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Monosized stationary phases for chromatography

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ABSTRACT

Polymer particles with a highly monodisperse particle size distribution were produced by a two-step microsuspension method. This process is based on the activation of monosized polymer seed particles by the introduction of a low-molecular-weight material, which leads to a large increase in the monomer swelling capacity of the seed particles. The versatility of the process allows the preparation of polymer monosized compact or macroporous particles of predetermined particle size in the range 1–100 μm and with application of a wide selection of polymeric materials. Underivatized, rigid, porous particles were developed for size-exclusion chromatography in organic solvents. The uniform packing that may be achieved with monosized particles has resulted in chromatographic columns with unusual efficiency and separation capacity. By coating the particle surface with a hydrophilic cross-linked polymer, supports for aqueous phase ion-exchange and size-exclusion chromatography may be produced.

INTRODUCTION

The conventional method of preparing polymeric packing materials is by suspension polymerization processes. Such methods involve the addition of monomer or monomer mixtures and a monomer-soluble initiator to a stirred reactor containing water with a small amount of steric stabilizer. Polymerization is effected by heating the reaction mixture, and the product particle size distribution is determined by parameters such as speed of stirring, polymerization temperature and type and amount of stabilizer. A broad particle-size distribution is a typical result of this method.

Monodisperse, small latex particles of diameter 0.5 μm may be produced by well known emulsion polymerization methods if the reaction conditions and the purity of the reagents are carefully controlled. The preparation of larger monosized beads has been a challenge to polymer chemists for many years. A method of successive seeding was published by Kim *et al.* [1]. This method involves equilibrium swelling of the seed particles with monomer before polymerization is carried out. In order to obtain a substantial increase in particle size, the sequence of swelling and polymer-

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ization must be repeated several times, *e.g.*, five times to go from 1 to 10 μm. This is so because polymer particles in aqueous dispersion are capable of absorbing only a limited amount of monomer, *i.e.*, of the order of 2–5 times by volume [2]. The low swelling ratio implies that during most of the successive seeding process the particles are in "sticky-state" conditions with a high risk of particle coagulation. Moreover, the low swelling ratio means that the seed constitutes a substantial part by volume of the swollen particles. This may cause phase separation and particle deformation by the subsequent polymerization if the monomer applied in the final polymerization is different from that in the seed.

In a series of papers, Ugelstad and co-workers [3–6] described new processes by which highly monodisperse polymer particles may be prepared by a two-step activated swelling technique. These processes resulted from developments of methods by which the capacity of polymer particles to absorb the monomer is greatly enhanced.

THE ACTIVATED SWELLING METHOD

The main feature of the new processes is that one initially activates polymer seed particles so that in aqueous dispersion they are capable of absorbing monomer in an amount which far exceeds that of pure polymer particles. The activation of the seed particles results from the presence in the particles of a highly water-insoluble, relatively low-molecular-weight compound (Y), which is introduced under conditions that allow it to be transported through the aqueous phase to become absorbed in the seed.

For comparison of the swelling capacity of such activated particles with that of pure polymer particles the following equations should be considered:

(i) swelling of pure polymer particles with monomer (Morton equation [2]):

$$\ln \varphi_M + \varphi_P + \varphi_P^2 \chi_{MP} + 2\bar{V}_M \gamma / rRT = 0 \tag{1}$$

(ii) swelling of polymer particles containing compound Y:

$$\ln \varphi_M + (1 - J_M/J_Y)\varphi_Y + \varphi_P + \varphi_Y^2 \chi_{MY} + \varphi_P^2 \chi_{MP} + \varphi_Y \varphi_P (\chi_{MY} + \chi_{MP} - \chi_{YP} J_M/J_Y) + 2\bar{V}_M \gamma / rRT = 0 \tag{2}$$

where φ_i is the volume fraction of compound i, χ_{ij} is the interaction energy per mole of compound i, J_M/J_Y is the ratio of number of segments in the monomer, M, and the oligomer, Y, $\bar{V}_M$ is the partial molar volume of the monomer, γ is the interfacial tension and r is the radius of the particles at equilibrium.

The increase in swelling capacity when part of the polymer in the particles is replaced with an oligomer, Y, is illustrated in Fig. 1, which shows the relationship between the logarithm of the volume of monomer V_M absorbed by the total volume of the activated particles $V_Y + V_P$ and log γ/r_0, where r_0 is the radius of the polymer/oligomer particles at the start. The volume $V_Y + V_P$ is set equal to 1. Graphical curves are given for various compositions, *i.e.*, from pure polymer particles, $V_Y = 0$, $V_P = 1$, to pure oligomer, $V_Y = 1$, $V_P = 0$. The value of J_M/J_Y is set equal to 0.2, $\chi_{MY} = \chi_{MP} = 0.5$ and $\chi_{YP} = 0$.

