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# Capillary electrophoretic study of the complex formation between DNA and cationic surfactants

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**Abstract**

Due to the growing interest in the use of cationic surfactants for the construction of liposomal genetic delivery systems, the study of complex formation between DNA and quaternary ammonium detergents is of fundamental importance. In this context, we undertook the study of this complex formation using capillary zone electrophoresis (CZE) with suppressed electroosmotic flow, a technique that allowed us to both monitor the change in mobility of DNA as a function of added surfactant in a precise and reproducible manner and evaluate the potential of CZE to reflect the change in hydrodynamic friction upon binding. Nevertheless, CZE must be applied with caution for binding studies where strong cooperativity occurs, because of the presence of peak splitting at concentrations close to the half-point of binding. Also, a comparison between this experiment and Manning's polyelectrolyte transport properties theory on one hand and Tirado and Garcia de la Torre expression for hydrodynamic friction of rod-like molecules on the other hand is given. © 1998 Published by Elsevier Science B.V.

*Keywords:* Complexation; DNA; Surfactants

## 1. Introduction

The complex formation between polymers and oppositely charged surfactants has generated considerable interest in the last few years [1–15]. The interaction between DNA and oppositely charged amphiphilic molecules has attracted special attention due to the importance of such systems in the construction of non-viral genetic delivery systems [16]. Some previous reports [17] of binding isotherms of N-alkyltrimethylammonium bromides with DNA indicate that the cationic surfactant binds to the negatively charged DNA in a strongly cooperative manner at a specific concentration called the critical aggregation concentration [17,18] which depends on the concentration of NaCl in the medium. This binding is usually followed by phase separation, and, in the case of high-molecular-mass DNA in very dilute solution, by chain collapse [19]. However, many aspects of surfactant interactions with DNA are not yet completely understood. In particular, although the binding of surfactants to oppositely charged polymers is generally considered to be a micelle formation process around the polymer molecule [2,5], this structure is still hypothetical because the precipitation of complexes currently precludes structural investigations. In a previous work [20], we showed that the use of short fractionated fragments of DNA at very low concentration allowed us to study this complexation by dynamic light scattering (DLS) without precipitation of the complexes formed. This study confirmed a two stage binding process where, in the first stage, surfactant cations exchange with condensed sodium counterions creat-

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ing micelle-like structures on the DNA surface without change in the DNA apparent charge. In the second stage, more surfactant binding causes a progressive charge neutralisation of DNA and phase separation.

In principle, CZE is well suited to study interactions between oppositely charged molecules. Large changes in electrophoretic mobility are expected as the result of binding. Also, fundamental information about electrostatic effects in the binding mechanism can be extracted. In the present work, we assessed the potential of CZE to investigate this complex formation. We examined the change in the electrophoretic mobility of DNA fragments as a function of added surfactant in order to see if the change in the hydrodynamic friction upon complexation resulted in a similar change in electrophoretic mobility. In this context, we undertook the study of this complex formation using capillary zone electrophoresis with suppressed electroosmotic flow, a technique that allowed us to monitor the change in mobility of DNA as a function of added surfactant in a precise and reproducible manner. Nevertheless, capillary electrophoresis must be applied with caution for binding studies where strong cooperativity occurs. In this case, the presence of peak splitting at concentrations of ligand close to the half point of binding limits the applicability of this otherwise very sensitive method. Also a comparison between experiments and some theoretical concepts was made in an attempt to explain the degree of mobility change upon complexation. We used expressions derived by Tirado and Garcia de la Torre [21,22] for the estimation of the hydrodynamic translational diffusion coefficient and frictional coefficient of rod-like molecules. The hydrodynamic frictional coefficient is not sufficient to describe the mobility of polyions over a broad range of ionic strengths due to electrostatic interactions between the DNA fragment and its surrounding ionic atmosphere. On the other hand, a set of equations giving account of the electrophoretic behaviour of polyions with high charge density was developed by Manning on the basis of his counterion condensation theory [23,24]. Although the ionic strength dependence of electrophoretic mobility was satisfactorily described, the expressions were derived for infinitely long thin polyions at low ionic strength, where electrostatic effects dominate and hydrodynamic friction is neglected. We propose that to describe the change of electrophoretic mobility upon surfactant complexation the hydrodynamic friction has to be included.

