Design and application of new (sub-micronsize particles based) stationary phases for capillary electrochromatography (CEC) within a pharmaceutical application perspective 'Biopharma separation techniques'- BL/03/C36

Context and objectives

Capillary electrochromatography, a hybrid technique between high-performance liquid chromatography and capillary electrophoresis, is a relatively new separation technique that has been studies for several years in research environments. However, on the level of column technology, especially the development of stationary phases, specifically designed for CEC applications, much work is still needed. Due to a lack of commercially available columns and stationary phases, the analyst has to prepare columns in-house, which can be detrimental for the between-column reproducibility repeatability. Most CEC analyses up till now have been performed using particle-based stationary phases inside the capillary, with functionalized particles of phases designed for HPLC. However, column lifetime and column performance will be fairly reduced because these phases are not developed to apply an electrical field on. Another disadvantage associated with particle-based stationary phases is the need for frits to fix the stationary phase inside the capillary, which can be responsible for column fragility, bubble formation during analysis leading to current disruption, current breakdown and noisy baselines. They also can loosen under the influence of an electrical field, leading to column failure.

Therefore, the main aims of this project were:

- Design and application of new stationary phases for capillary electrochromatography
- Application of these synthesized stationary phases for the separation and assay of drugs
- Development of chiral separation methods on the above stationary phases.
- Development of bio-analytical assays for drugs on the above stationary phases

Methodology

- Design and application of new stationary phases for capillary electrochromatography
  - The submicron-sized particle based phases were expected to be designed by the Chinese partner
  - The monolithic columns were to be synthesized by the Flemish partner and occasionally by the Walloon partner
- Application of earlier synthesized stationary phases for the separation and assay of drugs
  - The phases were provided by the Chinese partner
  - Separation methods were to be developed at the VUB and ULg
- Development of chiral separation methods on the above stationary phases.
  - Strategies for drug compounds related to drug development were to be defined at the department of the VUB
  - Chiral separation methods for bioanalysis were to be developed at the department of the ULg
- Development of bio-analytical assays for drugs on the above stationary phases (ULg)

Results

- Up till now, experiments with 3 micron particles were executed with the Chinese partner. The future work will lie in the development of 1.5 micron particles, preferably smaller, and to develop packing techniques for these particles.
- Dr. Qu has successfully transferred an existing HPLC separation to 1 um silica particles in pressurized CEC mode at the VUB. He also compared his results with columns which were packed with 3µm particles, and the 1µm columns gave better results. However, the obtained efficiencies pointed out that extra optimization of the packing procedure is needed. A paper about the results is submitted for publication.
- Preliminary experiments on methacrylate monoliths were performed at the VUB in CEC and p-CEC mode to evaluate their performance for the separation of drug molecules. It was seen that the composition of the polymerization mixture that was tested results in columns that are not optimally suitable for regular CEC experiments. In p-CEC the used columns provide relatively good results. In the next step, different polymerization mixtures were tested to determine the "ideal" composition and the "ideal" column. The influence of a change of composition on the retention, peak asymmetry and efficiency, is studied for the column performance in both CEC and p-CEC. The influence of mobile phase composition (organic modifier content, type of organic modifier) was already briefly studied and will be further optimized. Three papers about the above are either submitted or in preparation.
- The ULg started cooperation with the department of prof. Chankvetadze (Tbilisi, Georgia) They already received 1 regular HPLC column, containing 3 µm particles. Initial HPLC experiments revealed that the columns do not perform well in reversed-phase mode; in POSC better results were obtained. Later, capillary columns will be tested.
- The ULg also has a beginning of cooperation with prof. Tanaka (Japan), who is well known for the production of silica monoliths. The separation of a test mixture on a hybrid type of monolith is tested. When a successful transfer can be done, more columns will be obtained.

Products and services

Published
- “Preparation and evaluation of C18 bonded 1 µm silica particles for pressurized capillary electrochromatography”
Qishu Qu, Xiao Lu, Xiaojing Huang, Xiaoya Hu, Yukui Zhang, Chao Yan, Electrophoresis 27 (20) (2006) 3981 – 3987.

Submitted
- “Pressurized capillary electrochromatographic separation of trimethoprim and its impurities using 1 µm silica particles”
Q. Qu, D. Mangelings, F. Shen, X. Hu, C. Yan, Y. Vander Heyden, Submitted to J. Chromatogr. A

- “Evaluation of polymeric methacrylate-based monoliths in capillary electrochromatography for their potential to separate pharmaceutical compounds”
D. Mangelings, V. Meert, I. Tanret, S. Eeltink, P.J. Schoenmakers, W.Th. Kok., Y. Vander Heyden, Submitted to Journal of Chromatographic Science

In preparation (status?)
- ”Assessment of the influence of the polymerization mixture composition on the separation performance of methacrylate-ester-based monolithic capillary columns in CEC”
I. Tanret, D. Mangelings, Y. Vander Heyden

-“Pressure-assisted capillary electrochromatography using methacrylate-based monolithic capillary columns: influence of the polymerization mixture composition on the separation performance and comparison with capillary electrochromatography.
I. Tanret, D. Mangelings, Y. Vander Heyden

Execution

Period: 01/07/2005-31/12/2007

Laboratory/network:

Research team Belgium:
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Discipline

Material Sciences
Pharmaceutical Sciences