As shown in Fig. 1, the swelling capacity at a given value of γ/r_0 increases with

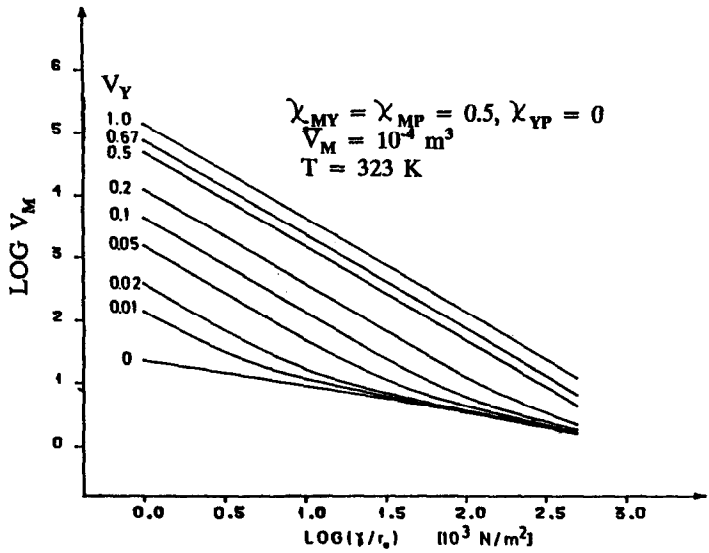

Fig. 1. Volume of monomer, V_M, which may be absorbed per unit volume of seed particles as a function of γ/r_0 at different values of V_Y. r_0 = radius of the polymer/oligomer particles prior to swelling. $V_Y + V_P = 1$.

increasing ratio of compound Y to polymer in the seed particles. Fig. 1 also shows that the swelling increases towards lower values of γ/r_0, *i.e.*, as the particle radius increases and the interfacial tension decreases.

As an example, with an interfacial tension of 10 mN/m and seed particles of 1-μm radius, containing 50% of compound Y, one would expect a swelling capacity of the seed particles which is 200 times higher than that of pure polymer.

Three methods have been applied to introduce oligomeric (Y) material into the particles in the first step of the process:

(1) Polymer seed particles are swollen with monomer containing a chain-transfer agent, followed by polymerization.

(2) Polymer seed particles are swollen with an oil-soluble initiator and monomers of types and in such a ratio that the subsequent polymerization leads to the formation of oligomeric material.

(3) Polymer seed particles are swollen with a relatively low-molecular-weight, highly water-insoluble compound (Y). To facilitate the diffusion process by which Y is transported through the water phase to the particles, compound Y may be added in the form of a finely divided aqueous dispersion.

In method 3, the swelling of the particles with Y in the first step then takes the form

$$\ln \varphi_Y + \varphi_P + \varphi_P^2 \chi_{YP} + 2\bar{V}_Y \gamma / rRT = 2\bar{V}_Y \gamma / r_Y RT \qquad (3)$$

where r_Y is the radius of the droplets of Y. An oil-soluble initiator may be used as compound Y, which then acts boths as an initiator for the polymerization and as an activator for monomer absorption. Also, the second-step swelling (eqn. 2) may be

facilitated by addition of the monomers in the form of an aqueous emulsion [4].

Starting with monosized seed particles, these swelling processes, when properly performed, secure an equal swelling of each particle, maintaining the monodispersity during the process. By combination of methods 1 and 2 with method 3, one may obtain monodisperse product particles which are more than 1000 times larger by volume than the original seed particles.

A characteristic feature of this process is that, when the polymerization of the monomer or monomer mixture is started, all necessary ingredients are present in the highly swollen seed particles. This implies that the activated swelling method is especially suitable for the preparation of cross-linked polymer particles. Moreover, this method is equally suitable for the preparation of porous macroreticular particles, in which case the second-step swelling is carried out by applying inert diluents in addition to monomers, including cross-linkers.

MONOSIZED POLYMER PARTICLES

Examples of monomers which have been applied to prepare monosized polymer particles by the activated swelling process are styrene and styrene derivatives, vinylbenzyl chloride, vinylpyridine, divinylbenzene (DVB) and alkyl acrylates and methacrylates such as hydroxyethyl acrylate and methacrylate (HEMA), glycidyl methacrylate (GMA), ethylene glycol dimethacrylate (EDMA) and trimethylolpropane trimethacrylate (TRIM). In addition, we have synthesized monomers with special functionalities for application in this process.

A wide range of applications for monosized polymer particles in various sizes and materials have been developed during the last few years. Some of these applications are illustrated in Fig. 2.

Fig. 3 shows an example of a scanning electron micrograph of 10-μm compact and cross-linked polystyrene (PS)–DVB particles. These particles were prepared directly from a 1-μm oligomeric seed latex. The high swelling ratio, 1000 times by volume, ensures a spherical shape and a smooth surface. The high degree of monodispersity causes the particles to arrange themselves in an ordered array on the microscope slide.

Fig. 4 shows a histogram of the size distribution of the 10-μm particles in Fig. 3, measured with a high-precision Coulter instrument. A relative standard deviation of the diameter of $< 1\%$ was obtained. The ordinary Coulter technique with an electrical sensing zone may not always be suitable for the characterization of the size distribution of highly monosized spheres, as an additional peak may appear in the histograms, in addition to the normal peak [7].

By an extension of the process, the preparation of monosized beads containing magnetic material is achieved [8]. For this purpose, porous particles are produced which in a subsequent step are made magnetizable by *in situ* deposition of magnetic iron oxides in the particle pores. Examples of applications of magnetic beads are selective cell separation processes and magnetic DNA technology [9,10].

