Journal of Chromatography, 609 (1992) 49–59
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 357

Application of non-porous silica ultramicrospheres to high-performance liquid chromatographic column packings

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(First received January 31st, 1992; revised manuscript received April 29th, 1992)

ABSTRACT

Silica–composite particles were made from silica ultramicrospheres and polyethylene microspheres by a dry impact blending method for application as high-performance liquid chromatographic column packings. A column packed with silica–composite particles has a double packing structure, which consists of the spatial configuration of silica–composite particles in the column and a dense arrangement of silica on the polyethylene microsphere surfaces. This structure enables silica ultramicrospheres to be utilized as the stationary phase while simultaneously maintaining the flow of the mobile phase. Proteins with MW from 6500 to 669 000 were separated satisfactorily on a column packed with *n*-octadecyldimethylchlorosilane-coated silica–composite particles.

INTRODUCTION

In the separation of polypeptides and proteins by reversed-phase high-performance liquid chromatography (HPLC), many studies have been reported on the relationship between column configurations and column performance [1–8]. The column configuration includes the length and bore, the average particle diameter, the pore diameter of the packings, the accessible surface area and the type of ligand and the column performance includes resolution, retention time, peak capacity and theoretical plate number. In these reports, however, there were considerable differences between experimental results and the theories on the basis of analyses of small molecules. These differences are believed to be due to the following effects. First, the diffusion coefficient of proteins with large molecules is decreased as the protein diameters approach the pore diameter in the column packing [4,9,10]. Second, solutes are bound to silanol groups on column packings [3,4, 11]. Third, equilibrium rates between different forms (native *vs.* denatured) are slow [4,12,13]. These effects contribute significantly to the increase in band width and the spread of band shape, and lead to still lower values of *N*. Therefore, recent studies of retention behaviour have involved mainly small peptides [14–18]. In protein analysis, wide-pore silica packings were applied in order to promote the free diffusion of solutes in the internal and external region of the pores [3,7]. Wide-pore silica packings, however, must have increased particle diameters, when the pore diameters required are large [19], and

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0021-9673/92/$05.00

an increase in particle diameter leads to a decrease in N. Hence the increase in pore diameter is a limiting factor. In addition, wide-pore silica packings have a lower packing stability [20].

In view of the above, the application of non-porous, very small particles has been proposed as an alternative to totally porous silica particles [21–27]. Non-porous particles were designed for the employment of the external surfaces of the particles instead of the internal surface of pores. Short columns packed with non-porous particles should permit the rapid separation of large molecules such as proteins, and the recovery of proteins is increased [27]. However, the disadvantages of non-porous silica packings are that the column length is restricted because high pressure is required for an adequate mobile phase flow and it is difficult to pack a column densely and homogeneously [28]. Guiochon and Martin [29] indicated that it would be almost impossible to use a high-efficiency column packed with less than 1-μm diameter particles. This suggests that the decrease in the particle diameter reaches a limit if particles are to be packed into columns directly.

On the other hand, Hersey [30] reported that fine particles could be made to adhere to the surface of coarse particles by a binary powder mixture. Koishi and co-workers [31–33] developed a binary mixture as composite particles by using a dry impact blending method. This procedure has been employed for the improvement of powder properties and in mixing homogeneously a small amount of material such as drugs and pigments with a large amount of excipient, etc. [34,35]. It was confirmed experimentally that small particles were fixed on large particles when the ratio of the diameter of the large particle to that of the small particle was larger than 10:1. Fig. 1 shows a schematic diagram of the processes. The small particles fixed on large particles were stable in spite of being dispersed and stirred in liquid phases.

This paper described the application of these composite particles to HPLC column packings utilizing the orderly and the close-packed arrangement of small particles. As small particles, we employed non-porous silica ultramicrospheres, which have rarely been utilized for column packings. We investigated whether the orderly arrangement formed by silica ultramicrospheres can be utilized effectively as a stationary phase and whether the orderly arrangement is suitable especially for the separation of proteins, taking advantage of the non-porous and ultramicrosphere aspects. First, these silica-composite particles were examined with respect to some basic properties as column packings, and then low- and high-molecular-weight proteins were studied to examine the ineffective separation in columns packed with composite particles.

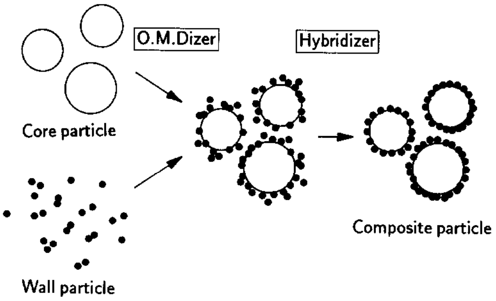

Fig. 1. Schematic diagram of wall particles adhering to core particles in a dry blending and a dry impact blending.