## 2. Experimental

### *2.1. Capillary electrophoresis apparatus*

All experiments were carried out on a P/ACE 2100 system (Beckman, Fullerton, CA, USA) monitored by a PS/2 computer (IBM), using Gold software (Beckman). Data collection was performed with the same software. Samples were loaded by a 3 s pressure injection at the cathodic end of a fused-silica capillary of 27 cm×75 μm I.D. At the temperature of the experiments (25°C), this injection time was estimated to correspond to a plug length of 6 mm. The UV detection was performed through the capillary at 20 cm from the inlet at 254 nm. In order to ensure a good dissipation of the Joule effect, the voltage applied, while constant for a set of experiment, was adjusted so as the power dissipated did not exceed 0.3 W or 1.1 W/m. It is well known [25] that cationic surfactants have a tendency to adsorb on the negatively charged silica surface, leading to a change in the rate and direction of the electroosmotic flow. In order to avoid some non-reproducibility in the DNA elution time due to this partial electrostatic adsorption of the cationic surfactant on the capillary surface, we decided to opt for the radical approach of coating the capillary with linear polyacrylamide using a technique derived from Hjerten [26]. This coating procedure ensured a very low electroosmotic flow which was estimated to be lower than $7.5 \cdot 10^{-10}$ $m^2\ V^{-1}\ s^{-1}$. Due to the strong electrophoretic mobility of DNA, this elimination of the electroosmotic flow had the added advantage of leading to small elution times, resulting in a good detection sensitivity.

### *2.2. Reagents and buffer preparation*

Buffer and sample solutions were prepared with distilled water deionized and filtered using a Milli-Q RG system (Millipore, Watford, UK). Decyltrimethylammonium bromide (DTAB) and dode-

cyltrimethylammonium bromide (DoTAB) were of analytical grade (purity of 97% or higher from Lancaster MTM Research Chemicals) and were recrystallised at least twice in acetone prior to use. All buffer reagents were of analytical grade from Sigma or Aldrich (Sigma-Aldrich, Dorset, UK) and were used as received. In order to study the complex formation in different ionic strength buffers, we prepared a stock concentrated buffer [25 m*M* 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), 10 m*M* EDTA, 50 m*M* NaBr] at the $pK_a$ of HEPES (pH=7.5) and diluted it accordingly. The dilution did not result in any shift in the pH value of the buffer, ensuring constant electrolyte composition throughout the experiments.

### *2.3. Preparation and characterisation of DNA fragments*

The DNA used was the sodium salt of calf thymus DNA Type I (Sigma), and was prepared by dissolving in a citrate buffer to a concentration of 3.5 mg/ml (50 m*M* trisodium citrate, 10 m*M* EDTA, pH=7.5). After dissolution, proteins and excess buffer salts were removed by phenol extraction and cold ethanol precipitation. The sample was then dialysed for 2 days against citrate buffer to remove residual phenol and ethanol. Short DNA fragments were prepared by sonication at low intensity using an ultrasonic processor VC50 (Sonics and Materials, Danbury, CT, USA) with titanium probe. The solution was kept chilled in an ice/water bath under a nitrogen atmosphere. The progress of fragmentation was monitored by capillary gel electrophoresis (CGE) and the sonication was stopped after 16 h, at which time the DNA distribution was as follows:

$M_n = 1.17 \cdot 10^5$ (178 bp),

$M_w = 1.62 \cdot 10^5$ (245 bp),

$M_w/M_n = 1.38$

where $M_n$ = number-average molecular mass, $M_w$ = mass-average molecular mass and bp = base pairs.

