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Design and implementation of an array of micro-electrochemical detectors for two-dimensional liquid chromatography—Proof of principle

Jude A. Abia[a,b], Joel Putnam[a,b], Khaled Mriziq[a,b,1], Georges A. Guiochon[a,b,*]

[a] Department of Chemistry, The University of Tennessee, Knoxville, TN 37996-1600, USA
[b] Division of Chemical Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

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ABSTRACT

Simultaneous two-dimensional liquid chromatography (2D-LC) is an implementation of two-dimensional liquid chromatography which has the potential to provide very fast, yet highly efficient separations. It is based on the use of time × space and space × space separation systems. The basic principle of this instrument has been validated long ago by the success of two-dimensional thin layer chromatography. The construction of a pressurized wide and flat column (100 mm × 100 mm × 1 mm) operated under an inlet pressure of up to 50 bar was described previously. However, to become a modern analytical method, simultaneous 2D-LC requires the development of detectors suitable for the monitoring of the composition of the eluent of this pressurized planar, wide column. An array of five equidistant micro-electrochemical sensors was built for this purpose and tested. Each sensor is a three-electrode system, with the working electrode being a 25 μm polished platinum micro-electrode. The auxiliary electrode is a thin platinum wire and the reference electrode an Ag/AgCl (3 M sat. KCl) electrode. In this first implementation, proof of principle is demonstrated, but the final instrument will require a much larger array.

1. Introduction

The purpose of liquid chromatography is the separation of the components of a plug of a sample into a string of bands of its components during the migration of this plug along a porous bed that is percolated with a suitable solution. This separation requires the proper selection of the material for making of the porous bed and of the solution. Plates covered with a thin porous layer and columns filled with a porous bed have successively been the preferred tool to implement liquid chromatography [1–8]. Both approaches have been successfully used to achieve the analysis of most complex mixtures, using appropriate methods of two-dimensional chromatography [9,10]. Today, however, the field is dominated by column-based separations and planar chromatography is no longer the widely used method as it was 50 years ago.

The main reasons for the preference of column over plate chromatography are (1) the fact that columns are eluted, a process easier than plate development; (2) the detection and quantitation of the bands of separated compounds exiting the columns with the stream of mobile phase is much easier than that of bands spread on plates of porous materials that scatter light; and (3) column separations were easily instrumentalized while considerable difficulties are still encountered to do so for plate separations [10,11]. These considerable advantages have obscured the potential qualities of planar separations. The latter explain why two-dimensional thin layer chromatography [12] has been reduced to practice 60 years before two-dimensional liquid chromatography [10,11]. While only one single sample can be separated at any given time on a given column, numerous samples can be separated in the same time on a given plate, which could provide a considerably higher analytical throughput.

Although thin layer chromatography (TLC) was the first practical and for a long time the most popular method of liquid chromatography, its traditional implementation suffered from serious drawbacks: (1) capillary forces acting between the mobile phases and the dry particles of the layer cause this liquid to percolate through the bed. With passing time, however, the hydraulic resistance increases while the capillary forces remain constant and the mobile phase velocity decreases. This does not affect the retention factor nor the rate at which bands broaden [2,4,5,13]. So, the velocities of the bands decrease and, eventually, band broadening becomes faster than the differential migration rate between adjacent zones, limiting the separation performance [13,14] and (2) the thin layer is in contact with a gas phase that promotes evaporation of the mobile phase in some regions of the plate and adsorp-

* Corresponding author at: University of Tennessee, Knoxville, TN 37996-1600, USA. Tel.: +1 865 974 0733; fax: +1 865 974 2667.
E-mail address: guiochon@utk.edu (G.A. Guiochon).
[1] Present address: Eksigent Technologies, 5875 Arnold Road, Dublin, CA 94568, USA.

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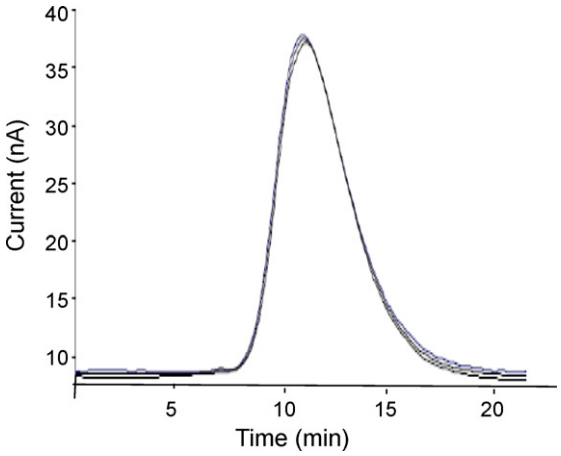

Fig. 1. Overlay of four chromatograms recorded at 2 h intervals, at the center of the exit of the flat wide column; flow rate of 1.0 mL/min.

tion in others, affecting the separation [4,13,15]. To palliate these drawbacks and those listed above overpressurized thin layer chromatography (OPTLC) methods were developed [15–17,9,18–20]. In these new approaches, the mobile phase flow rate is precisely controlled. Eventually, the design of the bed evolved toward a thin wide column operated much like traditional cylindrical columns. The bed is fully wet and there is no exchange of eluent components with a gas phase. Bands migrate at a constant velocity and resolution increases with passing time. However, on-line detection remains a serious problem.

