

Influence of frictional heating on temperature gradients in ultra-high-pressure liquid chromatography on 2.1 mm I.D. columns

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Received 10 November 2005; received in revised form 24 January 2006; accepted 27 January 2006

Available online 14 February 2006

Abstract

The effects of viscous heat dissipation on some important HPLC parameters, such as efficiency (N) and retention factors (k), using 2.1 mm columns at pressures up to 1000 bar have been investigated from both a theoretical and experimental point of view. Two distinct experimental set-ups and their respective influences on non-homogenous temperature gradients within the column are described and discussed. In the first instance, a still-air column heater was used. This set-up leads to approximate 'adiabatic' conditions, and a longitudinal temperature gradient is predicted across the length of the column. The magnitude of this gradient is calculated, and its occurrence confirmed with experimental measurements also indicating that no appreciable loss in efficiency occurs. Secondly, when a water bath is used to thermostat the column, a radial temperature gradient is prevalent. The extent of this gradient is estimated, and the loss in efficiency associated with this gradient is predicted and demonstrated experimentally. It is also observed that approximate adiabatic conditions can lead to floating retention factors. The implications of temperature gradients for routine HPLC analysis at ultra-high pressure are discussed.

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Keywords: Ultra-high-pressure liquid chromatography; Viscous heat dissipation; Temperature gradients

1. Introduction

Amongst the various methodologies employed in liquid-phase separations to provide increased efficiencies, the use of smaller particles in packed-column LC is currently most prevalent. It is well-documented, however, that the drawback of using small particles in LC is the increased backpressure required to operate these columns. Aside from the obvious demands placed on HPLC hardware, working at very high pressures affects various chromatographic parameters [1]. Moreover, the formation of non-uniform temperature gradients within the column due to viscous heat dissipation under these conditions has to be taken into account [2,3].

When considering temperature gradients within the column, two situations, themselves dependant on the experimental conditions, are distinguished. In the first instance, when the HPLC column is isolated or in an environment where slow heat trans-

fer occurs, an adiabatic situation is approximated. In this case, a longitudinal temperature gradient will occur inside the column, with the outlet temperature being higher than that of the inlet. Halász et al. [2] calculated the magnitude of this longitudinal gradient for an adiabatic system. However, as also pointed out in this paper, a truly adiabatic situation is rarely reached. In reality, measured values for the longitudinal gradient are often more than 50% lower than the calculated values. This effect can be ascribed to some heat transfer characteristics of the stainless steel column and connecting tubing as well as back-flow of heat over the column wall and heat consumption due to decompression of the mobile phase [2,4].

On the other hand, under well-thermostatted conditions, as pointed out by Horváth and Lin [3], a radial temperature profile will develop, with the centre of the column at higher temperature than the wall. The radial temperature profile was calculated by Poppe et al. [5]. This profile is expected to directly influence chromatographic efficiency as a result of the temperature-dependence of the mobile phase viscosity, analyte retention factors and analyte diffusion coefficients. Although the exact manner in which the peak profile is affected is complicated by

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the interaction of these factors, the effect has been measured as an increase in plate height under non-adiabatic conditions [4]. These authors also demonstrated how pre-cooling of the mobile phase can be used to compensate for the radial gradient formed under well-thermostatted conditions, allowing more efficient analysis under otherwise identical conditions.

As a quantitative measure of frictional heating generated when pumping a liquid through a packed LC column, the amount of power to be dissipated can be calculated using the equation:

$$\text{Power} = \Delta PF \quad (1)$$

where ΔP is the pressure drop over the column in N/m^2 , and F is the volume flow rate in m^3/s .

From this equation several ways to avoid, or at least minimise, the formation of temperature gradients in HPLC are evident. The first, and most simple, is to work at low pressure, since the power to be dissipated is directly proportional to the operating pressure. However, as mentioned, a major trend in modern HPLC is the reduction of particle size and column length to allow fast separations. Small particles will provide higher optimal flow rates in conjunction with a faster mass exchange. This will tempt the analyst to increase the flow rate beyond its optimum value, which will lead again to high pressure even on short columns.

