

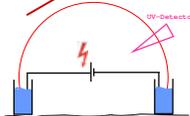
Design and evaluation of a modified ITP instrument for interfacing capillary isotachopheresis-zone electrophoresis (ITP-CZE) in a column-coupling format with electrospray mass spectrometry (ESI-MS)

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ITP-CZE is > 100 times more sensitive than CZE

Read clockwise from here - if you feel like it...



Conventional (single-capillary) CZE

V [eff] ~ 2,200 nI
V [inj] ~ 50 nI
Capillary 0.075 mm * 50 / 57 cm

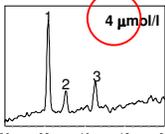
The Case for ITP-CZE

Introduction / Background

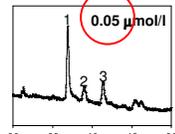
Capillary (zone) electrophoresis (CE or CZE) offers complementary approaches to gas, liquid, and ion chromatography, its advantages include excellent mass detection limits, different selectivity, and that there is usually no need to derivatize polar/charged analytes.

One of the major disadvantages of CZE is its relatively poor concentration sensitivity, which limits the use of CZE in trace analysis, e.g., of complex biological samples.

Poor concentration detection limits are a consequence of short optical path lengths, and, particularly, of small injection volumes - typically tens of nanoliters in CZE, compared with a few microliters in LC.



single-capillary CZE

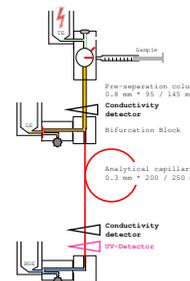


column-coupling ITP-CZE

- 1 = terephthalic acid
- 2 = isophthalic acid
- 3 = phthalic acid (UV at 254 nm)

How does it work ?

Large injection volumes can be realized by combining isotachopheresis with zone electrophoresis in coupled capillaries (ITP-CZE): during ITP in the first capillary, the analytes of interest are concentrated and separated from sample macro-components, and then transferred as sharp isotachopheretic bands to the second capillary, where they are separated in zone-electrophoresis mode and detected. ITP-CZE is 2-3 orders of magnitude more sensitive than conventional CZE, and it enables trace components to be analyzed in complex matrices with little or no sample pre-treatment.



Column-coupling ITP-CZE

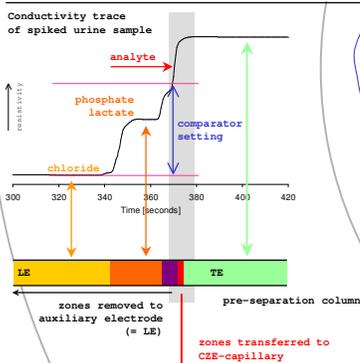
inj. valve: V [inj] ~ 30,000 nI
pre-column: V [tot] ~ 73,000 nI
analytical: V [eff] ~ 14,000 nI
desirable V [inj] < 350 nI

The Big Picture

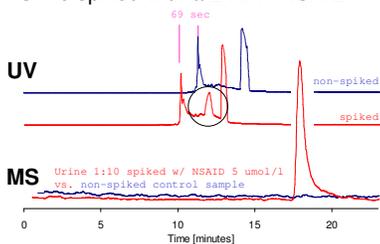
We are doing pretty good - and would be even better in positive mode ?!

The ITP Pre-Separation

Conductivity detector controls column switching



ITP-CZE-UV-MS: Urine spiked with a 2-APA NSAID



Proof of concept: switching to the additional electrode with a more complex electrolyte system. LE: HCl 10 mM His 18 mM, TE: MES 5 mM His 4 mM, BGE: acetic 10 mM amm. acetate 10 mM pH 4.75. CZE capillary 100 µm 15-22 µA → 8-9 kV.

Evaluation with more complex systems

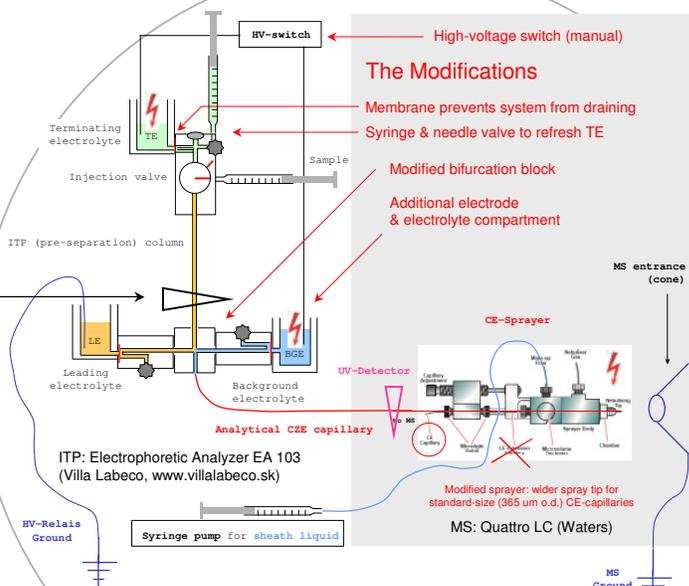
More complex electrolyte systems were used to prove the concept of switching to the additional electrode and to look at a wider range of analytes. These included 2-arylpropionic acid (2-APA) NSAIDs, vanillylmandelic (VMA) and homovanillic acid (HVA), and a range of small organic acids, many without UV-absorbing chromophores. The results demonstrate both the potential of MS-coupling for highly specific trace analyses of complex biological matrices and the significant gain in sensitivity over conventional CE-MS.