The sonicated solution was then centrifuged to remove titanium particles, and dialysed against HEPES buffer (0.5 m*M* HEPES, 0.1 m*M* EDTA, pH=8.1) at 4°C for 2 days. After dialysis the DNA solution concentration was determined to be 1.8 mg/ml and it was kept at −20°C. In order to obtain monodisperse fragments, we then proceeded to the fractionation of the DNA sample by size exclusion chromatography on a 50 cm×2.5 cm I.D. glass column filled with Sephacryl S-500 HR gel (Sigma). The mobile phase was a buffered 10 m*M* HEPES, 10 m*M* EDTA, 50 m*M* NaCl aqueous solution at pH 7.5. Detection was performed with a Knauer differential refractometer A0298 operating at 950 nm. Two ml of a 9 mg/ml solution of sonicated DNA was injected and eluted at a flow-rate of 2 ml/min. Six DNA fractions were collected and the fraction with an elution volume between 190 and 200 ml was kept for further work.

The characterisation of this fraction, as well as of the unfractionated sonicated DNA was then performed by CGE. Coated capillaries (27 cm×75 μm I.D.) were filled with a solution of 3% (w/w) linear polyacrylamide in 100 m*M* Tris–boric acid, 2 m*M* EDTA (pH=8) electrolyte (TBE buffer). The DNA samples, as well as the DNA ladder (step ladder 50 bp 50–3147 bp from Sigma) were diluted to 50 μg/ml in the TBE buffer and samples were injected electrokinetically at the cathodic end of the capillary (5 kV for 5 s) and separated at 5.4 kV (200 V/cm) at 25°C. UV detection was performed through the capillary at 7 cm from the anodic end at 254 nm. By comparing the elution pattern of the fraction with the step ladder fragments, the DNA distribution of the retained fraction from SEC was calculated as follows: $M_n = 1.33 \cdot 10^5$ (201 bp), $M_w = 1.50 \cdot 10^5$ (228 bp), $M_w/M_n = 1.13$. This treatment of sonication followed by fractionation allowed us therefore to work on quite monodisperse DNA fragments of 200±20 bp.

## 3. Results and discussion

### *3.1. Complex formation and electrophoretic mobility*

In order to evaluate the potential of CE for detecting the complex formation between short DNA fragments and cationic surfactants, we undertook the study of this complexation in the case of DoTAB at different ionic strengths. As shown in Fig. 1, a

