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Development of a 1.0 mm ID temperature-assisted focusing precolumn for use with 2.1 mm ID columns

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ABSTRACT

On-column solute focusing is a simple and powerful method to decrease the influence of precolumn band spreading and increase the allowable volume injected increasing sensitivity. It relies on creating conditions so that the retention factor, k' , is transiently increased during the injection process. Both solvent composition and temperature control can be used to effect solute focusing. In the case of temperature, the release of the transiently delayed solute band requires increasing the temperature rapidly and with a minimum of radial thermal gradients. Thus, the focus of attention in temperature-based efforts to carry out on-column focusing has been on capillary columns. As a result, the benefits of this simple and reliable approach, temperature-assisted solute focusing or TASF, are not available to those using larger diameter columns, in particular the highly successful 2.1 mm inside diameter columns. Based on considerations of thermal entrance length at the volume flow rates used with 2.1 mm inside diameter columns, TASF would not be effective with such columns. However, we determined that the thermal entrance length for a 1.0 mm inside diameter precolumn is sufficiently short, about 2 mm, that it could work as a precolumn before a 2.1 mm inside diameter analytical column. Finite element calculations demonstrate that a 1.0×20 mm precolumn packed with $5 \mu\text{m}$ reversed phase particles is effective at a flow rate of $250 \mu\text{L}/\text{min}$, suitable for the 2.1 mm inside diameter column. Eight 1-cm^2 Peltier devices are used to heat (and cool) the precolumn. The computed axial temperature profile shows that the center of the column heats more rapidly than the ends. Based on the changes in back pressure, the full temperature transient from 5°C (focus) to 80°C (release) takes about 10 s.

Experimental van Deemter curves indicate that the reduced velocity in the precolumn at $250 \mu\text{L}/\text{min}$ flow rate is about 50. Nonetheless, about 1000 theoretical plates are generated. When operating as a precolumn, clear advantages are seen for solutes across a range of modest k' values (2.2–23.4 at the separation conditions at 65°C) using TASF alone (5°C) with $50 \mu\text{L}$ injection volumes of methyl through *n*-butyl parabens, and with $100 \mu\text{L}$ injections that also include solvent-based focusing (90:10 aqueous/acetonitrile sample, 80:20 mobile phase).

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1. Introduction

The Jorgenson group has catalyzed advances in reaching the limits of liquid chromatography notably through the development of very high pressure capability [1–3] and small particles with good size distributions and chromatographic properties [4–6] and effective packing protocols [7,8]. Over approximately the same timeframe, there have been several important developments in the understanding of the effects of temperature on liquid chromatographic separations and, indeed, the problems posed by viscous

dissipation when using small particles and high velocities [9–15]. Certainly column temperature has long been recognized as an important variable in liquid chromatography, from fine tuning the selectivity of a separation [16] to the use of temperature well above ambient to realize dramatic improvements separation speed [17]. But dynamic control of temperature is also interesting and powerful.

Temperature gradients were carried out in the mid-80s independently by the Jinno [18], Hirata and Sumiya [19] and McNair [20–22] groups. As well as executing longer gradients, both the Jinno and McNair groups demonstrated high-speed separations. More recently, the Cortes group developed a low thermal mass system with potentially very high rates of temperature change and demonstrated fast separations with a $100^\circ\text{C}/\text{min}$ rates. In broad

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terms, each of these groups demonstrated 1–2 min separations with a peak capacity of about 5–10/minute. Aside from simple linear gradients, step-changes in temperature can be used for altering selectivity, as demonstrated by the Hilder group [23]. Controlling selectivity on-column at any time during a separation is a powerful idea and one not easily mimicked with solvent changes.

Perhaps the use of temperature that has gained the most attention is related to solute trapping/focusing or modulation. The idea is simple and akin to solvent-based focusing [24]. Decreasing k' prior to launching a sample down the column (focusing) or onto a second column (modulation) narrows the solute zones on column to achieve a better separation. For many solutes under reversed phase conditions, the enthalpy accompanying the sorption process is negative, thus decreasing temperature increases k' .

Verstraeten et al. [25] developed a modulation apparatus based on capillary columns (200- and 300- μm inside diameter (i.d.)) containing porous graphitic carbon (PGC) particles. The column's temperature is controlled by either resistively heating a tube surrounding the column or forcing air across the column to cool. Samples of 150 nL from a 1×150 mm column were focused and released in a two-minute cycle. Later Creese et al. [26] developed a larger, 800 μm i.d. ($\times 81$ mm) PGC column using a similar approach, by physically moving the tube to alternately heat and cool the first half of the column while simultaneously cooling and heating the second half in a one-minute cycle. In this work, the modulator was shown to be effective in a two-dimensional LC system, a most promising development. [27] van der Ven et al. [28] developed a backflushing approach using a column heater and ice water to motivate and trap solutes.

Focusing based on injecting samples while the head of the column is cooled was successfully demonstrated with capillary columns already in 1999 by the Greibrokk group. [29] End-column trapping has also been demonstrated [30]. In both cases, considerable advantages in sensitivity were realized. Our group has developed an instrumental method similar to one published earlier by Collins et al. [31] in which thermoelectric coolers (Peltier devices, or TECs) were used to improve on-column focusing and thereby improve separation quality as well as sensitivity [24,32–34]. We call this set of methods temperature assisted solute focusing or TASF.

