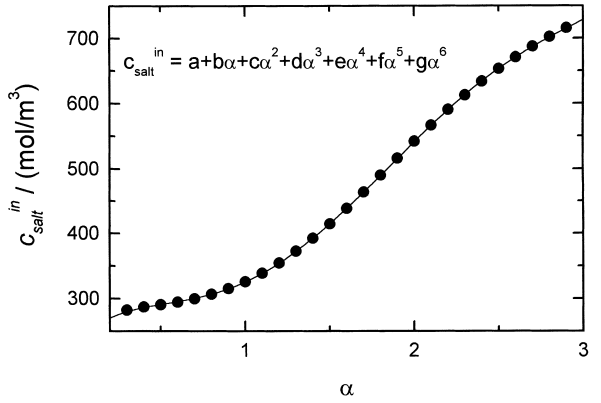

Fig. 5. Salt concentration, $c_{\mathrm{salt}}^{\mathrm{in}}$, inside the inner domains versus the expansion coefficient,α, where $P_{\mathrm{w,PEG}} = 182$, $c_{\mathrm{PEG}} = 50\ \mathrm{kg/m^3}$, $x_{\mathrm{methanol}} = 0\ \mathrm{kg/m^3}$, $c_{\mathrm{salt}} = 0.5$ M, $T = 25$°C, $a = 234.60$, $b = 259.14$, $c = -474.97$, $d = 436.01$, $f = -145.30$, and $g = 16.42$.

and

$$F_{\mathrm{out}} = F_{\mathrm{mix}}^{\mathrm{out}} + F_{\mathrm{trans}}^{\mathrm{out}} \tag{39}$$

From these we calculate the chemical potentials $\mu_i = \partial F/\partial N_i|_{i\neq j,V,T}$. of the PEG-molecules and the salt. At the equilibrium state it holds $\mu_{\mathrm{PEG}}^{\mathrm{in}} = \mu_{\mathrm{PEG}}^{\mathrm{out}}$ and $\mu_{\mathrm{salt}}^{\mathrm{in}} = \mu_{\mathrm{salt}}^{\mathrm{out}}$ so that

$$\frac{\partial\left(F_{\mathrm{mix}}^{\mathrm{in}}/(n_{\mathrm{DNA}}V_{\mathrm{dom}})\right)}{\partial\phi_{\mathrm{PEG}}^{\mathrm{in}}} = \frac{\partial(F_{\mathrm{mix}}^{\mathrm{out}}/(1-n_{\mathrm{DNA}}V_{\mathrm{dom}}))}{\partial\phi_{\mathrm{PEG}}^{\mathrm{out}}} \tag{40}$$

and

$$\frac{\partial\left(F_{\mathrm{trans}}^{\mathrm{in}}/(n_{\mathrm{DNA}}V_{\mathrm{dom}})\right)}{\partial c_{\mathrm{salt}}^{\mathrm{in}}} = \frac{\partial(F_{\mathrm{trans}}^{\mathrm{out}}/(1-n_{\mathrm{DNA}}V_{\mathrm{dom}}))}{\partial c_{\mathrm{salt}}^{\mathrm{out}}} \tag{41}$$

- (2) The osmotic pressure, Π, is defined as

$$\Pi = \sum_i \frac{\partial(F/V)}{\partial\phi_i}|_{i\neq j,V,T}\cdot\phi_i - \frac{F}{V} \tag{42}$$

For the inner domains we find

$$\Pi^{\mathrm{in}} = \frac{\partial\left(F_{\mathrm{mix}}^{\mathrm{in}}/(n_{\mathrm{DNA}}V_{\mathrm{dom}})\right)}{\partial\phi_{\mathrm{DNA}}^{\mathrm{in}}}\phi_{\mathrm{DNA}}^{\mathrm{in}} + \frac{\partial\left(F_{\mathrm{mix}}^{\mathrm{in}}/V_{\mathrm{dom}}\right)}{\partial\phi_{\mathrm{PEG}}^{\mathrm{in}}}\phi_{\mathrm{PEG}}^{\mathrm{in}} + \frac{\partial\left(F_{\mathrm{trans}}^{\mathrm{in}}/n_{\mathrm{DNA}}V_{\mathrm{dom}}\right)}{\partial c_{\mathrm{salt}}^{\mathrm{in}}}c_{\mathrm{salt}}^{\mathrm{in}} - \frac{F_{\mathrm{total}}^{\mathrm{in}}}{n_{\mathrm{DNA}}V_{\mathrm{dom}}} \tag{43}$$

