Chiral Separation of Nefopam using cyclofructans and chiral ionic liquids in CE

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Abstract

A chiral center in many pharmaceutical compounds gives rise to optical activity which can make a large difference between the two enantiomers in terms of pharmacokinetics, activity and toxicity. The widely accepted limit of enantiomeric impurity in the testing of a single enantiomer is 0.1% (m/m), which, in turn, requires that analytical methods have large enantiomeric-separation power and high-detection sensitivity.

Capillary Electrophoresis (CE), a well-established and unique type of analytical technique has many advantages, among them versatility and efficiency. In this study, the chiral separation of nefopam hydrochloride, a centrally-acting non-opioid analgesic drug of the benzoxazocine chemical class, is demonstrated. Chiral analysis of the drug has already been reported by using the universal and well-known chiral selectors (CSs), cyclodextrins (CDs), in capillary electrophoresis.

This is the first report of nefopam enantioseparation, in which, a new and promising category of chiral selectors, the cyclofructans (CFs), is utilized, as well as chiral ionic liquids (CILs), a new class of non-molecular solvents with unique properties in different areas of chemistry. The growing interest in CILs has also been observed in separation techniques, where they are used as either BGE additives or as sole CSs.

In this study, a comparison between SCF_6 and SCF_7 was made, and the effect of the CIL, L-Alanine tert butyl ester lactate (L-AlaC_4Lac) on both resolution and efficiency was examined. Moreover, the combined use of L-AlaC_4Lac (as a BGE additive) and SCF_6 (as a CS) was investigated.

Other parameters that affect the enantioseparation were also examined, such as BGE type and concentration, pH, CS type and concentration, CIL concentration and applied voltage. The optimum separation conditions were determined to be 2 mM SCF_6, in 100mM Tris/10mM Borate (pH 8.00) and the time of analysis was 3.5 min. However, in order for the method to be applied to biological and pharmaceutical samples for qualitative and quantitative analysis, the uncertainty of the method has to be evaluated, in regard to precision, accuracy, detectability and linearity.

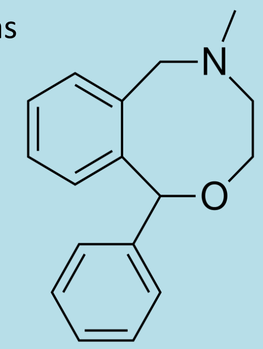


Figure 1. Nefopam (NEF) Structure.

Experimental

- ✓ Agilent System equipped with DAD detector (λ=200 nm).
- ✓ Fused-silica capillary column (L_{tot} = 38.5 cm, L_{eff} = 30 cm, 50 μm i.d.).
- ✓ A new capillary was washed with H_2O (10 min), 1 M NaOH (60 min), H_2O (10 min), BGE (30 min).
- ✓ **BGE**: 100 mM Tris/10 mM Sodium tetraborate decahydrate
- ✓ **T** = 20 °C, ***V= 20 kV**, **pH = 8.00.**
- ✓ **Injection size**: 50 mbar for 3 sec.
- ✓ **Analyte**: 0.4 mg/mL racemic mixture of Nefopam in MeOH.
- ✓ Examined concentrations of the **CS:** 1 mM - 10 mM.
- ✓ Examined concentrations of the **CIL:** 10 mM - 100 mM.

