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Review

3-Dimensional X-ray microtomography methodology for characterization of monolithic stationary phases and columns for capillary liquid chromatography - A tutorial

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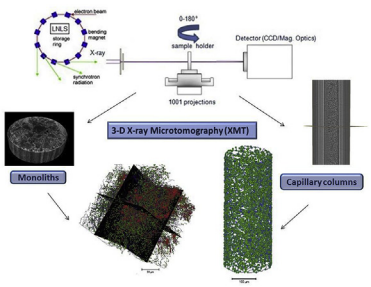
HIGHLIGHTS

- Capillary monolithic organic and inorganic columns were prepared.
- The chromatographic materials and columns were characterized by 3-D X-ray Microtomography (3D-XMT)
- The results were compared with 2-D imagining techniques: SEM and FESEM and with chromatographic profile.

GRAPHICAL ABSTRACT



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ABSTRACT

In this tutorial we describe a fast, nondestructive, three-dimensional (3-D) view approach to be used in morphology characterization of capillary monoliths and columns by reconstruction from X-ray microtomography (XMT) obtained by acquiring projection images of the sample from a number of different directions. The method comprises imaging acquisition, imaging reconstruction using specific algorithms and imaging analysis by generation of a 3-D image of the sample from radiographic images. The 3-D images show the morphological data for bulk macropore space and skeleton connectivity of the monoliths and were compared with other images from imaging techniques such as scanning electron microscopy (SEM) and field emission scanning electron microscopy (FESEM) and with chromatographic performance. The 3-D XMT methodology is applicable for organic and inorganic capillary chromatographic monolithic materials and it allows the acquisition of many hundreds (in our case 1001 projections) of longitudinal and cross-sectional images in a single session, resolving morphological details with a 3D-view of the monolithic structure, inclusive inside the column in a sectional structure with volume (three dimensions) when compared to the sectional structure area (with only two dimensions) when using SEM and FESEM techniques.

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Contents

1. Introduction

High performance liquid chromatography (HPLC) is the most used analytical separation technique due to its versatility for both quantitative and qualitative analyses of a wide range of compounds, especially in the pharmaceutical industry. For this reason, one of most promising fields in HPLC research and development is related to the synthesis and design of new stationary phases (SP), generating an increasing number of column types, both available commercially and labmade [1,2].

Miniaturization in chromatography has emerged with capillary liquid chromatography (cLC) and nano-liquid chromatography (nano-LC), as alternatives to HPLC. Columns in both cLC and nano-LC have reduced dimensions, with internal diameters reduced to the order of micrometers (10–500 μm) [3,4], containing the selected stationary phase, or, recently, confined in noncylindrical conduits used in microfluidic chips [5], in contrast to their larger counterparts for conventional HPLC. Besides miniaturization, cLC or nano-LC presents some advantages as short analysis times, high mass detectivity, low sample volume and reduced use of SP and mobile phases (MP) as well as easy coupling with mass spectrometry [6–8] and they have been applied for determinations in different areas such as pharmaceuticals, biomedicine, environmental analysis and recently in food control and agricultural research. Recently, porous polymer monoliths, with a hierarchical structure comprising a porous structure (microscale) and a polymer gel structure (nanoscale), created by specific linking chemistry relying on (free) radical cross-linking (co)polymerization, have enabled fabrication of complex chemical analysis systems for "lab-on-a-chip" separations. These micro-devices, presents additional advantages in comparison with cLC and nano-LC using system that are not miniaturized, especially related to coupling with mass spectrometry via porous-polymer assisted nanospray. However there still remain some difficulties related to the preparation of materials under spatial confinement and in accessing their nanostructural heterogeneity by imaging characterization techniques [5,9].

SP for capillary columns can be derived by organic [10] or inorganic materials [11]. Inorganic materials are mainly represented by silica, the most popular support used in HPLC SP; although materials based on zirconia [12–14] and titania [15–18] have already been explored in cLC separations. The organic materials are mainly represented by porous polymers, which can be based on organic polar (methacrylate/acrylate based monoliths, styrene divinylbenzene based monoliths and hypercrosslinked monoliths) or organic non-polar (methacrylate-derived monoliths modified with charged groups, e.g., neutral, anionic, cationic, and zwitterionic monoliths) [19,20]. According to the type of SP, columns for cLC can be classified as using particles, as in conventional HPLC columns, or monolithic materials [7]. Although a variety of approaches described in the literature for packing particles inside chromatographic columns by HPLC, features of this technology have proven difficult to implement on the capillary scale, particularly because of the technical challenges associated with packing and retaining beads in narrow-bore capillary columns [21,22]. In this way, capillary columns for cLC use, in the majority of applications, monoliths as SP, are prepared by *in-situ* reactions. These take into account the fact that downward scalability of monolithic

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