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Pore size characterization of monolith for electrochromatography via atomic force microscopy studies in air and liquid phase

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Abstract

This paper investigates the use of scanning electron microscopy (SEM) and atomic force microscopy (AFM) for the characterization of monoliths used in capillary electrochromatography (CEC) while focusing on the nature of the information available from both techniques. SEM imaging revealed a compact structure of non-porous micrometer sized particles homogeneously agglomerated. With a simple AFM methodology, we found by direct observation that the same material exhibits mesopores in the nanometer range while SEM showed non-porous surfaces. These results obtained by AFM clearly showed that micrometer sized particles shrank and micrometer sized pores increased in the monolith when wetted. Thus, AFM was capable of demonstrating the morphological differences between wet and dried monolithic materials that are not possible by other imaging methods at micrometer resolution.

Keywords: Atomic force microscopy; Capillary electrochromatography; Monolithic stationary phase; Porosity; Surface characterization

1. Introduction

The development of new monolithic supports used in capillary electrochromatography (CEC) and liquid chromatography (HPLC) has gained significant interest since the first published example by Hansen and Sievers [1]. Motivated by the numerous problems associated with the fabrication and use of packed beds, many research groups have worked since then on the development of novel organic and silica-based monolithic materials for the separation of both small analytes and macromolecules [2,3]. Based on in situ polymerization, these monoliths offer very flexible and easy-to-prepare media that, by selecting specific monomers, can be used as bioreactor supports [4], separation columns [5], concentration membranes [6] or sample extractors [7]. One of the main advantages of monolithic materials over packed beds is their higher permeability to liquid flow resulting in enhanced mass-transfer rates.

Techniques such as scanning electron microscopy (SEM) [8], mercury intrusion porosimetry (MIP) [9], Brunauer-Emmet-Teller (BET) and Barrett-Joyner-Halenda (BJH) gas adsorption–desorption isotherms and high-resolution optical microscopy (HROM) [10] have increased the understanding of how the chemistry affects the monolith morphology. They are also the primary methods for the characterization of novel polymeric separation supports used in electrochromatography since stationary phase surface morphology plays an important role in the separation process. However, the main drawback of SEM, MIP, BET and BJH stems from their inability to test wet material, thus providing no direct information concerning the monolith in a state similar to its functional state. It is important to mention that the non-imaging characterization techniques determine the material's physical characteristics based on the filling of available pores by probe species. For this reason, differences in results are often due to the size or nature of the probe used in the individual techniques [11]. For example, MIP only allows the determination of macropores (width > 50 nm) size while techniques such as BJH permit characterization of mesopores (2 nm < width < 50 nm). Micropores (width < 2 nm) usually stay inaccessible and therefore, to date, cannot be characterized. Therefore, the results from these methods are semi-quantitative and not always indicative of the polymer's behaviour when used for chromatography [12].

Marůska and Kornyšova [13] demonstrated that HROM has resolution down to 1–2 μm in both dry and wet conditions and

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can access macropore details in porous media. They showed, it also has the advantage of not requiring complex sample preparation compared to other intrusive techniques.

Due to its simplicity, SEM, a non-contact imaging technique, is the most widespread imaging method used for studying the physical aspects of new monoliths. With SEM, features down to 500 nm are readily imaged. However, detailed structures smaller than about 50 nm cannot be resolved. In this technique, a focused electron beam scans the surface leading to the emission of secondary-electrons that are collected by electro-optical lenses. For the best resolution the scanned surface must be thermally stable, relatively flat and conductive to reduce surface charge inhomogeneity. In the case of the polymeric monolith, the surface possesses none of these features, but sufficient conductivity for imaging can be achieved by sputtering a thin layer of either gold or carbon onto the surface but the thermal sensitivity of the polymer limits the voltage that can be applied for imaging.