EXPERIMENTAL

Chemicals and reagents

The composite particles were low-density spherical polyethylene (PE) beads (average diameters 5 and 10 μm, density 0.92 g/cm^3) and high-density PE (average diameter 20 μm, density 0.958 g/cm^3) as core particles, supplied by Sumitomo Seika (Osaka, Japan). Monodisperse silica ultramicrospheres (average diameter 0.3, 0.6 and 0.9 μm, density 2.01 g/cm^3, specific surface area 6.0 m^2/g) as wall particles were supplied by Mitsubishi Kasei (Tokyo, Japan). n-Hexane, isopropanol, acetonitrile, trifluoroacetic acid (TFA), toluene and nitrobenzene of analytical-reagent grade were purchased from Wako (Osaka, Japan). All proteins were purchased from Sigma (St. Louis, MO, USA). These proteins were aprotinin from bovine lung, cytochrome *c* from horse heart, bovine serum albumin, fibrinogen from bovine plasma, apoferritin from horse spleen and bovine thyroglobulin.

Shimpack CLC-SIL (150 mm × 4.6 mm I.D., average particle diameter 5 μm, pore diameter 10 nm, specific surface area 300 m^2/g) (Shimadzu, Kyoto, Japan) and Vydac 214TP54 (250 mm × 4.6 mm I.D., average particle diameter 5 μm, pore

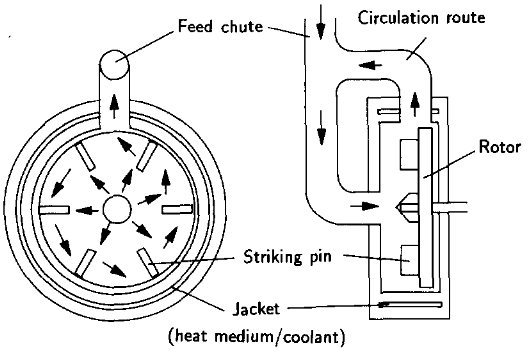

Fig. 2. Schematic diagram of hybridizer. Arrows indicate the direction of movement of powder particles.

diameter 30 nm, specific surface area 90 m^2/g) (Separations Group, Hesperia, CA, USA) were used as totally porous silica packed columns.

Dry impact blending method

For the preparation of silica–composite particles, an O. M. Dizer and a Hybridizer (type NHS-0; Nara Machinery, Tokyo, Japan) were used. Schematic diagrams of the machine for producing mechanical impact are shown in Fig. 2. The machine is surrounded by a jacket through which heat medium or coolant is circulated.

PE and silica powders were blended (1400 rpm, 10 min) by the O.M. Dizer at room temperature. The total amount was fixed at 15 g. The mixing ratios are shown in Table I. These mixing ratios were calculated on the assumption that silica ultramicrospheres were closest arranged on these PE particle surfaces [36]. After preparing the interactive mixture, the mixtures were treated by the dry impact blending method using the Hybridizer, with a rotational speed of 16 000 rpm and treatment time of 10 min at room temperature.

This hybridization process can be summarized as follows: the powder particles placed in the Hybridizer are mixed in a high-speed air stream caused by the rotation of the rotor at 16 000 rpm, and are hit repeatedly by the striking pins on the rotor. As a result of these mechanical actions, the smaller particles become fixed to the surface of the larger particles. Further details of this method were described in previous papers [31,32].

Observation of the particle surface by scanning electron microscopy (SEM)

Each of the silica–composite particles was coated with gold by an ion sputtering apparatus (JFC-1100; JEOL, Tokyo, Japan) and observed by SEM (JSM-T220; JEOL).

Measurement of spcific surface area

The specific surface areas of the silica-composite particles were determined by the BET method (Autosorb 6; Quantachrome, Syosset, NW, USA).

Chromatographic procedures

All chromatographic tests were performed on an LC-6A gradient system with a Rheodyne 7125 injection valve, connected to an SPD-6A UV spectrophotometric detector and to a Chromatopac C-R6A (Shimadzu). The silica–composite particles were packed into a stainless-steel column (250 mm × 4 mm I.D.) by tapping and equilibrated with *n*-hexane containing 0.5% of isopropanol at a flow-rate of 0.2 ml/min. Sample solution (1 μl)

TABLE I

COMPOSITION OF SILICA–COMPOSITE PARTICLES

Composite particle	Polyethylene particle size (μm)	Silica	
		Particle size (μm)	Mixing ratio (wt. %)
03/5	5	0.3	35
0.3/10	10	0.3	20
0.3/20	20	0.3	11
0.6/10	10	0.6	35
0.9/10	10	0.9	46

