ELSEVIER

Journal of Chromatography B, 706 (1998) 3–11

JOURNAL OF CHROMATOGRAPHY B

Capillary electrophoretic behavior of milk proteins in the presence of non-ionic surfactants

Run Juan Xu, Claire Vidal-Madjar*, Bernard Sébille

Laboratoire de Physico-Chimie des Biopolymères, CNRS, Université Paris Val-de-Marne, 2 Rue Henri Dunant, 94320 Thiais, France

Abstract

The electrophoretic behavior of α-lactalbumin and β-lactoglobulins (A and B) in the presence of non-ionic surfactants was studied by capillary electrophoresis (CE), using a poly(ethylene glycol) coated capillary column. The surfactants (Tween 20, Brij 35 and 78) were used as buffer additives. The separation is based on the difference in the strength of protein–surfactant association complexes, which results in a change of the effective electrophoretic mobility. The modification of the electrophoretic mobilities of proteins was observed and this variation permitted the estimation of the interaction between protein and surfactant. The effect of surfactant type and concentration on the migration behavior of protein in CE is discussed. It is found that the retention behavior of the milk proteins (the α-lactalbumin and the β-lactoglobulins) in CE is very different. The pH of the buffer and the surfactant type influence significantly the protein–surfactant interactions.

Keywords: Non-ionic surfactants; Proteins; α-Lactalbumin; β-Lactoglobulins

1. Introduction

Capillary electrophoresis (CE) is a relatively new analytical technique which allows rapid and efficient separation of macromolecules such as proteins. In principle, CE measures the mobility of the charged species under the influence of an electric field gradient. The observed migration time of a species is determined by a combination of its electrophoretic mobility and the mobility of electroosmotic flow (EOF). In CE non-ionic long-alkyl-chain surfactants such as Brij and Tween have been successfully used as agents of capillary coating in order to eliminate the adsorption of protein on the capillary wall [1,2]. The surfactants have been used only to modify the properties of the silica capillary surface, i.e., to passivate the residual silanol groups. In the separation of proteins by micellar electrokinetic chromatography (MEKC) these non-ionic surfactants have been mixed with other ionic surfactants such as sodium dodecyl sulfate (SDS) to form mixed micelles [3]. It was shown that the addition of non-ionic surfactants improved the selectivity by decreasing the surface charge in mixed micelles. In the separation of the variants of β-lactoglobulin the presence of low Tween 20 concentration as buffer additive improves the resolution of CE with silica tubings [4,5]. The proteins show different electrophoretic mobilities under the influence of surfactants. It was supposed that hydrophobic interaction between Tween 20 and proteins modified the resolution of separation. In this case the modification of the capillary wall by adsorption of surfactant also takes place.

In CE, surfactants are often used as buffer addi-

*Corresponding author.

0378-4347/98/$19.00
PII S0378-4347(97)00498-2

tives for improving the resolution of separation. They have also been used to separate ionic polymers with passivated columns [6]. The non-ionic surfactants have several advantages in CE, including low conductivity and good compatibility with proteins. In contrast to ionic surfactants, non-ionic surfactants bind relatively weakly to proteins. However, to our knowledge, studies of the interactions of proteins with surfactants by CE have not yet been reported.

Interactions of proteins with surfactants have been a subject of extensive study for many years. These interactions are of importance in a wide variety of industrial, biological, pharmaceutical and cosmetic systems. Common techniques, such as surface tension, rheology and dialysis, are used for characterizing the protein–surfactant interactions [7]. In food industry, e.g., the distribution of proteins and low-molecular-mass surfactants between the interface and bulk phases is of importance in the control of formation, stability and texture of multiphase foods. Proteins (from milk, eggs, etc.) and small-molecule surfactants are two kinds of molecular species present in food colloids that have a strong tendency to adsorb at the oil–water interface. Both the protein and surfactant not only compete for interfacial area, but in some cases can interact non-covalently with each other [8]. The resulting complexes can have completely different functional behavior compared to that of the individual species. The vast majority of surfactants used as food ingredients are either non-ionic or zwitterionic. Most studies of the binding of surfactants to proteins have focused on the interactions with non-ionic surfactants.