PREPARATION OF POROUS MATRICES

Porous matrices are obtained when polymerization and cross-linking take place

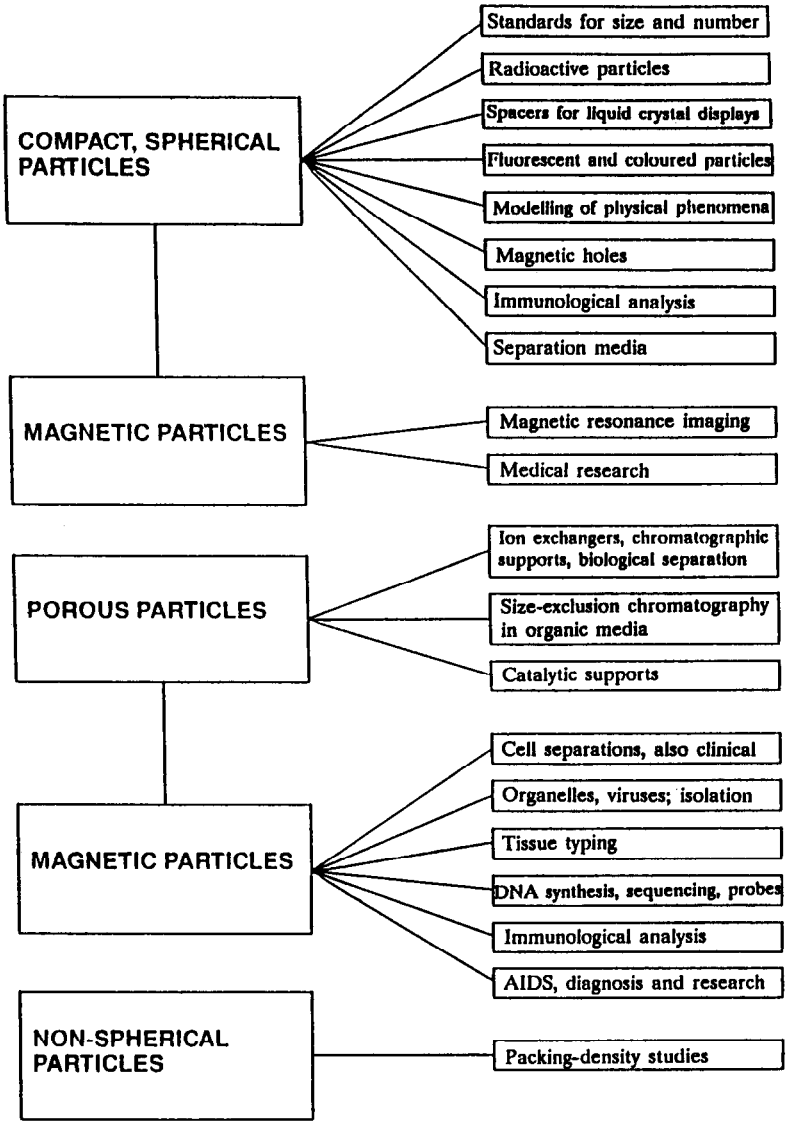

Fig. 2. Schematic illustration of various applications of compact and porous monosized polymer particles.

in the presence of inert diluents, which lead to the formation of permanent pores in the material after removal of the diluent. The most important parameters for the construction of special pore sizes are monomer type and reactivity, degree of cross-linking, amount of diluent and diluent solvency for the polymer (solvent–non-solvent).

By increasing the amount of polyfunctional monomer (cross-linker) in the reaction mixture, micropores (< 50 Å) are formed in the material, while larger pores may

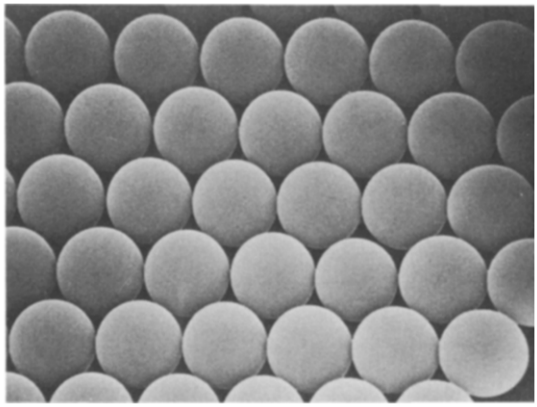

Fig. 3. SEM of 10-μm compact and cross-linked PS–DVB particles.

be obtained by increasing the amount of diluent and/or reducing the diluent solvency. Solvent–non-solvent mixtures are frequently applied to design tailor-made pore-size distributions for special applications, such as supports for liquid chromatography.

Cross-linked and porous PS–DVB materials have been described in the literature for over 25 years [11,12] and are still a focus of attention of both scientists and producers. PS–DVB matrices may be developed into highly rigid and mechanically and chemically stable chromatographic packing materials which are able to operate over a wide pH range. Acrylic porous particles have been less widely described [13,14]. Although acrylates contain ester linkages that are somewhat labile, methyl-substituted acrylates are far more stable than the unsubstituted derivatives towards hydrolysis. Methacrylic macroporous polymers can be used in many applications where prolonged exposure to high pH is not required.

