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Evaluation of electro-osmotic markers in aqueous and non-aqueous capillary electrophoresis

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AIM

The aim of this study was to evaluate and compare some compounds as EOF markers in aqueous and non-aqueous background electrolytes (BGEs). Different electrolyte system as well as BGEs with chiral selectors and micellar additives has been investigated.

Introduction

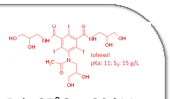
It is always necessary to know the electro-osmotic flow (EOF) and the reproducibility of the EOF during method development or troubleshooting in CE and sometimes it can be important to more accurately determine the EOF.

The most common was to measure the EOF is through the injection of a neutral marker.

There is a lack of a more general comparative studies of EOF markers in the literature. The previously published comparative studies only include one aqueous system at a specific pH [1] and micellar systems [2].

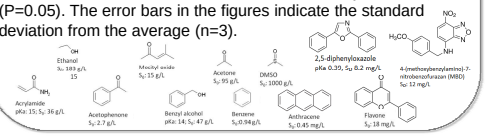
Experimental

Capillary electrophoresis: HP^{3D}CE



L_{det}:59.5 cm L_{tot}: 68.0 (50 µm I.D., 360 µm O.D.), 25°C, +30 kV, λ_{abs} : 200, 245, 310, 355 and 488 nm
Conditioning between runs: 0.1 M NaOH (0.5 min, 1 bar), BGE (10 min, 1 bar), 30 kV for 30 s. Injection:6 sec 35 mbar

One-way ANOVA (P=0.05) was used to identify the markers that differed from the others. Outliers were identified by Dixons Q-test (P=0.05). The error bars in the figures indicate the standard deviation from the average (n=3).



Influence of injection medium

Some of the hydrophobic markers were difficult to dissolve in the BGE, which caused distorted peaks. The peak shape for these markers are illustrated by acrylamide in different injection medium.

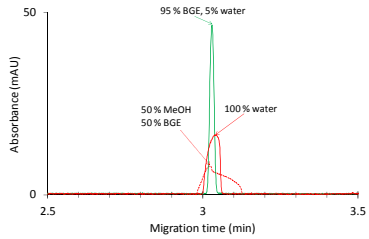


Figure 1. The peak shape for acrylamide in different injection medium
BGE: borate buffer pH 10.0 (I= 0.02 M). Green: 1 mM acrylamide (AA) in 50:45:5 MeOH BGE: H_2O, red: 1 mM AA in water, red dashed: 1 mM AA in 95:5 BGE: H_2O

Influence of SDS

•Acetone, acrylamide, DMSO and ethanol can be used as EOF markers.
•Anthracene, MBD, diphenyloxazole and flavone can be used as micellar markers.

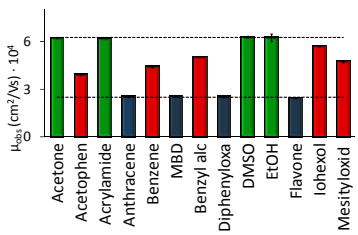


Figure 2. Effect on observed mobility in SDS-based BGE
BGE:0.02 M borate buffer pH 9.0 with 0.05 M SDS. The dashed lines indicates the two subgroups.

Influence of pH and buffer type

Sodium borate buffer

All markers seem to be useful in borate buffer except:

• *Iohexol who* is known to complexate with borate, and was used as a marker for complexation.
• *Anthracene* that differed in borate buffer at pH 8, 9 and 10.
• *MBD* had a lower observed mobility (μ_{obs}) in borate buffer.
Interestingly, the decrease is more pronounced at pH 10.

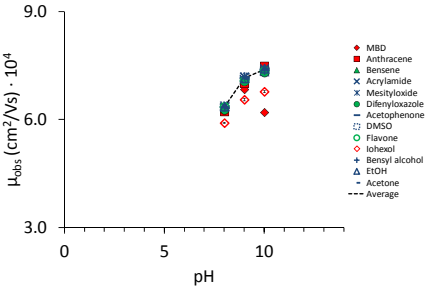


Figure 3 The observed mobility for the markers in borate buffer
0.02 M sodium borate buffer.The average values for all markers are connected by a dashed line.

