The impact of pressure and frictional heating on retention, selectivity and efficiency in ultra-high-pressure liquid chromatography

David V. McCalley *

Centre for Research in Biosciences, University of the West of England, Frenchay, Bristol BS16 1QY, United Kingdom

A B S T R A C T

The effects of pressure and frictional heating deserve serious consideration in ultra-high-pressure liquid chromatography (UHPLC) separations, as the pressures used can be three times greater than those in conventional high-performance LC (HPLC). We show that the effects of pressure alone can give useful selectivity effects, especially when separating molecules of different size. Frictional heating effects can cause serious losses in column efficiency and may also give changes in the selectivity of the separation. Nevertheless, the detrimental effect of frictional heating can be reduced, for instance by the judicious selection of column thermostat and particle type. In practical situations, pressure and heating effects occur simultaneously and can cause problems in transferring methods from conventional HPLC to UHPLC. In reversed-phase separations, the typical effect of increased retention with increasing pressure is counteracted by the reduction in retention that usually occurs at elevated temperatures.

© 2014 Elsevier B.V. All rights reserved.

Contents

1. Introduction

Consideration of the effects of high pressure and/or the concomitant effects of frictional heating have become increasingly important since the advent of commercial ultra-high-pressure liquid chromatography (UHPLC) systems capable of inlet pressures of 1300 bar or higher. These maximum pressures are more than three times higher than those achieved by typical systems that existed a decade previously. High pressures can cause unexpected changes in retention, selectivity and peak shape. Nevertheless, the effect of pressure has been recognized since the early days of the development of LC. For example, Giddings showed theoretically that pressure could induce equilibrium shifts in the distribution of solutes between stationary and mobile phase [1]. While these shifts might be considered

detrimental in modern applications, e.g., in the transfer of methods from classical HPLC to UHPLC systems (operating with smaller particles at higher pressure), Giddings recognized that benefits might accrue. For example, adjustment of pressure was considered to be a simple means of improving the selectivity of separations over the range of 100–1000 bar. An early practical study [2] indeed demonstrated that the separation of two azo dyes, methyl and ethyl orange, using adsorption chromatography on a silica column, was much improved at a pressure of ~2800 bar compared with 500 bar. The authors considered that increased ion-pair formation at high pressure might contribute to the selectivity change.

It is possible to study the effects of pressure more or less in isolation from concomitant heating effects. This can be done by attaching narrow capillaries of different lengths to the exit end of a column packed with relatively large particles while operating the column at a constant flow rate [3]. In this way, the pressure drop across the column remains small and approximately constant if the effect of pressure on the viscosity of the mobile phase is ignored

* Tel.: +44 1173282469.
  E-mail address: david.mccalley@uwe.ac.uk.

[4]. Thus, frictional heating effects are limited. Another approach is to use narrow-bore packed capillary columns (e.g., 0.2-mm internal diameter) in conjunction with the restriction-capillary method, such that rapid dissipation of heat occurs due to the relatively large surface area to volume ratios of such columns [5,6]. Most of the work discussed in sub-sections 2.1 and 2.2 uses these approaches to study the effect of pressure alone. Alternatively, the effect of pressure can be studied by varying the flow rate [7]. Such an approach gives rise to the superimposed effect of frictional heating as the power generated within the column increases according to the equation:

$$Power = \Delta P \times F \tag{1}$$

where ΔP is the pressure drop (e.g., in SI units N/m^2) and F the volumetric flow rate (m^3/s) through the column [8,9]. The power is often expressed in watts per unit length of the column [10]. As the applied pressure is approximately proportional to the flow rate, the power generated is proportional to the flow rate squared. While it is possible (although often technically demanding) to measure temperature changes within and at the column walls, it is often difficult to isolate the effects of frictional temperature changes from the effect of pressure changes, as these quantities are clearly interconnected, as shown by Equation (1). Indeed the concomitant influence of pressure changes has sometimes been overlooked in the studies of frictional heating discussed in sub-section 2.3. Nevertheless, some studies have at least compared the effects of pressure alone with the combined effects of pressure and frictional heating, as discussed in sub-section 2.4. These studies are valuable, as they attempt to interpret the situation encountered in practice by many chromatographers, e.g., when changing the flow rate in a separation, or switching from one stationary-phase particle size to another.

While variation in retention can be seen as a favorable effect or an unfavorable effect of pressure alone, frictional heating effects are almost always regarded as detrimental. Exceptionally, temperature variation along the axis of the column may give rise to fortuitous changes in selectivity of the separation, although such changes can be difficult to predict and control. However, temperature variation along the column radius generally gives rise to detrimental effects on column efficiency, particularly with wider bore columns, as the central portion of the column loses the heat generated at a slower rate than the material close to the column walls. This temperature gradient leads to a range of mobile-phase velocities across the axis of the column, giving a spread of retention times to a band of molecules, and in some cases causing serious band broadening.

