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(54) POLYSACCHARIDE BEADS

(71) We, TAKEDA YAKUHIN KOGYO KABUSHIKI KAISHA also known as TAKEDA CHEMICAL INDUSTRIES LTD. a joint stock company organised under the laws of Japan of 27 Doshomachi 2-chome, Higashi-ku, Osaka, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:

This invention relates to a matrix comprising a water-insoluble β-1,3-glucan gel in the shape of beads. The term "matrix" as used in the present specification and claims means any carrier for *inter alia* immobilized enzymes, affinity chromatography, gel filtration or ion exchange, or like applications, unless otherwise specified.

As the matrix materials hitherto employed, there may be mentioned polysaccharides. Among them are cellulose, dextran, starch and agarose as well as their derivatives. In preference, matrix materials for use in packed columns for such immobilized enzyme and affinity chromatography applications should *inter alia* be (1) insoluble in water, (2) chemically and microbiologically stable, (3) mechanically rigid, (4) hydrophilic, (5) possessed of adequate chemical functionality, (6) ready to couple with enzymes and ligands, (7) spherical in shape to let liquids pass freely over them, (8) not non-specifically absorptive and (9) available at low cost.

Although none of the materials heretofore known satisfies all the above requirements, agarose gel alone is relatively satisfactory. This material, however, is disadvantageous in that it is (1) unstable against acids, (2) intolerent of high temperatures and (3) unsuitable for the preparation of large-capacity columns of great packing heights because of its high compressibility.

We have sought to overcome these disadvantages and have found that water-insoluble β-1,3-gucans are comparatively well equipped with the above properties. Furthermore, such a β-1,3-glucan, in the shape of gel beads and when used as a matrix for immobilized enzymes and affinity chromatography, displays excellent flow rate properties as will be described in the Reference Example below, and also has properties which are very much desirable in carriers for gel filtration, ion exchange and other applications. The present invention is based on these above findings.

Thus, this invention provides a matrix comprising a water-insoluble β-1,3-glucan gel in the shape of beads which is useful for immobilized enzymes, affinity chromatography, gel filtration, ion exchange and other applications.

The present invention also provides a method of producing a water-insoluble β-1,3-glucan gel in the shape of beads with diameters within the range of from 5 to 1000 μ inclusive.

Other features will become clear hereinafter as the disclosure proceeds.

Among the water-soluble β-1,3-glucans employable according to this invention are the polysaccharides elaborated by microorganisms of the genus *Alicaligenes* and of the genus *Agrobacterium*, such as the polysaccharide elaborated by *Alcaligenes faecalis var. myxogenes* 10C3K [Agricultural Biological Chemistry, Vol.30, pages 196 et seq. (1966) by Harada *et al.*], the polysaccharide elaborated by the mutant strain, NTK-u (IFO 13140, ATCC 21680) of *Alicaligenes faecalis var. myxogenes* 10C3K (U.S. Patent Nos. 3,754,925 and 3,822,250) (hereinafter referred to as PS-1), the polysaccharide elaborated by *Agrobacterium radiobacter* (IFO 13127, ATCC 6466) and its mutant strain U-19 (IFO 13126, ATCC 21679) (U.S. Patent Nos. 3,754,925 and 3,822,250) (hereinafter referred to as

PS-2), pachyman which occurs in the crude drug known as *Poria cocos* (Agr. Biol. Chem. Vol.32, No.10, P.1261(1968)), laminaran which is an ingredient of brown algae, the glucan which is a cell wall constituent of yeasts, and so on.

As a preferred method for producing beads of such a glucan gel, there may be exemplified a method which comprises extruding, dripping or spraying a fluid containing a water-insoluble β-1,3-glucan into a heated oil bath and, thereby, causing the glucan to undergo gelation (Japanese Patent Application As Laid Open No. 52953/1973); the method which comprises dissolving a water-insoluble β-1,3-glucan in an aqueous solution of sodium hydroxide and feeding the resulting solution through a drip nozzle into an aqueous solution of hydrogen chloride to neutralize and cause the glucan to undergo gelation (U.S. Patent No. 3,899,480), and the method which comprises adding an alkaline aqueous solution of said water-insoluble β-1,3-glucan dropwise to an organic solvent which is not freely miscible with water, and neutralizing the resulting dispersion with an organic acid.

In the last method, as examples of said alkaline aqueous solution which is able to dissolve water-insoluble β-1,3-glucans, there may be mentioned aqueous solutions of sodium hydroxide, potassium hydroxide, barium hydroxide, calcium hydroxide, lithium hydroxide, ammonia and so on.

The pH range of the alkaline aqueous solution is usually from 9 to 14, preferably from pH 11 to 13.

The concentration of the water-insoluble β-1,3-glucan thus employed has an influence upon the average diameter of the resulting beads. Normally, it is within a range of from 0.5 to 10 percent, inclusive. Generally speaking, the lower the concentration β-1, 3-glucan the larger is the diameter of the resulting beads.

