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The role of silver nitrate as additive in non-aqueous capillary electrophoresis

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In this study, effect of use of silver nitrate as additive on non-aqueous capillary electrophoresis (NACE) separations of some structurally related compounds belonging to antidepressants, neuroleptics or sulfonamides, was examined. The presence of silver nitrate was found to enhance these NACE separations. The use of silver nitrate provided a successful method of improving the separations of antidepressants, neuroleptics and sulfonamides. The use of cyanomethyl-calix$_{[4]}$arene (CMCX$_{[4]}$) in the presence of silver nitrate for the separation of sulfonamides has significantly affected the separation.

1. Introduction

Capillary electrophoresis (CE) has experienced rapid advancement since its introduction in 1981 (Jorgenson 1981). The technique possesses both high resolving power and efficiencies with fast analysis times.

Organic solvents as modifiers have been shown to significantly affect both CE and MEKC separations (Janini et al. 1993; Shi and Fritz 1994; Lee et al. 1995). The first report of 100% non aqueous CE (NACE) separation appeared in 1984 (Wahlbroehl et al. 1984). In addition to the enhanced solubility of the hydrophobic analytes and additives, organic solvents offer greater flexibility in selectivity adjustment and permit a wider range of acid/base strength than in water, leading to extending the range of compounds that can be ionized. Chemical and physical properties of the organic solvents are much different from those of water and can be exploited in the optimization of the CE separations. NACE does not always lead to better solubility or selectivity than aqueous CE, but the large choice of solvents and solvent mixtures makes it very likely to achieve its intended purpose (Geiser and Veuthey 2007). Other advantages include the reduced interaction of hydrophobic compounds with the capillary wall, ion pairing capabilities and the ability to invoke various forms of chemical equilibria in order to place a charge on compounds and thereby allow the electrophoretic separation (Salimi-Moosavi and Cassidy 1995; Miller et al. 1997).

As alternative form of chemical equilibria Wright and Dorsey (1996; 1998) applied Ag(I) ion complexation to improve the selectivity in aqueous and non-aqueous CE depending on several previous metal ion complexation in CE studies (Cohen et al. 1987; Snopek et al. 1988; Taverna et al. 1993). They termed this technique argentation CE. They have applied this technique to separate some sulfonamides and some N-containing heterocyclics. They have found that the used analytes, acting as electron donors, can make charge-transfer interaction with Ag(I) ions, serving as electron acceptor, leading to enhancement of the separations. They have concluded that the stronger the complex the shorter the analyte migration time due to the positive charge of the complexed solute. Their results indicated that the migration order of N-containing heterocyclics is the same in both aqueous and non-aqueous CE but it is not the same for the sulfonamides.

An alternative method to enhance the selectivity in NACE can be the use of calixarenes (Karbaum 2000; Sokoließ et al. 2003). Karbaum (2000) was the first to examine the addition of calixarenes to NACE for the separation of structurally related compounds. This was followed by the examinations by Sokoließ et al. (2003) to separate the geometric isomers of neuroleptics using calixarenes as additives in NACE. Although calixarenes are widely used in CE and HPLC, its use in NACE was described only in the two works previously mentioned. Calixarenes can interact with the analytes through hydrophobic interaction, hydrogen bond formation, charge-transfer-complex formation and/or inclusion complex formation (Arimura et al. 1991; Shinkai et al. 1991; Shinkai 1993).

NACE separations of neuroleptics, beta-blockers, antidepressants and sulfonamides were previously described in a few papers (Karbaum and Jira 1999; Wang et al. 2000; Geiser et al. 2002; Servias et al. 2002; DelmarCantu et al. 2004).

In this study we have reported about the use of $AgNO_3$ to enhance the NACE separations of neuroleptics and antidepressants. The effect of calixarenes on the argentation electrophoresis separation of sulfonamides is also described. As far as we know, no other investigations in this respect exist up to now.