Conclusions

Our results indicate that ITP-CZE-MS has great potential for complex and trace analysis, and that it should be possible to build a next-generation CE-instrument that would combine column-coupling technology with mass-selective detection. Clearly, the potential gains in specificity and sensitivity are too promising to leave this possibility unexplored.

The Modifications

- High-voltage switch (manual)
- Membrane prevents system from draining
- Syringe & needle valve to refresh TE
- Modified bifurcation block
- Additional electrode & electrolyte compartment



ITP: Electrophoretic Analyzer EA 103 (Villa Labeco, www.villalabeco.sk)

$$c_1 \cdot V_1 = c_2 \cdot V_2$$

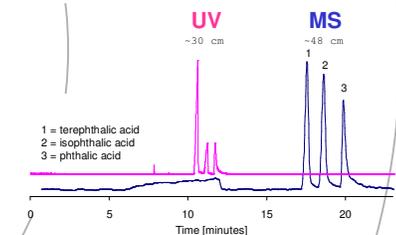
| The (theoretical) detection limit in ITP-CZE | | |
|--|----------------------------|--|
| $c_1 \cdot V_1$ | 50 nI * 1 µmol/l | |
| $c_2 \cdot V_2$ | 30,000 nI * 0.00167 µmol/l | |

- 1 = CZE, injection volume 50 nI, LOD 1 µmol/l
- 2 = ITP-CZE, same amount of analyte in 30 µI injection volume

A novel approach to ITP-CZE-MS

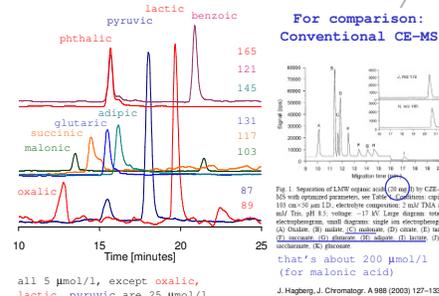
Interfacing ITP-CZE with mass spectrometry would be advantageous, for very much the same reasons as in single-capillary CE-MS, i.e., increased specificity, selectivity, and possibly sensitivity. Previous attempts to hyphenate ITP-CZE with MS have used experimental configurations (termed 'injection-split' technique and 'comprehensive ITP-CZE'). We have followed a different approach and modified a 2-dimensional ITP-instrument for ITP-CZE-MS.

ITP-CZE-UV-MS: Phthalic acid isomers



Initial testing with a simple model system. The BGE co-ion (acetate) matches the TE (acetic acid); no need to switch to the additional electrode. LE: HCl 5 mM β-alanine 30 mM, TE: acetic ac. 3.5 mM, BGE: acetic ac. 22 mM amm. acetate 8 mM pH 4.3. CZE capillary 150 µm [sic] 24 µA → 12-13 kV.

ITP-CZE-MS: Small organic acids



all 5 µmol/l, except oxalic, lactic, pyruvic are 25 µmol/l

Another potential application, small acids, many with no chromophores, compared with published CE-MS data. Same electrolyte system as for the NSAIDs (see left side), CZE capillary 100 µm, 25 µA → 10-9 kV. Single-ion recording (SIR) of 8 channels.

Kylie ♥ ITP-CZE-UV-ESI-MS

- Novel, multidimensional, and hyphenated
- Lots more sensitive than your old CE
- Environmentally friendly (uses only water)

Acknowledgements We would like to thank Marian Koval (Villa Labeco, Spisska Nova Ves, Slovak Republic) for discussing, implementing & improving the modifications we suggested; Gwyn Lord (Birkbeck College) for invaluable advice and help with a fractious CE-sprayer; John Briffa (KCL) for literally fixing one hundred little things, and Anna Przyborowska, Norman W. Smith, & Mark Parkin (all KCL) for advice on 'scientific plumbing' and other issues.

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* Our inspiration and muse Kylie Minogue, currently appearing larger-than-life on the British Film Institute IMAX cinema, London Waterloo, to promote a greener analytical chemistry.