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Characterisation of gel permeation chromatography column by fractal geometry

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Summary

General aspects of fractal geometry are treated, and a mathematic model for characterisation of chromatographic gel behaviour is presented. The fractal dimension of the porous gel within a Waters Styragel HT 5 chromatography column, $d_f = 2.3$, as well as the linear dimension of pores, $L = 0.3\ \mu m$, have been determined.

Introduction

Fractal geometry is a mathematical tool for dealing with complex systems which have no characteristic length scale. The notion of non-integer (fractal) dimensions and several basic properties of fractal objects have been studied as long ago as the last century by G. Cantor, G. Peano, and D. Hilbert, and in the beginning of this century by Helge von Koch, W. Sierpinski, C. Julia, and F. Hausdorff. Mandelbrot (1) showed the relevance of fractal geometry to many systems in nature, and presented many important features of fractals. Fundamental to these fractal structures is the concept of self-similarity, by which spatial relationships are translated to higher (or lower) dimensions (2). The porous media are of much importance in different aspects of technology, e.g. oil recovery, catalysis, drinking water treatment, high pressure liquid chromatography. In some cases, porous materials, aggregates, and branched structures are known to frequently exhibit self-similarity. A comprehensive review of the problems of fractal porous media is due to Birdi (3).

In view of the discussion of experimental data, we have to recall some basic results about fractal morphology and measuring the fractal dimensions. A mass fractal is defined (4) as an object such that the minimum number of cubes of edge / needed to cover it scales as:

$$N(l) \sim l^{-d_m} \qquad (1)$$

where $d_m \leq 3$ is the mass fractal dimension. In real fractals, the scaling (1) holds only between two characteristic length l_{min} and l_{max}, the so called lower and upper cutoffs, respectively. The mass fractal dimension is usually not an integer, $d_m = 3$ being the limiting case of an ordinary solid. Fractal concepts may be also applied to self-similar surfaces. The surface fractal dimension d_s can be defined by the relation (4):

$$N(l) \sim l^{-d_s} \qquad (2)$$

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where N(l) is the minimum number of squares of side l needed to cover the fractal surface. The surface fractal dimension varies from 2, the value for ordinary smooth surfaces, up to 3. Similarly to the case of mass fractals, for a real fractal surface (2) is valid for $l_{min} \leq l \leq l_{max}$. We may call a surface fractal (4) any solid object ($d_m = 3$) bounded by a fractal surface.

In the past (2), the measurement of surface areas and pore volumes of porous solids like real catalyst by gas adsorption/desorbtion and liquid intrusion (i.e. Hg porosimetry method) has led to variable and often ambiguous results, inconsistent with an idealized three-dimensional fractal like the Menger sponge. However, it has been extensively reported that there is good reason to expect fractal structure in artificial porous media.

Cherkashinin and Drozdov (5) obtained a simple relation between the extent of filling of pore volume and the adsorption potential from the equation describing vapor adsorption in micropores with nonuniform size distribution. The formula obtained was used to process the experimental nitrogen-adsorption isotherms (77 K) for a number of carbon sorbents. The values of the fractal dimensionality for the micropore systems of the carbon materials studied, range from 2.61 to 2.72.

Neimark and co. (6) investigated the distribution of a highly viscous fluid in porous media by the example of polybutadiene-coated on porous silica LiChrospher Si 300 for liquid chromatography columns. The values of the surface fractal dimensions for the parenteral support and the samples with varying polymer loading have been obtained from low-temperature nitrogen adsorption isotherms, by using the thermodynamic method. The fractal dimensions for polymer-coated samples do not essentially differ from the value of 2.22, inherent for the parenteral support, over the scale range of about 10 - 150 Å. The authors concluded that the thermodynamic method of the fractal analysis based on adsorption and/or capillary experimental data is recommended for the investigation of high-viscosity liquids or other host phase distributions in different porous systems.