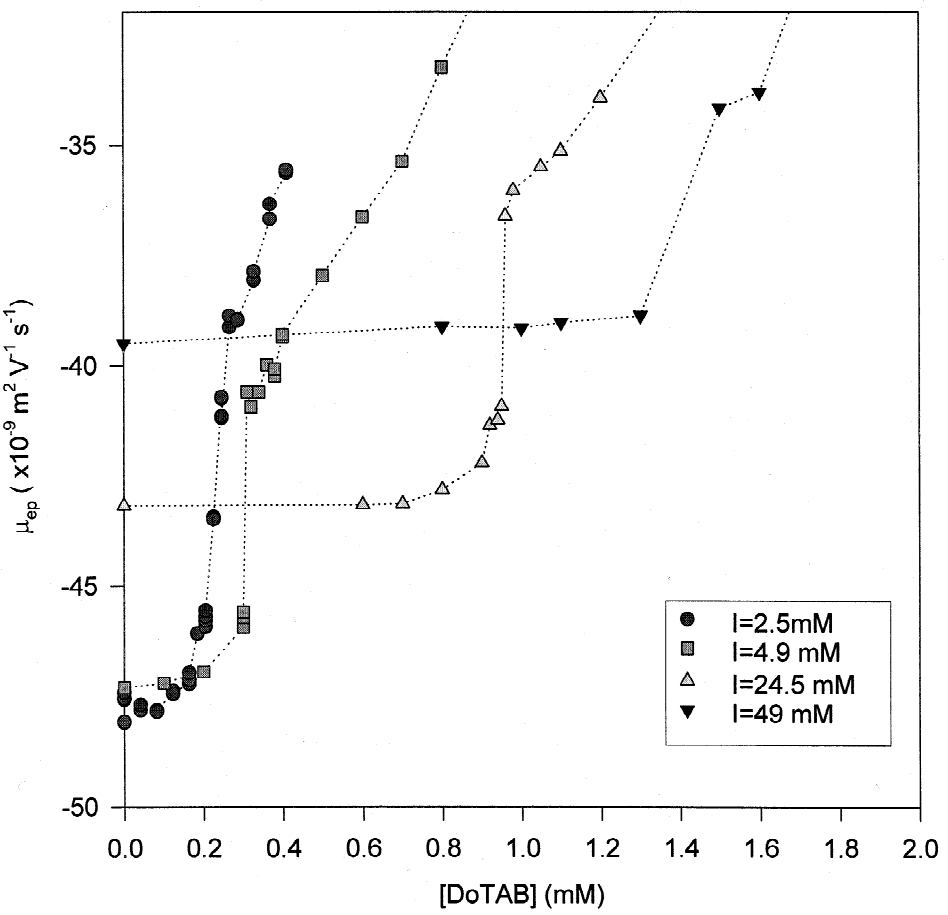


Fig. 1. Evolution of electrophoretic mobility of DNA as a function of DoTAB for background electrolytes of various ionic strengths.

dramatic change in the DNA mobility is observed upon addition of surfactant to the electrolyte. This change occurs at a specific concentration depending on the ionic strength of the background electrolyte and is in good agreement with the literature data [17].

This sharp change in mobility reflects the highly cooperative nature of the first stage of binding.

### *3.2. Peak splitting: a reflection of cooperativity*

If we focus on the binding region around the critical aggregation concentration (Fig. 2), in the case of HEPES 5 buffer (5 m*M* HEPES, 2 m*M* EDTA, 10 m*M* NaBr, pH 7.5, ionic strength $I = 24.5$ m*M*), we can observe an interesting phenomenon of peak splitting.

This phenomenon was described by Ermakov et al. [27] for the zone electrophoresis of a weak base in a buffer with a pH close to the p$K_a$ of the base. The large concentration of a weak base in the sample plug in a relatively dilute buffer of pH close to the dissociation constant of the sample resulted in a 'lock-in' effect where a portion of the sample eluted with the electrophoretic mobility of the base fully protonated while some residual concentration of the sample was not neutralised and eluted under the influence of the electroosmotic flow alone. In our case, the concentration of DNA in the sample is always lower by at least an order of magnitude than the concentration of the surfactant in the buffer (case

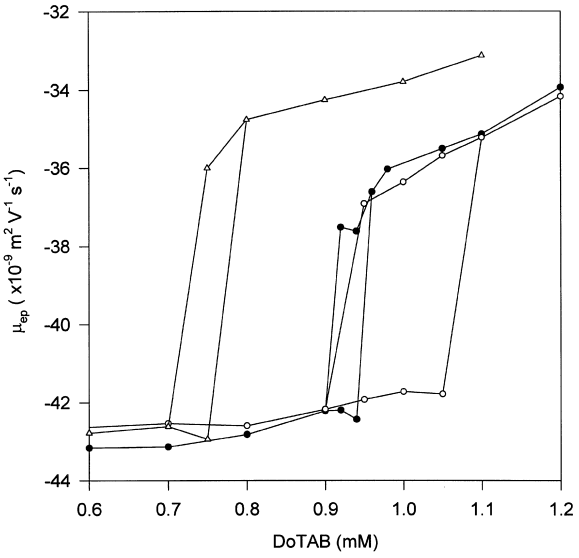


Fig. 2. Concentration range of peak splitting as a function of DNA concentration. (● 10 μg/ml; ○ 50 μg/ml; △ 50 μg/ml in 1.24 m*M* DoTAB; see text for details).