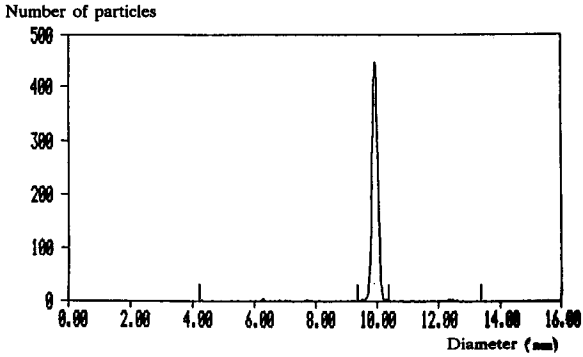

Fig. 4. Histogram of the size distribution of 10-μm PS–DVB particles.

PORE-SIZE CHARACTERIZATION

For applications in the chromatographic field it is important to have a good knowledge of the porous particle characteristics, the specific surface area, the total pore volume and the pore-size distribution. The nitrogen adsorption–desorption method, which covers the pore radius range 10–250 Å, is commonly used to characterize fine- and medium-pore-sized materials, whereas mercury porosimetry is to be preferred when the materials contain medium and large pores, 100 Å–1 μm. A complete pore-size distribution curve will in most instances require characterization by both methods.

The BET method and the mercury intrusion method both require drying of the porous materials. With nitrogen adsorption the measurements are performed at very low temperatures. Drying and lowering of the temperature may cause shrinkage of porous materials that are not completely rigid. A newly described characterization method which does not require drying is thermoporometry [15]. Inverse gel permeation chromatography (GPC) is a chromatographic pore-size determination method [16] in which narrow-molecular-weight polymer standards are applied to determine the pore-size distributions and the exclusion limit of chromatographic supports. For porous matrices of high rigidity we have found a good correlation between pore-size measurements by the mercury intrusion method and inverse GPC.

Fig. 5. shows the cumulative pore volume curves for 5-μm monosized porous PS–DVB particles with 50, 60 and 70% porosity. The curves were drawn by over-

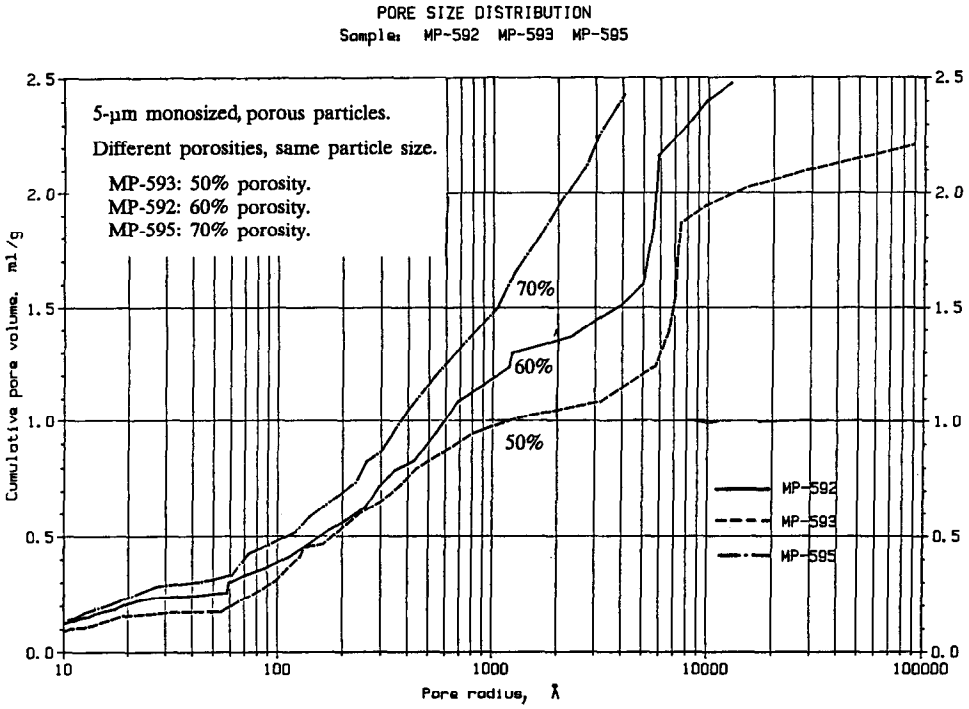

Fig. 5. Cumulative pore volume curves of 5-μm monosized porous particles.

lapping measurements from nitrogen adsorption–desorption and mercury intrusion. These polymer matrices were prepared by increasing the amount of inert diluent in the monomer mixture. As is evident from the curves, this leads to higher total pore volume and more of the larger pores in the particle matrix. When mercury is forced into a sample of porous spherical material, the inter-particle void volume will be measured in addition to the particle pore volume. The steep rise of the curves in the range 5000–7000 Å is specific for 5-μm particles and is due to registration of the void volume between the monosized spheres. For larger particle sizes this rise will move to higher inter-particle size ranges. The particle porosities and surface areas were measured using a Carlo Erba Sorptomatic 1800 and a Carlo Erba Model 1500 porosimeter.

Excellent additional information about the particle morphology, sphericity and particle size distribution may be obtained from scanning electron microscopy (SEM). A scanning electron micrograph of the 5-μm monosized particles with 50% porosity is shown in Fig. 6.