and for the outer domains it holds

$$\Pi^{\mathrm{out}} = \frac{\partial\left(\dfrac{F_{\mathrm{mix}}^{\mathrm{out}}}{1-n_{\mathrm{DNA}}V_{\mathrm{dom}}}\right)}{\partial\phi_{\mathrm{PEG}}^{\mathrm{out}}}\phi_{\mathrm{PEG}}^{\mathrm{out}} + \frac{\partial\left(\dfrac{F_{\mathrm{trans}}^{\mathrm{out}}}{1-n_{\mathrm{DNA}}V_{\mathrm{dom}}}\right)}{\partial c_{\mathrm{salt}}^{\mathrm{out}}}c_{\mathrm{salt}}^{\mathrm{out}} - \frac{F_{\mathrm{total}}^{\mathrm{out}}}{1-n_{\mathrm{DNA}}V_{\mathrm{dom}}} \tag{44}$$

At the equilibrium state both pressures are equal, i.e. $\Pi^{\mathrm{in}} = \Pi^{\mathrm{out}}$.

Eqs (40) and (41), and $\Pi^{\mathrm{in}} = \Pi^{\mathrm{out}}$ build a system of three equations. This system can be solved and used to determine the three unknown parameters α, $n_{\mathrm{PEG}}^{\mathrm{in}}$, and $c_{\mathrm{salt}}^{\mathrm{in}}$. Obviously, this cannot be done analytically, but numerically. The program used is again 'Maple'.

3. Results and Discussion

The calculation procedure is as follows. First, we solve Eq. (40) numerically for a number of values of c_{salt}, $P_{\mathrm{w,PEG}}$, c_{DNA}, and c_{PEG}. The output is a relationship between the DNA-expansion coefficient, α, and the number of PEG-molecules, $n_{\mathrm{PEG}}^{\mathrm{in}}$, inside the inner domains. Fig. 4 shows a

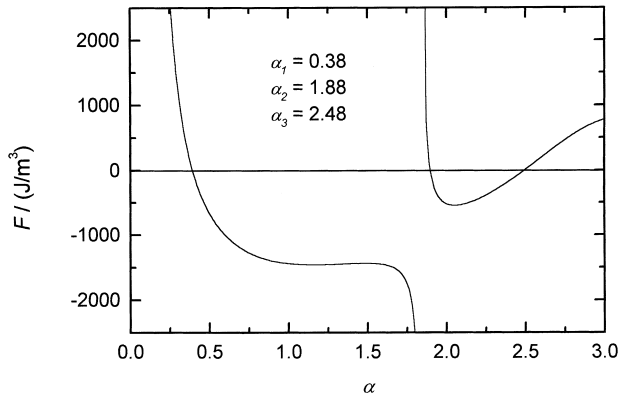

Fig. 6. Free energy versus the expansion coefficient, α, where $P_{w,PEG} = 182$, $c_{PEG} = 50$ kg/m^3, $c_{salt} = 0.5$ M, $x_{methanol} = 0$ kg/m^3 and $T = 25$°C.

typical result. There, c_{PEG} is 50 kg/m^3, $x_{methanol}$ is 0 kg/m^3,$P_{w,PEG} = 182$, and $c_{salt} = 0.5$ M. The curve obtained can be well described by the polynomial fit

$$n_{PEG}^{in} = a + b\alpha + c\alpha^2 + d\alpha^3 \quad (45)$$

where the coefficients *a*, b, *c* and d are -17.85, -85.31, 30.89 and 73832.5. It should be pointed out that these values are dependent on the interval chosen for α. For certain α-values n_{PEG}^{in} becomes negative or complex. Such n_{PEG}^{in}-values are unphysical and thus it is important to specify the α-region for which the n_{PEG}^{in} are physically reasonable. Here, this α-regime is $0.36 \leq \alpha \leq 1.92$.

The second calculation step is the determination of the salt concentration, c_{salt}^{in}, inside the inner domains. It is found by solving Eq. (41). Again the result can be quite well fitted by a polynom. Fig. 5 shows an example.