of [DNA]=50 μg/ml corresponds to $1.5 \cdot 10^{-4}$ *M* or 6 times less than [DoTAB]). Nevertheless, the cooperativity of the DNA-surfactant binding is very high compared to the dissociation equilibrium in [27] and this could be an important factor contributing to this lock-in effect. In order to minimise this effect, we tried firstly to inject the DNA sample in a buffer containing a concentration of surfactant (1.24 m*M*) higher than the critical aggregation concentration (Fig. 2, open triangles). This mode of injection resulted in an opposite effect whereby the DNA–DoTA complex was locked-in and the binding curve was displaced towards lower surfactant concentrations. The decrease of injection time from 3 to 1 s, which corresponds to a decrease in the injection plug length from 6 to 2 mm, did not lead to a substantial improvement (data not shown). Also, the electrokinetic injection of DNA, an approach previously rejected by us on the grounds that we wanted to control the DNA concentration throughout the experiment, did not eliminate the lock-in effect, whether or not the DNA sample contained DoTAB (data not shown). Finally, and following the conclusions of Ermakov, we tried to inject DNA in pure buffer in decreasing concentrations down to 10 μg/ml ([DNA]=$3 \cdot 10^{-5}$ *M*), the concentration which corresponds to the minimum concentration for a good detection of the DNA sample. As can be seen in Fig. 2 (black circles), this decrease in DNA concentration leads to an improvement in the sense that the range of DoTAB concentration over which peak splitting occurs is approximately decreased from 0.15 to 0.04 m*M*. Ermakov et al. presented a theory describing the peak splitting phenomenon when the pH of the buffer is close to the known value of p$K_a$ of the solute molecule. The possibility of extracting information about the binding curve from peak splitting in the present case of complex interaction remains to be seen.

### *3.3. Polyelectrolyte condensation and electrophoretic mobility*

In order to apply his counterion condensation theory [24] to the transport properties of polyelectrolytes, Manning [23,24] developed the Kirkwood theory of the translational friction coefficient for macromolecules composed of identical subunits. His major assumption in this derivation was the representation of the polymer as a linear array of point charges of negligible hydrodynamic friction. His final result is shown in Eq. (1):

$$\mu = \frac{4\varepsilon_0 \varepsilon k_B T}{3\eta q |z_1|} |\ln(\kappa b)|[\alpha/\beta] \quad (1)$$

where $\varepsilon$ is the dielectric constant of the solvent, $\varepsilon_0$ is the vacuum permittivity, $k_B$ the Boltzmann constant, $T$ the absolute temperature, $\eta$ the solvent viscosity, $q$ the elementary charge and $|z_1|$ the valence of the counterion. The logarithmic term in Eq. (1) shows that the interaction between each pair of subunits is attenuated not only by the inverse dependence on distance b between charge points but also by the oppositely charged Debye-Huckel atmosphere of length $\kappa^{-1}$ that surrounds each charge and moves in the opposite direction under the influence of an electric field. The ratio $[\alpha/\beta]$ expresses the asymmetry field or relaxation field due to the distortion of the Debye-Huckel ionic atmosphere by the external electric field. This equation reflects well the electrophoretic behaviour of long DNA fragments as a function of ionic strength in particular and the notable independance of the mobility on polymer

length. In order to evaluate this theory for the case of small rigid rod-like DNA fragments, we proceeded to the evaluation of the electrophoretic mobility of our monodisperse fragments as function of the ionic strength (Fig. 3).

As predicted by Manning's theory, the linear dependence on the logarithm of ionic strength is observed experimentally, proving that the electrostatic contribution to the mobility is accurately described by this model. Nevertheless, this representation of the electrophoretic mobility of the polyelectrolyte cannot predict any change in hydrodynamic characteristics.