AFM is a direct probing technique that allows the study of surface morphology, regardless of conductivity. It is based on the deflection of a flexible tip during its scanning movement on a surface [14]. Three operational modes prevail: contact, non-contact and intermittent (tapping) modes. In contact mode, the tip is engaged directly on a surface and dragged along a scan pattern to measure surface topography. To minimize noise due to adsorption of sample onto the tip in contact mode, the tip can be modulated such that the tip only touches the surface intermittently. This latter mode is called intermittent contact, or tapping mode, and is capable of resolving structures of 20 nm depending on surface hardness and morphology. Interactions between a derivatized or non-derivatized AFM tip and the surface can also be monitored by applying an oscillation to a tip close to the sample surface. The frequency of oscillations is influenced by Van der Waals forces between the sample and the tip. However, this technique is not readily used to study morphology characteristics of rough surfaces since this technique needs relatively flat surfaces in order to avoid any artefacts due to contact with the scanned sample surface.

AFM permits a higher resolution in the depth, or z, direction than SEM leading to more accurate surface roughness values. Care must be taken when imaging structures that are similar in size, or smaller, than the tip radius because the side of the tip can make contact with the structure leading to an erroneous measurement. A distinct advantage of AFM is its ability to analyze the surface of the monolith in a wetted state. Thus, conditions similar to those used in a chromatographic separation may be reproduced.

A limited number of other methods can also be used to describe monolithic polymer materials in wetted conditions. Chromatography-based techniques such as inverse size-exclusion chromatography (ISEC) and inverse size-exclusion electrochromatography (ISEEC) measure porosity parameters in the swollen state [15,16]. These parameters are calculated from the retention behavior of molecules with known hydrodynamic radii separated through the monolithic stationary phase. Chromatography-based techniques are able to access three-dimensional porosity characteristics over imaging techniques which are limited to characteristics at, and close to, the probed surface. However, they use operating conditions (e.g. strong solvents such as THF) that are not representative of typical chromatographic conditions and that may affect the morphology of the polymer. Additionally, the limited size range and nature of the standards available hampers its application.

Rathore et al. [17] and Chen et al. [18] described the calculation of porosity values extrapolated from electrokinetic measurements. This approach has the main advantage of providing information on the monolithic polymer material in the same conditions, i.e. same solvent and under an electrical field, as it is used normally in CEC separation experiments. However, this approach only allows the calculation of a factor representative of the total porosity. It does not allow the distinction between intra- and inter- skeleton sizes as available from imaging techniques.

In previous publications, Pesek and coworkers [19,20] showed a good comparison between information obtained by SEM and AFM for silica surfaces modified for open-tubular capillary electrochromatography (OTCEC). The higher resolution of AFM in the z-direction leads to more precise determination of surface roughness than by SEM. AFM allowed measurement of the surface area as well as the surface-tip forces of attraction that are not possible by the latter technique. In another paper, Peterson et al. [4] imaged a novel self-assembled monolithic support, i.e. a polyrotaxane-based monolith, by both AFM and SEM together with HROM. They demonstrated successfully with all these techniques that an increase in ionic strength during polymerization resulted in a net increase of channels size. Nonetheless, the monolithic media had to be dried prior to both AFM and SEM experiments and no experiments were performed in wetted conditions. Few studies have been published assessing the effect of pore size of monolithic media on chromatographic behaviour in part due to difficulties of measuring their true porous properties in their wetted state [21].

1.1. The aim

In this study, we show the capability of AFM as a method for the characterization of a previously described photo-polymerized monolith [22,23] in both wetted and dried state. A comparison of the information available by SEM and AFM will also be detailed.

2. Experimental

2.1. Materials and reagents

The photo-polymerized methacrylate-based monolithic stationary phase, containing C4 functionalities and sulfonate moieties was prepared in fused silica capillaries using the Methods and Materials as described previously by Hilder et al. [21]. Briefly, the monolithic stationary phase is prepared in-situ by the copolymerization of butylacrylate and 2-acrylamido-2-methyl-1-propanesulfonic acid in presence of butanediol diacrylate as a crosslinker. The polymerization is performed in a porogenic solvent consisting of a 60/20/20 (% v/v/v) mixture of acetonitrile/ethanol/5 mM phosphate buffer pH 7.0 and is photoinitiated through the cleavage of benzoin methyl ether through direct