The last step is the computation of α. For this purpose we insert $n_{PEG}^{in}(\alpha)$ and $c_{salt}^{in}(\alpha)$ into the condition $\Pi^{in} = \Pi^{out}$ and then we vary α as long as this condition is realised. Fig. 6 shows a typical result. We find three solutions for α, a fact also reported by Vasilevskaya et al. [29], but not all of these three α-values are physically reasonable. Here we get the solutions $\alpha_1 = 0.38$, $\alpha_2 = 1.88$, and

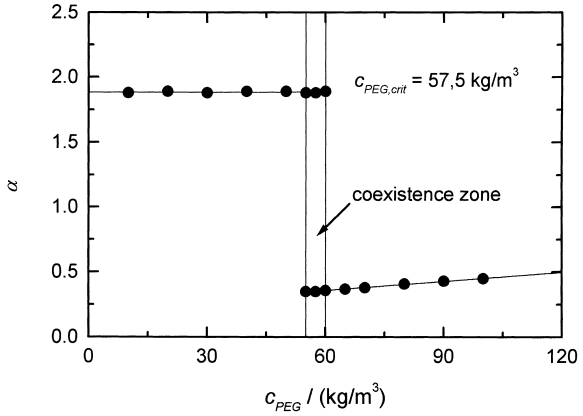

Fig. 7. The expansion coefficient, α, versus the PEG-concentration, c_{PEG}, where $P_{w,PEG}$ is 182, $c_{salt} = 0.5$ M, $x_{methanol} = 0$ kg/m^3 and $T = 25$°C.

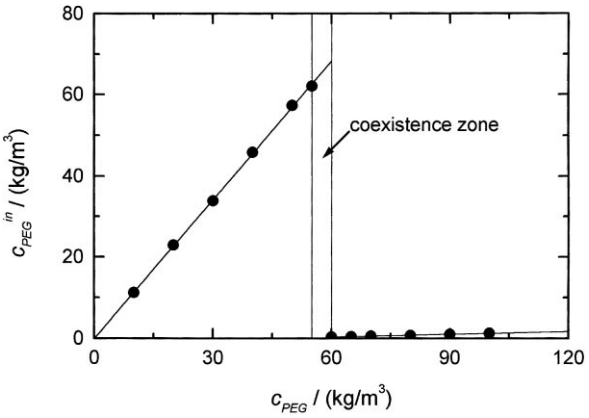

Fig. 8. The PEG-concentration inside a DNA-domain versus the total PEG-concentration with $P_{w,PEG} = 182$, $c_{salt} = 0.5\,M$, $x_{methanol} = 0$ kg/m^3 and $T = 25$°C.

$\alpha_3 = 2.48$, but α_3 lies outside the definition region of α. At α_3, the n_{PEG}^{in}-value becomes negative, so that α_3 is unphysical. That is, there remain only two α-values, α_1 and α_2, where α_1 describes the collapsed state and α_2 the coil state. That means, in this special case we have two DNA-conformations present simultaneously, but this is not generally the case. Mostly, we obtain only one solution for α, i.e. one DNA-conformation, either the coil or the globule state.

A more illustrative interpretation can be given as follows. We distinguish three cases: (1) A α-value of the order of 1 or larger predicts a good compatibility between the PEG and DNA-molecules. PEG-molecules can, practically, freely penetrate inside a DNA-domain. The DNA has a swollen coil conformation and, therefore, the PEG-concentration within the DNA-domain is nearly as large as in the outer domain. (2) α is much smaller than 1. Then the DNA is in its condensed state, and we have practically a perfect segregation between the DNA and the PEG-molecules. (3) We have two α-values, i.e. we are in the transition or coexistence region. Two DNA-conformations coexist side by side,