Scanning electron micrographs of two high-porosity 15-μm monosized materials are shown in Fig. 7. The pore-size distributions of these materials are presented in Table I. Material A is a matrix of high surface area and broad pore-size distribution with 70% porosity. When applied in chromatography, this material will combine a high load capacity with easy accessibility to the pores. Material B is a typical wide-pore matrix with 73% porosity.

RIGIDITY OF POROUS MATRICES

Investigation of the rigidity of a porous matrix is easily performed with monosized particles. Especially for chromatographic applications in organic solvents there is a high demand for packing materials that are non-compressible and able to withstand high pressures. Compressibility is closely related to the swelling of the porous matrix. The volume swelling of a monosized porous material may be observed and

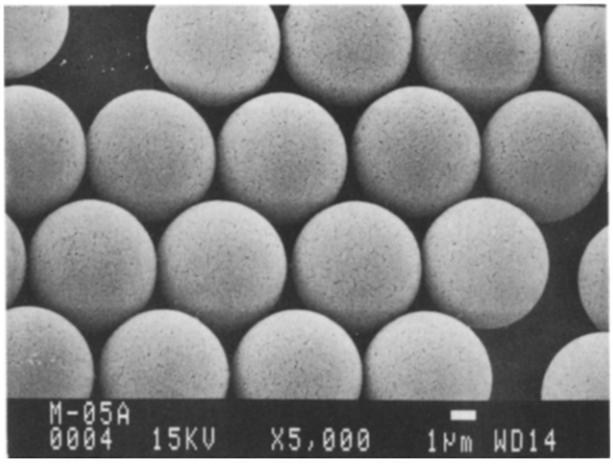

Fig. 6. SEM of 5-μm porous particles of 50% porosity.

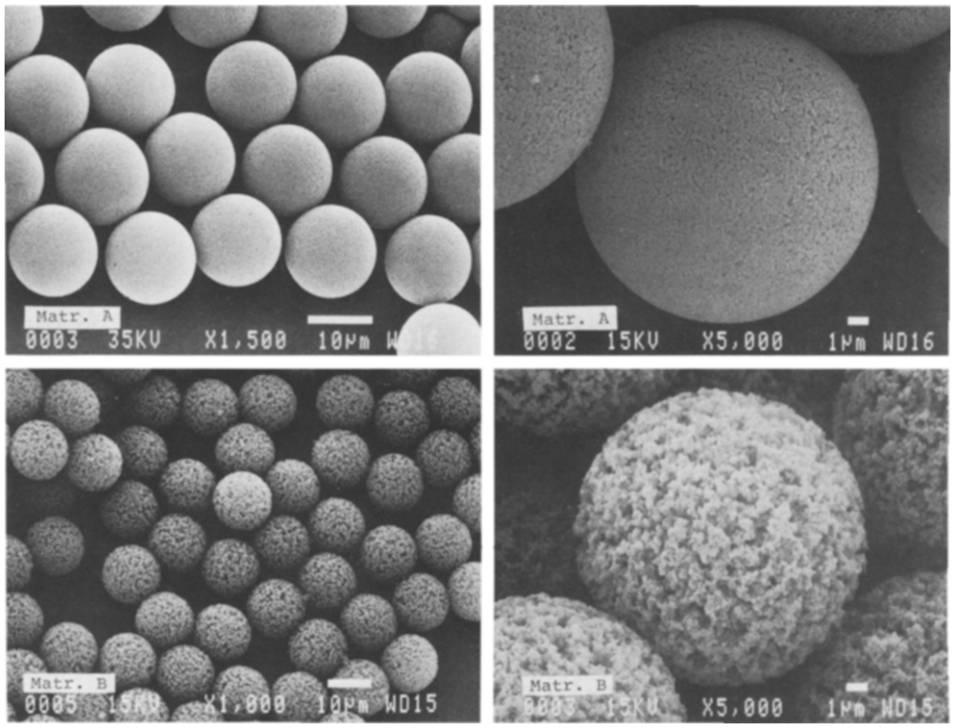

Fig. 7. Morphology studies by SEM of highly porous materials. (A) Monosized porous particles of high surface area and broad pore-size distribution. (B) Monosized porous particles with mainly large pores.

calculated from optical micrographs of dry and swollen particles. Table II shows the results from measurements of swelling of 15-μm highly porous, monosized PS–DVB particles in methanol, tetrahydrofuran (THF) and toluene. It is evident that the degree of cross-linking (% DVB) is a highly important factor. High-percentage DVB monomers have been prepared in our laboratory by purification of a commercial 63% technical-grade DVB [17]. Other parameters that influence the swelling of porous matrices are particle diameter (smaller particles swell more than larger particles), matrix porosity (higher porosity leads to increased swelling) and diluent solvency (reduced solvency gives less swellable matrices) [18].

TABLE I

PORE-SIZE DISTRIBUTIONS OF THE POROUS MATERIALS SHOWN IN FIG. 7

Sample	Surface area (BET) (m^2/g)	Pore volume (ml/g)	Pore-size distribution (radius) (ml/g)					
			< 50 Å	50–100 Å	100–300 Å	300–500 Å	500–2000 Å	2000–5000 Å
A	660	2.22	0.32	0.18	0.36	0.26	0.68	0.42
B	387	2.55	0.18	0.08	0.22	0.19	1.26	0.62

TABLE II

CHARACTERISTICS OF 15-μm, HIGHLY POROUS PS–DVB PARTICLES: EFFECT OF CROSS-LINKING ON TOTAL SURFACE AREA, PORE VOLUME AND SWELLING IN ORGANIC SOLVENTS.