Damme and Co. (7) tried to elucidate the adsorption process of homologous series of "molecules" on deterministic fractal curves in the whole range $1 < D < 2$. They simulated the process considering that the molecules have to reach the curve, corning from one side only, and are not allowed to cross it. Three series of molecules were used: (i) circles of increasing diameter, r; (ii) rectangles of increasing height, t; (iii) rectangles of high aspect ratio (almost lines) and increasing length, l. The fractal exponents D_r, D_t, and D_l were derived from ln N_m vs ln r, ln t, and ln l plots, respectively, N_m, being the number of molecules necessary to reach "monolayer" coverage. This provides the basis for predicting the size dependence of N_m, for molecules of any aspect ratio. This model has been extended to adsorption on fractal surfaces. The prediction is that the maximum fractal dimension which could be measured without error is $D = 2.5$, for molecules with constant aspect ratio. Using molecules of a different shape, would lead to considerable errors.

Various methods have been used to estimate the fractal dimension of a surface.

The fractal dimension of aggregates obtained by numerical simulations (8) of the process of particle-cluster aggregation, limited by diffusion, carried out using 2-dimensional square lattices with square "particles" having a variable number of active interaction sites (from 3 to 8), was calculated by the so-called "box counting" method.

The fractal nature of latex particles and their aggregates was characterised by image analysis in terms of fractal dimensions (9). The fractal dimensions were estimated for polystyrene latex aggregates formed by flocculation in citric acid/phosphate buffer solutions by the dimensional analysis method, which is based on power law correlation between aggregate perimeter, projected area and maximum length. The measured three-dimensional fractal dimension for such latex aggregates was found within the fractal dimension range 1.6-2.2 expected for aggregates formed through a cluster-cluster mechanism.

Morphological characterisation of individual particle surfaces was explored by off-line image processing of data obtained by scanning electron microscope – microanalyzer (10). The fractal geometry was studied by two methods, the power spectrum and the variogram approach. Both methods were theoretically evaluated by a series of numerically simulated surface profiles, as well as experimentally on a set of pre-recorded secondary electron images of particle surfaces exposing characteristic textures. It was shown that the fractal approach could stand as a base of the methods enlarging the application of electron probe X-ray microanalyzers for individual particle characterisation.

Controlled-porosity polymer absorbents have wide applications in separation technologies and in purification processes.

For polymer sorbents the fractal dimension was determined by sorption of water vapour by polymers (11), using a 3-parameter sorbtion isotherm equation, which takes into consideration the sorbent fractal properties in the domain of the relative change of water vapour pressure.

Hall and co. used the contrast matching small-angle neutron scattering to monitor the presence of closed porosity in the non-swellen state (12) and the effects of solvents with various Hildebrand solubility parameter (13) on the swellen pore structure of styrene-divinylbenzene resins. The porous structures of the type of resins examined are considered to be made up of interconnected crosslinked microgel particles typically ca. 1000Å in diameter and formed by phase separation during polymerization. The porosity normally accesible to non-swelling media falls in a broad size range, and can vary from microporous dimensions (< 20Å), through the mesoporous range (20-500Å) to macroporous range (> 500Å). The pore structure was believed to be associated with voids and crevices located between the microgel particles. It has been shown that swelling induced by solvents considerably alters the pore structure of the PS resins. The changes involve an increase in the surface area as well in the pore size distribution. The fractal dimension measured by scattering increased from 2.5, corresponding to a mass fractal of the dry samples to 2.95 for a high convoluted surface of the swollen samples.

Porous spherical particles of styrene-divinylbenzene copolymers specially designed for gel permeation chromatography (GPC) were first described by Moore in 1964 (14). These particles were synthesized by suspension polymerization, where a mixture of the two monomers is dispersed by mechanical stirring as droplets suspended in a continuous second liquid phase in which both monomer and polymer are essentially insoluble. The monomer droplets are then polymerized while dispersion is maintained by continuous stirring. In the continuous phase (usually water) a steric stabilizer is added, thus avoiding the coalescence of the droplets during polymerization. The suspension polymerization process produces particles with diameters in the range 50 - 200 µm and a broad particle size distribution.

