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Characterisation of porous materials for bioseparation

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ABSTRACT

A set of chromatographic materials for bioseparation were characterised by various methods. Both commercial materials and new supports presenting various levels of rigidity were analysed. The methods included size-exclusion and capillary phenomena based techniques. Both batch exclusion and inverse size-exclusion chromatography were used. Gas adsorption, mercury porosimetry and thermoporometry were applied as well as a new method based on water desorption starting from the saturated state. When the rigidity of adsorbents is high enough, the agreement is reasonable between the values of the structural parameters that were determined (surface area, porosity, and pore size) by various methods. Nevertheless, a part of macroporosity may not be evidenced by inverse size-exclusion chromatography whereas it is visible by batch exclusion and the other methods. When the rigidity decreases, for example with soft swelling gels, where standard nitrogen adsorption or mercury porosimetry are no more reliable, two main situations are encountered: either the methods based on capillary phenomena (thermoporometry or water desorption) overestimate the pore size with an amplitude that depends on the method, or in some cases it is possible to distinguish water involved in the swelling of pore walls from that involved in pore filling by capillary condensation.

1. Introduction

The pore structure of chromatographic adsorbents has direct effects on efficiency in preparative bioseparations. The characterisation of porous materials is a field of intense research activity because of the difficulty to describe materials that are highly heterogeneous in pore size, pore shape or pore network organisation. Some methods like nitrogen adsorption–desorption or mercury intrusion–extrusion are more or less considered as standards and many models are available to derive pore size distributions or surface area from corresponding data [1,2]. These methods often give rise to hysteretic phenomena that can be used to give information on pore network organisation [3,4]. For example, Armatas and Pomonis [5] used Monte Carlo techniques to adjust a random network to fit nitrogen adsorption–desorption measurements on porous silica particles. With the generated network, tortuosity and connectivity were determined.

Gas adsorption is limited to pores with radii smaller than 100 nm, whereas mercury porosimetry gives access to a large range of pore size, i.e. from 3 nm to 400 μm. Unfortunately, these two methods are based on capillary phenomena which may induce stress on the walls of the material both during sample preparation (the sample must be preliminary outgassed for both experiments) and during experiments where cycles of adsorption/desorption (resp. intrusion/extrusion) create depressure (resp. overpressure) stress on the pore structure. Because many porous materials for bioseparation are soft gels, these two methods cannot be applied in a standard way since the porous structure may be different between the dry and wet state. This is generally due to the swelling of the walls.

Consequently, inverse size-exclusion chromatography (ISEC) [6] is more and more considered as the best method to characterize chromatographic supports because it is an in situ method mostly applicable at the same conditions as the separation process. Here, the pore size is deduced from the variation of the pore volume being unaccessible to a molecule of given size. The set of probe molecules, generally polymers, must not be adsorbed by the material. Advantages and drawbacks of this method were recently analysed by Yao and Lenhoff [7]. The main advantages are the conservation of sample integrity, the easiness to carry out experiments and the simple equipment. There are no drastic operating conditions (like high pressure, low temperature and drying conditions for gas adsorption or mercury porosimetry) and, as a consequence, less significant morphological changes occur because experimental conditions are

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similar to those of normal operations, which is especially important for swellable gels because their structure is greatly affected by the liquid content. It is also possible to observe the influence of salt concentration on pore size distribution [8].

The experimental drawbacks of ISEC are the duration of experiment, because the flow rate of the mobile phase in the column must be low enough to ensure equilibrium, and eventually the need of long columns for attaining appreciable resolution among different sized probes. Thus compression of the packed bed in the column may occur which could be a problem for soft materials. Nevertheless, the main difficulties are probably at the level of the interpretation of data and of the derivation of quantitative information on pore structure. Discrepancies may be observed when pore information derived from dextrans is applied to proteins, which is not surprising considering the appreciable property differences between these two classes of molecules [7].