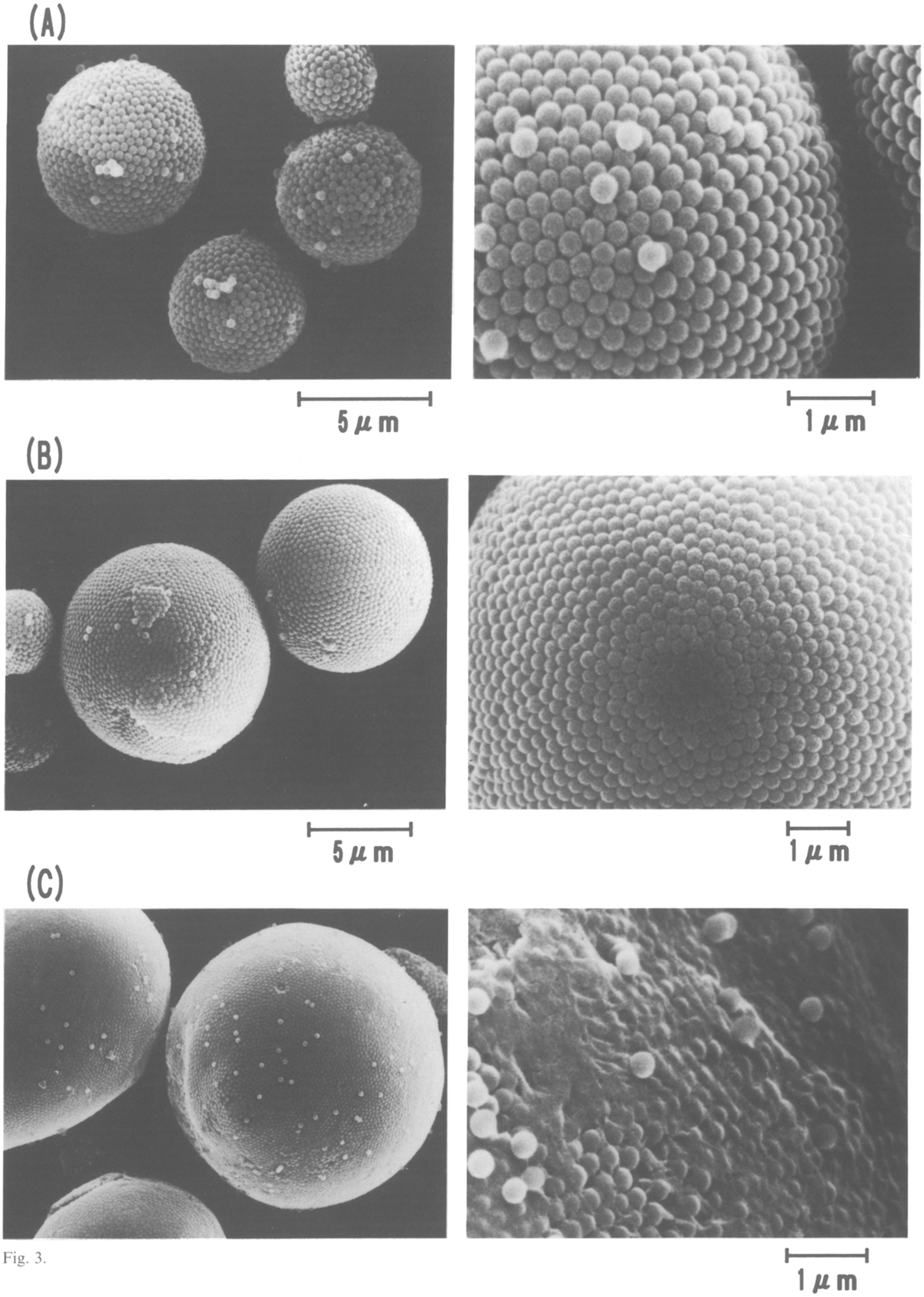

Fig. 3.