2. Investigations, results and discussion

Benzene was used as neutral marker to estimate the effect on EOF upon the use of $AgNO_3$ in 100% ACN (Table). Benzene does not have any mobility and will be trans-

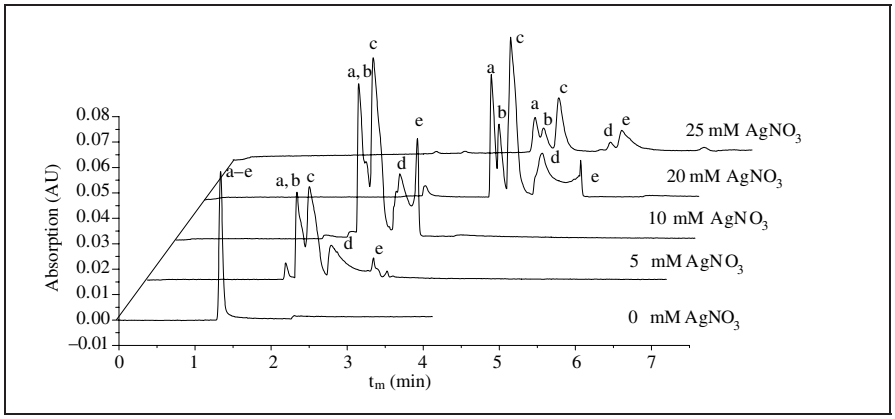

Fig. 1:
Conditions: $AgNO_3$/100% ACN, 20 kV, 27 °C, capillary 40/47 cm
Analytes: (a) levomepromazine (b) chlorpromazine (c) chlorprothixene (d) fluphenazine (e) flupentixol

Table: Conditions: $AgNO_3$ /100% ACN, 20 kV, 27 °C, capillary 40/47 cm

$AgNO_3$ (mM)	Migration time (min)	EOF ($cm^2\ min^{-1}\ kV^{-1}$)
0	1.30	72.25
5	2.67	35.22
10	2.93	32.05
20	4.50	20.91
25	6.68	14.07

ported through the EOF. It is clear that the addition of $AgNO_3$ leads to decreased EOF and hence elongate the migration time of benzene. With increasing concentration the migration time elongated and EOF decreased. EOF decreased through the interaction between Ag^+-ions and $Si-O^-$ on the capillary wall (Fu et al. 2000). Ag^+-ions can also build a charge transfer complex with the benzene ring (through π electrons) leading to initiation of a positive charge on the benzene ring (Wright and Dorsey 1996). This positive charge leads to make benzene migrate faster. So it is clear that there are two factors: one elongates the migration time (the interaction between Ag^+-ions and $Si-O^-$ on the capillary wall) and the other shortens the migration time (charge transfer complex formation). From the Table it is clear that the first factor is more dominant.

When 100% ACN without any addition was used as BGE for the separation of sulfonamides, neuroleptics or antidepressants, only one peak for all analytes in each group was obtained (Figs. 1, 2, and 3). When a very small amount of $AgNO_3$ (5 mM) was added to BGE, more than one peak could be seen but the concentration seemed to be not enough to achieve a significant improvement in separation.

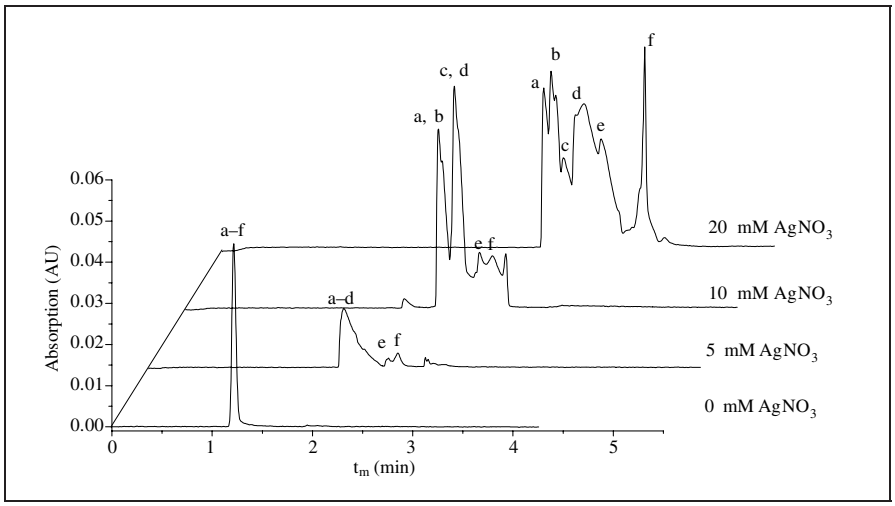

Fig. 2:
Conditions: s. Fig. 1
(a) amitriptyline (b) imipramine (c) mianserine (d) citalopram (e) maprotiline (f) nortriptyline

