

CHIRAL SEPARATIONS IN CAPILLARY ELECTROPHORESIS

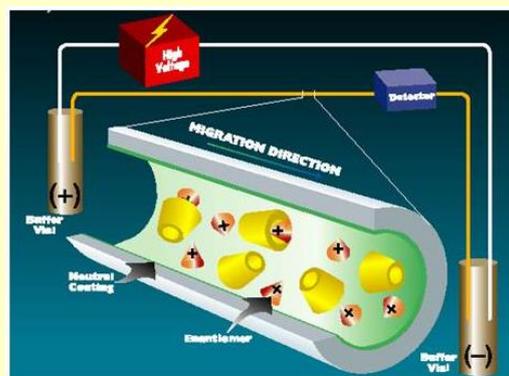
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CE apparatus

We carried out different research projects by using our expertise in the field of the study of equilibria in solution, the synthesis and the NMR characterisation of cyclodextrin derivatives, capillary electrophoresis techniques, and electrospray mass spectrometry for the detection and the characterisation of the involved analytes. The most of our work concerns the separation science, and particularly the chiral separations

Keywords: capillary electrophoresis, cyclodextrins, mass spectrometry, development of nano-separation methods

1. EKC separations in the presence of SID cyclodextrins

The electrokinetic chromatography (EKC) is a technique developed in the field of capillary electrophoresis, by the addition of a selector in the BGE (background electrolyte). The selector forms complexes with the analytes and modifies their apparent mobility, thus permitting separations otherwise not possible, as when the analytes are present as neutral species. An important application of EKC is in the field of the chiral separations. In fact, enantiomeric analytes, when interacting with a chiral selector, give rise to diastereoisomeric complexes that can show different mobilities each other, both by a different degree of formation, and by a different mobility of the complexes, allowing their separation.

As chiral selectors, we use cyclodextrins (CDs), a family of naturally occurring cyclic oligosaccharides characterized by a high solubility and the simultaneous presence of an apolar cavity. To optimise the separations in the context of a rational approach to these techniques we had to home synthesize the requested single isomer derivatives (SID), i. e. the pure regioisomers of cyclodextrins. In fact, generally only mixtures of different derivatives are commercially available, owing to the equivalence of the possible reaction sites of cyclodextrins. Thus, for our studies, it was necessary to synthesise and accurately characterise the selectors, either by NMR spectroscopy and by thermodynamic investigations concerning the equilibria of protonation and of complexation that the selectors undergo in aqueous solution.

Two different kinds of derivatives were synthesised in our laboratory, open monoderivatives, and capped derivatives, where a bridge moiety is added to the cyclodextrin cavity. Our capped derivatives are characterised by the presence of a trehalose unit, a disaccharide, and we have

called this class of compounds "hemispherodextrins". In all the synthesised SID CDs, the functionalising chains include groups showing weak acid-base properties, thus changing their electric charge as a function of the pH value. Typically, an amino group is present which on lowering the pH undergoes a protonation and thus gives rise to a cationic species. Different enantiomeric pairs were separated, among which the dansyl derivatives of amino acids, reaching values of resolution as high as 18 at selector concentrations lower than 3 mM (complete separation even at 0.05 mM).

2. LECE separations in the presence of copper(II) complexes

A specific technique of chiral separation is based on the process of ligand exchange. In this case, besides the chiral selector, an inorganic moiety, either cationic or anionic, able to give rise to complexed species, is added to the BGE. If the analyte has ligand properties, ternary species (including the inorganic moiety, the chiral analyte and the chiral selector) are formed during the electrophoretic run. Similarly to the EKC experiments, the complex formed by one of the enantiomer is diastereoisomeric with respect to that formed by the other one, and thus a separation is possible both on the basis of differences in the formation degree and in the mobility between the two complexes. However, native cyclodextrins have poor coordinating properties towards metal ions and thus they are not suitable for LECE. Thus we synthesized new SID CDs with good coordinating properties and accurately characterized the pertinent equilibria in solution, being able to appropriately choose the experimental conditions of the electrophoretic run (pH, analytical concentrations of all the concerned components) realizing "a rational approach to separation science". As inorganic moiety, the copper(II) ion was used, and as chiral selectors we used both 6- and 3- substituted SID CDs. The analytes separated were amino acids, without any need of preliminary derivatisation.

3. Mass spectrometry as a tool for the separation and the characterisation of inorganic species

A new method of separation of two metals, namely iron and cobalt as their PAR (4-(2-pyridylazo)resorcinol) complexes by capillary electrophoresis with mass spectrometry detection was developed. The experimental approach included the hyphenation of CE instrumentation with a mass spectrometer with an ESI (electrospray) source, allowing to obtain a satisfying separation of the two metals as well as to observe some peculiar phenomena occurring during the spray formation in the source.

ESI-MS was also used to characterise the species formed by 13 copper(II)-dipeptide complexes. The data obtained confirm the presence of the species singled out by previous thermodynamic approaches, showing the ability of electrospray mass spectrometry to give evidence of the presence of a water molecule in the copper(II) in-plane coordination.