Many non-ionic water-soluble surfactants used in food colloids are produced synthetically, e.g., polyoxyethylene sorbitan esters (Tweens) from reaction of sorbitol with first fatty acids and then ethylene oxide [9]. In bovine milk, the two major whey proteins are β-lactoglobulin (β-Lg) and α-lactalbumin (α-Lac). Both proteins are globular, comparatively small and they are broadly used in the food industry. The Tween 20/β-lactoglobulin system is an example of a model system which has often been studied [8,10–12]. The interaction of Tween 20 with β-Lg was investigated by fluorescence titration or by the measurement of dynamic surface properties. It was found that the Tween 20 and β-Lg form a 1:1 molar ratio complex and that a low concentration of Tween 20 can have a dramatic effect on β-Lg surface rheology [10]. The dissociation constants were calculated for the complexes formed with the native, A and B variants, respectively [12]. The system of Tween 20 with another whey protein, α-Lac, was also studied by comparing with the system Tween 20/β-Lg [13,14]. It is demonstrated that the presence of Tween 20 in the solution has distinct effects on the behavior of β-Lg and α-Lac.

In this paper we investigate the potential of CE in the study of the protein–surfactant interactions, and present the results of some preliminary studies on the electrophoretic behavior of milk proteins in the presence of non-ionic surfactants. Table 1 shows the structural characteristics of the three surfactants selected: Tween 20, Brij 35 and Brij 78. The Brij surfactants differ in alkyl chain length and hydrophilic head-group size. The influence of some factors, such as surfactant type and buffer pH, on the protein analysis was investigated.

Table 1
Nonionic surfactants selected in this study

Surfactant[a]	*M* (g/mol)	*m*[b] (oxyethylene units)	*n*[b] (alkyl chain length)	CMC (m*M*) in water, 25°C[c]
Tween 20	1227.54	20	12	0.045 [8]
Brij 35	1199.57	23	12	0.100 [1]
Brij 78	1151.57	20	18	–

[a] Tween 20, polyoxyethylene (20) sorbitan monolaurate; Brij 35, polyoxyethylene (23) lauryl ether; Brij 78, polyoxyethylene (20) stearyl ether.

[b] The values of *m* and *n* are from [1].

[c] CMC: Critical micelle concentration. Our experiments were conducted at 23°C. The CMC values listed are given at 25°C for comparison.

2. Experimental

2.1. Chemicals

All substances used in this work were of analytical grade. Sodium acetate, sodium nitrate and acetic acid were from Prolabo (Paris, France). The surfactants Brij 35, 78 and Tween 20 were obtained from Fluka (Buchs, Switzerland). All bovine milk proteins were purchased from Sigma (St. Louis, MO, USA): β-lactoglobulin B (p*I* 5.2), β-lactoglobulin A (p*I* 5.1) and α-lactalbumin (calcium depleted, p*I* 4.8).

Deionized water obtained from a Milli-Q system (Millipore, Bedford, MA, USA) was used for the preparation of the buffer solutions. Acetate buffer was made with sodium acetate and adjusted with acetic acid to the desired pH values. The concentration of acetate buffer was 50 m*M* for all analyses. In the case of the addition of surfactants both the buffer and sample solutions contained the same surfactant concentration. The protein samples were prepared by dissolving the appropriate proteins in the running buffer solution at a concentration of 0.5 mg/ml for each protein. The concentration of $NaNO_3$ in the sample of nitrate was also made 0.5 mg/ml. Before use, these solutions were filtered and degassed.

2.2. Apparatus and method

CE was carried out with a Spectra Phoresis 1000 system (Spectra-Physics, CA, USA), controlled by an IBM personal computer (PC) using OS/2 software. The apparatus is equipped with a reversible power supply and, in our study, the operation voltage was 20 kV for protein separation with the detector positioned near the cathode. A reverse voltage was used for the electrophoresis of NO_3^- (−20 kV). The hydrodynamic mode was used for the sample injections and the injection time was 5 s. The UV detector was set at 220 nm and all experiments were performed at 23°C. The capillary column with a neutral hydrophilic coating (50 μm I.D. DB-WAX with the poly(ethylene glycol) coating, purchased from J&W Scientific, Folsom, CA, USA) was used throughout this study to prevent adsorption of proteins on the silica wall. The total length of the capillary was about 43 cm with an effective separation length (distance from injection to detection position) of 35 cm. The EOF in this coated capillary was almost totally suppressed. The EOF was measured by using benzyl alcohol as a neutral marker and no peak appeared within 150 min in this experiment. This sets a limit to the EOF which is smaller than $0.8.10^{-5}$ $cm^2/(Vs)$ with this buffer. Thus, with the PEG coated columns used the effect of EOF on binding measurements is negligible. In this work, the measurement of EOF with a neutral marker became difficult because of the long migration time of a neutral solute. To estimate the variation of EOF during the analysis, the migration time of NO_3^- was measured for each running buffer by applying the same voltage but in the opposite direction [15]. In all runs the migration time of NO_3^- was almost constant and the variation of EOF in μ_{eo} was about 2%. It was supposed that the influence of EOF was negligible in our experimental conditions. Before analysis, the capillary was preconditioned by flushing through the running buffer and it was rinsed with buffer for 2 min between runs to improve the separation reproducibility.