It is no surprise that capillary columns are prominent in the foregoing cited works. The rate of heat transfer is an important design criterion for a chromatographic system employing temperature changes. Heat transfer is faster over shorter distances. However, a considerable fraction of LC separations takes place on larger columns, particularly those with a 2.1 mm i.d. We therefore sought a way to deploy TASF for the masses. Below, we first describe briefly the theoretical considerations that led to a design of a 1 mm precolumn intended for use with a 2.1 mm analytical column. We then simulated the performance of the precolumn using finite element calculations and demonstrate its effectiveness experimentally.

2. Theory

To realize the benefits of TASF, column dimensions must be carefully chosen to balance thermal entrance length and radial temperature distribution while also accommodating an acceptable sample volume. We established the maximum allowable i.d. for the precolumn by calculating the thermal entrance length [35] as a function of column diameter. Thermal entrance length is defined as the distance mobile phase entering the column at a uniform temperature, T_1 , must travel down the column with walls at temperature T_2 before the radially averaged temperature of the mobile phase has increased (or decreased) by 99% of the difference between T_1 and T_2 . At this distance the radial temperature gradi-

ent can be assumed to be insignificant. An example will make this clear, the approximate thermal entrance length [36] for a 150 μm i.d. fused silica capillary column is 46 μm . The interstitial velocity for this calculation was 1.5 cm/s (10 $\mu\text{L}/\text{min}$), a value typical for a reasonably brisk separation. As column diameter is increased we would expect the thermal entry length to increase as the distance between the fixed temperature column walls and incoming mobile phase increases. For a 2.1 mm i.d. column under the same conditions as the 150 μm i.d. capillary the entrance length is 9.0 mm. This is quite long. At a column diameter of 1.0 mm, the thermal entry length is a more acceptable 2.0 mm.

The disadvantage to decreasing column diameter is decreasing the loading capacity of the column. A column with a 1 mm i.d., has a void volume of 5 $\mu\text{L}/\text{cm}$ of column length while a 2.1 mm i.d. column has a void volume of 22 $\mu\text{L}/\text{cm}$. If a 1 mm precolumn could focus adequately low-retention solutes in an injection volume of the order of 50–100 μL (approximately 50–100% of the void volume of a 2.1 \times 50 mm analytical column) it would be effective.

In consideration of these factors and the desired sample volumes, we arrived at the conclusion that a 1 mm \times 20 mm long column with 5 μm particles may be useful as a precolumn for sample focusing. This precolumn device must be both heated and cooled. Columns are typically heated using external resistive heaters but have also been heated by resistive heating of the column wall [22] or a coating on the column wall [37,38] or with Peltier elements [24,31–34]. Peltier elements are heat pumps that create a heat flux across the device to from a heat sink or source. The maximum heat transfer rate across a commercial Peltier element that we are aware of is 10.9 W/cm². Columns are also cooled using submersion or cryogenic methods [28,30]. Submersion requires the manual transfer of the column from an ice bath into a column heating module. Cryogenic cooling involves jacketing the column in a device where cold or ambient air is circulated. While this is effective, the heat transfer from air to solids is not as fast as direct contact. We chose Peltier devices because of their fast rates of both heating and cooling, their small size, and their ability to be integrated into an instrument.

A 1 \times 20 mm column (21.2 mm packing due to fittings) has a total volume of 16.7 μL while the void volume is 10.7 μL (total porosity value was assumed to be $\epsilon_{\text{tot}} = 0.64$). The volume of steel comprising the precolumn without end fittings is 800 μL . Adding the volume of the end fittings (frit, frit nut, and viper fittings for both ends) brings the volume of steel that needs to be heated and cooled to 2020 μL . In considering the problem of rapid heating and cooling, it is clear even without knowing specific material thermal properties that the stainless-steel portion of the column has the dominant thermal mass. That is, for approximate calculations, the actual packing material, mobile phase, and flow rate are not important. Considering the density and specific heat of the stainless steel (8000 kg/m³ and 490 J/kgK, resp., azom.com), the precolumn itself without endfittings could be raised one degree K using 3.1 J. To raise the temperature 75 K in 5 s would thus require

$$3.1 \frac{\text{J}}{\text{K}} \cdot \frac{75 \text{ K}}{5 \text{ s}} = 46.5 \text{ W} \quad (1)$$

Recall that each Peltier is capable of a heat flux of 10.9 W/cm². Thus, at least five Peltiers are necessary. There will be axial heat loss into the fittings, heat loss due to heated mobile phase leaving the column, and there must be additional thermal mass from a flat substrate to support the Peltiers (they cannot be wrapped around the column). Thus, we decided to design a device with eight Peltier elements. The Peltier elements are arranged on a pair of substrates like biplane wings around a fuselage (the column), Fig. 1. There are four elements (shown in gold) on each side of the column in a 2 \times 2 array. The stainless steel “wing” substrates (shown in gray) are shaped to minimize their mass, without sacrificing significant

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