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SIMPLIFIED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY PHASE CHARACTERIZATION: PARTICLE SIZE, SURFACE AREA, MEAN PORE DIAMETER, AMOUNT OF BONDED PHASE AND ACTIVITY

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SUMMARY

Techniques which do not require expensive instrumentation are described for evaluating particle size, surface area, mean pore diameter, amount of bonded material and activity of high-performance liquid chromatography silica gel phases.

INTRODUCTION

There are already a large number of high-performance liquid chromatography (HPLC) phases on the market[1], but a difficulty is their unsatisfactory characterization. Points which are usually considered to be important in this respect are as follows:

(a) for silica gel: particle size and particle size distribution, surface area, mean pore diameter and pore size distribution, impurities, caking or clogging properties;

(b) for derivatized phases: as for silica gel, plus amount and nature of bonded organic phase, eventual deactivation of active sites.

Data for these quality criteria of commercial samples are scarce and are usually accepted as such although it is known that there can be large deviations and even batch to batch differences.

To evaluate these quality criteria, measuring instruments and established techniques include the Coulter Counter, mercury intrusion, neutron activation analysis, BET gas adsorption, thermogravimetric analysis and elementary analysis. The necessary (expensive) instrumentation for such measurements is normally not available in a chromatography laboratory.

In this paper more readily available alternatives for some of these analyses are presented.

PARTICLE SIZE

The most commonly used particle size in HPLC is 10 μm. A silica gel or derivatized phase labelled as such, normally contains particles down to 7–8 μm and up to 12–13 μm. Closer sizing does not improve the results, or at least not markedly, but the largest particles should not be more than twice as large as the smaller ones and

most important, the average value should be correct. Commercial 10-μm materials may well have a mean size of 12 or 7 μm in our experience. On comparing HETP figures for different columns, the particle size value should be known.

When spread out thinly on a glass plate, the particles can be seen clearly under a microscope magnification of $\times 400$. Without a graduated scale (normally not available) it is difficult or even impossible to evaluate the true dimensions of a particle observed under the microscope. An easy solution is to compare the particles with red blood cells (Fig. 1). The smallest drop of blood, when smeared on a glass plate, will produce a pattern as shown. The red blood cells are only slightly reddish under the microscope. Their particle size distribution is very close and between 7 and 10 μm. Silica gels of 5 μm diameter can also be compared with red blood cells for size evaluation. Microscopic evaluation tends to overestimate (*ca.* 15%) particle size as the particles, lying on a glass plate, present their largest cross section to the observer.

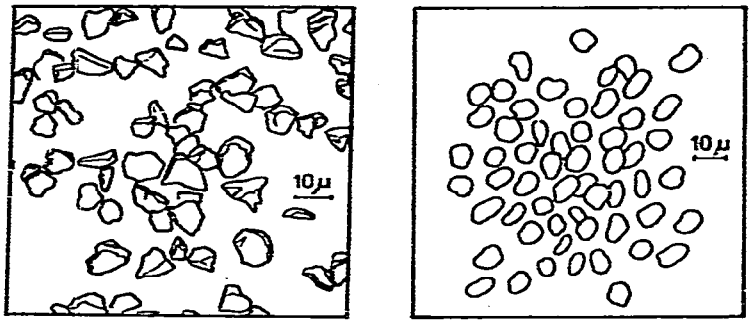

Fig. 1 Evaluation of particle size of silica gel (left) comparison with red blood cells (right) under a microscope.

The appearance of derivatized silica gels under a microscope is the same as that of underivatized material. Microscopic examination can also reveal the presence of ultra-fine material and possibly the reason for permeability problems.

SURFACE AREA

The surface area of fine particles is measured by the Brunauer, Emmett and Teller (BET) gas adsorption technique[2]. Many variations and instrumental arrangements of relative complexity have been described and commercial (expensive) instrumentation for BET analysis exists. We feel that this determination should be more readily available to chromatographers. A very simple instrument which can easily be constructed and which allows one determination of surface area (S) in a few hours is described below. It is based on the more complicated device of Harkins and Jura[3], and was developed in the Laboratory of Physical Chemistry of this University. It has been used there in practical training courses for students for many years. The technique is described here in sufficient detail to allow duplication by the interested reader.