Reduction in the column diameter greatly reduces the power to be dissipated. This is due to improved heat dissipation of narrow bore columns, as well as reduced flow rates dictated for downsized dimensions. It is for this reason that the few groups performing ultra-high-pressure liquid chromatography (UHPLC) on sub- $2\ \mu\text{m}$ particles have employed packed capillaries (30–100 μm I.D.) [6–8]. However, the use of these column formats places extreme demands on the instrumentation in terms of system volumes, injection and detection. Most routinely used HPLC instruments today are not suited to perform analysis on columns of internal diameter below 2.1 mm.

The use of eluent temperatures lower than that of the column wall has been proposed as an effective way to compensate for radial temperature gradients and thus to avoid the associated loss of efficiency [4,9,10]. Practical implementation of this method is, however, not feasible in most routine laboratories.

Colón et al. [11] have argued that columns with dimensions of 250 mm \times 4.6 mm and 150 mm \times 4.6 mm packed with 5 and 3 μm particles, respectively, and operated at a flow rate of 1 mL/min are considered “standard practice” in HPLC. They calculated that the power generated on these columns amounted to 167 and 280 mW, respectively, and they made the statement (from observation) that a power limit of 280 mW will not detrimentally affect chromatographic results. They subsequently showed that 150 mm \times 1 mm I.D. columns packed with 1.5 μm particles can be operated at pressures up to 1380 bar (measured flow rate 0.085 mL/min; generated power amounting to 196 mW) without leading to excessive heat generation and loss in chromatographic performance. They also indicated that it might be possible to achieve the same behaviour on 2.1 mm I.D. columns. However, thus far, the possibility of operating 2.1 mm

I.D. columns at high pressures, and the effect of radial and longitudinal temperature gradients on the measured efficiency, has not been clearly demonstrated.

In the light of this discussion, and the recent availability of commercial instrumentation capable of providing 1000 bar pressure on small-particle packed columns [12], we considered evaluating the possible effects of viscous heat dissipation under various experimental conditions. The aim of this contribution is in the first place to calculate the magnitude of temperature gradients expected under adiabatic and non-adiabatic conditions using this hardware and in the second place to obtain experimental evidence to demonstrate their effect on chromatographic parameters. To this end, a test sample was analysed at high pressures on 2.1 mm columns, under conditions where either the radial or longitudinal temperature profile is expected to dominate. The implications of temperature gradients for high-pressure LC are discussed.

2. Experimental

2.1. Materials

HPLC grade acetonitrile and water were obtained from Sigma-Aldrich (Bornem, Belgium). All mobile phases were filtered through 0.2 μm nylon filters before use (Alltech, Lokeren, Belgium). Acquity BEH C18 columns, 50 or 100 mm in length with an internal diameter of 2.1 mm and packed with 1.7 μm particles were used throughout this study (Waters, Zellik, Belgium).

2.2. Instrumentation

Analyses were performed on an Acquity UPLC system equipped with a Binary Solvent Manager, Sample Manager, Sample Organiser and Tuneable UV detector (TUV), from Waters. The system was used as delivered, with the exception that a 2.3 μL sample loop was installed in order to minimise system extra-column volume. Water bath experiments were performed using a Julabo model F10-UC bath (Analisis, Namur, Belgium).

2.3. Chromatographic method details

2.3.1. General chromatographic conditions

In all experiments the sample consisted of uracil (unretained marker, 0.4 mg/L (ppm)), caffeine (2 mg/L), pyridine (40 mg/L), aniline (2 mg/L), phenol (2 mg/L), benzene (10 mg/L) and toluene (10 mg/L), dissolved in 30/70 acetonitrile/water. The sample was prepared fresh daily from stock solutions of the individual compounds in acetonitrile. The mobile phase used throughout was 30/70 acetonitrile/water (pre-mixed). The same eluent was used as weak needle wash solvent on the Acquity system to avoid possible focusing of sample components. Injections were performed in full loop mode. Detection was at 210 nm with a data acquisition rate of 40 Hz. Unless otherwise stated, the column was thermostatted at 40 °C using the Acquity column heater (ACH).

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