None of the markers (except iohexol) seems to complexate with borate, Figure 4

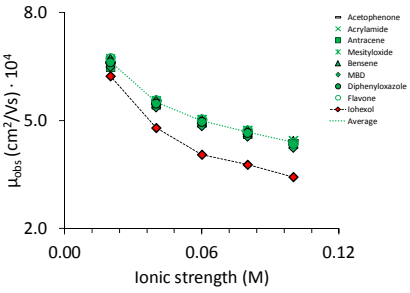


Figure 4. Observed mobility as a function of ionic strength
BGE: borate buffer pH 8.5

Sodium phosphate buffer

All markers seem to be useful in phosphate buffer except:

• *Anthracene* at pH 8.0 (lower μ_{obs} than the other)
• *MBD* at pH 6.0 and 8.0 (lower μ_{obs})
• *Diphenyloxazole* at pH 2.0 (higher μ_{obs}) and pH 8.0

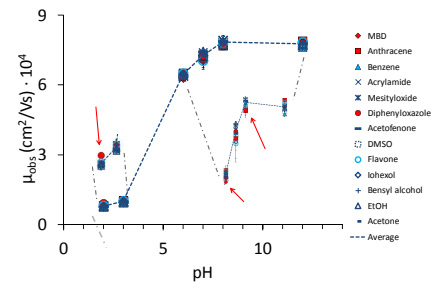


Figure 5. The observed mobility for the markers as a function of pH
0.02 M sodium phosphate buffer, The average value for all markers is connected by a dashed line.

References:
[1] Beckers, J. L., Everaerts, F. M., Ackermans, M. T., *J. Chromatogr.* 1991, *537*, 407-428
[2] Fuguet, E., Rafols, C., Bosch, E., Roses, M., *Electrophoresis* 2002, *23*, 56-66.

Ammonium acetate buffer

All analytes seem to be useful in acetate buffer except:

• *MBD at* pH 8.0 and 9.0 (lower μ_{obs} than the other)
• *Diphenyloxazole* at pH 8.0 and 9.0

As expected, the RSD(%) for μ_{obs} for all markers within one pH is higher at pH 4-6 (1-4%) than for pH 8-9 (0.5-0.9 %)

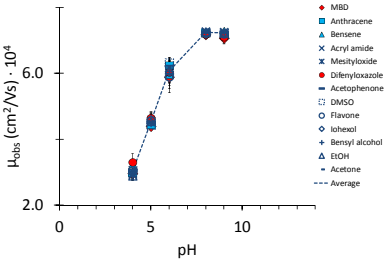


Figure 6. The observed mobility for the markers as a function of pH
BGE: 0.02 M ammonium acetate buffer. The average value for all markers is connected by a dashed line.

Sulphated β-cyclodextrin

Surprisingly, none of these structurally different markers seems to interact with the cyclodextrin. Thus all 13 analytes are suitable as EOF markers in a 20 mM phosphate buffer pH 6.0 that contains this sulphated β-cyclodextrin.

EOF-markers in NACE

All markers seem to be useful in NACE except:

• *Antracene* (lower μ_{obs} in one of the BGEs)
• *DMSO* (higher μ_{obs} in two out of three BGEs)
• *Iohexol* (lower μ_{obs} in one of the BGEs)

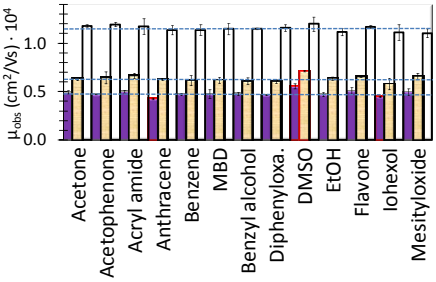


Figure 7. Effect on observed mobility of non-aqueous BGE
BGE: purple:50 mM NH_4Ac, 14 mM NH_4OH in MeOH
yellow:100 mM HAc, 50 mM NH_4Ac in MeOH
white:100 mM (-)-DIKGA (chiral selector) and 40 mM NaOH in MeOH
The average for each series is marked with the blue dotted line.

Conclusion

Recommended EOF markers

• **In ammonium acetate, sodium phosphate sodium borate based BGE**: acetone, acetophenone, **acrylamide**, benzene, benzyl alcohol, DMSO, **flavone,** EtOH and mesityloxide
• **In a SDS containing BGE:** acetone, **acrylamide**, DMSO and ethanol
• **NACE**: **acrylamide**, MBD, benzyl alcohol, diphenyloxazole, EtOH, **flavone** or mesityloxide

Acrylamide is, due to its low volatility, high solubility, detectability and sharp, symmetrical peaks. recommended as a ***general EOF-marker*** *when UV detection is used.*