The principle of the gas adsorption method is that the amount of adsorbed gas

is dependent only on the surface area of the adsorber and the amount of gas needed to establish a monolayer is determined. Vessel V_A in Fig. 2, filled with 0.5–1 g of silica gel, is de-gassed thoroughly by a high vacuum for 2 h to remove adsorbed air, water and possibly other small molecules. The vessel is then cooled in liquid nitrogen (77° K) and the manometer is brought to zero by moving the mercury reservoir to the desired level. This zero point is where the capillary tubing begins. In the other arm of the mercury-filled U-tube the mercury level (h_0) is noted. By manipulation of the appropriate three-way stop cocks B and C, a known amount of nitrogen is introduced into vessel V_S. The water locks maintain atmospheric pressure in V_S. Stopcock A is closed and B is opened between V_S and V_A. The gas sample is partly adsorbed on the silica gel. The mercury reservoir is moved so as to obtain the right-hand mercury arm level again at zero. The left-hand mercury arm level is now at h_1. A new volume of gas is introduced and h_2 is measured. This operation is repeated until about 40 cmHg pressure difference between the two arms of the U-tube is obtained.

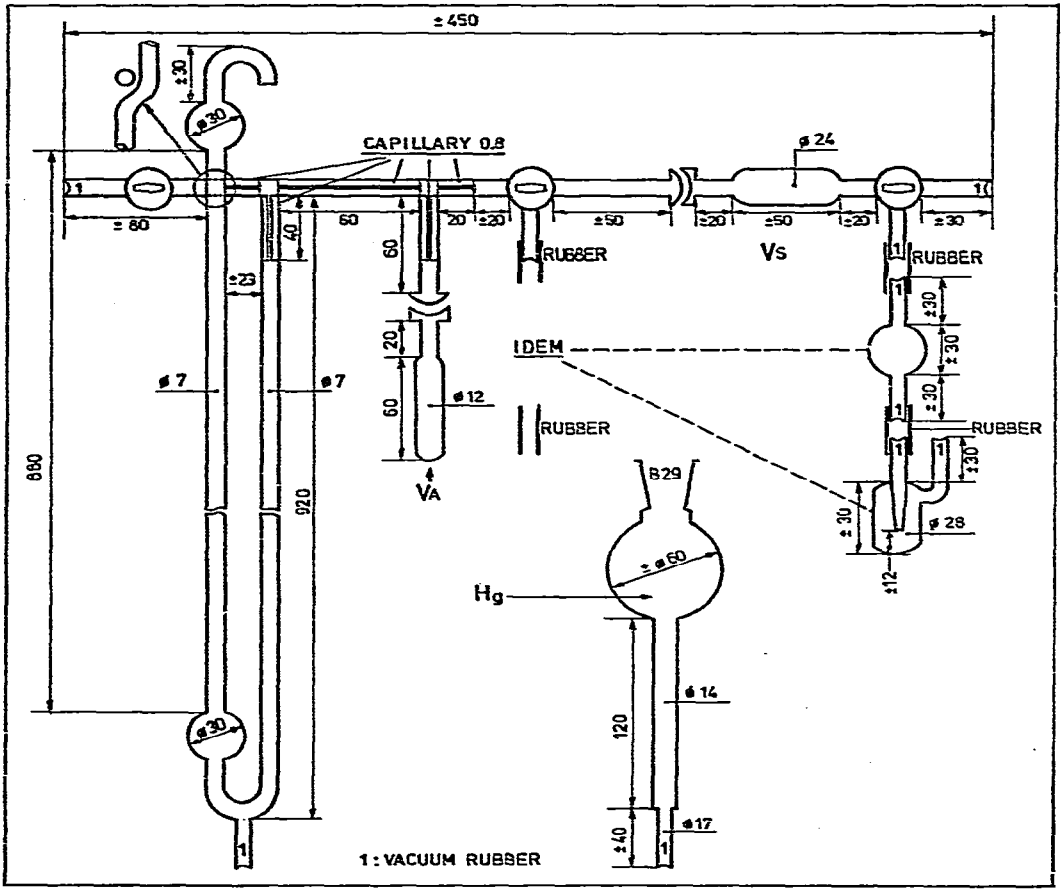

Fig. 2 Apparatus for the determination of specific surface area of powders through nitrogen adsorption (BET principle).