An instrument for planar chromatography was recently described that includes a pressurized column with a rectangular cross-section (100 mm × 1 mm) and methods to access the bed (for sampling and detection), to pressurize the column, and to control the mobile phase flow rate [21]. This instrument did not incorporate an on-line detector. The purpose of this work was to design and build an array of micro-electrode sensors and to assess its potential performance for on-line detection of eluent at the exit of a pressurized flat wide column.

2. Experimental

2.1. Materials

HPLC grade water and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The samples used in this study, *p*-benzoquinone, epinephrine and norepinephrine, were purchased from Aldrich (Milwaukee, WI, USA). Unless otherwise stated, the mobile phase flow rate was 0.50 mL/min. The operation of the instrument was investigated primarily with samples of 0.04 M solution of *p*-benzoquinone dissolved in the mobile phase; this solute had a retention factor of 1.75. The sample injection size was 10.0 μL. This sample was stable and the injection reproducible (see Fig. 1).

The column was packed with silica gel (average particle size, 5 μm; surface area 500 m^2/g; pore volume, 0.75 cm^3/g; average pore size, 60 Å) purchased from Sigma (Sigma–Aldrich, St. Louis, MO, USA). Calcium sulfate hemihydrate from Sigma (Sigma–Aldrich, St. Louis, MO, USA) was added to consolidate the bed during operations.

2.2. Instrumentation

The instrument, which is similar to one previously described [17,18,21] was built in-house. It incorporates some key features of instruments developed for OPTLC [15,19,20]. It differs from our earlier instrument [21] by the addition of a detector, which consists of an array of electrochemical microsensors placed across the exit slit of the mobile phase stream. Analyte signals are acquired using a CHI900B scanning electrochemical microscope (CH Instruments, Austin, TX, USA) operated in the amperometric mode. An HP1050 microprocessor-controlled standalone pump unit with full programming capabilities (Agilent Technologies, Palo Alto, CA, USA) was used to deliver the mobile phase.

2.2.1. Column housing

The instrument is made of two 15 cm × 15 cm × 2.5 cm blocs separated by a 0.24 mm Mylar sheet purchased from Fisher Scientific. Schematics of these blocs are shown in Fig. 2a and b. The system is designed to control the mobile phase stream through very thin and wide channels, for which there is no convenient valves. The top plate is made of plexiglass (Fig. 2a), the bottom one of stainless steel (Fig. 2b).

The top plate contains three chambers used for pressurizing the Mylar sheet against the lower plate [21]. Applying pressure to the column bed by pumping water into the central cavity of the top bloc (Fig. 2a) compresses strongly the Mylar sheet against the packing material contained in the central chamber of the lower bloc, prevents particles to drift downstream, and, by sealing the packing material inside its cavity, forces the mobile phase to percolate through the bed in the central cavity of the lower bloc during the whole experiment (Fig. 2b), avoiding that it by-passes the bed. The flow of mobile phase in and out of the flat wide column is controlled by applying pressure in the other two chambers (inlet and outlet chambers) of the top bloc, which function as valves. Applying pressure in these chambers compresses the Mylar sheet against the bottom plate, preventing the mobile phase from flowing through the bed. When the pressure is relieved, the Mylar sheet is pushed back into the compression chamber and the mobile phase flows freely. A Gilson pump model 302 (Fisher Scientific) was used to deliver the water needed to pressurize the column bed and to switch the mobile phase stream on and off through the valves V_{inlet}, $V_{central}$, and V_{outlet}.

The mobile phase is delivered by an HP1050 microprocessor-controlled standalone pump, into the column through an inlet groove parallel to the column edge, through the valve $V_{solvent\ inlet}$ in Fig. 2b. The packing material is contained in a 10 cm × 10 cm × 0.1 cm cavity, with two thin, long rectangular frits inserted in the steel block, at the opposite sides of this cavity, one at the inlet, the other at the outlet, to avoid that any particle of the bed move downstream. The mobile phase entering the inlet groove flows first through the inlet frit, which has a relatively low permeability (compared to those of the inlet valve and the column bed). This enables a constant pressure to be established along the groove, which ensures a uniform flow velocity across the bed. The distribution of the mobile phase velocity across the bed width and depth is comparable to that achieved in the stream of mobile phase entering a standard 4.6 mm i.d. packed column [21]. Then the mobile phase percolates through the exit frit.

2.3. Column bed preparation

The procedure for preparing the flat bed is similar to that previously described [21]. The bed cavity is entirely filled with a thick slurry of the packing material, mechanically homogenized and composed of 15.0 g of silica gel with an average particle size of 5 μm, 2.0 g of a gypsum binder (calcium sulfate hemihydrate), and 32.0 mL of water. A thick flat metal bar is slid over the bloc to eliminate the excess of slurry while making sure that the cavity is filled. After a few minutes the slurry solidifies, and left overnight to dry, and dried for 30 min in an oven at 110 °C, to activate the silica.