### *3.4. Hydrodynamic friction and electrophoretic mobility*

The dramatic change in mobility of around 25% upon complexation coincides in the case of the lowest ionic strength buffer ($I=2.2$ m$M$) with a similar increase in diffusion coefficient as evaluated by dynamic light scattering. Calculations based on the expression of translational diffusion coefficient derived by Tirado and Garcia de la Torre [21,22] allowed us to deduce [20] that this phenomenon could be attributed to the formation of a micelle-like structure on the surface of the DNA, the surfactant tails protruding into the solvent while the ionic groups are close to the DNA surface. This interpretation explains quite successfully the change in diameter from 2.7 to 6.3 nm of the polymer during complexation which corresponds to an increase in diameter equal to the length of two surfactant molecules.

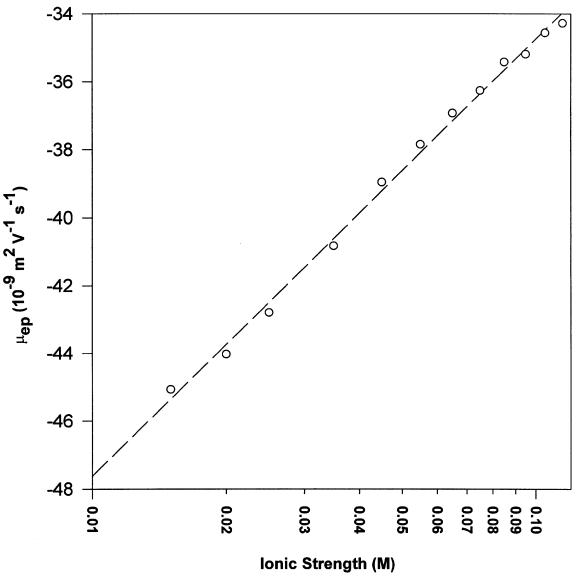


Fig. 3. Evolution of electrophoretic mobility of DNA as a function of ionic strength in background electrolyte.

In the limit of low ionic strength, the electrophoretic mobility can be described as $Q_{\mathrm{eff}}/f_{\mathrm{H}}$ where $Q_{\mathrm{eff}}$ is the effective charge of the polyion and $f_{\mathrm{H}}$ its hydrodynamic friction coefficient as defined according to Kirkwood's theory by Tirado and Garcia de la Torre (Eq. (2)).

$$f_{\mathrm{H}} = 3\pi\eta L/[\ln(p) + \gamma] \tag{2}$$

where $p$ is the aspect ratio (length over diameter), and $\gamma$ is the end effect correction. If the effective charge of the DNA fragments is calculated according to Manning's condensation theory [23], i.e.:

$$Q_{\mathrm{eff}} = 4\pi\varepsilon\varepsilon_0 k_B TNb/q|z_1| \tag{3}$$

we can write the electrophoretic mobility as:

$$\mu = \frac{2\varepsilon\varepsilon_0 k_{\mathrm{B}} T}{3\eta q|z_1|}[\ln(p) + \gamma] \tag{4}$$

Surprisingly, the electrophoretic mobility measured at the lowest ionic strength buffer is well represented by this calculated value (Fig. 1). It is not surprising then, that the amplitude of the decrease of electrophoretic mobility upon binding (Fig. 1) also agrees well with the increase in diffusion coefficient observed by dynamic light scattering. In Fig. 1 a relative decrease in the amplitude of the electrophoretic mobility change upon binding as the ionic strength increases is shown. This observation can be qualitatively explained by the fact that the expression for the electrophoretic mobility in Eq. (4) is no longer valid and that electrostatic screening can no longer be neglected when the ionic strength of the background electrolyte is increased.

### *3.5. Complexation of DNA with DTAB: a case of mixed regime*

Finally, we undertook a study of the complex

formation between the same short DNA fragments and DTAB.