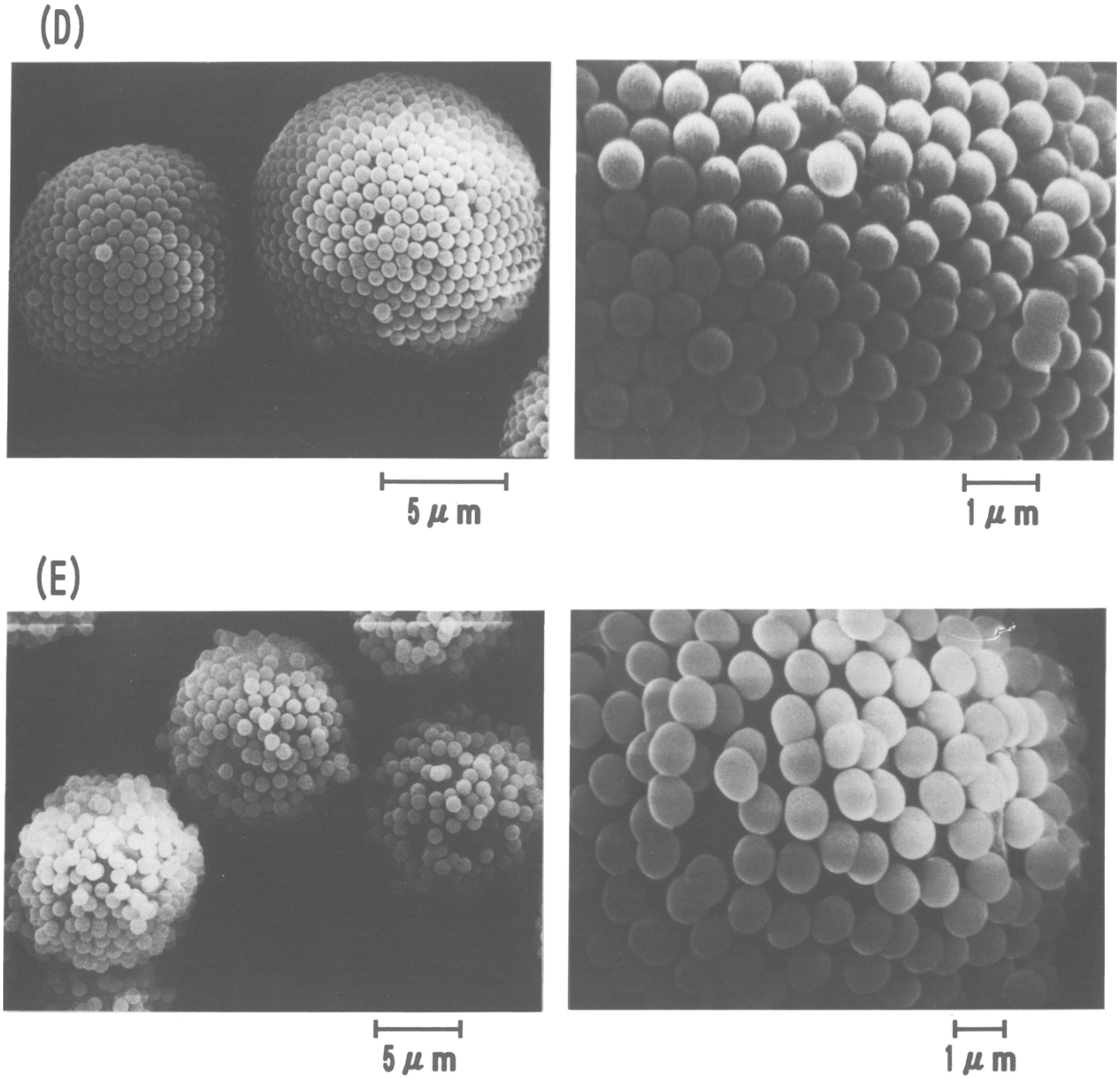

Fig. 3. Typical SEM photographs of silica–composite particles: (A) 0.3/5; (B) 0.3/10; (C) 0.3/20; (D) 0.6/10; (E) 0.9/10 combinations.

containing toluene (2000 ppm) and nitrobenzene (16 ppm) was applied to the column, and elution was followed using the UV detector set at 220 nm. The experiments using CLC-SIL were performed in a similar manner.

Separation of proteins

Silica 0.6 μm in diameter was converted into its *n*-octadecyl-bonded derivative with *n*-octadecyldimethylchlorosilane according to the procedure of Kinkel and Unger [37] and then end-capping was carried out with trimethylchlorosilane (Shinetsu Chemical Industry, Tokyo, Japan) once. The ODS-coated silica ultramicrospheres and 10- μm PE were prepared as composite particles as described under *Dry impact blending method.* The 0.6-ODS/10 composite particles, which consist of ODS-coated 0.6-μm silica ultramicrospheres and 10-μm PE, were slurry-packed into a stainless-steel column (50 mm × 4 mm I.D.) with 50% acetonitrile at a pressure of 50 kg/cm^2.

Standard proteins were separated with a linear gradient of acetonitrile in 0.1% TFA and detected 220 nm. The Vydac 214TP54 column (250 mm × 4.6 mm I.D.) was employed for the separation of the proteins in a similar manner. The recovery of proteins from both columns was determined by rationing the peak areas found for the injected proteins with and without the column. For controls, the column was replaced with an empty stainless-steel tube (0.8 mm I.D.) which was cut to a suitable length corresponding to the void volume of the columns.

RESULTS AND DISCUSSION

Observations of silica–composite particles

SEM photographs of silica–composite particles are shown in Fig. 3. On each particle except the combination of 0.9-μm silica and 10-μm PE (0.9/10), it was observed that the silica ultramicrospheres were arranged and fixed densely on the surface of the PE particles. In the dry impact blending method, it was empirically confirmed that this fixing and arrangement of small particles were achieved when the size of the large particles was more than ten times that of the small particles. With the 0.9/10 combination, it is considered that an orderly arrangement would not be achieved because the size ratio is near to the critical value.

