Visualizing and studying frictional heating effects in reversed-phase liquid chromatography using infrared thermal imaging

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HIGHLIGHTS

- We used an infrared camera to observe longitudinal frictional heating on column.
- IR camera was calibrated; no reflections; camera sensitivity was within 0.02 °C.
- IR allowed us to see data-rich temperature profiles in real time with different solvents.
- Camera achieved temperature agreement with theory within 1% for non-compressible eluent.
- Data-rich temperature profiles allowed for insights related to column packing.

GRAPHICAL ABSTRACT
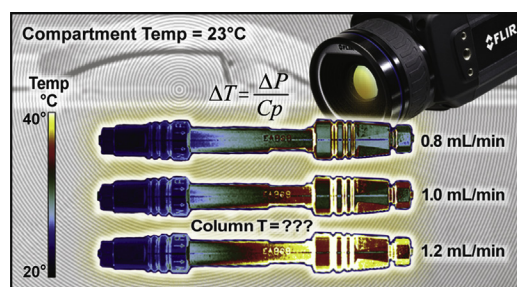
ABSTRACT

Column temperature control is a fundamental component of liquid chromatography experiments. However, it is typically monitored indirectly by tracking the temperature of an adjacent heating element that exchanges heat with the column in a controlled environment. The practice of not directly measuring the column temperature means that uncontrolled contributions of heat, such as frictional heating inside the column, can be overlooked. The present work describes the use of a high-resolution infrared thermal imaging camera to directly measure the column heat map during mobile phase flow. The approach was used to measure the longitudinal temperature gradient formed with three common mobile phases: water, methanol, and acetonitrile, in two 50 mm reversed-phase columns, a 1.7 μm particle-packed column and a polystyrene divinylbenzene monolith. In a close approximation to an adiabatic environment, the temperature gradients (ΔT) observed with the 1.7 μm particle column at a linear velocity of 5.8 mm/s were up to +16.6 and + 12.8 °C above an ambient temperature of 23 °C for water and acetonitrile, respectively. In the case of water, the measured temperature gradient values (ΔT) were within 1% difference of theoretically-calculated values and on average within 10% for acetonitrile. By contrast, the ΔT observed in the monolith was negligible. The elevated temperatures that are generated through friction in sub-2 μm particle columns may be particularly important to consider for the design of experiments that measure structural features of temperature-sensitive analytes, such as biomolecules. While frictional heating is one important application of the thermal imaging approach described, the

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technique can be used to provide a data-rich profile of heat exchange in numerous experimental configurations, chromatographic or otherwise.

1. Introduction

In chromatographic separations, temperature is a fundamental contributor to the chemical potential of an analyte within a given phase, and it is therefore recognized as one of the principal drivers of analyte retention and elution [1]. Primary contributions to column temperature include the local external environment, the mobile phase temperature before it reaches the column, and the frictional heating that arises from the flow of mobile phase adjacent to the inner wall of the column and across the surface of the stationary phase. Both the ambient temperature and the mobile phase temperature at the inlet are routinely controlled with modern chromatographic instrumentation, but solutions to control frictional heating are not widely implemented, in part because it remains difficult to measure frictional heating in real time.

The development of reversed-phase liquid chromatography (RPLC) stationary phase materials has undergone significant evolution over the last half century. Improvements of chromatography packing materials have been significant in the areas of particle synthesis, stationary phase bonding chemistry, mechanical stability and diverse selectivity [2–4]. Improvements to column stationary phases have translated into dramatic improvements of efficiency/peak capacity, shorter columns, and faster methods [5–12]. The reduction in particle diameter size over the past decades has led to increased column backpressure [13], and there has been a renewed interest in understanding the significance of frictional heating contributions with the current sub-2 μm particles used in ultra-high pressure liquid chromatography applications [5,14–18].

The progression of ever-smaller particles has led to the development of LC instruments that can withstand inlet pressures up to about 1500 bar, roughly 3 times higher than the previous generation of instruments [19]. Frictional heating (Equation (1)) [20] in ultra-high pressure liquid chromatography (UHPLC) separations has been observed to impact both separation efficiency and analyte retention [14,15].

$$Pf = F \times \frac{\Delta P}{L} \tag{1}$$

where P_f is the heat power friction generated inside the column per unit length measured in W/m, ΔP is column pressure drop across the column (Pa), F is flow rate (m^3/s), and L is column length (m). The frictional heating generated on the chromatographic column by mobile phase flow leads to the formation of a longitudinal temperature gradient under adiabatic conditions. This longitudinal temperature gradient (ΔT) can be calculated using Equation (2) [21] in case of incompressible eluent:

$$\Delta T = \frac{\Delta P}{Cp} \tag{2}$$

where Cp is the specific heat capacity of the solvent (J/m^3 °C). However, to correct for eluent compressibility Gritti and Guiochon suggested using Equation (3) [22]:

$$\Delta T \approx (1 + \overline{\alpha p T}) \frac{\Delta P}{Cp} \tag{3}$$

where α_p is the expansion coefficient of the eluent and $\overline{\alpha p T}$ is the average of the product from column inlet to outlet (i.e. −0.37 for acetonitrile [22]). The non-uniform longitudinal temperature gradients generated inside the column due to frictional heating and viscous heat dissipation can lead to a significant decrease in analyte retention factor [16,23–27].

The heat generated by friction may be dissipated radially and along the column. It has been reported that the heat generated from the use of elevated flows under high pressure in packed capillary columns can be transported readily out of the capillary if small column diameters are used [28]. The increased surface area-to-volume ratio may explain in part why micro bore columns have been preferred in separating complex biological samples [29]. In columns that are unable to effectively dissipate heat at the rate it is generated, the separation efficiency may be negatively affected by radial heat dissipation [5].

There are many reports about frictional heating effects on chromatographic efficiency and peak broadening [14,30], and there have also been efforts to measure the longitudinal temperature gradient on column [18,23]. However, measuring longitudinal temperature gradient (ΔT) on column has been one of the most challenging tasks in chromatography. Indeed, previous reports illustrated measured values within 50%−70% agreement with the theoretically estimated ΔT values [17,18,23].

Building on previous efforts to measure the axial temperature gradient generated as a result of frictional heating, this work describes the use of infrared thermal-imaging to obtain data-rich temperature measurements in real time. In this study we surveyed the applicability of IR thermal-imaging for studying frictional heating effects in reversed-phase liquid chromatography columns.

2. Experimental section

2.1. Reagents and chemicals

Ultrapure water was obtained from a Milli-Q Gradient A10 from Millipore (Bedford, MA, USA). Acetonitrile (MeCN) and methanol (MeOH) HPLC grade were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Instrumentation

An Agilent 1290 UHPLC with a binary pump and a photodiode array detector was used for all experiments (Agilent, Santa Clara, CA, USA). Agilent OpenLab ChemStation Edition C.01.05 was used for data acquisition and processing.

All experiments were performed with isocratic elution. Mobile phase A (MP A) was water, or acetonitrile, or methanol.

Experiments were performed with particle-packed BEH C18-130 Å, 1.7-μm particles, 2.1 × 50 mm columns from Waters (Milford, MA) and Chromolith High Resolution RP-18e silica column, 4.6 × 50 mm (Merck Millipore, Billerica, MA, USA).

Infrared camera FLIR A6701-sc with Indium Antimonide (InSb) detector and built-in cryo-cooler (Wilsonville, Oregon, USA) was