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# Effect of sodium dodecyl sulphate in protein samples on separation with free capillary zone electrophoresis

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ABSTRACT

The addition of sodium dodecyl sulphate (SDS) to samples of proteins (conalbumin and ovalbumin), but not to the carrier electrolyte (borate, 0.1 mol/l, pH 10) leads to a total loss in resolution of the analytes in free capillary zone electrophoresis, even at SDS concentrations of 0.1% (w/w). Simultaneously, a sharpening effect on the single peak obtained from the two proteins is observed, simulating favourable dispersion properties of the separation system (apparent plate number of 1 500 000). No such effects are found if SDS is added to the carrier electrolyte. It is demonstrated that the effects observed are not caused by binding of SDS onto the proteins but seem to be generated by conductivity gradients present in the system.

## INTRODUCTION

Capillary zone electrophoresis (CZE), carried out either in open or in gel-filled capillaries, is a high-performance separation method with special interest for the separation of proteins and oligonucleotides. While gel-filled capillaries can be applied in a similar fashion to sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) for the separation of proteins according to their molecular weight, in free zone electrophoresis the migration of the analytes depends on their effective mobilities.

In electrophoresis the matrix components as well as the analytes may determine the electrical properties of the sample zone, namely its conductivity. Ionic matrix components in high concentrations especially affect and even dominate these properties to such an extent that the electrophoretic behaviour of the analytes in the sample differs significantly from that derived from standard solutions.

SDS is often present as a matrix component in protein samples either to increase the protein solubility, or when the samples are isolated from gels after SDS-PAGE on microscale. Although in these cases SDS is often regarded as not interfering with subsequent electrophoretic separation using classical methods (zone electrophoresis or isoelectric focusing), no investigations have yet been carried out with respect to potential interferences in free CZE. Therefore, the influence of SDS present in protein samples on the zone electrophoretic patterns of the analytes was investigated.

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## EXPERIMENTAL

### *Chemicals*

Proteins applied were ovalbumin (recristallized five times; Serva, Heidelberg, Germany) and conalbumin (research grade; Serva). All other chemicals used were of reagent grade (E. Merck, Darmstadt, Germany). Water was redistilled twice from a quartz apparatus.

The carrier buffer was boric acid–sodium borate (0.1 mol/l), pH 10.

The concentration of the proteins (dissolved in the borate buffer) was 1 mg/ml.

The different SDS concentrations in the buffers and in the samples are given in weight percentages.

### *Apparatus*

Zone electrophoresis was carried out with a P/ACE System 2000 instrument (Beckman, Palo Alto, CA, U.S.A.), equipped with a fused-silica capillary (50 cm distance to the detector, 75 $\mu$m I.D.). The field strength was 350 V/cm and the electric current was 120 $\mu$A. The time of the hydrodynamic injection was 1 s, carried out pneumatically. Two washing steps were programmed: in the first step, rinsing with sodium hydroxide (0.1 mol/l) was carried out for 3 min; in the second step, the capillary was flushed with working buffer for the same time.

A UV detector was used at 214 nm, positioned at the cathode side of the capillary.

Data were recorded and processed with a computerized system (System Gold, Beckman).

## RESULTS AND DISCUSSION

In Fig. 1 the capillary zone electropherograms of conalbumin and ovalbumin, selected as analytes, are shown. In all cases the carrier buffer was borate in aqueous solution, which did not contain SDS. When the two proteins were dissolved in SDS-free buffer, the electropherogram indicated by 0% in Fig. 1 was obtained. It can be seen that the two proteins are well resolved and exhibit migration times of 5.5 and 6.8 min, respectively, under the given conditions (obtained by anionic separation and an electro-osmotic flow of the bulk liquid directed towards the cathode).

The ovalbumin peak is relatively broad. This is not caused by low efficiency of the separation system but by the (well known) inhomogeneity of the protein due to the various subunits ovalbumine consists of, which lead to several bands in PAGE also (see, *e.g.*, refs. 1–4).