Further examination of the surface of the silica–composite particles indicated that an orderly arrangement of silica particles approximated to the closest packing structure of spheres on a plane. In the 0.3/20 combination, which consists of high-density PE particles, the silica particles are deeply embedded in the surface of the PE particles and a large part of the silica surface is lost, although in the 0.3/5, 0.3/10 and 0.6/10 combinations, consisting of low-density PE particles, the silica particles are superficially fixed.

Such differences are attributed to the mechanisms of the fixing of the small particles by the dry impact blending method. The mechanisms assume that the local area of large-particle surfaces are deformed and melted by pressure and friction forces because of collisions between the large and small particles or between particles and the striking pins. Consequently, small particles are embedded and fixed on the surface of large particles.

The embedded depth was evaluated by measuring hole diameters on large particles. The holes arose as a result of small particles being deliberately torn off. The ratio of hole depth to the small particle diameter in the 0.3/5, 0.3/10, 0.6/10 and 0.9/10 combinations were 0.23, 0.10, 0.18 and 0.21, respectively. However, with the 0.3/20 combination, silica particles would not be torn off because they are too deeply embedded. As a result, it is suggested that the depth ratio is influenced by the ratio of particle diameters rather than the PE and silica particle diameters.

The physical properties and diameters of the core particles affect the depth ratio, as compared with the 0.3/10 to 0.3/20 combinations. The difference in embedding mechanisms between 5- and 10-μm PE and 20-μm PE is assumed to be follows. With 5- and 10-μm PE particles, the deformation and the melting in the local area of the core particle surface (PE) occur proportionally, and silica particles are fixed at an adequate depth. In contrast, with 20-μm PE particles of high density, the energy generated by collision with silica particles and the striking pins is large because of the large mass, and the PE particle is relatively hard. These effects result in most of the energy being used to melt the local surface area rather than for deformation and the wall particle is thus embedded more deeply. As 20-μm PE particles of low density were not available, we could not compare the embedded depth ratio directly. However, as 0.6- and 0.9-μm silica particles were used to make composite particles with 20-μm PE of high density, the composite particle surfaces showed evidence of melting and large depth ratios were obtained. From these results, the large embedded depth ratio such as that obtained with the 0.3/20 combination is attributed not to the size ratio of PE particles to silica particles, but to the PE particles themselves.

In summary, to achieve an orderly and dense arrangement with well proportioned embedding it is necessary to deform and melt in the local area of the PE particle surfaces proportionally. Accordingly, it is confirmed that the sizes of the large and the small particles, the size ratio of the large to the small particle and the physical proporties of materials affect the configuration of silica–composite particles.

TABLE II

THEORETICAL SPECIFIC SURFACE AREA (S) OF SILICA ON COMPOSITE PARTICLES AND THE TOTALLY POROUS SILICA PARTICLE

Composite particle	S	
	m^2/g	m^2/ml
0.3/5	2.58	1.20
0.3/10	1.79	0.792
0.3/20	0.510[a]	0.256
0.6/10	1.38	0.610
0.9/10	1.16	0.547
CLC-SIL[b]	300	150

[a] The embedded depth ratio was assumed to be 0.5 (not measured).

[b] Totally porous silica packing of diameter 5 μm.

Specific surface area

The theoretical values of the specific surface area of silica in the silica–composite particles were calculated as described below.

When silica ultramicrospheres with a radius r are arranged and fixed on a PE particle surface with a radius R in closest order, the number of silica ultramicrospheres (A) on the PE particle is given by

$$A \approx \frac{4\pi[R + r(1 - 2k)]^2}{\pi r^2} \cdot \frac{\pi}{\sqrt{12}} \tag{1}$$

where k is the embedded depth ratio and $\pi/\sqrt{12}$ is the coating ratio of the PE surface area due to the silica monoparticle layer. Therefore, the silica specific surface area (S) of composite particles of unit weight is given by

$$S = \frac{4\pi r^2(1 - k)A}{\frac{4}{3}\pi R^3 \rho_p + \frac{4}{3}\pi r^3 \rho_s A} = \frac{3r^2(1 - k)A}{R^3 \rho_p + r^3 \rho_s A} \tag{2}$$

where ρ_p and ρ_s are density of PE and silica, respectively. Calculated S values are given in Table II.

Using the BET method, the S values were 0.31–2.32 m^2/g for all silica–composite particles. These experimental results support the calculated values in Table II. The order of these experimental data, however, were near to the experimental error range of the BET method, because silica–composite particles have lower S values than totally porous silica. Therefore, the calculated values were used in subsequent considerations.