The calculations are then as follows:

$$n_1 = [h_1 V_S - (h_0 - h_1)\, V_d] \cdot \frac{1}{760} \cdot \frac{1}{RT_R}$$

$$n_2 = [(h_1 + h_2)\, V_S - (h_0 - h_2)\, V_d] \cdot \frac{1}{760} \cdot \frac{1}{RT_R}$$

$$n_i = [(\Sigma_1^i h_i)\, V_S - (h_0 - h_i)\, V_d] \cdot \frac{1}{760} \cdot \frac{1}{RT_R}$$

where

R = 82.06 $cm^2 \cdot atm \cdot °K^{-1} \cdot mole^{-1}$;
T_R = room temperature (°K);
V_d = determined by an experiment without adsorbent sample, corrected for the sample volume and thus equal to

$$V_S \cdot \frac{p_0 - p}{p} - \frac{w}{d_S} \cdot \frac{T_R}{77};$$

V_S = *ca.* 10 ml and is determined exactly by filling with carbon tetrachloride (or another liquid) and weighing;
p = pressure in V_A or $h_0 - h_i$ (h in mmHg);
p_0 = atmospheric pressure;
w = weight of adsorbent sample;
d_S = sample density (2.2 for silica gel);
V_A = *ca.* 7 ml

In the above equations a correction factor for the difference in mercury density at 0°C and room temperature has been omitted because for our purposes it is negligible.

n_i and the appropriate pressures p ($p = h_0 - h_i$) fit the following BET equation:

$$\underset{y}{\frac{p}{n(p_0 - p)}} = \underset{b}{\frac{1}{n_m c}} + \underset{a}{\frac{c - 1}{n_m c}} \cdot \underset{x}{\frac{p}{p_0}}$$

where c is a constant.

A plot of $p/n(p_0 - p)$ against p/p_0 for all of the measurements made reveals a linear relationship for the middle values and from this a and b can be deduced. In this BET equation n_m is the amount of nitrogen in moles required to obtain a monolayer on the adsorbent surface. The surface area covered by n_m is the surface area of the adsorbent. With a and b and the above equation it follows that $n_m = 1/(a + b)$ and this leads to

$$S = \frac{n_m s N_A}{w}$$

where s = surface area covered by one nitrogen molecule (16.3 Å²) and N_A = Avogadro's number. $sN_A \approx 10^5$ if S is measured in square metres. Using the values of a and b of the BET equation, this leads to

$$S = \frac{10^5}{(a + b)\, w} \text{ (m}^2\text{/g)}$$

A small computer speeds up the calculations considerably.

The surface areas of other materials can be measured in the same way. The volume V_A may have to be adapted in order to accept a larger sample.

MEAN PORE SIZE

The pore size distribution of silica gels is normally very wide. The material mostly advocated for chromatographic purposes is that with a mean value of 6 nm (60 Å). When such silica gel is washed, acid treated or activated by a wet process, the mean pore size increases to 8–10 nm. This has been mentioned before in the literature, but without an explanation. Most silica gel chromatography is therefore carried out on material with a mean pore diameter of 8–10 nm (rather than 6 nm).

Pore size, surface area and total pore volume (TPV) are related according to the following approximation equation of Halpaap[4]:

$$\bar{d} = \frac{4\text{TPV}}{S} \cdot 10^3$$

where S (m^2/g) is the total surface area per gram, TPV is measured in ml/g, and $\bar{d}$ (nm) is the mean pore diameter.

A good approximation of TPV can be obtained from the direct titration of dry silica gel with water, according to Fisher and Mottlau[5] and Innes[6]. This titration procedure succeeds much better than is generally assumed. The procedure is very simple.