4. Development of CE-MS methods in the field of clinical toxicology

The ethylglucuronate is one of the most important metabolites of ethanol present in the urines. Unfortunately, it is a non-volatile substance, and thus GC is not suitable for its determination, and neither it is an UV-active substance, thus not permitting its detection by optical methods. We are developing a method in CE which, by combining procedures for an on-line concentration and ESI-MS detection, can permit the determination of this metabolite at concentrations sufficiently low to be used for toxicological analysis, particularly during the two-three days after the ethanol-taking.

Salsolinol is another important metabolite of ethanol, and it is also found in the urines after ethanol-taking. Besides to be a new alcoholism biomarker, its importance is related to its ability to modify the dopamine metabolism, rendering possible to correlate the tendency to become alcoholic with a genetic predisposition. However, the abuse of ethanol is not directly correlated to the salsolinol concentration, but rather with the ratio between its two enantiomers. By combining a SPE pre-treatment with a phenylboronate stationary phase with the hyphenated CE-MS technique and by using a new SID CDs which is able to interact with the diol functionality of salsolinol, we succeeded in the determination of enantiomeric ratio of salsolinol in the urine.

5. Study of chemical aspects in the purification and in the control of underground and waste waters

Our group has been involved in the development of new methods in the purification of waste waters, realizing a prototype which uses ultrafiltration for the purification. Our contribution mainly concerned the analytical monitoring of the treated and untreated waters. Further developments are in progress to improve this process.

In this field we have also been involved in the project for the development of a prototype for the denitrification of the underground waters. In fact, in Sicily, as well as in many other Italian regions, the major part of the drinkable waters come from underground water tables and thus show a too high concentration of nitrates. The prototype developed for the denitrification plant follows new procedures, in order to reduce the cost and to simplify the process.

Collaborations and Research Grants

Prof. Alejandro Cifuentes, Institute of Industrial Fermentations (CSIC), Madrid, Spain

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Selected Publications

CUCINOTTA V, GIUFFRIDA A, MACCARRONE G., MESSINA M, VECCHIO G (2006). Ligand exchange capillary electrophoresis by cyclodextrin derivatives, a powerful tool for enantiomeric separations. ELECTROPHORESIS, vol. 27; p. 1471-1480, ISSN: 0173-0835

CUCINOTTA V, GIUFFRIDA A, MACCARRONE G., MESSINA M, PUGLISI A, VECCHIO G (2007). SYNTHESIS AND NMR CHARACTERISATION OF β -ALANINA-BRIDGED HEMISPHERODEXTRIN A VERY EFFICIENT CHIRAL SELECTOR IN EKC. ELECTROPHORESIS, vol. 28; p. 2580-2588, ISSN: 0173-0835

CUCINOTTA V, GIUFFRIDA A, GRASSO G, MACCARRONE G., MESSINA M, VECCHIO G (2007). HIGH SELECTIVITY IN NEW CHIRAL SEPARATIONS OF DANSYL - AMINO ACIDS BY CYCLODEXTRIN DERIVATIVES IN ELECTROKINETIC CHROMATOGRAPHY. JOURNAL OF CHROMATOGRAPHY A, vol. 1155; p. 172-179, ISSN: 0021-9673

CUCINOTTA V, CARUSO R, GIUFFRIDA A, MACCARRONE G., MESSINA M, TORRISI A (2008). SEPARATION AND QUANTITATION OF METAL IONS BY 4-(2-PYRIDYLAZO)RESORCINOL COMPLEXATION IN CAPILLARY ELECTROPHORESIS-ELECTROSPRAY IONISATION MASS SPECTROMETRY. JOURNAL OF CHROMATOGRAPHY A, vol. 1179; p. 17-23, ISSN: 0021-9673

MACCARRONE G., CARUSO R, CONTINO A, GIUFFRIDA A, MESSINA M, CUCINOTTA V (2009). THE CONTRIBUTION OF ELECTROSPRAY MASS SPECTROMETRY TO THE STUDY OF METAL COMPLEXES: THE CASE OF COPPER(II)-DIPEPTIDE SYSTEMS. EUROPEAN JOURNAL OF INORGANIC CHEMISTRY, vol. 2009; p. 2612-2620, ISSN: 1434-1948

GIUFFRIDA A, CONTINO A, MACCARRONE G., MESSINA M, CUCINOTTA V (2009). The 3-amino-derivative of γ -cyclodextrin as chiral selector of Dns-amino acids in electrokinetic chromatography. JOURNAL OF CHROMATOGRAPHY A, vol. 1216; p. 3678-3686, ISSN: 0021-9673

CUCINOTTA V, CONTINO A, GIUFFRIDA A, MACCARRONE G., MESSINA M (2010). APPLICATION OF CHARGED SINGLE ISOMER DERIVATIVES OF CYCLODEXTRINS IN CAPILLARY ELECTROPHORESIS FOR CHIRAL ANALYSIS. JOURNAL OF CHROMATOGRAPHY A, vol. 1217; p. 953-967, ISSN: 0021-9673