DVB (%)	Surface area (BET) (m^2/g)	Pore volume (ml/g)	Swelling in organic solvents (%, v/v)[a]		
			Methanol	THF	Toluene
40	269	2.35	49	49	49
50	431	2.55	26	26	21
60	463	2.63	14	10	14
80	644	2.80	3	3	3
97	674	3.00	*ca.* 0	*ca.* 0	*ca.* 0

[a] % (v/v) swelling = $(V_{sp} - V_p)/V_p \cdot 100$, where V_{sp} = volume of swollen particle and V_p = volume of dry particle.

SIZE DISTRIBUTION OF PACKING MATERIALS

It is generally known that in chromatography the large particles in a packing material with a broad particle-size distribution will limit the resolution whereas the presence of fines will reduce the column permeability and necessitate high pressure to produce reasonable flow-rates. Particle segregation may occur during packing, and this will produce variations in the packing density. Variable resistance to flow across the column will be the result. The optimum size distribution, considering both back-pressure and resolution, should therefore be monodisperse.

Dawkins *et al.* [19] demonstrated the superiority of a narrow particle-size distribution in size-exclusion chromatography by using mechanically sieved fractions of a material obtained by suspension polymerization. Their results showed that the height equivalent to a theoretical plate (HETP) was proportional to the particle diameter (d_p) according to the relationship HETP $\approx d_p^{1.95}$. It has been deduced theoretically that HETP should be proportional to the square of the particle diameter [20].

Packing of spheres of identical size in a hexagonal close packing gives a packing density of 0.7405, regardless of the size of the spheres. This implies that a void volume as low as 26% is theoretically attainable. It is our experience that in practice a well packed chromatographic column of monosized beads will have a void volume of about 35%.

MONOSIZED STATIONARY PHASES

The advantages of monosized chromatographic supports are a uniform column packing, uniform flow velocity profile, no fines, low back-pressure, high resolution and high speed of separation compared with materials of broad size-distribution. For porous supports, the monodisperse technology ensures equal bead-to-bead morphology.

Optical micrographs of 20-μm monosized macroporous particles and a commercial liquid chromatographic material of size 12–28 μm are shown in Fig. 8. There

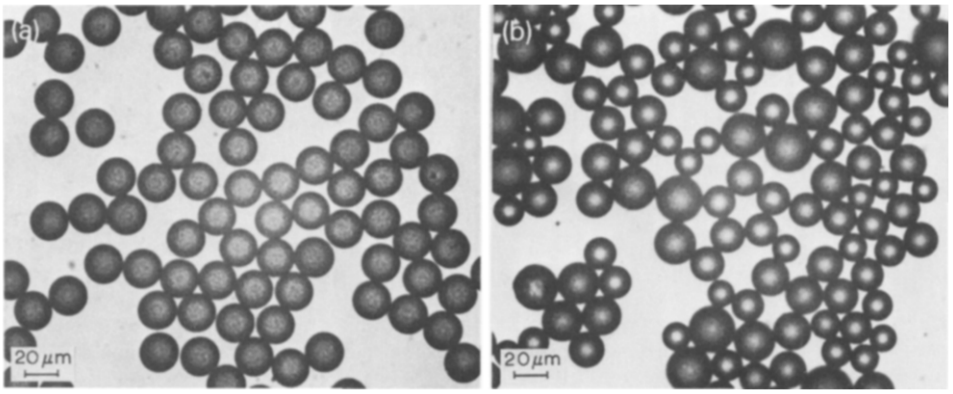

Fig. 8. Optical micrograph of macroporous chromatographic column materials. (a) Monosized particles of 20 μm. (b) Commercial column filling of 12–28 μm.

is a clear difference in size distribution between the monodisperse particles and a traditional column material.

Special types of 10-μm monosized porous particles, prepared by our activated swelling method, were the basis for the widely used Fast Protein Liquid Chromatographic (FPLC) system, introduced by Pharmacia in 1982 [21,22]. The MonoBeads made rapid and high-efficiency chromatography of labile biomolecules possible.

For chromatographic purposes we have prepared macroporous, spherical, monosized particles with a wide range of particle sizes and pore-size distributions. Underivatized porous particles, applied in size-exclusion chromatography (SEC) in organic solvents, have resulted in columns with unusual efficiency and separation capacity. By coating the particles with hydrophilic polymer, monosized chromatographic supports for aqueous phase ion-exchange and size-exclusion chromatography were developed. Examples of applications are given below.

Non-aqueous SEC

Monosized porous PS–DVB particles of three different particle sizes, 20, 10 and 5 μm, were tested by SEC in toluene [23]. Chromatographic calibration was performed with polystyrene standards with narrow molecular-weight distributions in the range 450–$2.7 \cdot 10^6$ dalton.

Results from the SEC calibrations are presented in Table III. The plate counts are averages from several chromatograms with each particle-size material. A considerable increase in plate count with decreasing particle diameter was obtained, giving more than 50 000 plates per foot for the 5-μm monosized particles. In this instance, the HETP was almost as small as the particle diameter (HETP = 0.006 mm). The results in Table III lead to the proportionality HETP $\approx d_p^{2.06}$.

