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Dynamic electric field assisted multi-dimensional liquid chromatography of biological samples

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Abstract

Complex biological samples require very high resolution separation strategies. The platform introduced here capitalises on the hyphenation of liquid chromatographic (LC) and electric potential gradient electrochromatographic multi-dimensional separation genres. First-dimension selectivity is provided by simultaneous size exclusion (SEC) and strong cation exchange (SCX) chromatography modes, while the second dimension comprises reversed phase (RP) characteristics in a dynamic (time-variant) electric field. The time-variant potential gradient with reversal of polarity is applied across the second dimension monolithic capillary throughout the duration of the solvent strength gradient elution. Hence, the platform offers comprehensive on-line sample clean-up (matrix depletion, analyte enrichment), fractionation (first dimention LC), and separation (second dimension LC) with the prospect of altering selectivity via polarity reversal dynamic electric field tuning.

Keywords: Multidimensional liquid chromatography; Size exclusion chromatography; Strong cation exchange chromatography; Reversed phase chromatography; Affinity chromatography; Tailor-made sorbents; Monolithic capillary columns; On-line sample clean-up; Human hemofiltrate; Human plasma; Electrically assisted liquid chromatography; Polarity reversal; Dynamic electric field; Transport mechanism

1. Introduction

Complex biological samples demand separation strategies with very high resolving power. A multitude of sophisticated methodologies is currently available to address the desire for (sufficient) appropriate separation space to accommodate representatively the whole sampled universe from a large universe of a small sample quantity [1–6].

The complexity of biological samples in general, and proteomic questions in particular, have paved the way for multi-dimensional liquid chromatography (MDLC) to be established as a complementary tool to two-dimensional polyacrylamide gel electrophoresis (2D PAGE) among the arsenal of analytical equipment to meet the requirements of the post-genomic era. The assets and drawbacks of many of these platforms have recently been documented [7–11]. The versatility of MDLC approaches is unparalleled as the known facets of selectivity provided by tailor-made sorbents may be combined to a multitude of off-line tandem processing systems [12,13]. The more elaborate on-line arrangements are marginally reduced in their resourcefulness paying tribute only to the requirement of compatibility between the hyphenated dimensions (with respect to column hardware dimensions, selectivity and operational features) [4,14], whereby the most popular two-dimensional sequential combination to date is represented by strong cation exchange (SCX) and reversed phase (RP) selectivity [15]. Automated sample preparation and sample clean-up steps are introduced with increasing frequency to serial analytical separation strategies [16,17]. As a consequence, the development of separation

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strategies associated with a particular analytical goal rather than a specific sample should be a cherished creed for separation scientists [18].

In analogy to gradient elution in pressure-driven high performance liquid chromatography (HPLC), gradient systems for electromigration techniques in general, and capillary electrochromatography (CEC) in particular, materialise to be essential to target complex samples. A variety of different gradient installations have been reported [19–24]. International Union of Pure and Applied Chemistry (IUPAC) recommendations on the terminology for analytical capillary electromigration techniques are available [25]. Despite the fact that theoretical approaches to quantitatively describing and modelling electrokinetic techniques vary significantly in complexity and mass transfer involved [26–29], and moreover general theoretical certainty with respect to mutual interactions of the analysis system's protagonists lacking abundance [30], cross-over applications/techniques emerge with their evolution driven by the quest for separation space and resolution within. Irrespective of their particular individual contribution and in arbitrary sequence the authors are inspired by the following publications [31–34]. For the feasibility study in hand, the data recorded by pressure-driven solvent strength gradients under the influence of an electric field applied across the separation capillary obviously proved most prominent [35].

The initial campaign propagated here is hyphenation of SCX LC with integrated sample clean-up via SEC on a bimodal functionalised chromatographic material in the first dimension and electrically assisted RP LC in the second dimension. While the two-dimensional LC system with integrated sample clean-up is well established [17] the electric field fine-tuning capacity in the second dimension is a novelty to separation science literature. The separation system set-up may be byzantine, but in return offers the total scope of versatility provided by the individual hyphenated analysis machinery to create the novel separation platform. Previously [35,36], electrically assisted LC (in one dimension) was associated with the tag 'selectivity fine-tuning' in particular in the mixed mode operation status. Superimposing two gradients (in one dimension or the second of two dimensions) of thoroughly different origins (pressure-driven solvent strength gradient and electrically-driven voltage gradient with reversal of polarity) defiantly may be associated with 'selectivity tuning' as the result may prompt drastic amendments to the resulting raw-data profiles.

The approach documented in this paper combines the features of reducing sample complexity by adopting the strategy of integrated sample clean-up by simultaneous SEC and SCX via the choice of the bifunctional spherical particle based sorbent in the first dimension and orthogonal, complementary analyte separation by simultaneous two-pronged gradient-driven mass transport through the endcapped RP monolith serving as the second dimension of a potentially comprehensive on-line multi-dimensional separation platform.

2. Experimental

2.1. Samples

Hemofiltrate was generously provided by IPF PharmaCeuticals, Hannover, Germany, while the human plasma was obtained from the DRK (German Red Cross) Frankfurt, Germany from healthy volunteers undergoing routine blood sampling. Blood was collected in sampling tubes, inhibited with citrate and centrifuged prior to screening against HIV and Hepatitis viruses, respectively. Citrate-plasma was pooled from viral-negative blood donors and stored frozen at −80 °C.

2.2. Equipment

A contemporary two-dimensional liquid chromatography platform resembles the backbone of the equipment hyphenated with a state-of-the-art capillary electrophoresis (here, more precisely: electrochromatography) apparatus utilised in the present case to enhance the achievable resolution of the individual separation tools. The entire platform is set up from Agilent Technologies (formerly Hewlett Packard) modules namely the 1100 series system comprising solvent trays, degassers, binary pumps, autosampler, diode-array detector (DAD), instrument control (ChemStation) and the HP^{3D}CE likewise equipped with a DAD. The individual systems are both ChemStation controlled and communication of the individual systems occurs via a remote control cable. Hyphenation of the autonomous dimensions is executed by a T-splitter set to ground potential. The splitter serving the purpose of assimilating the flow between the hyphenated separation dimensions and representing the flow-through electrode generating ground potential to create the potential difference for the application of the (dynamic) electric field with optional reversal of polarity in the second dimension. Column switching and analyte transfer, respectively occur by way of a two-position six-port valve from Rheodyne. All tubing and connectors are PEEK (poly ether ether ketone) from Upchurch Scientific. All presented (electro)chromatograms are recorded by the DAD of the HP^{3D}CE apparatus. A chart of the system is detailed in Fig. 1.

2.3. Columns

All sorbents utilised in these studies are either Merck KGaA, Darmstadt, Germany products or – at the time of manuscript preparation – research specimens from the afore named company.

First dimensional separations are performed on restricted access material (RAM) columns 25 mm × 4 mm length and inner diameter (i.d.) respectively, packed with 25 μm spherical beads with an average pore size of 60 Å (LiChrospher® 60 XDS (SO_3H/Diol) 25 μm). A bimodal surface functionalisation provides diol groups bonded to the outer surface and