Combined use of cyclofructans and an amino acid ester-based ionic liquid for the enantioseparation of huperzine A and coumarin derivatives in CE

Ioannis Stavrou[a], , Constantina Kapnissi-Christodoulou[a]

[a] *Department of Chemistry, University of Cyprus, 1678 Nicosia, Cyprus; stavrou.ioannis@ucy.ac.cy*

Abstract

Cyclofructans (CFs) and their derivatives have recently been proven to be efficient chiral selectors (CSs) for the enantioseparation of several analytes in CE, HPLC, and GC. In this study, the chiral separation ability of a number of native and derivatized CFs was examined in CE. Particularly, six different CFs, with different derivatization groups and cavity sizes [native CF-6 and CF-7, isopropyl cyclofructan-6 (IPCF-6), IPCF-7, sulfated cyclofructan-6 (SCF-6), and SCF-7] were used as CSs for the enantioseparation of huperzine A, warfarin, and coumachlor. Almost all of the examined CFs, except from SCF-6 & -7, demonstrated relatively low and sometimes no chiral separation ability for huperzine A. In an effort to improve both resolution and efficiency, the chiral ionic liquid D-Alanine *tert* butyl ester lactate (D-AlaC_4Lac) was added into the BGE. In most of the cases, the combination of CF with D-AlaC_4Lac resulted in an improvement in peak efficiency and/or resolution. When CF-6 was utilized with D-AlaC_4Lac, a resolution of 1.4 was obtained, while the use of IPCF-6/D-AlaC_4Lac provided a baseline enantioseparation. Although the combination of SCF-7 and 40 mM D-AlaC_4Lac did not affect resolution, it dramatically increased peak efficiency from 24 000 to 117 000. In the case of warfarin and coumachlor, IPCF-6 and IPCF-7 proved to be the most effective CSs. It is, therefore, concluded that the size of the cavity and the CF derivatization are the key parameters for the chiral separation capability. It is also clear from this study that D-AlaC_4Lac is necessary for improved peak efficiencies and resolutions. The method was then validated by estimating the run-to-run and batch-to-batch repeatability of the method, at the optimum conditions. Finally, by estimating the uncertainty of measurements, the particular method could be suggested for the routine analysis of pharmaceutical compounds.

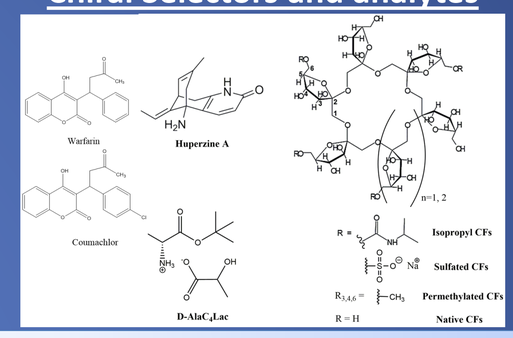
Experimental and Methods

Separation Conditions

- Agilent CE System equipped with UV-Vis detector (230 nm, 214nm).
- Fused-silica capillary column (l= 55.5 cm, l = 30.0 cm).
- A new capillary was washed with H_2O (5 min), 1 M NaOH (60 min), H_2O (10 min), BGE (30 min)

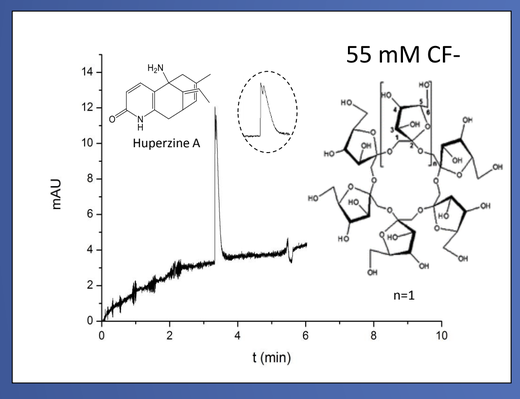
.**BGE:** i) Tris/Borate, ii) 4 mM Ammonium Acetate in H_2O/MeOH (95:5), pH = 4.0, iii) 4 mM Ammonium Acetate in H_2O/MeOH (80:20), pH = 4.0