The addition of SDS to the sample solution (but not to the carrier electrolyte) leads, however, to a significant change in the electropherogram: the two protein peaks are detected with a smaller difference in their migration times, even at an SDS concentration of only 0.05% (w/w). Further, both proteins are found in a single and extremely sharp peak at SDS concentrations in the sample of 0.1% and higher. It can be seen that this loss in resolution is caused by the fact that the selectivity of the separation (given by the relative difference of the migration times) is drastically decreased, although the apparent efficiency of the separation system (given by the peak widths) is enhanced. The apparent theoretical plate number of the peak with 0.5%

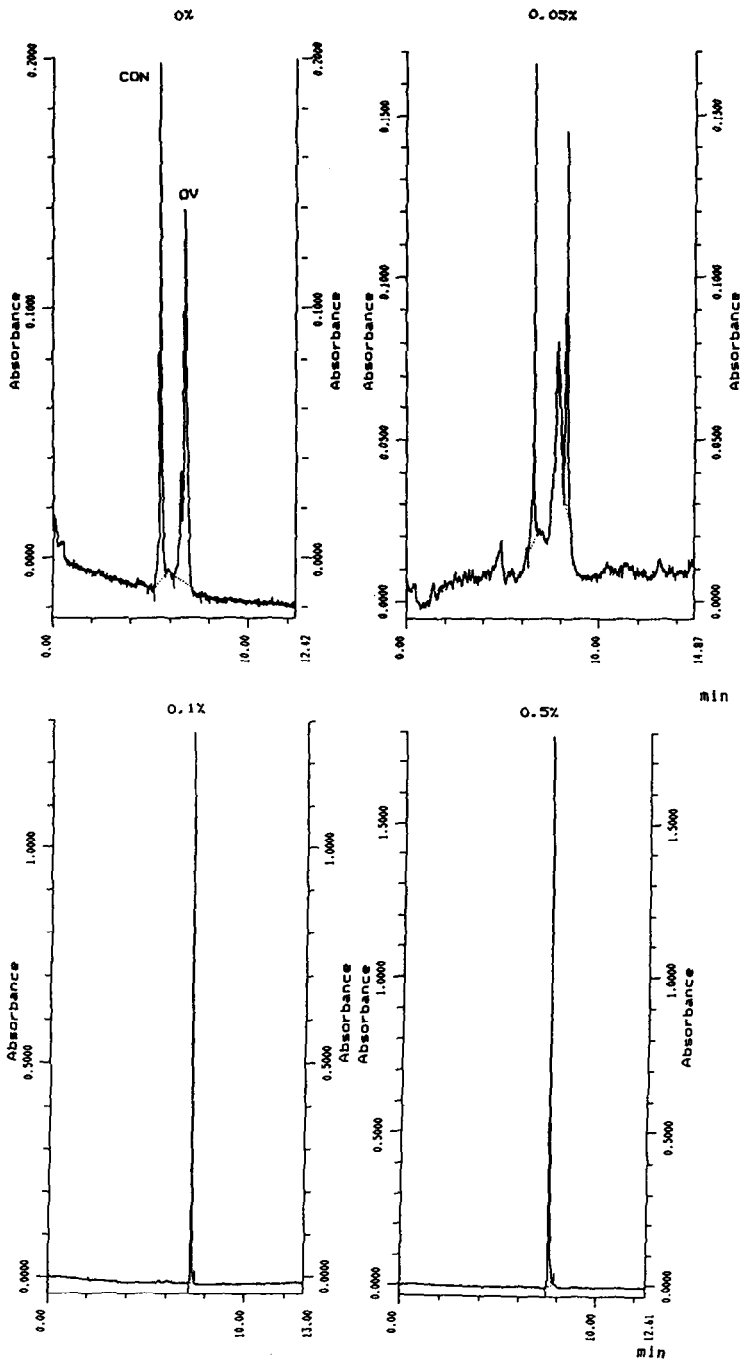


Fig. 1. Capillary zone electropherograms of conalbumin and ovalbumin at different concentrations of SDS added to the sample solution. Peaks: CON = conalbumin; OV = ovalbumin. The separations were carried out in aqueous borate buffer (0.1 mol/l) at pH 10 which did not contain SDS. The proteins were dissolved in buffer at a concentration of 1 mg/ml. The samples contained different concentrations of SDS (weight percents), indicated in the electropherograms by 0% (no SDS added), 0.05%, 0.1% and 0.5%. Instrumental conditions: fused-silica capillary (50 cm distance to the detector, 75 $\mu$m I.D.); field strength, 350 V/cm; electric current, 120 $\mu$A. Detection, UV at 214 nm.