The three batches of 50% porosity particles had nearly identical pore-size distributions. This resulted in calibration graphs which were similar for all three particle sizes and linear in the range 20 000–350 000 dalton. In a forthcoming study, we shall introduce wider pore sizes into the monosized SEC materials in order to extend the linear calibration range to higher molecular weights.

TABLE III

SIZE-EXCLUSION CHROMATOGRAPHY IN TOLUENE: PERFORMANCE OF 20-, 10- AND 5-μm COLUMNS IN THE SEPARATION OF POLYSTYRENE STANDARDS

Column, 300 × 7.8 mm I.D.; flow-rate, 1 ml/min.

Particle diameter (μm)	Plate count (plates/ft.)	Resolution factor, R_{sp}[a]
20.6	3000	2.4
10.5	13 000	2.8
5.3	>50 000	4.4

[a] $R_{sp} = 0.58/\sigma D_2$, where σ = peak standard deviation and D_2 = slope of calibration graph.

Fig. 9 shows the SEC results with application of 10- and 5-μm monosized particles. The pore-size distribution of the 5-μm material is the 50% porosity curve in Fig. 5.

Protein recovery studies

Underivatized PS–DVB particles are extremely hydrophobic and cannot be used in aqueous media. Acrylic polymers, such as copolymers of HEMA and EDMA,

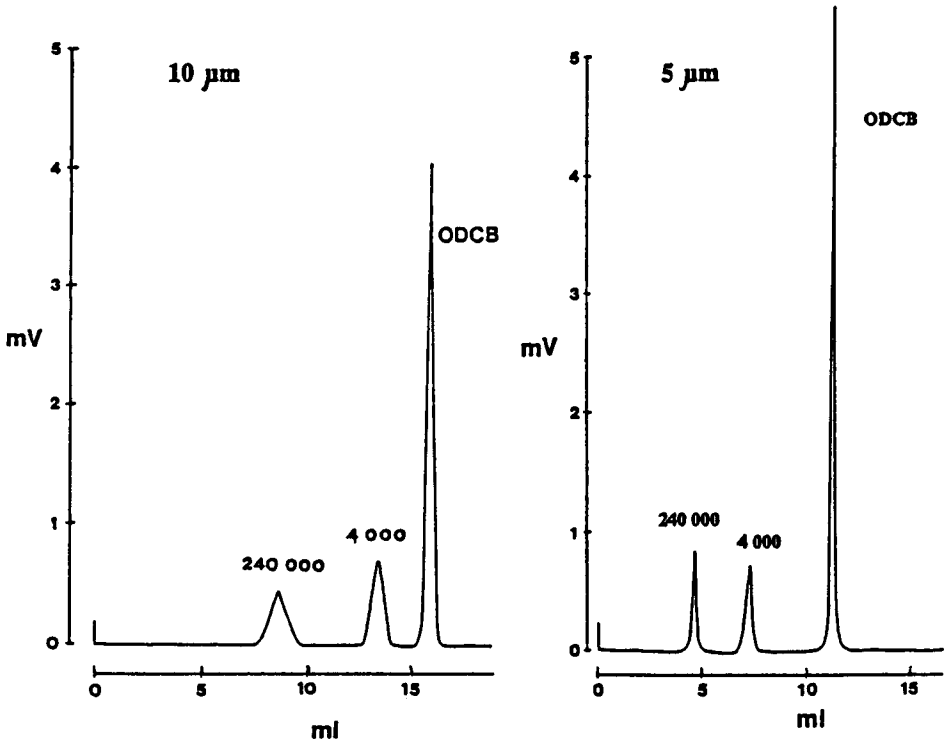

Fig. 9. Separation of polystyrene standards of molecular weight 240 000 and 4000 and *o*-dichlorobenzene by 10- and 5-μm monosized porous PS–DVB column materials.

are considerably more hydrophilic, but exhibit some hydrophobic character that leads to non-specific protein adsorption.

Hydrophilization of PS–DVB and acrylic polymers may be obtained by coating the particle surfaces with a hydrophilic polymer. A surface hydrophilic layer which is covalently bonded to the matrix is preferable to a physically adsorbed layer. A thin, uniform coating which does not permit any protein adsorption would be ideal.

By grafting of a hydrophilic polymer to residual double bonds in the cross-linked polymer matrix, we hydrophilized 20-μm monosized acrylic particles for the aqueous phase SEC of proteins. This acrylic matrix was HEMA–EDMA copolymer, possessing a hydrophobic character and a specific surface area of 141 m^2/g. Underivatized particles exhibited total non-specific adsorption of all proteins. Grafting of a linear hydrophilic polymer to this matrix reduced the surface area to 71 m^2/g, closed the matrix micropores and resulted in a considerable increase in the yield of proteins. In a last step the hydrophilic polymer layer was cross-linked with epichlorohydrin. After this treatment, the protein recovery was high for all proteins, including the smallest protein molecules. These results are presented in Table IV.

An example of the performance of a well hydrophilized, 15-μm monosized, porous PS–DVB matrix is shown in Table V. In this case cross-linking of the hydrophilic layer again led to an increased yield of the smallest molecules, *i.e.*, the peptides.