This surfactant possesses the same ionic head as DoTAB, but its hydrophobic tail is shorter by two methylene groups. This surfactant is therefore thought to interact less strongly and with less cooperativity than DoTAB with DNA because the gain in energy due to the inclusion of the hydrophobic tails in a micelle-like environment will be lower. Therefore, the critical aggregation concentration for DTAB should be relatively higher than for DoTAB and the influence of the salt on the bulk electrolyte ionic concentration more pronounced. In a very low ionic strength buffer, the overall salt concentration will then be dictated by the concentration of surfactant. In Fig. 4 the evolution of the electrophoretic mobility of DNA as a function of the added concentration of DTAB in a buffer of moderate ionic strength is shown (5 m*M* HEPES, 2 m*M* EDTA, 10 m*M* NaBr pH 7.5, $I = 24.5$ m*M*).

The general effect of addition of DTAB on the electrophoretic behaviour of DNA is a monotonic decrease of the electrophoretic mobility up to 7 m*M* where a quite abrupt change occurs. After the addition of 9.5 m*M* of surfactant, the DNA peak becomes multiple and irreproducible and reflects aggregation. In order to explain this general trend of decrease of mobility before the complexation at around 7.5 m*M* of DTAB, we have to take into account the change in background electrolyte ionic strength, as well as its composition and its possible influence on the electrophoretic friction coefficient. Indeed, by adding 10 m*M* to the background electrolyte, we have increased the ionic strength from

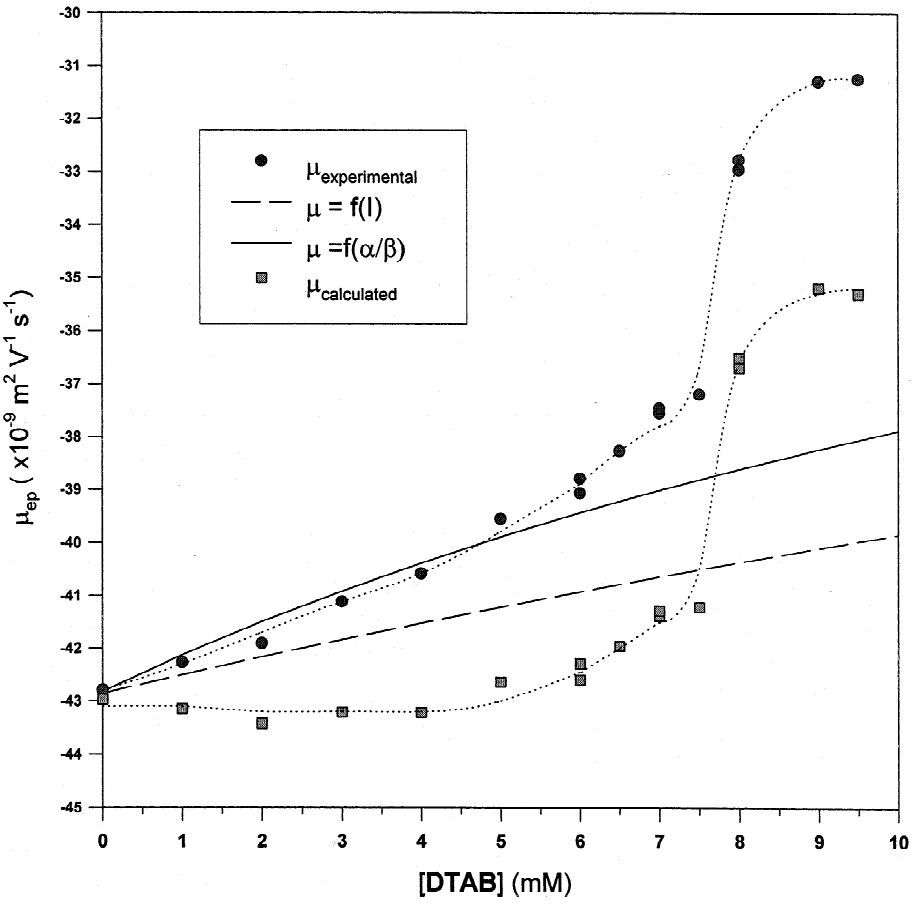


Fig. 4. Evolution of electrophoretic mobility of DNA as a function of DTAB.