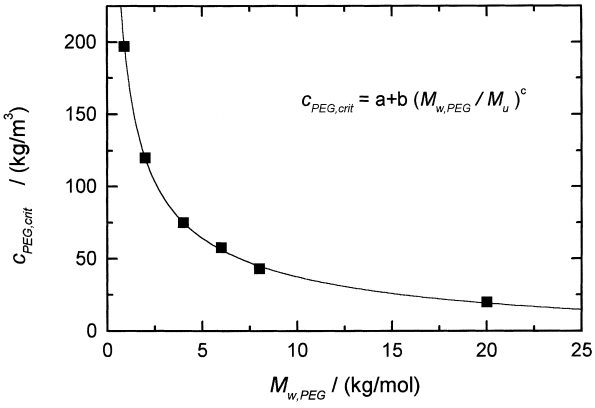

Fig. 9. The critical PEG-concentration, $c_{PEG,critical}$, versus the PEG molar mass where c_{salt} is 0.5 M, $x_{methanol} = 0$ kg/m^3 and $T = 25$°C. Additionally, it holds $M_u = 1$ kg/m^3, $a = 19.22$, $b = 9493$ and $c = 0.556$.

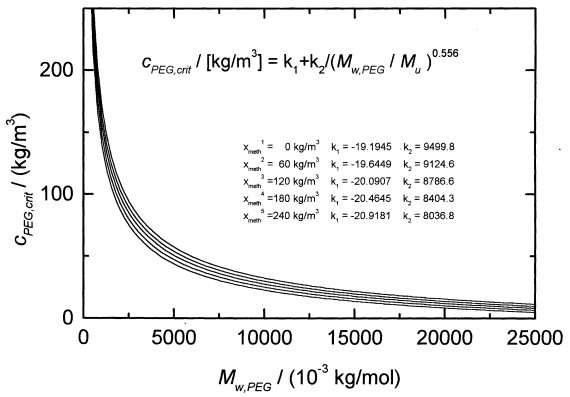

Fig. 10. The critical PEG-concentration, $c_{\mathrm{PEG,c}}$, versus $M_{\mathrm{w,PEG}}$ at various methanol fractions, x_{methanol}, where c_{salt} is 0.5 M and $T = 25°\mathrm{C}$. The upper curve correspondents with $x_{\mathrm{methanol}} = 0\ \mathrm{kg/m^3}$ and the lowest curve with $x_{\mathrm{methanol}} = 240\ \mathrm{kg/m^3}$.

namely expanded DNA-domains with $\alpha > 1$ and contracted DNA-globules with $\alpha < 1$.

The questions are now: (1) What are the influences of the system parameters n_{PEG}, c_{salt}, and $P_{\mathrm{w,PEG}}$ on DNA-condensation and (2) how do the theoretical predictions agree with the experimental results? We can only address the second question in this paper.

We start our discussion with the dependence of α on c_{PEG}. A typical plot is shown in Fig. 7. For PEG-concentrations smaller than $57.5\ \mathrm{kg/m^3}$ the regime of good DNA-PEG compatibility is realised. The values of α are of the order of 1.9 and do not depend on the absolute value of c_{PEG}. In the narrow range of $55.0\ \mathrm{kg/m^3} < c_{\mathrm{PEG}} < 60\ \mathrm{kg/m^3}$ we observe both DNA-conformations side by side. This is the coexistence zone. Unfortunately, theory says nothing about the prozentual ratio of both states. Therefore, we define the middle point of this region, i.e., the concentration $c_{\mathrm{PEG,c}} = 57.5\ \mathrm{kg/m^3}$ as the critical PEG-concentration. There, probably 50% of the DNA-molecules are in the coil-state and the other 50% in the contracted state.

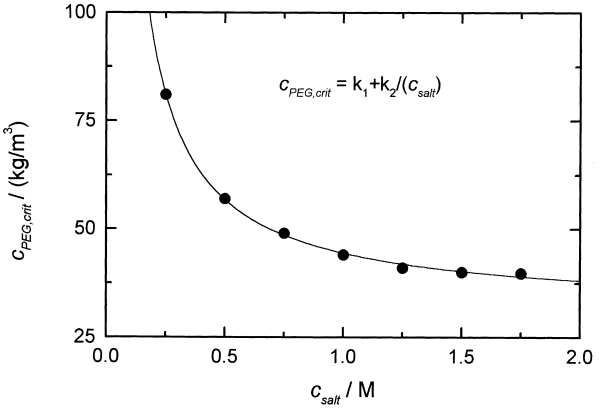

Fig. 11. The critical PEG-concentration versus c_{salt} for $M_{\mathrm{w,PEG}} = 6\ \mathrm{kg/mol}$, $x_{\mathrm{methanol}} = 0\ \mathrm{kg/m^3}$ and $T = 25°\mathrm{C}$. The parameters of the fit formula are $k_1 = 32.13\ \mathrm{kg/m^3}$ and $k_2 = 12260\ \mathrm{kg\ mol/m^6}$.