SDS in the sample is about 1 500 000. The plate number, $N$, is calculated as for gradient-free chromatography or zone electrophoresis from the migration time $t_M$ and the standard deviation $\sigma_t$ of the peak (given in time units), by the expression $N = (t_M/\sigma_t)^2$.

The electropherogram shown in Fig. 1 obtained with a concentration of SDS of, *e.g.*, 0.5% in the sample would lead to a misinterpretation of the result: the sample seems to consist of a single, pure protein on the one hand, which is not the case; on the other hand, a very high plate number is indicated for the separation system.

This high (apparent) plate number and reduction in selectivity are not observed when the sample and the carrier electrolyte contain the same amount of SDS, as shown in Fig. 2. It can be seen that (besides an increase in the migration times) the pattern of the peaks is nearly identical to that obtained from an SDS-free system (Fig. 1, 0%). Both proteins are separated and the ovalbumin peak is also relatively broad. From this electropherogram it can be concluded that the decrease in selectivity when SDS is

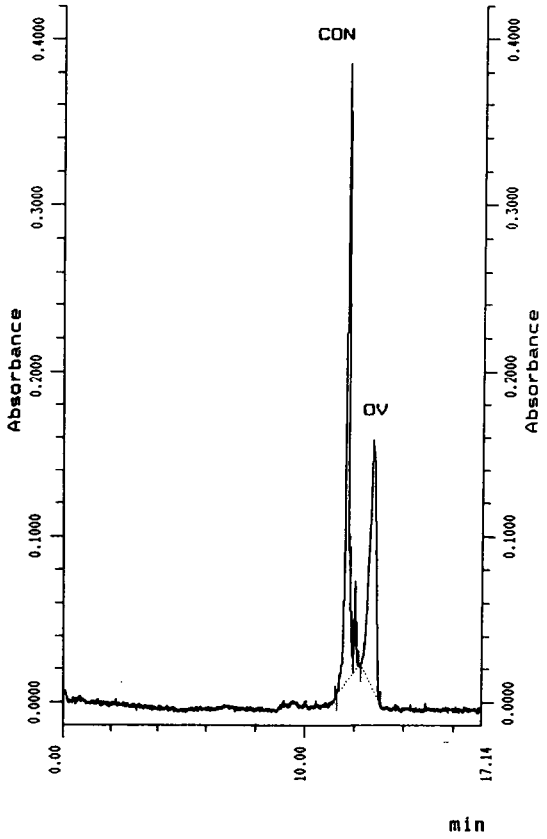


Fig. 2. Capillary zone electropherogram of conalbumin and ovalbumin obtained with a buffering carrier electrolyte containing the same SDS concentration as the sample. Peaks: CON = conalbumin; OV = ovalbumin. Both the sample and the carrier electrolyte had an SDS concentration of 0.5%. Buffer: borate (0.1 mol/l) pH 10. The analytes were dissolved in the buffer at a concentration of 1 mg/ml. Instrumental conditions were as in Fig. 1.

added to the sample is not caused by binding of SDS to the proteins, an effect which is used in SDS-PAGE and which is applied to eliminate mobility differences in order to separate proteins with respect to their molecular weight [5–8]. This binding is a reversible process and would therefore take place in a system totally filled with SDS rather than in one where SDS is present only in the sample and not in the carrier electrolyte.

It must be noted that the binding process in SDS-PAGE is supported by treating the protein–SDS mixture at elevated temperatures, as described in the literature [9]. In fact, this elimination of the mobility differences can also be observed in the electropherograms shown in Fig. 3, where SDS was added to the protein solutions and the mixture was kept at 100°C for 10 min prior to electrophoresis. It can be seen that even at an SDS concentration of 0.05% in the sample (a mass ratio of only 0.25 with respect to the proteins when it is known that SDS usually binds to most proteins with

