Effect of pressure pulses at the interface valve on the stability of second dimension columns in online comprehensive two-dimensional liquid chromatography

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ABSTRACT

Users of online comprehensive two-dimensional liquid chromatography (LCxLC) frequently acknowledge that the mechanical instability of HPLC columns installed in these systems, particularly in the second dimension, is a significant impediment to its use. Such instability is not surprising given the strenuous operating environment to which these columns are subjected, including the large number (thousands per day) of fast and large pressure pulses resulting from interface valve switches (on the timescale of tens of milliseconds) associated with very fast second dimension separations. There appear to be no published reports of systematic studies of the relationship between second dimension column lifetime and any of these variables. In this study we focused on the relationship between the lifetimes of commercially available columns and the pressure pulses observed at the inlet of the second dimension column that occur during the switching of the valve that interfaces the two dimensions of a LCxLC system. We find that the magnitude of the pressure drop at the inlet of the second dimension column during the valve switch, which may vary between 10 and 95% of the column inlet pressure, is dependent on valve switching speed and design, and has a dramatic impact on column lifetime. In the worst case, columns fail within the first few hours of use in an LCxLC system. In the best case, using a valve that exhibits much smaller pressure pulses, the same columns exhibit much improved lifetimes and have been used continuously under LCxLC conditions for several days with no degradation in performance. This result represents a first step in understanding the factors that affect second dimension column lifetime, and will significantly improve the usability of the LCxLC technique in general.

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1. Introduction

We continue to observe increasing interest in two-dimensional liquid chromatography (2D-LC), motivated by greater fundamental understanding of the potential and limitations of the technique, and increasing availability of sophisticated commercial instrumentation designed specifically for 2D-LC. Recently, both theoretical and experimental comparisons [1–3] of conventional 1D-LC and comprehensive 2D-LC (LCxLC) have shown that LCxLC quickly outperforms 1D-LC, starting at analysis times as short as 5 min, as measured both by peak capacity and the number of resolved peaks observed in separations of complex mixtures such as plant extracts. The demonstrated potential for LCxLC to outperform 1D-LC has attracted considerable attention recently from a variety of industries and application areas, including the pharmaceutical industry [4], surfactant analysis [5,6], and natural products analysis [7].

In spite of the demonstrated performance potential of LCxLC, however, a number of practical limitations of LCxLC must be resolved before the technique can be fully competitive with 1D-LC in terms of performance metrics other than peak capacity (e.g., detection limits [8,9], robustness, and ease of use). In our own experience, a significant practical limitation to the use of LCxLC in more extended experiments spanning tens of hours or several days has been the poor mechanical stability (e.g., failure in less than one day of use) of columns used in the second dimension of the various LCxLC systems we have used. In the early years of our work in 2D-LC we frequently prepared our own columns in-house using preparation conditions that undoubtedly are not as robust as those used by major column manufacturers, and this could be cited as the major reason for the instability of those second dimension (^{2}D)
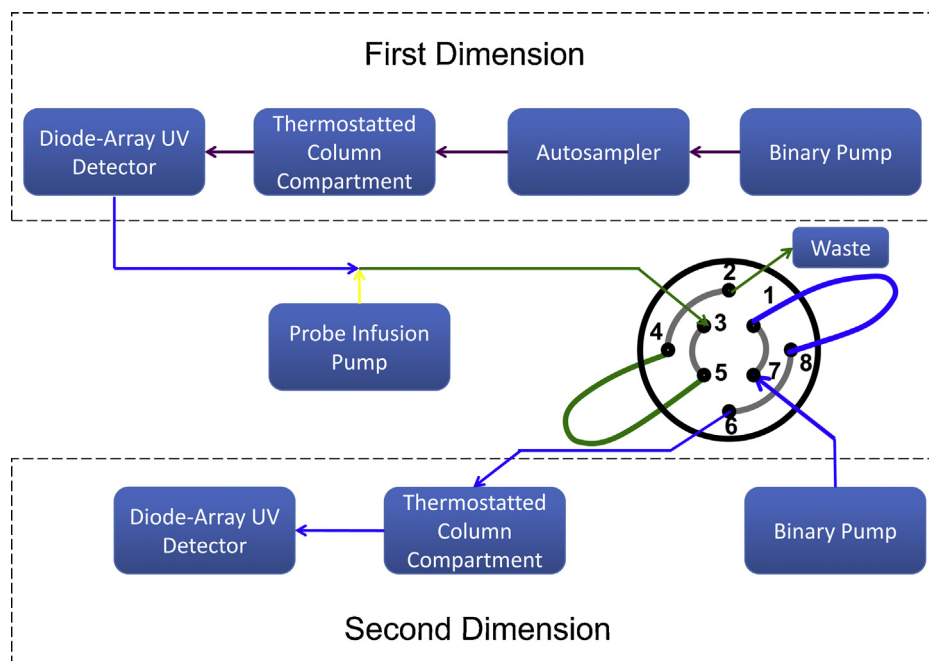
Fig. 1. Block diagram showing the main components of a system for online *LCxLC*.