Resolution and theoretical plate numbers

Silica–composite particles were packed into columns by the tapping method. The tapping densities of the 0.3/5, 0.3/10, 0.3/20, 0.6/10 and 0.9/10 silica–composite particles were 0.48, 0.47, 0.51, 0.46 and 0.48 g/cm^3, respectively. The packing porosities were 63.1, 58.6, 52.9, 64.5 and 66.5%, respectively. These large porosities suggest the presence of a void volume caused by physical particle aggregates in the columns, because the theoretical porosities of the closet and the loosest packing structure models of spheres are 25.95 and 47.64%, respectively. However, columns packed with the 0.3/10, 0.3/20 and 0.6/10 combinations had constant column pressures and did not show a decrease in the bed volume at a flow-rate of 1.0 ml/min. These results indicate that the packing structures formed are considered to be in stable states. In constrast, columns packed with the 0.3/5 and 0.9/10 combinations showed a sudden increase in column pressure and a decrease in bed volume at a flow-rate 0.5 ml/min. Therefore, the packing structures were considered to be in an unstable state. Further, although the slurry packing method was applied to pack the 0.3/5 and 0.9/10 combinations, the columns could not be employed in the analysis because of high pressure. The cause of such high pressure is attributed to blocking of the path of the mobile phase. With the of 0.3/5 combination, small particles, which were included in PE particles because the PE particles were not well classified precisely, may clog the path. The 0.9/10 combination has an irregular form, as shown in Fig. 3E, hence the particles may form aggregates in the column.

Consequently, the tapping method was employed for packing particles and the flow-rate was fixed at 0.2 ml/min in order to apply analysis under the same conditions for all silica–composite particles.

Silica–composite particles were packed into columns and their properties were investigated in adsorption chromatography. The resolution and theoretical plate number (N) calculated from the chromatograms of toluene and nitrobenzene are shown in Table III.

In comparison with the 0.3/5, 0.3/10 and 0.3/20, combinations, the resolution and N increase with a

TABLE III

THEORETICAL PLATE NUMBER AND RESOLUTION OF A COLUMN PACKED WITH SILICA–COMPOSITE PARTICLES

Composite particle	Theoretical plates per meter (*N*)[a]	Resolution
0.3/5	4080	1.26
0.3/10	2428	1.09
0.3/20	1356	–
0.6/10	6640	1.69
0.9/10	2716	0.884
CLC-SIL[b]	38600	6.25

[a] Calculated from peak of nitrobenzene.

[b] Totally porous silica packing of diameter 5 μm.

decrease in PE particle diameter. The specific surface area of silica particles increases with a decrease in the diameter of PE particles (see Table II). Therefore, the decrease in the PE particle diameter provides high resolution and *N* because of the large specific surface area.

The separation of two peaks was scarcely achieved with the 0.3/20 combination, owing to the loss of the silica surface area, as apparently shown in Fig. 3C.

When 10-μm PE was used as core particles, the 0.6/10 combination provided the highest resolution and *N*. Although there is not much differences in the *S* values between the 0.3/10, 0.6/10 and 0.9/10 combination, *N* for 0.6/10 is more than double that for 0.3/10 and 0.9/10. These results indicate that the performance represented by resolution and *N* is not determined only by *S* and the diameters of the silica–composite particles that form the packing structures in the columns. The performance is also affected by the size of the silica ultramicrospheres. With the 0.3/10 and 0.6/10 combinations, on the assumption that the spatial configuration of 0.3/10 in the column is nearly equal to that of 0.6/10, it is suggested that diffusion of solutes and mobile phases at the surface and the interstices of silica ultramicrospheres influences the column performance. With the 0.0/10 combination, it is explained by the disorder arrangement of silica particles on the PE particles in Fig. 3E as follows: the actual amount of silica particles fixed on PE particles is smaller than the calculated value, hence the value of the silica surface area is smaller than the theoretical value. Also, the randomness of the arrangement may cause an irregular flow of the mobile phase. Consequently, this leads the broadening of the peak and a decrease in resolution.

The resolution and *N* of CLC-SIL, which is a commercial column packed with totally porous silica of 5 μm diameter, are shown in Table III. The *N* values of CLC-SIL are 6–28 times higher than those of columns packed with silica–composite particles, so that a high performance of ultramicrospheres was not displayed with silica–composite particles. From the results, the specific surface area per unit column length is much less than that of totally porous silica (see Table II). Second, diffusion of mobile phases and solutes occurs at the large interstices of silica–composite particles, and this effect is further enhanced because of the loose packing structure of the silica–composite particles which is attributable to the slightly broader size distribution.

Consequently, silica–composite particles are not regarded as having the packing structures of ultramicrosphere silica, but regarded as composites which have much better configurations.