3. Results and discussion

Affinity capillary electrophoresis (ACE) provides a new approach to the study of protein–ligand interactions [16–18]. The separations are based on the difference in the strength of protein–surfactant association complexes, which results in the difference in effective electrophoretic mobility. A number of variables such as the surfactant type, pH and the protein nature affect the electrophoretic behavior.

The type of surfactant has a significant influence on protein mobility in CE. Polyoxyethylene-based non-ionic surfactants have a statistic homologous distribution of ethylene oxide units. Surfactants possess both hydrophilic and hydrophobic characteristics and their ability to form micelles is very different (Table 1). The chemical compositions of the hydrophobic moieties of non-ionic surfactants significantly affect their interactions with proteins. To study the effect of the surfactant type on the behavior of protein in CE, the separations with non-

ionic surfactants such as Brij and Tween present in the buffer were preformed under the same experimental conditions.

3.1. Electrophoretic behavior in the presence of surfactants

The electrophoretic behavior of β-lactoglobulin A and B and α-lactalbumin in the presence of surfactants was investigated below the critical micellar concentration (CMC) of the surfactants. The variation of the migration time of the proteins as a function of the concentration of Tween 20 is presented in Fig. 1. The migration time of β-Lg A and B increases gently as the concentration of the surfactant increases. At pH 4, an affinity pattern is observed; the plateau value is reached at 30 μ*M* of Tween 20. The migration pattern of α-Lac is very different from that of β-Lg A and B with an electrophoretic mobility continually decreasing with increasing Tween 20 concentrations. No asymptotic value is observed at the larger surfactant concentrations.

Compared with the results obtained in the presence of Tween 20, the variations of the migration times of β-Lg A and B are more important with Brij 35, while the migration time of α-Lac increases dramatically with the concentration of Brij 35 in solution (Fig. 2). In the electropherograms of Fig. 3 comparing the effects of Tween 20 and Brij 35 in the eluent, it is interesting to note that the protein separation is not markedly affected by the addition of Tween 20 in the buffer (Fig. 3a). Instead with Brij 35 (Fig. 3b), the migration times of α-Lac and β-Lg A, B increase with the concentration of surfactant but to different degrees, leading to important modifications of the electopherogram pattern. At large Brij 35 concentrations, an important increase of the migration of α-Lac relative to that of the β-Lg is observed.

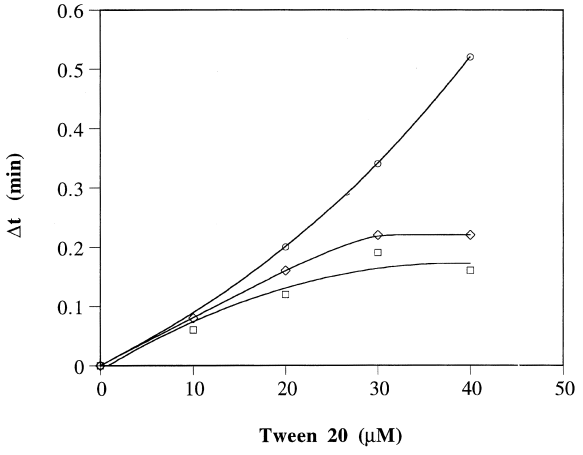

Fig. 1. Variation of the migration time of milk proteins as a function of Tween 20 concentration at pH 4.0. Conditions: PEG-coated capillary, 43 cm (35 cm effective length)×50 μm I.D.; Buffer, 50 m*M* acetate; applied voltage, 20 kV; hydrodynamic injection for 5 s; detection, 220 nm. Proteins: (◇) β-Lg A; (□) β-Lg B; (○) α-Lac.