columns. However, in more recent studies we have also observed that even some commercially prepared columns exhibit very short lifetimes when used in the second dimension of 2D-LC systems. Given the strenuous conditions (e.g., 60 to more than 100 sample injections and solvent gradients per hour) commonly employed in the second dimension of many *LCxLC* applications, perhaps significantly shortened column lifetimes should not be surprising, if not expected. For example, in our own work ^{2}D columns have been subjected to frequent, steep solvent gradients along with equally frequent injections (e.g., 180 per hour, 4320 per day, assuming 20-s cycles) of effluent from the first dimension (^{1}D) column into the ^{2}D column for further separation of sample constituents not resolved by the ^{1}D separation. Moreover, we have typically employed high column temperatures (e.g., 100–150 °C) to improve the throughput of the ^{2}D separation, and the overall productivity of the *LCxLC* separations. These factors taken together produce an environment in the ^{2}D column that has no analog in conventional 1D-LC.

While it is well known in the liquid chromatography community that columns from the 'early days' of HPLC were susceptible to failure as a result of all kinds of mechanical stress (e.g., during shipping, dropping on lab benches, evaporation of eluent leading to drying of the particle bed) [10,11], we have not found any peer-reviewed literature describing a systematic study of the stability of performance of HPLC columns as a function of variations in exposure to mechanical stresses during operation of the columns. In this paper we describe the execution and results of such a systematic study aimed specifically at measuring the impact of pressure pulses at the inlet of the ^{2}D column on the stability of these columns over extended periods of use under actual *LCxLC* conditions. We find that the designs of different valves used for the interface between the two dimensions of *LCxLC* systems, and the correspondingly different pressure pulses produced at the ^{2}D column inlet, have a significant effect on the lifetime of ^{2}D columns. Moreover, we find that implementation of a valve interface exhibiting minimal pressure fluctuations improves the ^{2}D column lifetime such that they are stable for several days of continuous *LCxLC* operation, even with very short switching cycles (e.g., 20 s/cycle). These results represent a first step in the quest to solve the ^{2}D column stability problem

in *LCxLC* and remove this as a major barrier to more widespread and routine use of the technique to solve challenging separation problems.

2. Experimental

2.1. Chemicals

The aqueous component of the mobile phases used in this work was 10 mM perchloric acid in water, prepared from concentrated perchloric acid (60%) from Fisher Scientific (Fair Lawn, NJ) and HPLC grade water prepared in-house using a Milli-Q Nanopure water purification system (Billerica, MA). HPLC grade acetonitrile (CHROMASOLV®, ≥99.9%) was from Sigma–Aldrich (St. Louis, MO). A solution of probe compounds containing nitropropane (≥98.5%), nitropentane (≥97%), and dipropylphthalate (≥98%), all from Sigma–Aldrich, was prepared by dissolving the compounds separately in ACN, and then diluting them in HPLC grade water.

2.2. Measurement of pressure profiles

Pressure measurements were made at five locations in close proximity to the valve that serves as the interface between the two dimensions of the *LCxLC* system depicted in Fig. 1. All instrument modules were from the Agilent Technologies (Waldbronn, Germany) 1290 Infinity Line, except for a HP1050 pump (Hewlett Packard, Palo Alto, CA) used to infuse probe compounds into the ^{1}D effluent prior to sampling in the interface valve (labeled 'Probe Infusion Pump' in Fig. 1). The modules used in the first dimension were a binary pump (Model G4220A), autosampler (Model G4226A), thermostated column compartment (Model G1316C), and diode-array absorbance detector (Model G4212A). The modules used in the second dimension were a binary pump (Model G4220A), thermostated column compartment (Model G1316C), and diode-array absorbance detector (Model G4212A). The ^{1}D column was an Agilent Poroshell 120 Bonus RP (150 mm × 1.0 mm i.d.). The ^{1}D flow rate and mobile phase composition were 0.075 mL/min and 30/70 (v/v) ACN/10 mM perchloric acid in water, respectively.