In conclusions, silica–composite particles, which

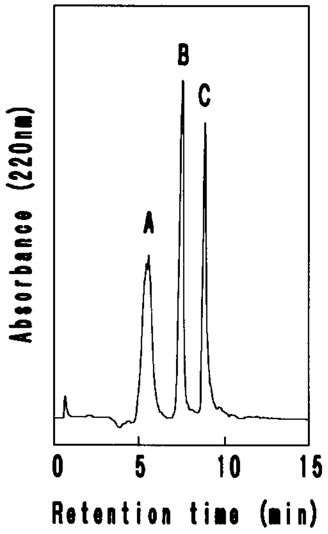

Fig. 4. Gradient elution of proteins on the column packed with silica–composite particles (0.6-ODS/10). Conditions: column, 50 mm × 4 mm I.D.; gradient, 5 to 70% acetonitrile in 0.1% TFA in 15 min; flow-rate, 0.5 ml/min; detection, UV at 220 nm.Peaks: A = aprotinin; B = cytochrome *c*; C = BSA.

TABLE IV

RECOVERY OF PROTEINS FROM COLUMNS PACKED WITH COMPOSITE PARTICLES

Protein	Recovery (%)	Protein	Recovery (%)
Aprotinin	100.1	Fibrinogen	96.7
Cytochrome *c*	99.4	Apoferritin	92.8
BSA	102.3	Thyroglobulin	70.1

consist of 0.6-μm silica ultramicrosphere arranged on the surface of 10-μm PE, showed the highest *N*, and the silica ultramicrospheres were arranged orderly and densely on PE. Therefore, it is confirmed that the 0.6/10 combination is the most appropriate for HPLC column packings.

Separations of proteins

As the 0.6/10 combination was ascertained to be the best composite particle for packings, ODS-treated silica–composite particles (0.6-ODS/10) were prepared using 0.6-μm silica ultramicrospheres with previously introduced ODS groups and 10-μm PE. 0.6-ODS/10 was packed into a column, and aprotinin, cytochrome *c* and BSA were separated (see Fig. 4). Three separated peaks were obtained within 10 min with an elution volume of 5 ml, and these three proteins demonstrated excellent recoveries, as shown in Table IV. From these results, it is clear that separations in a short time and with high recovery, which are features of non-porous silica packings, can be achieved by using silica–composite particles consisting of silica ultramicrospheres.

In another experiment, the separation of three high-molecular-weight proteins, fibrinogen (340 000) apoferritin (443 000) and thyroglobin (669 000) was attempted. As shown in Fig. 5, these proteins could be separated as sharp and symmetrical peaks within 20 min and the recoveries were also satisfactory (see Table IV).

The Vydac 214TP54 column was also employed to separate these three high-molecular-weight proteins (see Fig. 6). The column was packed with wide-pore silica of average pore diameter 30 nm. However, apoferritin and thyroglobulin could not be separated. This result indicates that specific hindrance factors, which limit the permeability of the solute in the pores [2], were unavoidable with such high-

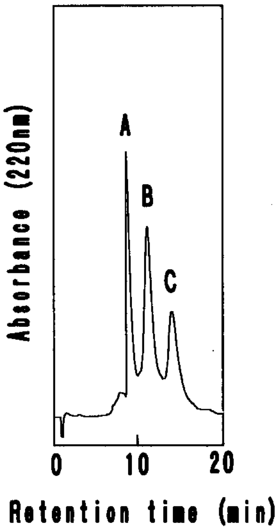

Fig. 5. Gradient elution of high-molecular-weight proteins on the column packed with silica–composite particles (0.6-ODS/10). Conditions: Column, 50 mm × 4 mm I.D.; gradient, 30 to 70% acetonitrile in 0.1% TFA in 15 min; flow-rate, 0.3 ml/min; detection, UV at 220 nm. Peaks: A = fibrinogen; B = apoferritin; C = thyroglobulin.

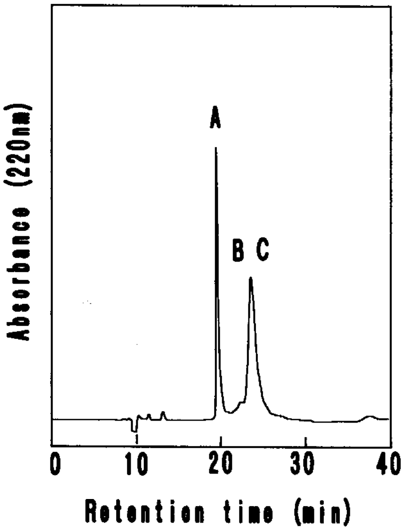

Fig. 6. Gradient elution of high-molcular-weight proteins on Vydac 214TP54. Conditions: column, 250 mm × 4.6 mm I.D.; gradient, 30 to 70% acetonitrile in 0.1% TFA in 20 min; flow-rate, 0.3 ml/min; detection, UV at 220 nm. Peaks: A = fibrinogen; B = apoferritin; C = thyroglobulin.