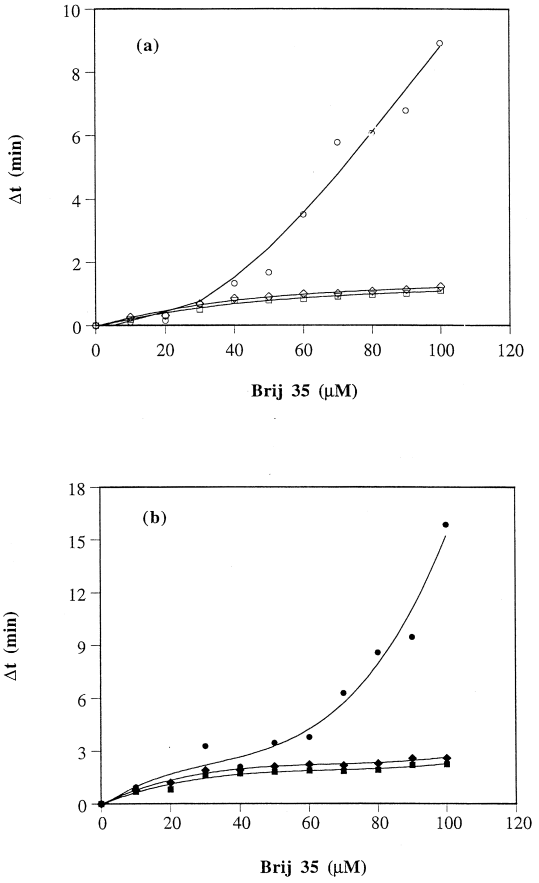

Fig. 2. Variation of the migration time of α-Lac, β-Lg A and B as a function of Brij 35 concentration (a) at pH 4.0 and (b) at pH 4.5. Electrophoretic conditions as in Fig. 1. Proteins: α-Lac (○) at pH 4.0 and (●) at pH 4.5; β-Lg A (◇) at pH 4.0 and (♦) at pH 4.5; β-Lg B (□) at pH 4.0 and (■) at pH 4.5.

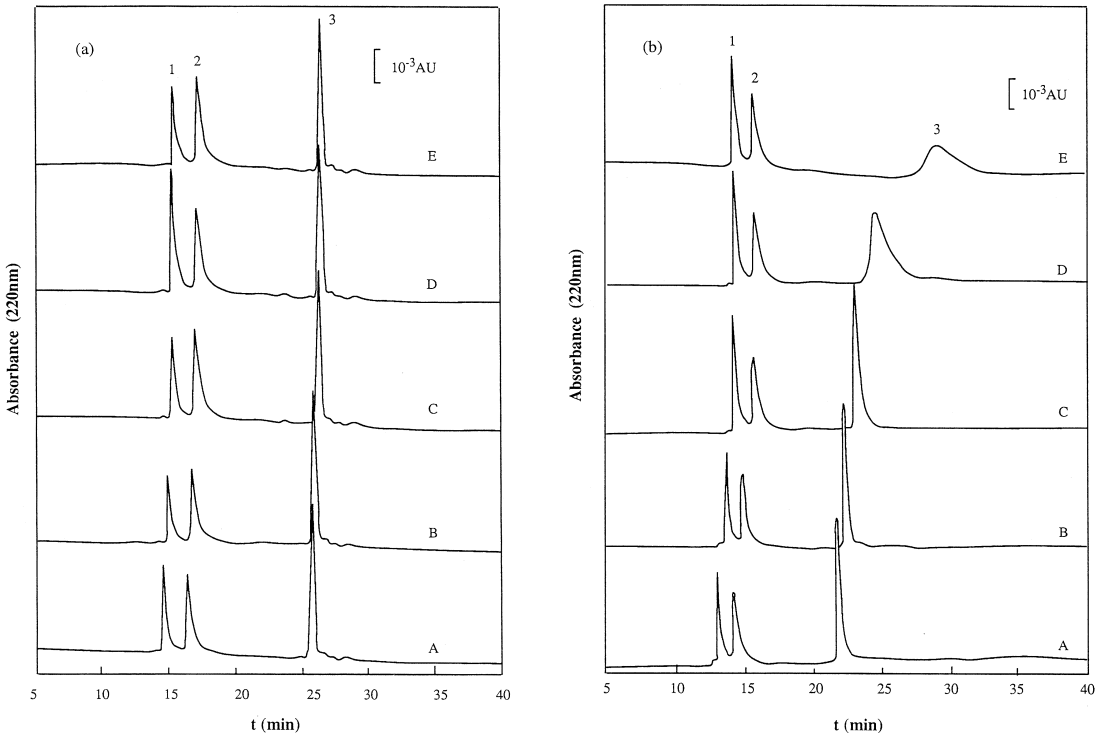

Fig. 3. Influence of the surfactant type on the CE separations of milk proteins; (a) in the presence of Tween 20 in the buffer (pH 4.5) with various concentrations: A, 0 μ*M*; B, 10 μ*M*; C, 20 μ*M*; D, 30 μ*M*; E, 40 μ*M*; (b) in the presence of Brij 35 in the buffer (pH 4.5) with various concentrations: A, 0 μ*M*; B, 20 μ*M*; C, 40 μ*M*; D, 60 μ*M*; E, 80 μ*M*. Electrophoretic conditions as in Fig. 1. Peak identifications: (1) β-Lg B; (2) β-Lg A; (3) α-Lac.