Chiral Selectors and analytes



Results

Use of native CF-6

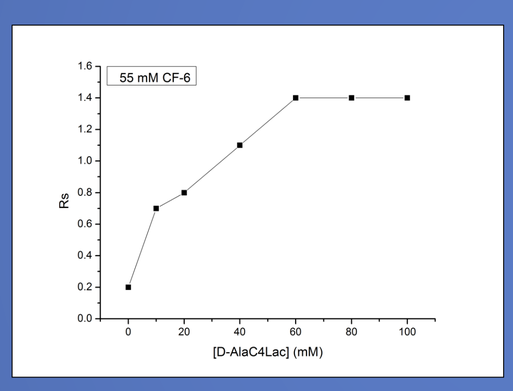


10, 25, 35 mM CF6 ➡ No separation

55 mM CF6 ➡ Slight splitting (R_S = 0.2)

No chiral separation of Hup.A

Combined use of CF-6 and D-AlaC_4Lac

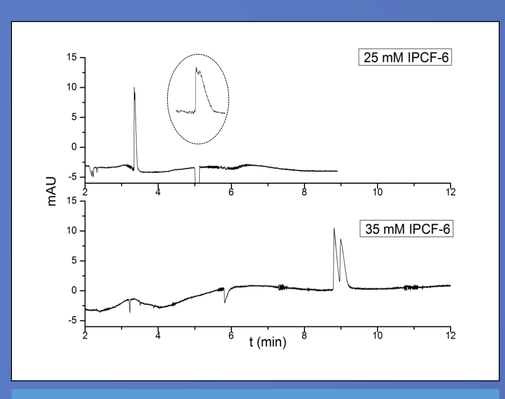


↑ [D-AlaC_4Lac] ➡ ↑R_S

60 mM D-AlaC_4Lac: MAX. **R_S = 1.4**

Addition of IL → Increase of R_S

Use of IPCF-6



Greater enantioselectivity than CF-6

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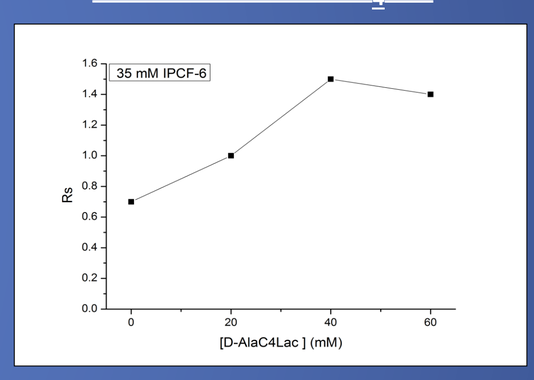
Isopropyl-groups provide more interaction sites for the enantioseparation

Addition of D-AlaC_4Lac



Addition of IL in the BGE caused increase of R_S

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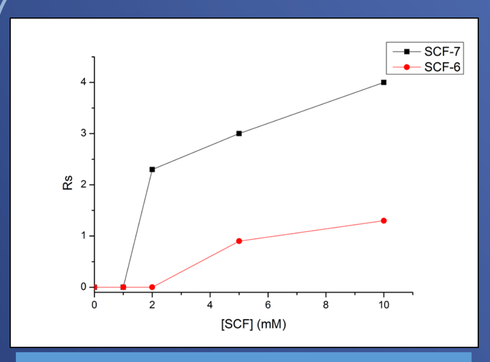
Synergistic effect