Effect of pH on Separation

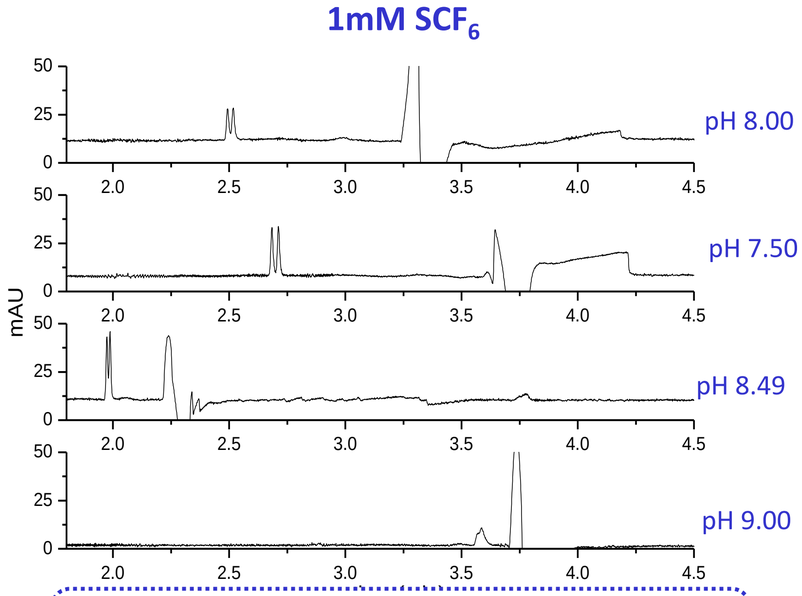


Figure 2. Electropherograms of racemic NEF obtained at different pH values with 1mM SCF_6.

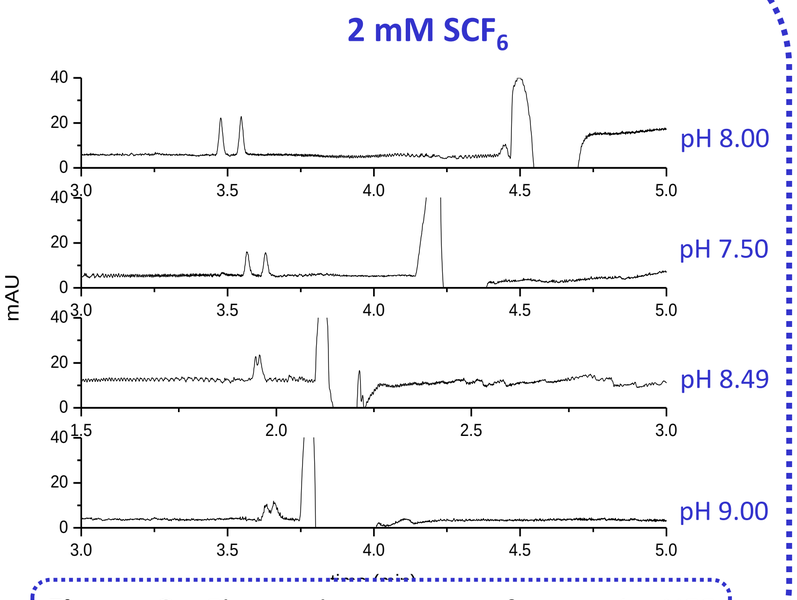


Figure 3. Electropherograms of racemic NEF obtained at different pH values with 2mM SCF_6.

Effect of SCF_6 Concentration

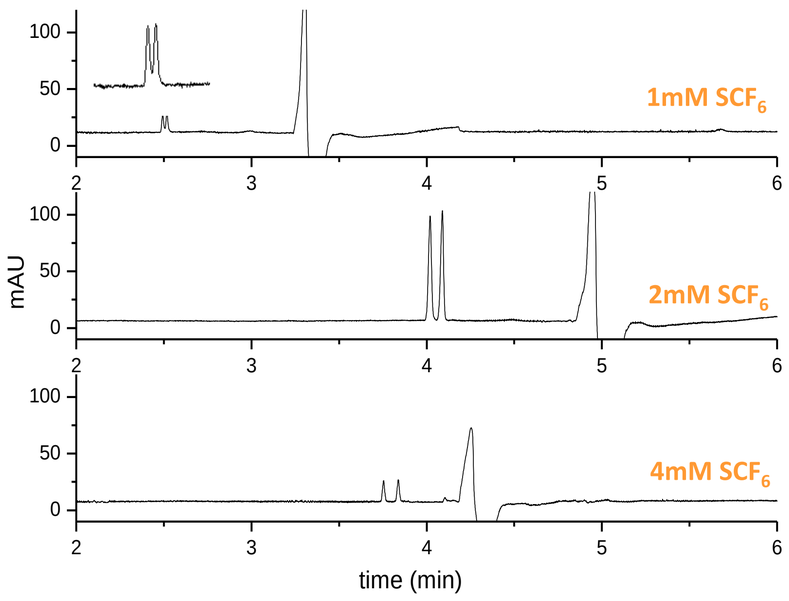


Figure 4. Effect of SCF_6 concentration on enantioseparation of NEF.