24.5 m*M* to 34.5 m*M* or nearly 41%. This increase will be reflected by a substantial decrease of the Debye length $\kappa^{-1}$ and therefore will lead to a decrease of the electrophoretic mobility as expressed by Eq. (1). The influence of the ionic strength so calculated is shown on Fig. 4 [dotted line $\mu = f(I)$]. However, it can be seen that this influence is quite small over the range of added surfactant studied and reflects only partially the experimental observation. Another aspect of the addition of surfactant in quite large concentration in the background electrolyte is the substantial change in buffer composition. While the co-ion bromide is still the predominant anion in the electrolyte, the relative composition in counterion will change from 100% sodium to 65% sodium 35% DTA. This significant change in buffer composition will affect the asymmetry field of the ionic atmosphere around DNA even if no binding occurs. According to Manning [24], the asymmetry field correction $\alpha/\beta$ in Eq. (1) takes the form, in the case of monovalent added salt:

$$\alpha = 1$$
$$\beta = 1 + \frac{1}{6}\,\mu_p\left(\frac{1}{\mu_1} + \frac{1}{\mu_2}\right) \qquad (5)$$

where subscripts 1 and 2 relate to co-ion and counterion respectively and $\mu_p$ is the polyelectrolyte mobility influenced by charged solvent but not by asymmetry. In order to assess the change in field asymmetry, we take the mobility of small ions as their respective mobility at infinite dilution instead of their value in the same electrolyte without polyelectrolyte. This simplification is of minor importance [24]. From literature data [28] we established the electrophoretic mobility of the co-ion $\mu_{Br} = 81 \cdot 10^{-9}$ $m^2\ V^{-1}\ s^{-1}$ and of the counterions $\mu_{Na} = 52 \cdot 10^{-9}$ $m^2\ V^{-1}\ s^{-1}$ and $\mu_{DTA} = 25 \cdot 10^{-9}$ $m^2\ V^{-1}\ s^{-1}$. As the average mobility of counterions in the ionic atmosphere will decrease upon addition of large concentrations of DTAB, the influence of field asymmetry on the electrophoretic mobility of DNA will increase (Fig. 4). Fig. 4 shows that this effect is largely responsible for the experimental decrease of the mobility prior to binding. In order to validate this approach, we then calculated the electrophoretic mobility of DNA without electrostatic influences due to the change in ionic strength and field asymmetry (squares of Fig. 4). This curve shows clearly the change in mobility upon binding alone and is similar in shape to the ones observed for DoTAB.

## 4. Conclusion

Capillary electrophoresis was applied successfully to evaluate the binding between short DNA fragments and cationic surfactants. A sharp decrease in electrophoretic mobility in a narrow range of surfactant concentration was observed, reflecting a cooperative binding of surfactant to DNA. The interpretation of the complex mobility close to the half point of saturation must be made with caution because peak splitting was observed in that region. Peak splitting was attributed to the 'lock-in' effect previously described in the literature.

At low ionic strength, an experimental electrophoretic mobility can be very satisfactorily calculated as a ratio of the effective charge of DNA to hydrodynamic friction coefficient, where the effective charge is calculated on the basis of counterion condensation theory and the hydrodynamic friction is calculated on the basis of the theory for diffusion of rod like molecules. The decrease in the electrophoretic mobility of DNA upon binding at low ionic strength can be then assigned to the change of hydrodynamic diameter due to the layer of surfactant bound, without change in effective charge. The experimental decrease of mobility at low ionic strength is well described by that representation.

At higher ionic strength, electrostatic screening must be taken into account, as seen from the decrease of mobility of bare DNA and the diminishing effect of binding on the mobility. The decrease in the electrophoretic mobility of DNA with increasing ionic strength is well described by the Manning's theory for electrophoretic mobility of infinitely long thin polyelectrolytes. However, the theoretical basis for the description of the mobility dependence on binding at intermediate ionic strength is less clear.

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