In order to perform high performance GPC, small copolymers beads (5 - 10 µm) with narrow size distribution are desirable (15). However, the decrease of particle diameter increases the system pressure drop, which requires the use of high pressure instrumentation and copolymers with high mechanical stability. Small spherical particles of styrene-divinylbenzene copolymers have been synthesized by modified suspension polymerization.

In that method, predetermined amounts of monomers, initiator, and solvents are uniformly mixed. The mixture is added to water having a suspension stabilizer dissolved therein and subjected to high speed stirring for a specified time to obtain the desired particle size.

Afterwards, the polymerization continues as a conventional suspension polymerization. The formation of porous structures during copolymerization can be controlled by addition of a porogen agent. Typical porogens employed in suspension polymerization can be classified into three types: solvating diluents (thermodynamically good solvents); non-solvating diluents (thermodynamically poor solvents); and soluble polymers or mixtures thereof. The type and concentration of the porogen used have a marked influence on the porous properties of the resulting packing material. During the copolymerization process the porogen agent may remain in the network phase (gel) or may separate out of the gel. These two situations will produce expanded networks or macroporous structures, respectively. Another variable that has a remarkable influence in the porous properties of macroporous polymers is the concentration of the crosslinking monomer. Typically, an increase of the divinyl monomer content results in polymers with larger surface area. However, little is known about the influence of these large surface area divinylbenzene copolymers on the chromatographic properties of the resulting porous beads.

The main purpose of our research is the characterization by fractal geometry of small heads of divinylbenzene copolymers employed as packing material for GPC columns.

Theory

Brochard-Wyart (16) has studied the properties of the porous media surfaces, concluding that if the surface irregularities increase, the fractal dimension of the surface also increase from 2 to 3. The value $d_f = 2$ corresponds to a smooth surface, and the values close to $d_f = 3$ correspond to a very porous solid. In this case, d_f is defined as the fractal dimension of the interface ($2 < d_f < 3$) and could be determined by generating spheres of radius ε with centres situated on an interface of a grain (or a pore) of a linear dimension L. The spheres situated on the surface of a pore occupy a volume Ω(ε), which is dependent on the number $\frac{L^{d_f}}{\varepsilon^{d_f}}$ of spheres of volume ε^{d}:

$$\Omega(\varepsilon) = L^{d_f}\,\varepsilon^{d-d_f} \tag{3}$$

where d = 3 is the space dimension. The definition can be easily verified for points ($d_f = 0$), straight lines ($d_f = 1$) or planes ($d_f = 2$).

The repulsive surfaces can be studied by the way of an external agent with a variable penetration length in interface, l_s. For linear polymers as external agents, this length l_s is given by R_G (the gyration radius of the polymer chain) for dilute solutions, and by ξ(C) (the correlation length between two macromolecules) for semi-dilute solutions:

$$l_s = R_G \qquad C < \frac{N}{R_G^3} = C^* \tag{4}$$

$$l_s = \xi(C) = a(Ca^3)^{-3\,4}, \qquad C > \frac{N}{R_G^3} = C^* \tag{5}$$

where *a* is the length of the polymer structural unit, C is the concentration of solution and C^* is the critical concentration at which the macromolecules start to overlap.

GPC is a frequently used method for the size separation of macromolecules. The separation is carried out inside columns packed with porous gels. Because the solid/liquid interface is repulsive to the polymer, every fraction of the polymer sample, corresponding to macromolecules with radius of gyration R_G, is separated at a retention volume, V_r, equal with the volume available for that fraction in the chromatographic column:

$$V_r = V_0 + K_d V_i \qquad (6)$$

where K_d is the partition coefficient; V_0 is the interstitial volume and V_i is the internal volume of pores inside of the column.