Instructive is also a plot of the PEG-concentration, $c_{\mathrm{PEG}}^{\mathrm{in}}$, inside the DNA domains versus the total PEG-concentration c_{PEG}. Such a plot is shown in Fig. 8. As long as the DNA and PEG-molecules are compatible, $c_{\mathrm{PEG}}^{\mathrm{in}}$ increases linearly with c_{PEG} where $c_{\mathrm{PEG}}^{\mathrm{in}}$ is nearly as large as c_{PEG}. Then, in the coexistence zone $c_{\mathrm{PEG}}^{\mathrm{in}}$ drops sharply down to a value of nearly zero. That is, the DNA-domains contract. There remain some PEG-molecules inside a DNA-domain, but their number is about two orders of magnitude smaller than in the expanded state.

Fig. 9 illustrates the influence of the PEG molar mass, $M_{\mathrm{w,PEG}}$, on $c_{\mathrm{PEG,c}}$. We see, the lower the molar mass the higher is the PEG-concentration at which the DNA contracts. Since $c_{\mathrm{PEG,c}}$ behaves as $M_{\mathrm{w,PEG}}^{-0.556}$ it is very difficult if not impossible to detect $c_{\mathrm{PEG,c}}$ for PEG samples, having molar masses smaller than $900 \cdot 10^{-3}$ kg/mol. Theoretically, $c_{\mathrm{PEG,c}}$ cannot be larger than the density of pure PEG. Thus, the smallest possible $M_{\mathrm{w,PEG}}$-value at which condensation can take place is $40.5 \cdot 10^{-3}$ kg/mol. This value is nearly as large as $44.05 \cdot 10^{-3}$ kg/mol being the PEG repeat unit molar mass. That is, theoretically also ethylene glycol should induce the transition, but at a very high concentration. The upper limit for $M_{\mathrm{w,PEG}}$ is achieved when $c_{\mathrm{PEG,c}}$ converges to zero. According to Fig. 9 this situation is given at $M_{\mathrm{w,PEG}} \approx 70$ kg/mol. However, the value of this upper molar mass, $M_{\mathrm{w,PEG}}^{\mathrm{upper}}$, depends on the actual value of α. When α converges to zero $M_{\mathrm{w,PEG}}^{\mathrm{upper}}$ converges to infinite. It is therefore not surprising that experiments [39] exist where DNA-condensation is observed with PEG-molecules having molar masses larger than 100 kg/mol.

The most interesting quantity that influences $c_{\mathrm{PEG,c}}$ may be the solvent quality, i.e. the solvent composition. Here, we use a mixture of water and methanol where we have varied the methanol content, x_{methanol}, between 0 and $240\ \mathrm{kg/m^3}$. This corresponds to mass fractions, w_{methanol}, between 0 and 25.2%. The calculation is somewhat more complicated than for pure water because methanol alters a number of parameters. They are among others the Flory–Huggins interaction parameters, the molar volume, the dielectric constant of the solvent, the DNA persistence-length, and the cell volume of the solution. A measure for the change of the cell volume is the ratio of the DNA radius of gyration in pure water to the corresponding radius in the methanol–water mixture. To this approximation it holds

$$V_{\mathrm{cell}}(x_{\mathrm{methanol}}) = V_{\mathrm{cell}}(x_{\mathrm{methanol}} = 0)\left(\frac{< S^2 >_{\mathrm{z,DNA}}^{1/2} t(x_{\mathrm{methanol}} = 0)}{< S^2 >_{\mathrm{z,DNA}}^{1/2} (x_{\mathrm{methanol}})}\right)^3 \quad (46)$$

Additionally, water and methanol interact with each other. They build cluster molecules so that in the statistical average the mass of a solvent–monomer unit increases while simultaneously the number of solvent particles per unit decreases. This coordination effect alters the degree