- ✓ 2mM SCF_6 resulted in baseline discrimination of NEF enantiomers (Rs = 1.92).
- ✓ Further addition of SCF_6 resulted in Rs decrease.
- ✓ 4mM SCF_6 (Rs of 3.84).
- ✓ At 10mM SCF_6 resolution was lost.

- ✓ Strong ionic interaction between the (+) charged NEF and the (-) charged SCF_6.

- ✓ **L-AlaC_4Lac as the sole CS in the BGE presented no enantioselectivity for NEF enantiomers.**

- ✓ The Study of the combined use of L-AlaC_4Lac and the SCF_6 follows, in an attempt to examine a possible **synergistic effect** between the CS and the CIL.

CD	CF
S-α-CD	S-CF_6 and S-CF_7
S-β-CD	n-CF_6 and n-CF_7
	IPCF_6 and IPCF_7
	CM-CF_6

Table 1. The CSs utilized in this project, CDs and CFs.

Effect of CIL on Separation

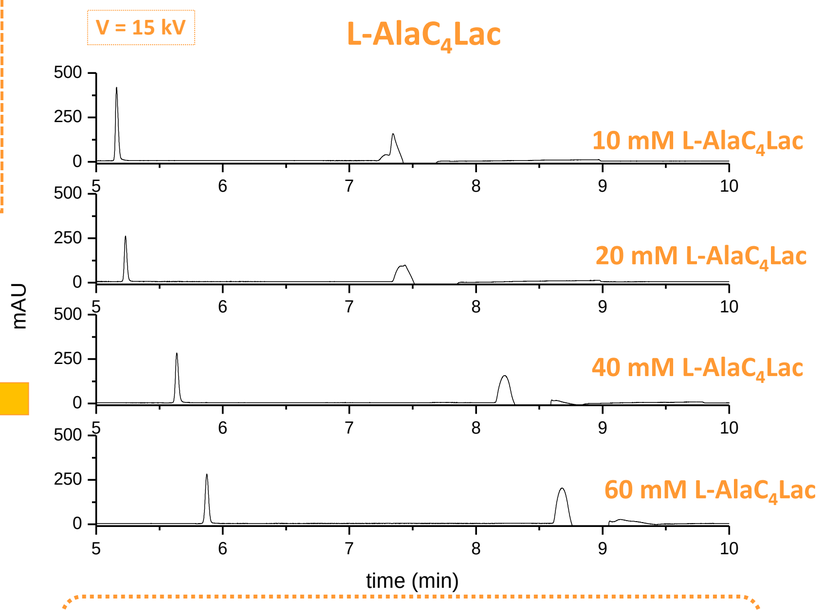
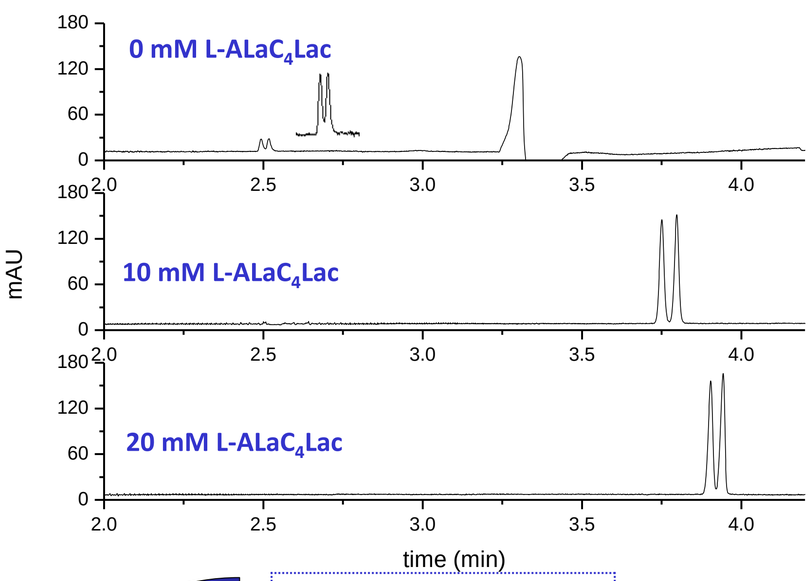