This result indicates the possibility of varying the selectivity of protein separations by careful selection of the surfactant type in the buffer.

In the presence of large concentrations of Brij 35, the shape of the β-Lg peaks remains unchanged but the peak of α-Lac loses its shape (Fig. 3b). Contrary to the separations obtained with Tween 20 added to the buffer (Fig. 3a), a significant band broadening of the α-Lac peak with an important tailing is observed when the Brij 35 concentration is increased. Similar effects are also obtained at large concentrations of Brij 78 in the buffer.

For the same concentration in the buffer, the different surfactants studied have different modifying powers. The degree of the increase in migration time for the same protein is determined by the chemical nature of the surfactant. For the Brij-series surfactants, both length of alkyl chain and number of ethylene oxide are varying (Table 1). For Brij 78 the hydrophilic head group is slightly smaller than that of Brij 35 but the alkyl chain length is increased from 12 to 18. With Tween 20, the small modifications of the electropherogram patterns are explained by a weak hydrophobic interaction due to the presence of three hydrophilic chains and one hydrophobic chain in the surfactant molecule.

The variation of the hydrodynamic radius of the protein–surfactant complex depends on the type of surfactant used and also on the protein nature. A possible explanation of the electrophoretic behavior of α-Lac compared to that observed with the β-Lgs is that the hydrophobic interaction leads to a modification of the protein structure in solution. Another possible reason for the broadening and tailing of the α-Lac peak is that the presence of Brijs favours protein–protein interactions and leads to the aggregation of proteins. The formation of protein aggregates would also produce a slower migration of the

species. These results suggest that further work is needed to clarify this point.

A few studies of the behavior of the individual whey proteins in the presence of surfactants have been done. From competitive adsorption experiments of whey proteins at pH 7, α-Lac was more difficult to displace from the interface by Tween 20 than the β-Lg [13]. Conductivity methods have also shown that the solutions of α-Lac in phosphate buffer at pH 7.0 were considerably less stable than those of β-Lg [14]. Moreover, the addition of relatively low concentration of the non-ionic surfactant Tween 20 causes destabilization of α-Lac. In our work, this difference was also observed with the capillary electrophoresis experiments. The migration times of the β-Lgs generally increase with the surfactant concentration in the system, but reach a plateau at large surfactant concentrations. A different behavior is observed with α-Lac, with a migration time continuously increasing with the surfactant concentration.

3.2. Determination of equilibrium constants

The basis of this application is the analysis of the changes in electrophoretic mobility of the protein when complexed with a surfactant present in the buffer. Compared with the protein alone, the complex formed between the non-ionic surfactant and protein has an increased hydrodynamic radius which decreases its electrophoretic mobility [19,20]. If the equilibrium is achieved during the electrophoretic experiment, the method allows calculation of the equilibrium constants from the changes in migration time at varying surfactant concentrations [16–18]. Determination of kinetic and equilibrium constants using ACE relies only on the changes in migration time of the peak due to protein complexation [17,18].

The multiple binding of ligands (L) by a protein (P) can be formulated by a series of equilibria [21,22]:

$$\begin{aligned} \mathrm{P} + \mathrm{L} &= \mathrm{PL}; & [\mathrm{PL}] &= k_1[\mathrm{P}][\mathrm{L}] \\ \mathrm{PL} + \mathrm{L} &= \mathrm{PL}_2; & [\mathrm{PL}_2] &= k_1k_2[\mathrm{P}][\mathrm{L}]^2 \\ \mathrm{PL2} + \mathrm{L} &= \mathrm{PL}_3; & [\mathrm{PL}_3] &= k_1k_2k_3[\mathrm{P}][\mathrm{L}]^3 \\ &\vdots & &\vdots \\ &\vdots & &\vdots \\ \mathrm{PL}_{n-1} + \mathrm{S} &= \mathrm{PL}_n; & [\mathrm{PL}_n] &= k_1k_2.....k_n[\mathrm{P}][\mathrm{L}]^n \end{aligned} \quad (1)$$

where k_1, k_2,...k_n are the equilibrium association constants and n the number of sites. The ratio of the moles of bound ligand to the total moles of protein is given by the general equation:

$$r = \frac{k_1C + 2(k_1k_2)C^2 + \cdots + n(k_1k_2\cdots k_n)C^n}{1 + k_1C + (k_1k_2)C^2 + \cdots + (k_1k_2\cdots k_n)C^n} \quad (2)$$

