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Exogenous factors contributing to column bed heterogeneity Part 1: Consequences of 'air' injections in liquid chromatography

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ABSTRACT

It has been shown that not only the packing homogeneity, but also factors external to the column bed, such as, frits and distributors can have important effects on the column performance. This current communication is the first in a series focusing on the impact of exogenous factors on the column bed heterogeneity. This study is based on several observations by us and others that chromatographic runs often, for technical reasons, include more or less portions of air in the injections. It is therefore extremely important to find out the impact of air on the column performance, the reliability of the results derived from analyses where air was injected, and the effect on the column homogeneity. We used a photographic approach for visualising the air transport phenomena, and found that the air transport through the column is comprised of many different types of transport phenomena, such as laminal flow, viscous fingering like flows, channels and bulbs, and pulsations. More particularly, the air clouds within the column definitely interact in the adsorption, i.e. mobile phase adsorbed to the column surface is displaced. In addition, irrespective of the type of air transport phenomena, the air does not penetrate the column homogeneously. This process is strongly flow dependent. In this work we study air transport both in an analytical scale and a semi-prep column.

1. Introduction

The heterogeneity of chromatography columns is now well documented [1–13] and it is clear that beds are heterogeneously packed in both the radial and the axial directions [1–14], and that such heterogeneities exist in all forms of Liquid Chromatography (LC) columns, be they low pressure open tubular columns, low to medium pressure (Fast protein liquid chromatography) FPLC columns, or High performance liquid chromatography (HPLC) and Ultra High Performance Liquid Chromatography (UHPLC) columns. Most important is, the radial bed heterogeneity because the variation in the fluid flow velocity across the column radial cross section causes severe distortion of the solute band, resulting in 'bowl' like elution profiles [15]. The development of the Active Flow Technology (AFT) column end fitting [16–19] has shown conclusively that band distortions that result from the heterogeneity in packing density can to a very significant degree be moderated since the flow from these AFT columns is collected from the more uniform radial central region of the column bed, hence effectively eliminating wall effects [13] and other aspects that contribute to radial flow heterogeneity. These fittings offer advantages at HPLC scale columns or prep scale columns, including columns utilised in low pressure environments such as FPLC.

While it is safe to say that column bed heterogeneity arises from the packing process [1–14], it is also clear that exogenous factors can lead to radial heterogeneity in the column bed. We detailed this in earlier studies that showed frits were often a contributing factor [20–22]. In this context it is worth stressing that not only the frit contributes to heterogeneous solute zone, but also dead volumes and sample transport through the injection loop [23–25]. From these earlier works there is little doubt that being able to see inside a chromatography column yields great clarity as to the hydrodynamic transport processes through the fluidised porous bed [12,13,15,20–22]. Usually chromatography is conducted using columns made from stainless steel tubes or other opaque materials, packed with a stationary phase that is also opaque. Being able to see inside these types of columns is therefore very difficult. Nevertheless there are a variety of techniques that enable on-column visualisation even for columns that are made in opaque tubes, for example, Currivan, Connelly and Paull used contactless conductivity detectors to study the uniformity of monolithic beds [26]. Also,

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columns made from plastic tubes can be placed inside an NMR magnet, and MRI allows for images of the flow hydrodynamics to be visualised [8–11]. Effectively the internal dimensions of the column can be viewed. The difficulty associated with this process is that complex pulse sequences must be developed, the NMR is an expensive 'detector' and adequate spatial resolution is limited.

Alternatively, a great deal of information can be obtained from columns that are packed in glass tubes [12,13,15,20–22], since high pressure glass tubing suitable for HPLC-type applications is available. If these glass tubes are filled with C18 silica and the mobile phase has the exact same refractive index then the otherwise opaque stationary phase becomes perfectly transparent to the eye. Coloured solutes, or solutes with a different refractive index to the C18 silica can then be injected, and their migration paths can be viewed through the column. Photography can be used to record their transportation [12,13,15,20–22]. Shalliker et al., has used this on-column visualisation process to detail the wall effect [13], transport through frits [21,22] and distributors [20], measure column bed heterogeneity [12], and detail viscous fingering phenomena [15,27–30]. All of which are processes that are difficult, if not impossible to describe without being able to see inside the chromatography column.