Figure 3. Effect of L-AlaC_4Lac concentration on the enantioseparation of NEF.

Synergistic Effect of SCF_6 and CIL on Separation

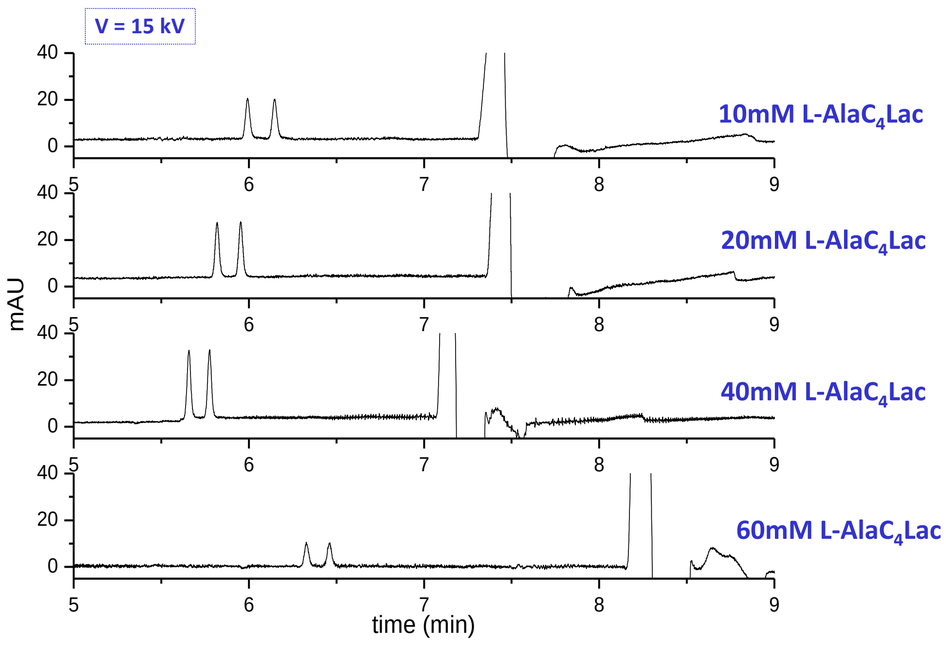
V = 15 kV

1mM SCF_6 + dC mM L-ALaC_4Lac



L-ALaC_4Lac (mM)	Rs	N
0	0.86	104807
10	1.18	255994
20	1.13	213903

2mM SCF_6 + dC mM L-AlaC_4Lac



L-ALaC_4Lac (mM)	Rs	N
10	2.07	103454
20	2.13	145471
40	2.41	157090
60	2.84	320112

Concluding marks

- ✓ A CE method was developed for the discrimination of NEF enantiomers using 100mM Tris/ 10mM borate as BGE solution.
- ✓ **Separation Conditions:** 2mM SCF_6 (added to the BGE), at pH 8.00,Rs = 1.61.
- ✓ UV detection at 200nm and the temperature was controlled at 20° C.
- ✓ Addition of L-AlaC_4LAc improves resolution and efficiency.
- ✓ SCF_7 was also tested as a CS but no enantioselectivity for NEF enantiomers was observed.

Future/Current Work

Evaluation of the uncertainty of the method in regard to:

- ✓ precision
- ✓ accuracy,detectability and
- ✓ linearity.

Acknowledgements: Special thanks to Dr. D. W. Armstrong's group for providing the cyclofructans.