In this review, we evaluate the effects of pressure and frictional heating mostly from a practical, rather than a theoretical, point of view. We consider how the effects of pressure may be beneficial in producing different selectivity or instead may give rise to unexpected changes in selectivity when experimental parameters are altered. We assess the origin of frictional heating, and the production of longitudinal and radial temperature gradients. We also evaluate approaches to reducing the generally detrimental effects of frictional heating.

2.1. Effect of pressure on retention

A comprehensive theoretical and practical study of the fundamental effects of pressure on retention was carried out by Guiochon and co-workers [11–14]. It was shown that all the experimental parameters that are supposed to be constant in LC (e.g., physical dimensions of the column, particle size and porosity, phase ratio, column hold-up volume, mobile-phase density and viscosity, diffusion coefficients, equilibrium constants, retention and efficiency parameters) depend on pressure to some extent [11]. Some surprising events, such as the breakage of a pump piston when operating with cyclohexane, causing it to solidify when compressed at a

pressure of ~200 bar, were also reported. This result was due to the increase in the melting points of many chromatographic solvents that occurs when increasing the applied pressure. Guiochon considered some of the most basic parameters that might be affected by pressure (e.g., the dimensions of the stainless-steel column tube). The tube might be subject to expansion due to pressure stress, causing it to inflate. However, calculations showed that the effect was negligible in practice, increasing the volume of the tube by less than 0.1% over the pressure range 1–400 bar. Nevertheless, silica-based monolith columns encapsulated within a PEEK tube suffer larger stress with pressure than stainless-steel columns, with pressure indeed tending to inflate the columns and to increase retention times appreciably at relatively low pressures [15].

The compressibility of the mobile phase must also be considered. Guiochon measured the compressibility of aqueous solutions containing 0–100% v/v of acetonitrile (ACN) and methanol over the pressure range 10–350 bar using a porous silica phase. The maximum change in elution volume of the void volume marker thiourea was 1.3% in pure water and 4.0% in pure methanol. The compressibilities of ACN-water mixtures were found to be about half those of methanol-water mixtures [12]. Results were somewhat influenced by increased retention of the void-volume marker thiourea, as shown by additional experiments substituting a non-porous silica with a low surface area, which gave negligible adsorption of thiourea.

A further study of the effect of pressure on C18 bonded columns (rather than bare silica) showed that the increase in the hold-up volume was again largely due to the compressibility of the mobile phase (0.9%, 2.3% and 4.8% increases for water, methanol and pentane, respectively, for an increase in average column pressure of 200 bar). However, this increase was also due in part to the compressibility of the C18 bonded layer on the silica (0.5–1.0%) and an increase in the retention of thiourea, which resulted from the difference between the partial molar volume of the solute in the stationary and mobile phases [14].

Desmet and co-workers [16] have studied the effect of pressure (and temperature) on the mobile phase where the results were not influenced by the presence of a column (i.e. its effect on the retention of thiourea, or the compressibility of the stationary phase). In these experiments, the mobile phase was placed in a pressure bomb, and the volume change (ΔV) to the original volume (V) caused by the pressure increase (δP) was measured. The isothermal compressibility χ_T (bar^{-1}) is given by the relationship:

$$\chi_T = -\frac{1}{V}\left(\frac{\delta P}{\delta V}\right)_T \tag{2}$$

For a pressure increase of 1000 bar, compressibilities were 3.700×10^{-5} bar^{-1}, 5.980×10^{-5} bar^{-1} and 5.760×10^{-5} bar^{-1} for water, 100% methanol and 100% ACN respectively. At 400 bar, the values were 4.139×10^{-5} bar^{-1}, 8.409×10^{-5} bar^{-1} and 7.786×10^{-5} bar^{-1} for water, 100% methanol and 100% ACN, respectively. While of the same order as the values reported by Guiochon, there was clearly considerably smaller variation reported in compressibility for methanol-water compared with ACN-water in the Desmet study.

Besides these effects of pressure on basic HPLC parameters, the effect of pressure on (retained) solute retention and chromatographic performance must be considered. The change in retention (k) with pressure (P) is expressed by the equation [5,17]:

$$\ln\left(\frac{k}{k_0}\right) = -\frac{\Delta V}{RT}P + \ln\left(\frac{\beta}{\beta_0}\right) \tag{3}$$

where k_0 and β_0 are the retention factor and the phase ratio under reference conditions (at atmospheric pressure), k and β the equivalent quantities at elevated pressure, R the gas constant, T the