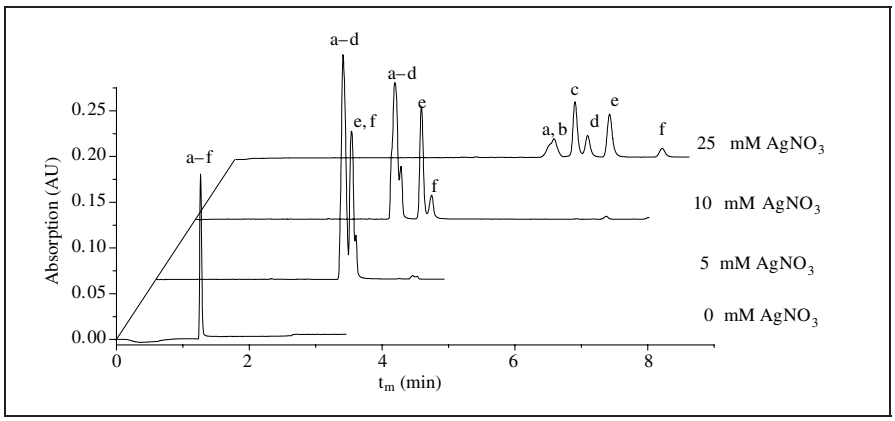

Fig. 3:
Conditions: s. Fig. 1
Analytes: (a) sulfamerazine (b) sulfamethoxypyridazine (c) sulfacetamide (d) sulfanilamide (e) sulfaguanidine (f) sulfacarbamide

So the concentration of $AgNO_3$ was increased gradually until 25 mM, which led to the best separation for all the three groups, although there are some analytes co-eluted or partially separated from each other. The $AgNO_3$ concentration was increased but with more than 25 mM the electric field was always too high leading to break-down of the analysis. Fu et al. (2000) found that washing of the capillary between runs with EDTA/NH_4OH solution helps in overcoming the problem of break-down of the analysis in the presence of high concentration of $AgNO_3$, which was tried in our investigations but did not help, so the concentration of $AgNO_3$ did not exceed 25 mM.

2.1. Separation of neuroleptics

By addition of 25 mM $AgNO_3$ the following migration order was seen (Fig. 1): levomepromazine < chlorpromazine < chlorprothixene < fluphenazine < flupenthixol. The number of the electron-donor-centers is in the following order: levomepromazine > chlorpromazine > chlorprothixene, which indicates the following order of the strength of the complex between each of these three neuroleptics and $AgNO_3$ (and the amount of the positive charge): levomepromazine > chlorpromazine > chlorprothixene. And this is why levomepromazine migrated faster than chlorpromazine and both of them migrated faster than chlorprothixene.
The bigger molecular weight of both fluphenazine and flupenthixol and hence the charge/mass ratio in comparison to the other three neuroleptics can be the reason for the longer migration times of fluphenazine and flupenthixol. Fluphenazine has a higher number of electron-donor-centers than flupenthixol leading to stronger complex with $AgNO_3$ and shorter migration time. Also the basicity of fluphenazine and flupenthixol lower than that of the other three neuroleptics can explain the weather interaction between silver ions and these two neuroleptics (fluphenazine and flupenthixol), which leads to their slower migration compared to the other three neuroleptics.

2.2. Separation of antidepressants

In case of addition of 20 mM $AgNO_3$ antidepressants had the following migration order (Fig. 2): amitriptyline < imipramine < mianserine < citalopram < maprotiline < nortriptyline. Maprotiline and nortriptyline differ from the other examined antidepressants in that they include a secondary instead of a tertiary amino group. The presence of one additional methyl group in the other antidepressants increases the electron intensity in the amino group and hence can form a complex between these analytes and $AgNO_3$ which is more stronger than in the case of maprotiline and nortriptyline leading to a more positive charge on amitriptyline, imipramine, mianserine and citalopram than on maprotiline and nortriptyline.
Also the presence of a hydrogen atom in the side chain of maprotiline and nortriptyline increases their ability to form hydrogen bonds and hence decreases the electron density on the nitrogen atom (Bell et al. 1994) leading to a weaker interaction with $AgNO_3$. For these two reasons maprotiline and nortriptyline have longer migration times than the other antidepressants. The formation of a six member ring through complexation between the two nitrogen atoms in both of amitriptyline and imipramine and silver ions can help to stabilize the formed complexes and hence increases the intensity of the positive charge. This can be also a reason why amitriptyline and imipramine migrated faster than the other antidepressants.

