ANALYSIS OF COMPLEX IONIC MIXTURES AND (ULTRA)TRACE ANALYSIS BY COUPLED COLUMN CAPILLARY ELECTROPHORESIS

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High efficiency column separation techniques are increasingly implemented into the (ultra)trace analysis schemes for ionogenic compounds. This approach is effective in several respects:

1. Systematic errors in the determinations are reduced;
2. Identification certainties are enhanced;
3. Less selective detection techniques can be employed.

At present we can see an explosive growth of interest in capillary zone electrophoresis. Extremely high separation efficiencies as reported for this technique (up to 10^6 theoretical plates per meter under favourable conditions) and very low mass detection limits (low attomole levels for favourable combinations analyte-detector) probably explain this fact. These features are very attractive as far as the use of CZE in trace analysis is concerned. In this context, however, some inherent limitations of this technique should also be mentioned:

1. Low load capacities of current CZE columns associated with low sample injection volumes (typically up to 10 nl). It is clear that this is a serious hindrance in achieving low concentration limits of detection (an essential requirement in trace analysis).
2. Problems associated with sample matrices are manyfold:

a) Reduced resolutions of the separands induced by matrix macroconstituents. Here, the macroconstituents can stack (focus) the separated constituents into a narrow band which may require a long separation time to vanish (refs.1,2).

b) Adsorption of some sample constituents on the surface of the capillary tube with a concomitant change of ζ-potential (ref.3). Problems of this kind can be expected in the analysis of biological samples containing proteinous material.

c) For example, environmental and biological matrices contain large numbers of sample constituents present at ng-μg/l concentrations. Here, CZE as any single column separation technique has practical limits in obtaining pure sample components (peaks) (ref.4).

Sample preparation and CZE

Following current concepts in column chromatography techniques it is logical to combine CZE with appropriate sample pretreatment techniques to eliminate the above limitations. Here, however, some specific features of CZE need to be taken into account:

1. Limited sample load capacity as outlined above restricts the use of the sample preparation procedures as developed, e.g., for column chromatography (see, e.g., ref.10 for an excellent review) when they do not reduce the ionic strength of the sample on the pretreatment.
2. Separation efficiencies of current sample pretreatment techniques (solid-phase extraction, liquid-liquid extraction, dialysis, etc.) are very low. This may be a serious drawback when physico-chemical properties of the analyte(s) and matrix constituents are close (problems in optimizing the pretreatment via the selectivity effects). In such situations the use of high efficiency sample pretreatment techniques is probably the only solution.

Considering the amount of the analyte currently needed for the CZE analysis (femtomoles-picomoles) it is apparent that the use of (sub)microscale sample preparation techniques can be very convenient. It is, however, mandatory that such techniques consist of small numbers of sample handling steps (reduced risks of sample contaminations, minimized losses of the analytes). In this context it is clear that on-line coupling of the sample preparation with CZE is favourable.

Which of the sample preparation techniques offer alternatives compatible with CZE?

1. Some variants of solid-phase extraction (SPE) and column chromatography;

2. various alternatives of liquid-liquid extraction;
3. dialysis and electrodialysis;
4. ultrafiltration and centrifugation;
5. electrophoretic techniques (isotachophoresis, isoelectric focusing and zone electrophoresis).

With various degrees of complexity of the instrumentation all of these techniques can be on-line coupled with CZE. The scope of practical applicability, however, can differ very much, especially, when the extent of sample clean-up is taken as a criterion for the evaluation. In this respect the use of electrophoretic techniques offer some advantages as they can be used to remove ionic sample macroconstituents, separate the analyte(s) from proteinous matrix and isolate the analyte(s) from other sample constituents. A theoretical predictability of optimum working conditions in electrophoresis is, in general, very high so that a search for suitable pretreatment conditions can be considerable reduced in terms of required experimental work.

On-line coupling of capillary electrophoresis techniques

Column-switching capillary electrophoresis equipments as developed for capillary electrophoresis some 15 years ago (refs.5,6) provide already existing alternative to couple CZE with electrophoretic sample pretreatment (ref.7). The question to be answered is which of the electrophoretic techniques is to be preferred for the sample pretreatment. The following criteria will determine the choice:

1. load capacity for a given column;
2. concentrating capabilities;
3. universal applicability to ionogenic compounds;
4. clean-up potentialities;
5. minimum dispersion on the injection of the analyte(s) fraction into the CZE column;
6. automation of the total electrophoretic run.

Although we cannot be conclusive in making unambiguous statements it appears that isotachophoresis (ITP) is meeting these criteria in a wide extent.

This lecture is focused on analytical potentialities of the ITP-CZE combination in (ultra)trace analysis. Practical examples and an accompanying theory will concentrate on the following aspects:

1. Clean-up potentialities from a general point of view. Here, it will be shown that a simple theory can be very useful in guiding the analyst in the choice of the electrolyte systems providing a combination with a minimum chance of the analyte peak overlap with matrix constituents.
2. Clean-up potentialities of the ITP-CZE combination will be illustrated on a practical example (analysis of sulfanilate in urine) (ref.8).
3. Determinations of cationic pesticides (paraquat and diquat) in water is intended to demonstrate (ref.2) capabilities of the ITP-CZE combination in ultratrace environmental analysis.
4. ITP need not be used only for the sample pretreatment but it provides analytical data relevant to the sample macroconstituents. These features of the ITP-CZE combination are illustrated on the determination of ionogenic anions when present in the samples at extremely differing concentrations (ref.9).
5. Capabilities of the ITP-CZE tandem in multicomponent trace analysis will be demonstrated on the determination of nitrophenols.

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