where C is the free concentration of the ligand. In the case of independent and equivalent binding sites, it may be shown from statistical considerations that Eq. (2) can be approximated by a very simple binding equation of the Langmuirian type:

$$r = \frac{nC}{K_\mathrm{d} + C} \quad (3)$$

where n is the maximum number of bound surfactants per protein molecule and K_d is an apparent dissociation constant related to the equilibrium association constant of the ith reaction by:

$$K_\mathrm{d} = \frac{n - i + 1}{ik_i} \quad (4)$$

Upon binding, the electrophoretic mobility of the protein–surfactant complex can be affected by the change of charge and by the change of the hydrodynamic radius. In the present study, where non-ionic surfactants are used, the variation of the electrophoretic mobility of the protein bound to the surfactant is explained by a change of the hydrodynamic radius. This variation can be used to calculate the binding constant if equilibrium is reached during the electrophoresis experiment. A linear relationship is assumed between the change in mobility of the surfactant–protein complex and the fraction of surfactant molecules bound to the protein [23]. At zero surfactant concentration ($C=0$), the protein is free in solution and migrates along the column to the detector with a time t_0. At saturation ($r=r_{\max}$), the protein is totally complexed with the surfactant and migrates in a time $t_{\max}$. Assuming a binding process with independent and equivalent binding site (Eq. (3)), one can determine by ACE the apparent dissociation constant, K_d, from the relationship:

$$\frac{r}{r_{\max}} = \frac{t - t_0}{t_{\max} - t_0} = \frac{C}{K_\mathrm{d} + C} \quad (5)$$

A non-linear least square fit program was used to determine K_d and $(t_{max}-t_0)$ from the variation of $(t-t_0)$ with the surfactant concentration in the buffer. This approach can be used to analyse situations where a well defined plateau is reached as for the binding of the β-Lg A and B with Brij 35 and Brij 78 surfactants. For the experiments of α-Lac in the presence of surfactants, this approach is not valid.

The theoretical model (full line) fits well the whole range of experimental data (Figs. 4 and 5) and the corresponding binding constants are listed in Table 2. The linearisation of Eq. (5) is generally carried out (Scatchard or Klotz plot) to determine the binding constant for the interaction. Instead, we fitted directly the raw data using a non-linear least square fit program in order to give the same statistical weight to all measurements. The relative standard deviation (R.S.D.) of the K_d determination is roughly 10%.

The migration time of the protein–surfactant complex is affected by several factors as the variation of the EOF with the concentration of the surfactant in the buffer or the interactions of the reactive species with the capillary walls. In this study, a PEG-coated [poly(ethylene glycol)] capillary was used. The EOF was so low that a direct measurement from the migration time of a neutral marker was not possible. In general, the suppression of EOF in CE provides higher migration time reproducibility. The change of EOF was monitored from the migration time of NO_3^- [15]. During analysis, the coefficient relating the EOF to the NO_3^- migration time was almost constant at different concentrations of surfactant. It is thus valid to assume that the influence of the EOF is negligible in this work so that the correction for EOF is not necessary.

Otherwise, with the PEG coating used, the EOF is so weak that the eventual surfactant adsorption is unlikely because of the very few residual silanol groups on the surface. The adsorption of the surfactant on the capillary wall should be very weak because no sign of interaction between polyethoxylated non-ionic surfactants and hydrophilic polymers as PEO (polyethylene oxide) is noticed [24]. More-

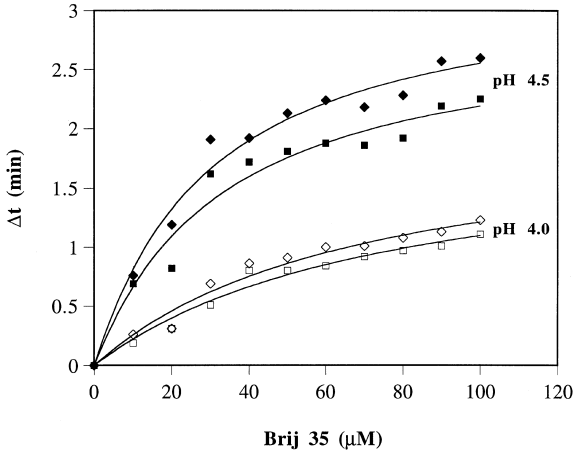

Fig. 4. Variation of migration time of β-Lg A and B as a function of the Brij 35 concentration at pH 4.0 and at pH 4.5. Electrophoretic conditions as in Fig. 1; (—) theoretical models (Eq. (5)). Proteins: β-Lg A (◇) at pH 4.0 and (♦) at pH 4.5; β-Lg B (□) at pH 4.0 and (■) at pH 4.5.