Use of SCF-6 and SCF-7



SCF-7 demonstrated the greatest enantioselectivity

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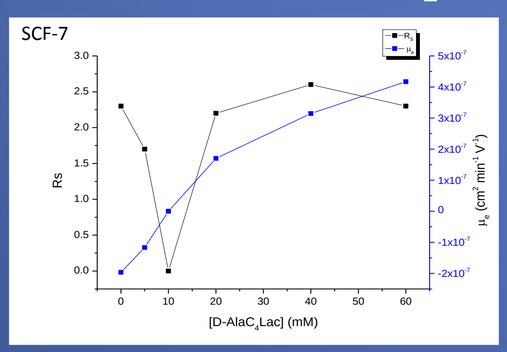
SCF-7: More sulphated groups → more electrostatic interactions

Effect of D-AlaC_4Lac on R_S and efficiency



Increase of IL concentration increased the mobility

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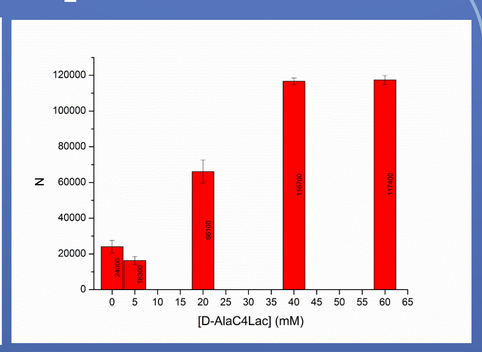
↑10 mM D-AlaC_4Lac → positively charged complex → different separation mechanism

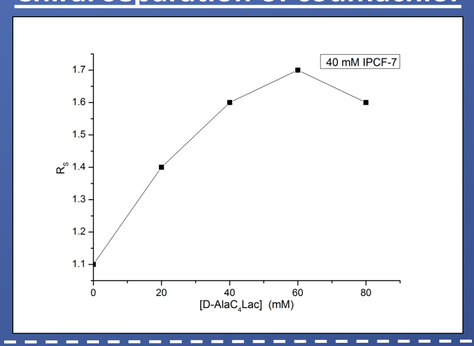


Increase of IL concentration increased the efficiency

Chiral separation of warfarin

	[D-AlaC4Lac] (mM)			
[IPCF-6] mM	0	10	20	40
40	0.5	0.8	0.9	0.6

Chiral separation of coumachlor



IPCF-6 and IPCF-7 in combination with D-AlaC_4Lac were the optimum chiral selectors for the enantioseparation of Warfarin and Coumachlor, respectively.

Reproducibility study

The run-to run and batch-to-batch reproducibility was evaluated by calculating the RSD values of the EOF and the migration time of the first eluted peak

Reproducibility	RSD_{EOF} (%)	$RSD_{(+)Hup.A}$ (%)
Run-to-run[a]	0.6	0.7
Batch-to-batch[b]	< 0.50	< 0.80

[a]The studies were done in the same capillary.

[b]The study was performed by synthesizing three batches of D-AlaC_4Lac.

Conclusions

- Cyclofructans demonstrated low enantioselectivity in CE.
- Sulphated CFs demonstrated the greater enantioselectivity towards huperzine A probably due to the electrostatic interactions, however poor peak efficiency was observed.
- The addition of the chiral ionic liquid D-AlaC_4Lac in the BGE increased, in the most cases, the resolution and peak efficiency.
- A synergistic effect between D-AlaC_4Lac and CFs probably takes place during the enantioseparation.
- Warfarin and coumachlor enantiomers were partially separated when IPCFs were used, while no enantioseparation was performed when SCFs were used. Therefore, the enantioseparation mechanism, in these cases, differs from that observed for huperzine A.
- The run-to-run and batch-to-batch reproducibility were evaluated and it can be concluded that the use of SCF-7 and the chiral ionic liquid, can provide excellent reproducibility for the enantioseparation of huperzine A.

Acknowledgments

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References

Stavrou, I. J., Breitbach, Z. S., Kapnissi-Christodoulou, C. P., Electrophoresis 2015, 36, 3061–3068