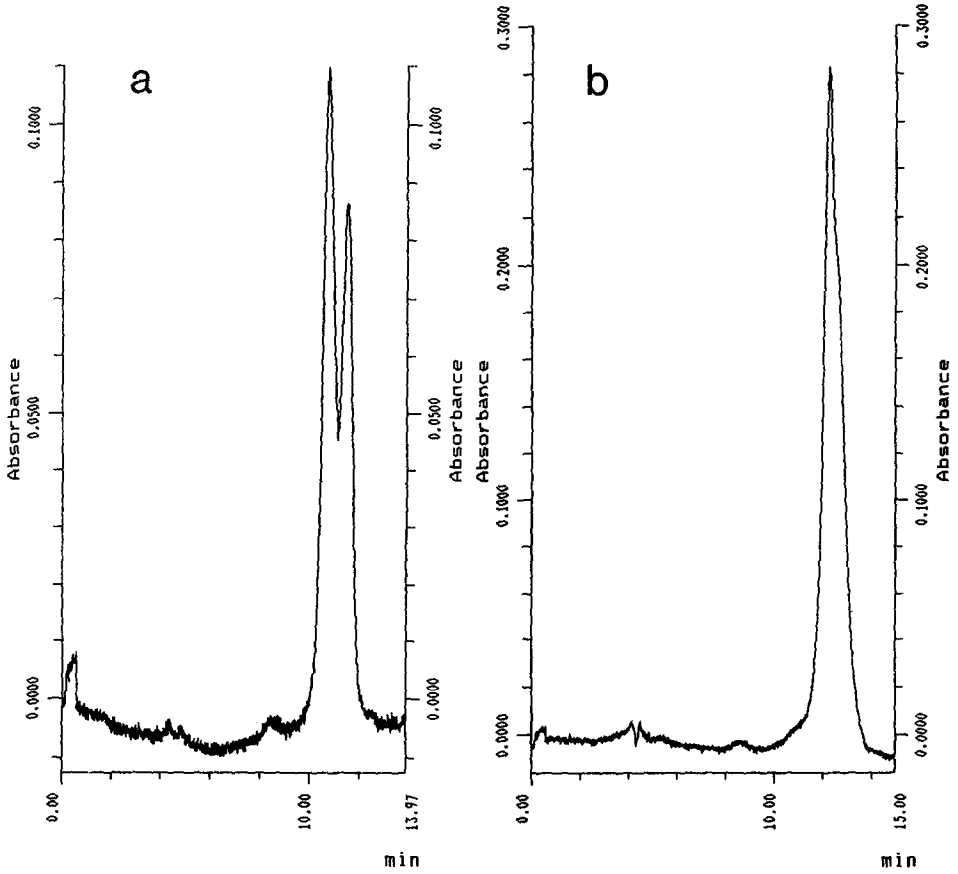


Fig. 3. Capillary zone electropherograms of conalbumin and ovalbumin in samples containing SDS, treated at elevated temperature prior to electrophoresis. The proteins were dissolved in borate buffer, and SDS was added at concentrations of 0.05% (a) and 0.5% (b), respectively. The mixtures were kept at 100°C for 10 min prior to injection. The separations were carried out at pH 10 with borate buffer (0.1 mol/l) containing SDS at the same concentration as the samples. Instrumental conditions were as in Fig. 1.

mass ratios between 0.9 and 1.4 [10]) the pattern of the electropherogram changes when compared with Fig. 2. Both peaks exhibit a similar migration time, which indicates that the mobility differences are increasingly eliminated due to the binding of SDS. This effect increases at an SDS concentration of 0.5% (2,5-fold with respect to the proteins), where both analytes are almost forming a single peak due to their virtually identical mobilities. This peak is much broader than that obtained under conditions where SDS was added in the same amount to the sample, but where no heat treatment was carried out, and where SDS was not added to the carrier electrolyte (Fig. 1, 0.5%).

From these results it can be concluded that the cause of the peak sharpening effect cannot lie in the binding processes between SDS and the proteins. It seems that the sharpening is originated by mobility gradients caused by SDS in the sample. In this case the additive acts in a similar fashion to the terminating ion in isotachophoresis, which is also termed displacement electrophoresis because of its analogy to displacement chromatography, where such sharpening effects are applied, *e.g.*, for the enrichment of trace analytes.

The assumption that the sharpening effect is caused by mobility gradients between the sample and the carrier electrolyte on the one hand, and between the analytes and SDS in the sample on the other hand, is supported also by the fact that peak sharpening is not observed when SDS gradients are not formed between sample and carrier electrolyte (Fig. 2).

As such effects, which depend on the mobilities of the analytes, of the additives (like SDS) and of the carrier electrolytes, are difficult to predict in real samples, electropherograms must be critically evaluated in practise to avoid misinterpretations. An unusual high plate number especially must be confirmed as it can be simulated by gradients present in the system.

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