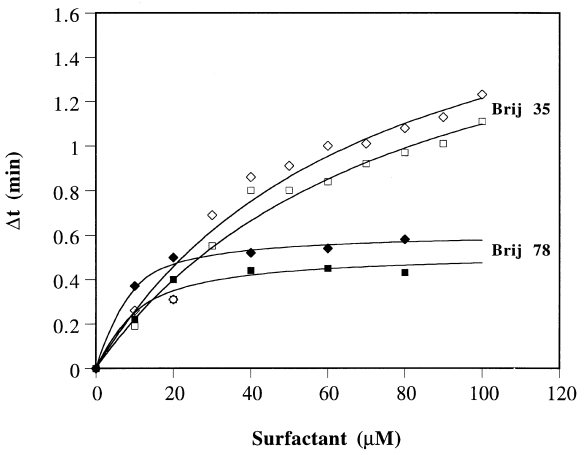

Fig. 5. Variation of migration time of β-Lg A and B as a function of Brijs surfactant concentration at pH 4.0. Electrophoretic conditions as in Fig. 1; (—) theoretical models (Eq. (5)). Proteins: β-Lg A in the presence of (◇) Brij 35 and (♦) Brij 78; β-Lg B in the presence of (□) Brij 35 and (■) Brij 78.

Table 2
Dissociation constants for the binding of β-lactoglobulin A and B with surfactants[a]

Protein	Dissociation constant K_d (μ*M*)		
	Brij 35		Brij 78
	pH 4.0	pH 4.5	pH 4.0
β-lactoglobulin A	69	30	6
β-lactoglobulin B	77	33	10

[a] Electrophoretic conditions are as in Fig. 1.

over the low ionic strength of the buffer minimizes the possible residual adsorption due to hydrophobic interaction. Therefore we can assume that the interaction of the protein with the surfactant adsorbed on the column is negligible.

The addition of Brij 35 to the buffer was quite effective in increasing the migration times of β-Lg A and B, i.e., decreasing their electrophoretic mobilities. Fig. 4 compares at pH 4.0 and pH 4.5 the changes of the migration times of β-Lg A and B as a function of the Brij 35 concentration. It is observed that the system Brij 35/β-Lg has the same affinity pattern at both pH values. At a higher pH (pH 4.5) the tendency to form complexes with the surfactant is more important as shown by the K_d values. This supports the notion that the protein is more hydrophobic when the pH value is close to its p*I* (isoelectric point).

The comparison of the dissociation constants determined with the Brij surfactants at pH 4.0 shows that the K_d is significatively lower with Brij 78, which reflects the differences in the hydrophobic interactions of the proteins with the surfactants. Fig. 5 displays the effect of the alkyl chain length of Brijs on the migration behavior of β-Lg A and B. At a low concentration of surfactant in the buffer, the variation of protein mobility is more important in the presence of Brij 78. The saturation plateau is then reached for a lower concentration of surfactant in the buffer. These results demonstrate stronger interactions of β-Lg A and B with Brij 78 than those observed with Brij 35. It also proves that hydrophobic interactions between the surfactant and the protein are responsible for the protein–surfactant complex formation, and the more hydrophobic surfactant has a stronger tendency to bind with the protein.

In the presence of Tween 20, the change in electrophoretic mobilities of the β-lactoglobulins is low (Fig. 2): the maximum ($t-t_{max}$) variation is around 10 s, much lower than the variation observed at the plateau (1–2 min) in the presence of the Brij surfactants. A reliable determination of the K_d constant is not possible with the experiments performed in the presence of Tween 20. Wilde and Clark [12] have reported that β-lactoglobulin bound to Tween 20 with a stoichiometry of 1:1 molar ratio in 10 m*M* phosphate at pH 7.0, with K_d values of 4.6 μ*M* and 6.8 μ*M* for the interaction of Tween 20 with respectively, the A and B variants of β-lactoglobulin. A direct comparison with the values reported in Table 2 is not possible because the nature of the surfactant used and the experimental conditions are different. At pH 7.0 the proteins selected in this study are negative in charge so that the separations have to be performed with a reverse polarity of the applied voltage. Under these conditions no reproducible result was obtained. Nevertheless, the K_d values determined in this work are of the same magnitude as those reported in Ref. [12], with a lower K_d found for the interaction of the surfactants with the A variant.