A 2–5-g amount of silica gel in a 25-ml erlenmeyer flask is stirred with a magnetic stirrer at about 3 turns per second. Titration is carried out with distilled water. When a few silica gel particles tend to adhere to the glass wall, the end-point is close. The addition of one or two drops more will reduce the speed of stirring as the silica gel is no longer free flowing but starts to agglomerate in lumps. This final titre is then the TPV, from which the mean pore diameter is calculated via the Halpaap equation above. Surface area and TPV can also be measured in the same manner for bonded phases. The TPV titration has to be carried out with a solvent, *e.g.*, methanol and precautions have to be taken to prevent evaporation.

Some typical results are as follows:

(1) Normal chromatographic silica gel: $S = 432$ m^2/g, TPV = 0.96 ml/g and $\bar{d} = 8.8$ nm. The silica gel manufacturer (Herrmann) indicates an S value of *ca.* 450 m^2/g.

(2) Silica gel (1) boiled with hydrochloric acid: $S = 284$ m^2/g, TPV = 0.90 ml/g and $\bar{d} = 13.0$ nm. It is well substantiated that acid (or water) treatment reduces S.

(3) Silica gels prepared according to the procedure described by Uyterhoeven *et al.*[7]: $S = 72$ m^2/g, TPV = 0.60 ml/g and $\bar{d} = 35.3$ nm. These figures agree with the data of Uyterhoeven *et al.*

(4) Silica gel (Herrmann, Dusseldorf, G.F.R.) with 0.3% of sodium carbonate added and heated for 7 h at 800°C (which reduces S and increases $\bar{d}$): $S = 82.8$ m^2/g, TPV = 0.80 ml/g and $\bar{d} = 38.0$ nm.

(5) Octadecyl-bonded (17% octadecyl) silica gel made from material (2) above: $S = 182$ m^2/g, TPV = 0.42 ml/g and $\bar{d} = 9.0$ nm. It is well known (see, for example, ref. 8) that derivatization of silica gel reduces S.

(6) Diol phase (8% diolchain) silica gel made from No. (2) silica gel: (a) $S =$

261 m^2/g, TPV = 0.59 ml/g and $\bar{d}$ = 9.0 nm; (b) S = 261 m^2/g, TPV = 0.59 ml/g and $\bar{d}$ = 9.0 nm. These measurements illustrate that the reproducibility can be excellent.

(7) Tenax 60–80 mesh: (a) S = 25 m^2/g, TPV = 1.60 ml/g and $\bar{d}$ = 297 nm; (b) S = 18 m^2/g. These data were obtained on the same Tenax batch. With Tenax the end-point is difficult to establish. Specific surface areas mentioned in the literature are, *e.g.*, 18.6 m^2/g (ref. 9) and 30 m^2/g (ref. 10).

(8) Porapak Q 50–80 mesh: (a) S = 364 m^2/g, TPV = 1.26 ml/g and $\bar{d}$ = 20 nm; (b) S = 358 m^2/g, TPV = 1.23 ml/g and $\bar{d}$ = 20 nm. See remarks under (6). The S value of Porapak Q given by the manufacturer (Waters Assoc., Milford, Mass., U.S.A.) is 500–600 m^2/g.

IMPURITIES

By impurities are meant organic contaminants and inorganic trace elements. What these are and how they can be largely eliminated by acid treatment was recently discussed by us[11].

AMOUNT OF ORGANIC MATERIAL BONDED TO DERIVATIZED SILICA GELS

To determine the amount of organic material bonded on a particular phase, thermogravimetric analysis (TGA) or C, H, N elemental analysis is mostly used. A much simpler way of obtaining a useful figure is by heating and weighing. The weight loss on heating a 100-mg sample for 2 h at 110°C gives the amount of water or of other solvents present. This can be high and for one commercial sample even attained 25%. Heating the dried samples at 600°C for 1 h in a crucible will remove all bonded organic material and weighing before and after leads to interesting figures. Some results are given in Table I.