If a porous solid is swollen with a polymer solution, the volume fraction occupied by the macromolecular chains is equal with the ratio between the volume occupied by the polymer spheres, with a linear dimension ls, and the volume of pore, $\frac{\Omega(l_s)}{L^3}$, and thus the partition coefficient is:

$$K_d = 1 - \frac{\Omega(l_s)}{L^3} \qquad (7)$$

Because only dilute solutions are used in chromatographic measurements, the pore volume available to be penetrated for macromolecules characterised by the radius of gyration $R_G = l_s$, is given by:

$$\Omega(R_G) = L^{d_f} R_G^{d-d_f} \qquad (8)$$

Equations (7) and (8) results in an equation for the determination of fractal dimension of the porous gel inside the chromatographic column:

$$K_d = 1 - \left(\frac{R_G}{L}\right)^{d-d_f} \qquad (9)$$

Experimental

For the determination of the volume V_i, standards of polystyrene with known molecular mass and narrow molecular mass distribution were used (17). The GPC system was a Waters HPLC equipped with a Waters 510 high-pressure pump. The sample injection system was a Rheodyne-valve type. The detector was a Waters 410 differential refractometer. Solutions of concentrations of 2 mg/ml for standards were used. The flow rate, through Waters Styragel HT 5 column was 1ml/min and the GPC temperature was 25°C. Using polystyrene standards with molecular mass between 1500 to 1800000 g/mole, a calibration curve was plotted (Figure 1).

The points in which the calibration curve appears to modify its slope, were correlated with the technical data of column Waters Styragel HT 5 (WAT 044213) for effective molecular weight range (18, 19), and the results are:

- for volume corresponding to elution of polystyrene with molecular mass of $4\text{x}10^6$ g/mole: $V_0 = 7.1$ ml;
- for volume corresponding to elution of polystyrene with molecular mass of $5\text{x}10^4$ g/mole: $V_0 + V_i = 11$ ml.
- for internal volume of the pores inside the column: $V_i = 3.9$ ml.

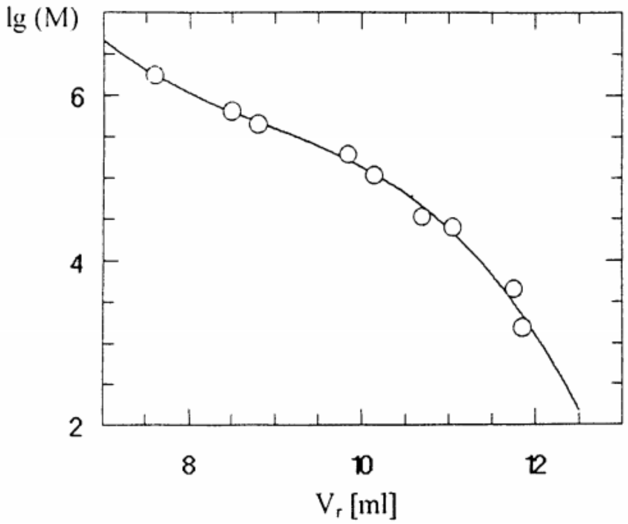

Figure 1. Calibration curve for Waters HT 5 column, for polystyrene standard at 25°C in tetrahydrofuran (THF).

Results and discussion

Determination of radius of gyration for polystyrene standards in THF

The radius of gyration for a polymer chain dissolved in a good solvent is:

$$R_G^3 = [\eta]\, M/\phi' \qquad (10)$$

where $[\eta]$ is intrinsic viscosity, M it is the molecular weight of the polymer chain, $\phi' = 6^{3/2}\phi$, and $\phi = 3.62\text{x}10^{21}$ is the Flory constant.