4. Conclusions

CE is a rapid analytical technique for study of protein–surfactant interactions. From this work it is clear that the presence of non-ionic surfactants has a considerable influence on the electrophoretic behavior of milk proteins. The interactions of surfactants at very low concentrations with proteins often lead to the formation of protein–surfactant complexes. The effect of surfactants on the electrophoretic mobility depends on the nature of proteins. There are distinct differences in behavior between α-lactalbumin and β-lactoglobulin in the presence of surfactants selected (Tween 20, Brij 35 and 78). β-lactoglobulin appears to form a complex with these non-ionic surfactants by hydrophobic binding, whereas some structural modifications of α-lactalbumin occur within the concentration range employed. The value of pH has an influence on the magnitude of the binding of the surfactant to the protein.

ACE can be used to determine the apparent dissociation constant of the protein–surfactant complex. The method requires large enough a change in migration time of the protein–surfactant complex and can only be applied to systems leading at saturation to asymptotic values of the electrophoretic mobilities. The use of a passivated capillary column permits to eliminate the main limitations of the ACE method, such as variations of the EOF and adsorption of the interacting species on the capillary walls.

References

[1] J.K. Towns, F.E. Regnier, Anal. Chem. 63 (1991) 1126–1132.
[2] X. Yao, D. Wu, F.E. Regnier, J. Chromatogr. 636 (1993) 21–29.
[3] H. Nishi, S. Terabe, J. Chromatogr. A 735 (1996) 3–27.
[4] G.R. Paterson, J.P. Hill, D.E. Otter, J. Chromatogr. A 700 (1995) 105–110.
[5] N.M. Kinghorn, G.R. Paterson, D.E. Ottter, J. Chromatogr. A 723 (1996) 371–379.
[6] J. Collet, C. Tribet, P. Gareil, Electrophoresis 17 (1996) 1202–1209.
[7] K.P. Ananthapadmanabhan, in E.D. Goddard and K.P. Ananthapadmanabhan (Editors), Interactions of Surfactants with Polymers and Proteins, CRC Press, Boca Raton, FL, 1993, pp. 319–365.
[8] M. Coke, P.J. Wilde, E.J. Russell, D.C. Clark, J. Colloid Interface Sci. 138 (1990) 489–504.
[9] E. Dickinson, G. Iveson and S. Tanai, in E. Dickinson and P. Walstra (Editors), Food Colloids and Polymers: Stability and Mechanical Properties, Spec. Publ. No. 113, R. Soc. Chem., 1993, pp. 312–322.
[10] J. Courthaudon, E. Dickinson, Y. Matsumura, D.C. Clark, Colloids Surfaces 56 (1991) 293–300.
[11] D.C. Clark, P.J. Wilde, D.J.M. Bergink-Martens, A.J.J. Kokelaar and A. Prins, in E. Dickinson and P. Walstra (Editors), Food Colloids and Polymers: Stability and Mechanical Properties, Spec. Publ. No. 113, R. Soc. Chem., 1993, pp. 354–364.
[12] P.J. Wilde, D.C. Clark, J. Colloid Interface Sci. 155 (1993) 48–54.
[13] J. Courthaudon, E. Dickinson, Y. Matsumura, A. Williams, Food Structure 10 (1991) 109–115.
[14] D.C. Clark, P.J. Wilde, D.R. Wilson, Colloids Surfaces 59 (1991) 209–223.
[15] G.M. Janini, G.M. Muschik, H.J. Issaq, J. Cap. Electrophoresis 1 (1994) 116–120.
[16] J.C. Kraak, S. Busch, H. Poppe, J. Chromatogr. 608 (1992) 257–264.
[17] L.Z. Avila, Y. Chu, E.C. Blossey, G.M. Whitesides, J. Med. Chem. 36 (1993) 126–133.
[18] K. Haupt, F. Roy, M.A. Vijayalakshmi, Anal. Biochem. 234 (1996) 149–154.
[19] B.J. Compton, E.A. O'Grady, Anal. Chem. 63 (1991) 2597–2602.
[20] T. Tadey, W.C. Purdy, J. Chromatogr. A 652 (1993) 131–138.
[21] I.M. Klotz, F.M. Walker, R.B. Pivan, J. Am. Chem. Soc. 68 (1946) 1486–1490.
[22] M.N. Jones, Chem. Soc. Rev. 21 (1992) 127–136.
[23] Y. Chu, L.Z. Avila, H.A. Biebuyck, G.M. Whitesides, J. Org. Chem. 58 (1993) 648–652.
[24] B. Lindman and K. Thalberg, in E.D. Goddard and K.P. Ananthapadmanabhan (Editors), Interactions of Surfactants with Polymers and Proteins, CRC Press, Boca Raton, FL, 1993, pp. 203–276.