Appropriate solvents relevant to practical use should be chosen, with additional considerations such as to minimize adsorption effects and favour optimal solute conformations. The total pore volume and interstitial space are typically measured by solute at opposite ends of the size spectrum of the standards. Considering the rigidity of the solutes, wall effects can affect precise evaluation of the exact values, with the significance depending on the relative abundance of pores. Dextran radii are calculated as if dextrans were hard spheres but in fact they are flexible and may penetrate pores smaller than their nominal size. Data are generally presented as partition coefficient (K) versus probe size. Partition coefficients do not reflect only the actual pore size but the interaction between the molecules and the walls too. Hubbuch et al. [8] showed the influence of salt concentration on ISEC data for an agarose grafted with dextran before coupling with sulfopropyl groups. At a low ionic strength, ISEC measurements show a low pore accessibility, because there are strong interactions between unshielded sulfopropyl groups. These might lead to a rather stiff network conformation which is difficult to penetrate for large molecules. At high ionic strength, the charges on sulfopropyl groups might be shielded and an increase in pore accessibility is observed. Finally, size-exclusion principle can be applied also in a batch mode where it is easier to get the equilibrium partition coefficient [9–11].

Like in other methods, the derivation of a pore size distribution from ISEC data is model dependent. A pore shape must be assumed and it must be kept in mind that usually only up to 15 points can be experimentally obtained, which can limit the detailed description of the pore size distribution. In the case of rigid samples, for example like silica monoliths, a good agreement is obtained with other techniques in the mesopore range (2–50 nm) whereas some differences may be observed in the macropore range, i.e. above 50 nm [12]. Recently, modelling methods of pore structure considering their connectivity based on ISEC data were developed [13,14].

Because it is always difficult to get reliable information from a single technique, it is useful to compare the results of ISEC with other techniques when possible. In this paper, comparisons will be made between the methods quoted above but also with two methods that are less common. The first one is thermoporometry [15], which is another method based on capillary phenomenology. Here the influence of confinement on melting/solidification of a fluid is studied. Thermoporometry has the advantage that a sample can be used in its application medium without drying step (only washing with a pure solvent). Its disadvantages are mainly due to the fact that melting–solidification in confined medium is less well understood than capillary condensation despite clear similarities [16]. Notably, the parameters used in the calculation models are not directly measurable, which means that a calibration needs to be done [15]. This method has often been proposed as a useful method for characterising soft materials, gels [17] or polymers [18].

Finally, the last method used for comparison is the liquid desorption method recently developed by Denoyel et al. [19], which is based on the determination of the desorption isotherm of a liquid from a porous medium starting in an excess of liquid. Pore size distributions are derived from capillary condensation theory like in the gas adsorption method. Because the various phenomena involved in these methods are of different nature, the comparison between the methods needs to choose a number of parameters that can be derived from all methods. Porosities and pore sizes will be compared. The determination of pore size or pore size distribution is a rather complex problem in any method because it needs a number of assumptions concerning pore shape and pore structure. This point will be discussed in detail for each method in Section 2 where thermoporometry and liquid desorption will receive more attention.

2. Materials and methods

2.1. Chromatographic stationary phases

Three ion exchange materials, one hydrophobic charge induction material, four protein A based affinity materials and four non-functionalized supports were analysed. The origin and the main initial characteristics of these materials are given in Tables 1 and 2.

2.2. Gas adsorption

When possible, nitrogen adsorption measurements were performed with a Micrometrics ASAP 2010 apparatus. Adsorption samples were first evacuated at a pressure lower than 10^{-3} Pa. The BET equation was applied to determine the surface area and the pore size distribution was calculated from the desorption branch

Table 1
Selected materials: origin and chemical composition.

Materials	Type and ligands	Supplier	Support
SP Sepharose Fast Flow	SO_3 ion exchange	Amersham Bioscience	Cross-linked agarose
Fractogel SE Hicap	SO_3 ion exchange	Merck	Cross-linked polyacrilic
S Ceramic HyperD F	SO_3 ion exchange	Ciphergen	Silica based ceramic
MEP HyperCEL	Hydrophobic Charge Induction Chromatography (HCIC) Sorbent: 4-mercapto-ethyl-pyridine	Ciphergen	Cross-linked cellulose
Mabselect	Protein A based affinity media	Amersham Bioscience	Cross-linked agarose
r ProteinA Sepharose FF	Protein A based affinity media	Amersham Bioscience	Cross-linked agarose
Poros 50A High Cap	Protein A based affinity media	PerSeptive	Polystyrene/divinylbenzene
Prosep vA High Cap	Protein A based affinity media	Millipore	Controled Pore Glass
Fractosil	Support	Merck	Silica
Fractogel	Support	Merck	Cross-linked polyacrylic
FractAIMs	Support	Merck	Cross-linked polyacrylic
Fractoprep	Support	Merck	Vinylic polymer