TABLE I

PERCENTAGE OF MATERIAL LOST FROM DERIVATIZED SILICA GEL SAMPLES UPON HEATING

SiO_2–C_{18} = octadecyl-bonded silica gel; SiO_2–amide = N-acetylaminopropyl-bonded silica gel, SiO_2–amine = aminopropyl-bonded silica gel; SiO_2–diol = glycerolpropyl-bonded silica gel.

Sample	*% Bonded determined by TGA*	*% Removed by heating at 600°C for 1 h*
SiO_2–C_{18}	18	18.5
SiO_2–amide	10	9
SiO_2–amine	6	8
SiO_2–diol	15	13

The differences between the two methods may seem large for SiO_2–amine and SiO_2–diol, but we have found repeatedly that amino phases give lower TGA values than expected from the derivatizing conditions while SiO_2–diol phase may easily be dehydrated during the drying step, thus leading to lower weighed pyrolysis figures.

Undoubtedly silica gel loses weight on heating at all temperatures up to 1000°C and precise analysis by thermogravimetry is therefore impossible. However, the figures obtained at 600°C are useful in practice since they give an indication whether the phase is heavily or lightly loaded or whether it is loaded at all!

DEACTIVATION OF REVERSED-PHASE SILICA GELS

Reversed-phase silica gel still contains a considerable number of silanol functions, some of which may be accessible to smaller (and even larger) molecules and are thus the site of chemical adsorption activity. This activity can be partially removed or the phase can be deactivated by treatment with silylating reagents. The silylation status is therefore a criterion that should be considered. A simple test often suggested to determine activity is to shake a small sample of the phase (30–100 mg) with 5 ml of a solution of methyl red (0.8 g/ml) in xylene (or benzene), according to the procedure of Shapiro and Kolthoff[12]. The xylene solution of methyl red is yellow-brown and the supernatant of the test solution should remain coloured with a deactivated phase. The silica gel should at most turn slightly reddish. With non-deactivated (too active) phases, the derivatized silica gel turns red and the supernatant xylene is colourless. This test, however, is unsatisfactory as too much depends on the end treatment of the silica gel, which can be more or less washed acid free; even a base treatment is sometimes applied. In other words the test is for residual acidity in the first place and only in the second place for free silanol functions.

An alternative way of checking the silylation status of a derivatized silica gel seems to be to ascertain whether additional deactivation by silylation changes the chromatographic properties of the material; however, this takes much time.

It has also been suggested[13,14] that chromatography of benzene and nitrobenzene could be used to evaluate the polarity of HPLC reversed phases. Sufficient deactivation is revealed when the capacity ratios of the two compounds are less than 0.1 and 0.5. In our hands however, this is not always possible and certainly difficult to reproduce. We feel that a simple test for the evaluation of the activity of reversed silica gel phases is still lacking.

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REFERENCES

1 R. Majors, *J. Chromatogr. Sci.*, 15 (1977) 334.
2 S. Brunauer, P. Emmett, and E. Teller, *J. Amer. Chem. Soc.*, 60 (1938) 303.
3 W. Harkins and J. Jura, *J. Amer. Chem. Soc.*, 66 (1944) 1366.
4 H. Halpaap, *J. Chromatogr.*, 78 (1973) 63.
5 N. Fisher and A. Mottlau, *Anal. Chem.*, 34 (1962) 714.
6 W. Innes, *Anal. Chem.*, 28 (1956) 332.
7 J. Uyterhoeven, M. Sleex and J. Fripiat, *Bull. Soc. Chim. Fr.*, (1965) 1800.
8 H. Colin and G. Guiochon, *J. Chromatogr.*, 141 (1971) 289.
9 K. Sakodynskii, L. Panina and N. Klinskaya, *Chromatographia*, 7 (1974) 339.
10 B. Versino, M. de Groot and F. Geiss, *Chromatographia*, 7 (1974) 302.
11 M. Verzele, M. De Potter and J. Ghysels, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, (1979) 151.
12 I. Shapiro and I. Kolthoff, *J. Amer. Chem. Soc.*, 72 (1950) 776.
13 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 142 (1977) 213.
14 K. Karch, I. Sebestian and I. Halász, *J. Chromatogr.*, 122 (1976) 1.