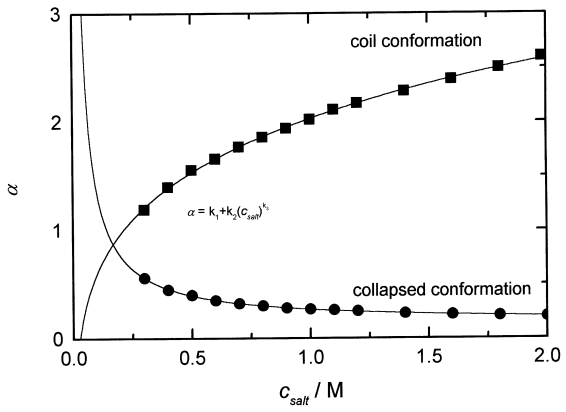

Fig. 12. The expansion coefficient, α, versus the salt concentration, c_{salt}, for both the coiled and the collapsed state. The system parameters are: $x_{methanol} = 0$ kg/m^3, $M_{w,PEG} = 6$ kg/mol and $T = 25$°C.

of polymerisation, P_s, of the solvent. For simplicity we assume that

$$P_s = 1 + x_{methanol}(M_{methanol}/M_{water}) \quad (47)$$

where $M_{methanol}$ and M_{water} are the molar masses of methanol and water, respectively. Further effects, such as the interactions between solvent and the small salt ions, are neglected.

Fig. 10 presents some results. We see a plot of $c_{PEG,c}$ versus $M_{w,PEG}$ where each curve describes a different methanol concentration. In comparison to $M_{w,PEG}$ the influence of $x_{methanol}$ on $c_{PEG,c}$ is rather small. This may be somewhat surprising, but it is in line with the results of measurements.

To describe the dependence of $c_{PEG,c}$ on $x_{methanol}$ as well as on $M_{w,PEG}$, a multiple regression fit is possible. For instance, for $c_s = 0.5$ M we have

$$c_{PEG,c} = k_1(x_{methanol}) + k_2(x_{methanol})\left(\frac{10^{-3}\ \text{kg/mol}}{M_{w,PEG}}\right)^{k_3} \quad (48)$$

with $k_1(x_{methanol}) = -19.2 + 0.00711x_{methanol}$, $k_2(x_{methanol}) = 9500 - 6.08x_{methanol}$, $k_3 = 0.556$, $M_{w,PEG} \in [0.9, 20$ kg/mol], and $x_{methanol} \in [0, 2\ 40$ kg/m$^3]$.

Finally, we come to the influence of the low molar salt. We can expect that c_{salt} alters not only $c_{PEG,c}$, but also α. The higher the salt concentration the stronger the DNA charges are electrostatically screened from each other so that α should decrease.

Fig. 11 shows a plot of $c_{PEG,c}$ versus c_{salt}. We see, $c_{PEG,c}$ decreases continually as c_{salt} decreases. That is, DNA compacting is generated at sufficiently high values of $M_{w,DNA}$ and low salt concentration. The transition point shifts to lower values of $c_{PEG,c}$ the higher c_{salt} becomes. The curvature is asymptotic and converges to the limit $c_{PEG,c} = 32$ kg/m^3. This is understandable because at high enough salt concentration the DNA charges are nearly completely neutralised so that c_{salt} has no influence any more. The same effect can be observed for the DNA-persistence length [40]. Above the limit $c_{salt} = 1$M, l_p no longer depends on c_{salt}.

The correlation between the expansion coefficient, α, and c_{salt}. is also quite interesting. For the expanded coil state (see Fig. 12) α increases as c_s increases while for the collapsed state α decreases or is nearly salt independent. The intersection point of both curves determines the limiting salt concentration below which no DNA condensation takes place. The electrostatic repulsion forces are then so strong that a DNA contradiction becomes impossible. A similar statement was given by Grosberg [25]. He points out that a minimal salt concentration is necessary to induce DNA condensation where we see that this concentration decreases with increasing methanol content.

According to Fig. 12 α rises as c_{salt} increases. This result is opposite to what will be observed when the DNA is dissolved in a solution free of PEG. Thus, it seems that a DNA-molecule may first expand before it collapses. Such an effect is not unusual. It is also observed for neutral polymers in mixed solvents [41].