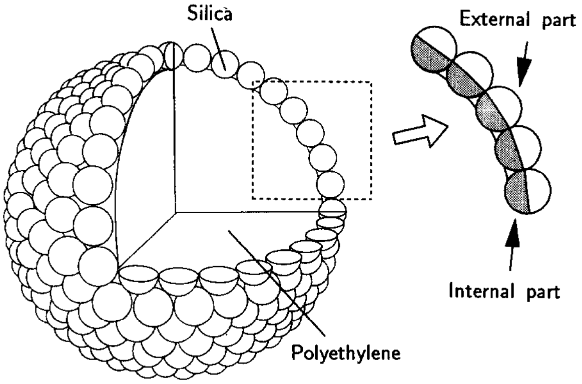

Fig. 7. Schematic diagram of a silica–composite particle and the internal and external parts of the silica surface.

molecular-weight proteins using a porous silica packing.

When porous packings are used for the separation of solutes, it is reported that the pore diameter must be ten times greater than the solute diameter for complete access of all solute molecules to the internal surface area of the pores [2]. We applied this theory for porous packings to silica–composite particles which have a fine structure on the surface.

The diameters of the three high-molecular-weight proteins that were separated in this study are estimated to be 10–15 nm [29]. Fig. 7 shows the silica surface of a composite particle where the stationary phase is divided roughly into two parts. One is the external part, which means the outer surface of silica on composite particles. The other is the internal part of composite particles, which means the surface of silica facing the PE particles. With the 0.6/10 combination, the cusp-shaped pore formed between close-packed silica ultramicrospheres approximates a triangle of 300 nm side, and the area of the pores is equivalent to a circle of diameter 136 nm. Approximately 2000 cusp-shaped pores are formed on each composite particle surface. The structure consisting of the interstices can also be regarded as an accurate pellicular porous structure which is formed on the 10-μm PE particles. This assumption suggests that the column packed with the 0.6/10 combination could be employed for the separation of solutes with molecular diameters up to 30 nm without specific hindrance factors.

These three kinds of proteins are able to access the external and the internal parts of the stationary phase, and similarly low-molecular-weight solutes are able to access both parts.

The arrangement of silica ultramicrospheres on the surface of 10-μm PE particles is valuable for improving the stationary phase utilization. It was found that 0.6-ODS/10 was able to separate proteins of MW up to 669 000.

Use of composite particles for HPLC packings

There are three aspects of using composite particles for HPLC packings. The first aspect is the arrangement of ultramicrospheres which are fixed orderly and densely, the second is a pellicular particle which has uniform pores and the third is a complex particle which consists of some kinds of materials.

In this study, a dense packing structure consisting of ultramicrospheres was utilized to form the rapid and homogeneous interaction system between the solute and the stationary phase and the mobile phase. It was found that the diameter of silica ultramicrosphere itself affects the N values. The structure of the composite particles led to the successful separation of proteins with a wide molecular weight range. For example, with the 0.6/10 combination, although the apparent particle diameter is 10 μm, the surface area of the composite particle is equal to that of a 3-μm non-porous silica microsphere per unit volume.

If silica–composite particles are regarded as pellicular particles, it should be noted that these particles were not prepared by the conventional method in which the pore diameters are regulated by controlling the conditions of synthesis, but by a novel method in which the pores are formed by arranging silica ultramicrospheres. It is possible to make any form of pores if the combination of core materials and wall materials are suitable for preparing composite particles. A homogeneous surface can be obtained easily by the employment of previously coated particles.

Finally, if silica–composite particles are regarded as complexes consisting of several particles, it suggests the possibility of preparing particles which have different properties. In this study, non-porous silica ultramicrospheres were utilized for HPLC column packings by being arranged on the surface of large particles. Some materials which have never been considered as column packings, *e.g.*, a needle-like, a plate-like or a fibre-like substance, may be proposed as column packings in a similar manner as described above. Also, several kinds of particles may be blended to prepare multi-functional particles.

It is suggested that silica–composite particles which possess unique properties due to the structure not only can be utilized for the separation of high-molecular-weight proteins but also represent a new concept for preparing column packings.

CONCLUSIONS

The double packing structure, which consists of the spatial configuration of silica–composite particles in the column and the dense arrangement of silica ultramicrospheres on the PE particle surface, enable silica ultramicrospheres to be utilized as stationary phases in HPLC while simultaneously maintaining the flow of the mobile phase. The diameters of the silica ultramicrospheres affected N; of the six kinds of composite particles examined the 0.6/10 combination gave the highest N. Silica–composite particles are non-porous and have a large surface area, which are the conditions required for column packings for protein analysis, and achieved the separations of proteins with a wide range of molecular weights.

ACKNOWLEDGEMENT

The authors are grateful to Mr. Eiji Hattori, Mitsubishi Kasei, for measuring specific surface areas by the BET method.

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