For a solution of polystyrene in THF, at 25°C, the Mark-Houwink relationship (20):

$$[\eta] = K\, M^a \qquad (11)$$

has the following constants: $K = 1.6\text{x}10^{-4}$ dl/g, $a = 0.706$, which gives the result that the radius of gyration for a polystyrene macromolecule with molecular weight M, in THF is:

$$R_G = 0.67\, M^{0.57} \qquad [\text{in Å}]\ (12)$$

The results for polystyrene standards used for the new calibration curve plot are given in Table 1:

Table 1. Experimental results for polystyrene standards solved in THF at 25°C The data correspond to the effective (linear) molecular weight range of the column Styragel HT 5.

No.	Standard	M	V_r [ml]	K_d	R_G [Å]	ln R_G
1.	PS 37000	36000	10.70	0.92	70	4.23
2.	PS 111000	111000	10.15	0.78	495	6.20
3.	PS 200000	196500	9.85	0.70	685	6.53
4.	PS 498000	452500	8.80	0.43	1100	7.00
5.	PS 670000	655000	8.50	0.35	1360	7.21
6.	PS 1800000	1750000	7.60	0.12	2380	7.77

Determination of fractal dimension of porous gel inside the HT 5 column

Relation (9) may be rewritten as:

$$1 - K_d = \left(\frac{R_G}{L}\right)^{d-d_f} \qquad (13)$$

and by taking logarithms, a linear relationship between radius of gyration and (1-K_d) results:

$$\ln(1 - K_d) = (d - d_f)\ln(R_G) - (d - d_f)\ln(L) \qquad (14)$$

where for three-dimensional space d = 3. The experimental data are plotted in Figure 2.

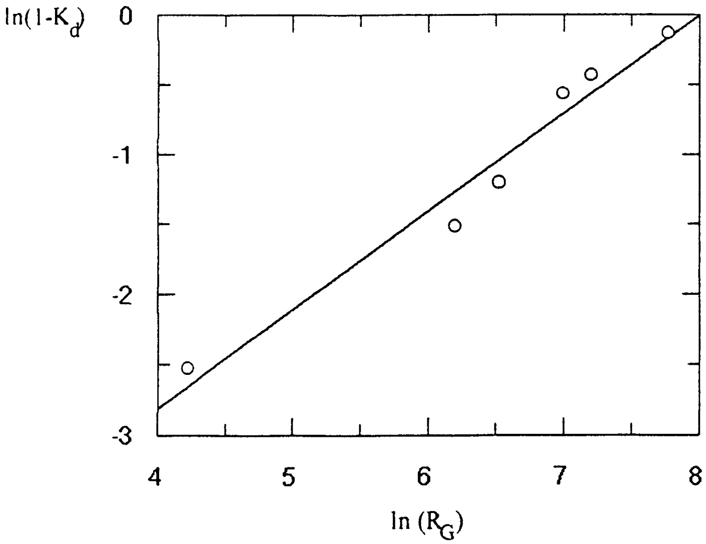

Figure 2. ln (1-K_d) vs ln (R_G) for polystyrene standards used for calibration plot in the linear range of Waters Styragel HT 5 column, solvent THF.

The coefficients of interpolation of the resulting straight line were determined by the least square method: ***gradient = 0.70;*** ***intercept = -5.6.***

By identification of these coefficients with terms of relationship (14), it results:

- the fractal dimension of porous gel: **d_f = 2.3;**
- the average linear dimension of pores: **L = 0.3 μm.**

Conclusions

a) The determined relationship between ln(R_G) and ln(1-K_d) is important because it gives an alternative way to obtain the universal calibration curve for a GPC system.

b) Characterisation of porous gel by two parameters with physical significance (d_f and L) could be useful in development of new packs for chromatographic columns. Thus, a smaller d_f could produce a better resolution for chromatographic columns, while the increase of d_f allows separation of macromolecules with a broad molecular mass distribution. Also L could be considered as the upper cutoff, l_{max}, for the fractal behaviour of porous gel.

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