We finish this section with some statements about the coexistence zone. According to the present model neither the methanol concentration nor the salt concentration have an influence on the width of the coexistence zone. Our experimental investigations do suggest this prediction, but other authors [29] propose that there is a slight increase in the width with increasing salt concentration. The quantity that alters the extension of the coexistence zone is the PEG molar mass. Its width increases as $M_{w,PEG}$ increases. However, the width of the coexistence zone is in all cases rather small. It exceeds never 3 kg/m^3. Therefore, we can state the process of DNA-condensation is a sharp transition, probably of first order.

4. Conclusions

The compacting of a single DNA-molecule dissolved in an aqueous–methanolic salt solution of PEG-molecules was investigated theoretically. A statistical thermodynamic model calculation is presented that directly yields the critical PEG-concentration, $c_{PEG,c}$, for the point at which the coil–globule transition takes place. The most important predictions are: (1) the critical PEG-concentration $c_{PEG,c}$, which is a function of the system parameters, such as the PEG degree of polymerisation, $P_{w,PEG}$, the methanol content, $x_{methanol}$, and the concentration, c_{salt}, of the added salt increases as $P_{w,PEG}$ decreases, it decreases as $x_{methanol}$ increases, and $c_{PEG,c}$ is the more smaller the higher c_{salt} is. (2) There exists a coexistence region where DNA-coils coexist side by side with collapsed DNA-globules. The width of this coexistence zone is very small and more or less independent on the system parameters. Thus, the transition is discrete and not gradual. (3) The average size of a condensed DNA is both independent on the solvent conditions as well as on the degree of PEG polymerisation. The latter influences only the position of $c_{PEG,c}$. The expansion coefficient, α, is of the order of 0.4 for the contracted state and of the order of

1.8 for the expanded coil. It decreases both as the salt concentration increases as well as the methanol content increases. These results are in line with experimental observations [42]. However, some critical comments are necessary.

The model calculations presented here are not of purely theoretical nature. They contain some empirical elements. For instance, we have used empirical relations to describe the influence of the methanol content on $[\eta]_{DNA}$ and $[\eta]_{PEG}$ in Eq. (20).

Another problem is the relatively large number of parameters that have to be known a priori. We mention only the DNA-persistence length, l_p, the Flory–Huggins parameters, χ_{ij}, or the DNA-eigenvolume per repeat unit. For some of these quantities such as l_p one can find true literature data, but these data are affected by errors of measurement and they depend on the method used for their determination. Other parameters, such as the Flory–Huggins parameters χ_{ij}, depend on the solvent quality and thus they are coupled with each other. Therefore, assumptions are necessary which oversimplify the real situation. For instance, it is unrealistic to assume that the PEG–DNA segment–segment interactions are equal for the expanded coil and the collapsed state. It is more likely that χ_{PD} depends on both $c_{PEG,c}$ as well as on $P_{w,PEG}$.

A principal problem is also the right choice for the cell-volume. We can use the volume of a naked, unsolvated DNA repeat unit, but we can also use a solvated DNA unit or something else. Since the DNA persistence length alters with the solvent composition it is also unclear how large such a repeat unit is. V_{cell} may be different for the coil and the collapsed state. Here, we have chosen V_{cell} so that the agreement between theory and experiment is the best possible, but this is scientifically inexact.

There is a further oversimplification. We have divided all quantities into two groups. One kind of quantities describe the inner DNA domains and the other refer to the outer domain. The only exception is the solvent. It is assumed that the solvent composition, i.e. the molar ratio of methanol to water is the same for both the inner domains as well as for the outer domain. This seems unrealistic. The system is not a four but a five component system (DNA, PEG, salt, water and methanol). It is likely that the methanol content in the inner domains is different from that of the outer domain. At the equilibrium state it would hold $\mu^{in}_{methanol} = \mu^{out}_{methanol}$, but it is very difficult if not impossible to formulate an expression for $\mu_{methanol}$ that is free of unknown parameters.

Additionally, there are several other effects that are neglected. For instance, there is no energy term that takes into account the electric interactions between the charged species. We have also neglected influences such as selective solvent adsorption, DNA surface tension, or DNA-molar mass.

In summary, the model calculations presented yield realistic predictions. The results correspondents with those observed experimentally, but to our taste the model contains to many parameters. The reason is the system, it is simply to complex.