In this study we have utilised this matched refractive index on-column visualisation process to detail the transport of air through a chromatography column, i.e. 'air' injections in liquid chromatography. In this situation, air has a different refractive index to the mobile phase, hence, as the air displaces the mobile phase from the stationary phase material the stationary phase then becomes visible and the air transportation can be viewed, much in the manner that the earlier viscous fingering experiments undertaken by Shalliker et al. [27,28] were visualised. A reasonable question to ask oneself is why study air injections? In fact, there are numerous reasons; first and foremost, analysts from time to time accidently undertake air injections, either from partially filling an injection loop with air as the fluid lines are compromised, or if the sample vial volume drops below the required level. Other basic operational mistakes may be made, such as incorrect flushing of a newly inserted sample loop or the like. We wonder whether, for example the anecdotal claims by many who practice liquid chromatography, that the first injection is often erroneous, is in fact a result of 'air-injections'? Other reasons to visualise 'air' injections may be found in chemical processes whereby gases are purged through fluidised porous beds, perhaps for the recovery of fossil fuels, or to study greenhouse gas emissions through a fluidised permafrost in a melting Arctic environment. Nevertheless, the injection of air inside a chromatography column raises the question as to the whether the homogeneity of the column bed has been exogenously affected by air transport into the column. We address this issue with detailed photographic data following the introduction of air into the column. The aim of this study is to experimentally investigate air transport through a chromatographic column. In this study we will investigate how the air transport is affected by changing: the column inner diameter, flow rate and injection volume. We will also demonstrate the effect of a column void on top of the column has on the air transport.

2. Experimental

2.1. Chromatography columns, mobile phases and reagents

Analytical scale (5 mm, i.d.) HR glass columns were supplied by Pharmacia (Sweden, Uppsala) and a 17 mm i.d. column, was supplied from Omnifit with in-house manufactured endfittings. These columns were packed using an axial compression process consistent with the manufacturer's guidelines. The packing material was Kromasil-100-5-C18 (5 μm d_p) AkzoNobel (Bohus, Sweden). The stationary phase was dispersed in acetone, stirred for 10 min, and then ultra-sonicated for a further 10 min, prior to being poured into the column blank. Once the stationary phase had settled the column end fitting was applied and the bed compressed. The packed bed height was 6 and 5.8 cm for the 17 mm and 5 mm i.d. column, respectively.

The HPLC mobile phase was a mixture of dichloromethane (DCM) and toluene, purchased from VWR International, in the proportion of 45:55 (DCM:Toluene). This composition had the exact same refractive index as the C18 silica. Flow rates, and sample injection volumes varied and are detailed as appropriate in the text.

2.2. Instrumentation

A Jasco PU 1580 HPLC pump (JASCO, Japan) was used for the delivery of all mobile phases. A Midas auto injector from Spark Holland (AJ Emmen, The Netherlands) was used for sample injection. Detection was achieved photographically using a Nikon D5100 SLR camera (Nikon Corporation, Japan) fitted with a variable focal length 18 to 300 lens (Nikon Corporation, Japan) fixed at 300 mm for image acquisition. The f-stop was set at 10, and the camera was operated in video record mode. Still photographs were taken as 'snap-shots' from these videos using Adobe Photoshop CS6. In order to minimise the cylindrical lens effect of the tubular column, the column was housed in a standard 30 cm × 40 cm × 30 cm tank, which was illuminated using an 8 Watt white fluorescent light tube (Diversa, Poland) located directly behind the HPLC column.

3. Results and discussion

Once the mobile phase having the same refractive index as the stationary phase has fully equilibrated with the stationary phase, the column and stationary phase become perfectly transparent to the eye, and we have reported this phenomenon on many occasions, and the reader is referred to references [12,13,15,20–22,27–30] for verification of the visual clarity of the technique. When a sample of dye is injected into the column the solute is transported through the bed and its migration path can be visualised, as evidenced by the photographic images reported in these references.

Likewise, migration through a packed bed that displays this 'invisible bed' phenomenon can be visualised if the injection plug has a different refractive index to the mobile and stationary phases. In that case, the injection plug appears as a dark shadow since the light illuminating the bed from behind cannot penetrate the bed. In this study our injection plug was air, which has a different refractive index to that of the C18 silica and hence, the presence of air inside the column can be seen as a shadow as it migrates along the column, since the presence of the air allows the bed itself to be seen. Throughout the course of this study we tested the effects of air injections at various injection volumes, flow rates and column formats (5 and 17 mm i.d.). The results from which are systematically discussed according the observations on 17 mm i.d. and then 5 mm i.d. columns.

3.1. Air injections on a 17 mm internal diameter column

Typical injection volumes on a 17 mm i.d. column in non-overloaded conditions are around 50 to 300 μL, while in overloaded conditions users would typically inject as much as 2 mL. Hence in this study we tested air injection effects within these limits. In the photographs shown in Fig. 1, a 1000 μL injection of air was made at the flow rate of 2 mL/min. The first of these photographs (1a) shows the bed prior to the air reaching the column, while photograph 1b shows the appearance of the air 40 s after injection, then