2.3. Separation of sulfonamides

With 25 mM $AgNO_3$ all sulfonamides were separated from each other except for sulfamethoxypyridazine (which was not used in the study of Wright and Dorsey (1998)) and sulfamerazine (Fig. 3). By combination of 25 mM $AgNO_3$ with 2 mM CXAI ($AgNO_3$ was added to the BGE while calixarene was applied through PFT), the separation was negatively affected (Fig. 4), as sulfamerazine and sulfamethoxy-pyridazine were still co-eluted with each other and sulfacetamide and sulfanilamide were not completely separated from each other or from sulfamerazine and sulfamethoxypyridazine. Upon increasing CXAI concentration to 4 mM the separation improved compared to 2 mM CXAI but was still not as good as in the case of 25 mM $AgNO_3$ alone. It was noted, that with increasing CXAI concentration the migration times increased indicating certain interactions between the analytes and CXAI or change of the interaction between the analytes and $AgNO_3$ through a certain interaction between CXAI and $AgNO_3$. When 2 mM $CMCX_{[4]}$ were used in presence of $AgNO_3$ the separation between sulfamerazine and sulfamethoxypyridazine could be initiated, which were co-eluted with each other in the absence of $CMCX_{[4]}$. But the separation between sulfamethoxypyridazine and sulfacet-amide/sulfanilamide was negatively influenced.
The increase in $CMCX_{[4]}$ concentration to 4 mM improved the separation of the first four sulfonamides but

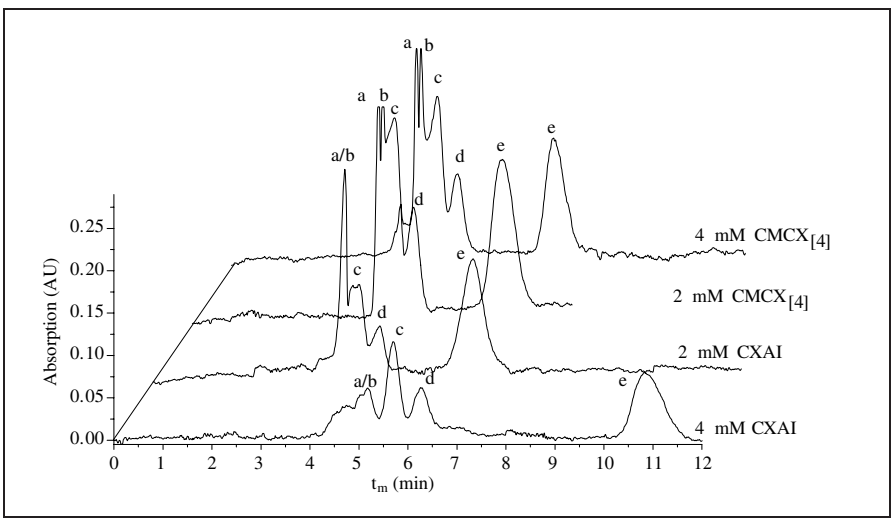

Fig. 4:
Conditions: 25 mM $AgNO_3$/100% ACN, 20 kV, 27 °C, capillary 30/37 cm
Analytes: (a) sulfamerazine (b) sulfamethoxy-pyridazine (c) sulfacetamide (d) sulfanilamide (e) sulfaguanidine