ION-EXCHANGE CHROMATOGRAPHY

Column materials for ion exchange in aqueous media were prepared by introduction of charged groups to the hydrophilized surface layer of the porous matrices. These new ion-exchange sorbents are based on monosized polymer particles having a uniform, rigid and chemically stable structure. The chromatograms in Fig. 10 illustrate the separation of standard protein mixtures on 15-μm monosized anion and cation exchangers. The charged group of the anion exchanger (AQ) is a quaternary

TABLE IV

PERFORMANCE OF 20-μm ACRYLIC MONOSIZED PARTICLES IN AQUEOUS GPC: RECOVERY OF STANDARD PROTEINS

Column, 5 cm × 5 mm I.D.; mobile phase, 0.1 *M* phosphate buffer (pH 7.1); flow-rate, 0.2 ml/min.

Protein	Mol. wt.	Protein recovery (%)		
		Uncoated particles	Linear polymer coating	Cross-linked coating
Ferritin	440 000	0	100	82
Aldolase	158 000	0	6	74
Bovine serum albumin	67 000	0	68	89
Ovalbumin	43 000	0	63	92
Chymotrypsinogen	25 000	0	100	91
Ribonuclease	13 700	0	100	92
Cytochrome *c*	13 000	0	0	85
Lysozyme	14 600	0	1	90

TABLE V

PERFORMANCE OF MONOSIZED 15-μm HYDROPHILIZED PS–DVB PARTICLES: RECOVERY OF PROTEINS AND PEPTIDES

Column, 5 cm × 5 mm I.D.; mobile phase, 20 m*M* phosphate buffer (pH 7.0); flow-rate, 0.2 ml/min; load, 1 mg/ml.

Protein/peptide	Mol. wt.	Recovery (%)	
		Linear polymer coating	Cross-linked coating
Thyroglobulin	669 000	85	76
Ferritin	440 000	65	59
Immunoglobulin G	160 000	97	100
Bovine serum albumin	67 000	100	100
Ovalbumin	43 000	94	76
Ribonuclease	13 700	89	100
Cytochrome *c*	13 000	100	96
Angiotensin I	1290	0	100
Angiotensin II	1050	58	90

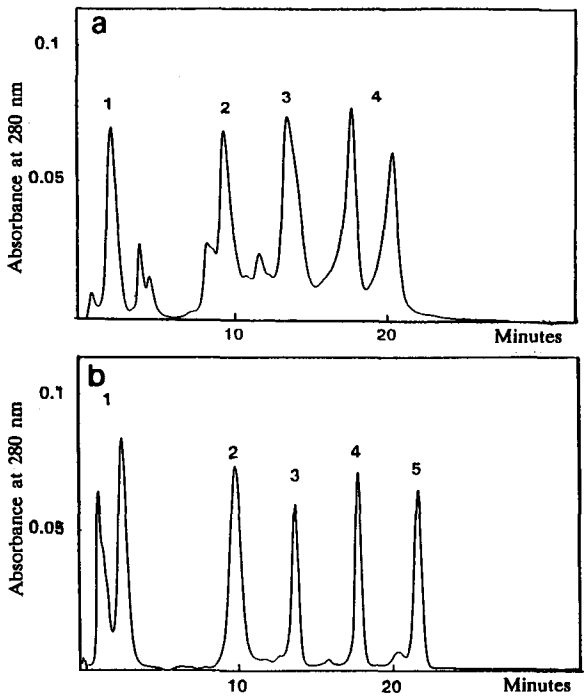

Fig. 10. Separation of protein mixtures by ion-exchange chromatography. Column, 5 cm × 5 mm I.D.; flow-rate, 5 cm/min (1 ml/min); gradient, 20 column volumes (20 ml); sample volume, 100 μl. (a) 15-μm monosized anion-exchange particles. Protein mixture (all from Sigma): 1 = carbonic anhydrase, 0.75 mg/ml (C-7500); 2 = ovalbumin, 3.00 mg/ml (A-7638); 3 = bovine serum albumin, 3.00 mg/ml (A-7638); 4 = β-lactoglobulin (A + B), 3.00 mg/ml (L-4756). Buffer: (A) 20 m*M* Tris–HCl (pH 8.0); (B) A + 0.5 *M* sodium acetate (pH 8.0). (b) 15-μm monosized cation-exchange particles. Protein mixture (all from Sigma): 1 = myoglobin, 0.5 mg/ml (M-1882); 2 = aldolase, 1.0 mg/ml (A-1893); 3 = chymotrypsinogen, 0.2 mg/ml (C-4879); 4 = cytochrome *c*, 0.2 mg/ml (C-7752); 5 = lysozyme, 0.2 mg/ml (L-6870). Buffer: (A) 20 m*M* N-(2-acetamido)iminodiacetic acid (ADA) (pH 6.3); (B) A + 0.5 *M* NaCl.

amine with ligand density of 0.18–0.22 mmol/ml gel. The charged group of the cation exchanger (CS) is sulphopropyl with ligand density of 0.16–0.20 mmol/ml gel.

CONCLUSION

New methods for the preparation of highly monodisperse particles have made available a number of different polymeric supports for chromatography. The monodisperse porous packing materials have proved to be very suitable in various liquid chromatographic applications. High-performance chromatographic materials were developed by careful control of important parameters, such as particle size, particle-size distribution, pore-size distribution and surface chemistry.

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