Acknowledgements

This work was supported by the ‘Fonds der Chemischen Chemie’. It is also a great pleasure to thank Prof. Lechner for sustained interest and making his laboratory facilities available.

References

[1] McGhee JD, Felsenfeld G. Ann. Rev Biochem 1980;49:1115.
[2] Riemer SC, Bloomfield V. Biopolymers 1978;17:785.
[3] Carroll D. Biochemistry 1972;11:421.
[4] Suwalsky M, Traub W. Biopolymers 1972;11:2223.
[5] Ong EC, Fasman GD. Biochemistry 1976;15:477.
[6] Gosule LC, Schellman JA. J Mol Biol 1978;121:327.
[7] Shin YA, Eichhorn GL. Biopolymers 1977;16:225.
[8] Whitlock JP, Stein AJ. Biol Chem 1978;253:3857.
[9] Geiduschek EP, Gray IJ. Am Chem Soc 1956;78:879.
[10] Gray DM, Taylor TN, Lang. Biopolymers 1978;17:145.
[11] Cheng SM, Mohr SC. Biopolymers 1975;14:663.
[12] Maniatis T, Venable JH, Lerman LS. J Mol Biol 1974;84:37.
[13] Lerman LS. Proc Natl Acad Sci USA 1971;68:1886.
[14] Griffith JD. Proc Natl Acad Sci USA 1976;73:563.
[15] Porschke D. Biochemistry 1984;23:4821.
[16] Porschke D. Biopolymers 1985;24:1981.
[17] Oosawa F. Polyelectrolytes, 1971.
[18] Manning GS. Biopolymers 1980;19:37.
[19] Manning GS. Biopolymers 1983;22:689.
[20] Manning GS. NATO ASI. SER. E. 1987;133:169.
[21] Post CB, Zimm BH. Biopolymers 1979;18:1487.
[22] Post CB, Zimm BH. Biopolymers 1982;21:2123.
[23] Grosberg AY. Biophysics 1979;24:30.
[24] Grosberg AY, Erukhimovitch IY, Shokhnovitch EI. Biopolymers 1982;21:2413.
[25] Grosberg AY, Zhestkov AV. J Biomol Struct Dyn 1986;3:859.
[26] Marquet R, Houssier C. J Biomol Struct Dyn 1991;9:159.
[27] Vasilevskaya VV, Khokhlov R. Macromolecules 1992;25:384.
[28] Vasilevskaya VV, Starodubtzev SG, Khokhlov AR. Adv Polym Sci 1993;109:123.
[29] Vasilevskaya VV, Khokhlov AR, Matsuzawa Y, Yoshikawa K. Chem Phys 1995;102:6595.
[30] Ebert G. Biopolymere. Stuttgart: Teubner, 1993.
[31] Periodic table of the elements. New York: Sargent-Welcher, Scientific Company, 1980.
[32] Flory PJ. Statistical mechanics of chain molecules. New York: Interscience, 1969.
[33] Kok CM, Rudin A. J Appl Polym Sci 1981;26:3583.
[34] Landolt-Börnstein, Neue Serie, Gruppe 4, Band 1b, K.-H. Hellwege. Berlin: Springer.
[35] Flory J. Macromolecules 1978;11:6.
[36] Yamakawa H. Modern theory of polymer solutions. New York: Harper and Row, 1971.
[37] Treloar LRG. The physics of rubber elasticity. Oxford: Clarendon Press, 1958.
[38] Manning GS. Q Rev Biophys 1978;11:179.
[39] Louie D, Serwer P. J Mol Biol 1994;242:547.
[40] Nordmeier E, Dauwe W. Polym J 1992;24:229.
[41] Nordmeier E, Lechner MD. Macromolecules 1991;24:2529.
[42] Kleideiter G, Nordmeier E. Polymer 1999;40:4025.
[43] Gehrke SH, Lee P. In Tyle P, editor. Specialized drug delivery systems: manufacturing and production technology. New York: Marcel Dekker, 1989:333.
[44] Ricka J, Tanaka T. Macromolecules 1984;17:2916.
[45] Siegel RA. Firestone E. Macromolecules 1988;21:3254.
[46] Char BW, Geddes KO, Watt SM. Maple 5, language reference manual. New York: Springer, 1991.