they were still not baseline separated. By using 25 mM $AgNO_3$ with or without calixarene additives the following migration order was obtained: sulfamerazine/sulfamethoxypyridazine < sulfacetamide < sulfanilamide < sulfa-guanidine < sulfacarbamide. Sulfamerazine and sulfamethoxypyridazine contain more electron-donor-centers than the other sulfonamides, what led to more intensive complexes between these two sulfonamides and $AgNO_3$ with stronger positive charge leading to shorter migration times of these two sulfonamides. These two sulfonamides can also form six a membered ring between the silver ions and two nitrogen atoms of sulfonamides (one of these nitrogen atoms is heterocylic and the other is attached to the sulfonyl group). This can increase the stability of the formed complexes and hence increases the intensity of the positive charge leading to faster migration. The presence of two or three amino groups with several hydrogen atoms in the side chains of sulfaguanidine and sulfacarbamide increases the ability of these two sulfonamides to make hydrogen bonds, what resulted in the lowering of electron density in the side chains (Bell et al. 1994) and hence the weaker interaction with $AgNO_3$ and the longer migration times. Sulfaguanidine is more basic than sulfacarbamide leading to better interaction between sulfaguanidine and $AgNO_3$ and shorter migration time of sulfaguanidine in comparison to sulfacarbamide. Sulfacetamide and sulfanilamide have a lower ability to form hydrogen bonds than sulfaguanidine and sulfacarbamide due to the presence of only one amino group in the side chain, so sulfacetamide and sulfanilamide can form stronger complexes with $AgNO_3$ and hence have shorter migration times than sulfaguanidine and sulfacarbamide. Sulfanilamide has less electron-donor-centers in comparison to sulfamerazine, sulfamethoxypyridazine and sulfacetamide and this can be the reason for the longer migration time of sulfanilamide in comparison to these three sulfonamides.

The effect of $AgNO_3$ on the NACE separations of some related compounds in the presence and absence of calixarenes was examined. $AgNO_3$ presented a method to change the selectivity in NACE. 25 mM $AgNO_3$ has strong positive effect on the separations. But it is important to find a method to overcome the breakdown of the analysis upon high concentrations of $AgNO_3$. The presence of calixarenes affects the separation of sulfonamides in the presence of $AgNO_3$ indicating the the presence of certain interactions between $AgNO_3$, calixarene and/or analytes. In a further study we will study the combination between calixarenes, $AgNO_3$ and surfactants for the separation of different analyte groups.

3. Experimental

3.1. Chemicals

HPLC grade acetonitrile (ACN) was purchased from Applichem (Darmstadt, Germany). Water was deionised and doubly distilled. $AgNO_3$ was obtained from Sigma (St. Louis, MO, USA). Cyanomethyl-calix$_{[4]}$arenes ($CMCX_{[4]}$) and calix$_{[4]}$arene (CXAI) were supplied by Syntrex GbR (Greifswald, Germany). Neuroleptics were friendly supplied by Tropon (Cologne, Germany). All of them were used as HCl salts except promazine as phosphate and perazine as hydrogenmalonate. Antidepressants amitriptyline, citalopram-HBr and nortriptyline (H. Lundbeck A/S, Kopenhagen, Denmark), imipramine-HCl, maprotiline-HCl and mianserine (Salutas Pharma GmbH, Barleben) Sulfonamides were obtained from Sigma (St. Louis, MO, USA).

3.2. Apparatus and separation conditions

CE was performed using a P/ACE 2100 capillary electrophoresis instrument (Beckman, Fullerton USA) equipped with an on-column UV-detector. GOLD software (Beckman) was used for data acquisition.

Analyses were performed at a detector wavelength of 214 nm with an applied voltage of 20 kV in fused silica capillaries (30/37 or 40/47 cm × 50 μm I.D.; CS-Chromatographie Service GmbH, Langerwehe, Germany) thermostated at 27 °C. The analytes were dissolved in MeOH (1 mg/ml) and before the injection the solution was mixed with electrophoresis medium (1 : 1) to prevent the breakdown of the current flow. Samples were injected hydrodynamically for 4 s under low pressure (0.5 psi). All the used solutions were filtered before use through a 0.45 μm filter.

Before the first use of the capillary, it was rinsed with 1 M HCl, bidistilled water, 0.1 M NaOH then finally with bidistilled water each of them for 10 min. At the beginning of the analysis each day, the capillary was rinsed with MeOH, 0.1 M NaOH then with bidistilled water each of them for 5 min. Between the runs, a rinsing was performed with MeOH for 2 min, 0.1 M NaOH for 2 min then with BGE for 4 min. After the last run each day the capillary was rinsed with MeOH, 0.1 M NaOH, bidistilled water then with air each of them for 2 min. All rinsing steps were applied under high pressure of 5 psi. In case of PFT, calixarene/BGE was injected under the low pressure (0.5 psi) for 2 min direct before injection of the sample. For each run three injections were performed.

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Abbreviations

$CMCX_{[n]}$	= cyanomethyl-calix$_{[n]}$arene
BGE	= background electrolyte
EOF	= Electroosmotic flow
t_m	= migration time
PFT	= partial filling technique
ACN	= acetonitrile.

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