As for the procedure of dispersing a solution of water-insoluble β-1, 3-glucan in said organic solvent which is not freely miscible with water, the solution is preferably added dropwise under stirring. While there are no particular limits to the duration of time over which the dropwise addition is made except that it should not be long enough to cause hydrolysis of the water-insoluble β-1,3-glucan, a duration of time from 10 to 90 minutes is generally appropriate. In this operation, a surface-active agent may be added to the organic solvent, if necessary. Generally, such surface-active agent is preferably a non-ionic surfactant, although this is not an exclusive choice. The concentration of the surfactant thus added with respect to the organic solvent may range from 0.1 to 20 percent and, preferably, 0.2 to 10 percent (W/V). While the organic solvent to be thus employed may be any solvent that is not freely miscible with water, there may be mentioned, as typical examples, aromatic hydrocarbons and their derivatives (e.g. benzene, toluene, xylene, etc.) aliphatic hydrocarbons and their derivatives (e.g. chloroform, carbon tetrachloride, dichloroethane, *n*-hexane, cyclohexane, etc.), ethers (e.g. diethyl ether, isoproypyl ether, etc.), esters (e.g. ethyl acetate, butyl acetate, etc.) and alcohols (e.g. *n*-butanol, isobutanol, etc.).

The rotational speed of the stirrer employed in dispersing the water insoluble β-1, 3-glucan in the above-mentioned organic solvent has significant effects on the diameters of the product beads. Generally, the higher the stirring speed, the smaller is the diameter of the product beads. A preferred procedure for gelling the water-insoluble β-1,3-glucan dispersion comprises adding an acid to the dispersion, the acid which is soluble in said organic solvent not freely miscible with water. Among the preferred acids are formic acid, acetic acid, propionic acid and benzoic acid. While they depend upon the conditions of manufacture, the diameters of water-insoluble β-1,3-glucan gel beads are generally within the range of 5 to 1000 μ. In the application of the gel beads according to this invention as a gel filtration matrix, generally, their diameters are desirably smaller to achieve higher separation efficiencies but, in consideration of pressure drop and other factors, the diameters of beads are preferably in the range of from 5 to 500 μ, inclusive, and, for still better results, in the range of from 30 to 150 μ, inclusive.

To solve the problems accompanying the application of a matrix or carrier for immobilized enzymes and affinity chromatography, we have further developed a method of encapsulating a core material of small diameter with the water-insoluble β-1,3-glucan. Thus, we have succeeded in the production of gel beads (hereinafter called microcapsules) comprising a fine core material and, by way of encapsulating material, the water-insoluble β-1,3-glucan, which may be made either to float on the water or to settle in water depending on the specific gravity of the particular core material chosen. As the core material, there may be employed pumice (e.g. SHIRASU (trade mark) beads), alumina, silica, glass beads, hollow glass beads, and so on. The material SHIRASU is described in U.S. Patent No. 3904377. Where the specific gravity of the core material is greater than 1, the micro-capsules as packed into a column show flow rate properties which are even superior to those of water-insoluble β-1, 3-glucan gel beads. Where the microcapsule has a specific gravity of less than 1, an enzyme or other active ingredient may be attached to the core to produce an immobilized enzyme preparation and, if this preparation is used in a batch

reaction system where the reaction product separates out as a precipitate, the immobilized enzyme will float onto the reaction mixture and, therefore, considerably facilitate separation of the reaction product. There is thus provided a commercially very advantageous process.

The known gel filtration techniques are compatible with the gel beads according to this invention. In an exemplary technique, a column is packed with the water-insoluble β-1,3-glucan gel beads and, then, a suitable buffer solution is passed through the column at an appropriate flow rate to wash the bed. A predetermined quantity of a similar buffer solution containing a sample is then applied to the column. Elution is carried out using a similar buffer solution at a predetermined flow rate and the eluate is collected in fractions. This procedure can be applied to the separation of mixtures and the molecular weight determination of proteins, polysaccharides and other substances.

Where the gel beads according to this invention are used as a carrier for immobilized enzymes or affinity chromatography, it has been found that the smaller the diameter of bead, the greater is the amount of coupled enzymes and ligands. However, when the beads are used as packed into a column, pressure drop must again be taken into consideration. In this connection, the diameters of the beads are preferably within the range of from 30 to 500 μ, inclusive, and, for still better results, within the range of from 30 to 300 μ, inclusive.

To prepare an immobilized enzyme or a carrier-ligand complex for use in affinity chromatography, the process described in German Offenlegungsschrift 25 51 438 may, for example, be utilized. Thus, one part of water-insoluble β-1,3-glucan gel beads is suspended in 50 volume parts of water, followed by the addition of 20 to 60 volume parts of water containing 0.1 to 3 parts of a cyanogen halide. Under stirring at an optional temperature within the range of from 0°C to 50°C, inclusive, the pH of the reaction mixture is increased to pH 11 by the dropwise addition of a 2N-aqueous solution of sodium hydroxide, care being taken not to cause the gel beads to dissolve (at a rate of about 0.5 pH units/minute). The mixture is maintained at pH 11 for an additional quarter of an hour, whereby the activation reaction is carried to completion. After the reaction has been completed, the solids are recovered by filtration and rinsed with 10 times their volume of water. The activated gel beads thus obtained are insoluble in water and in aqueous solutions of alkalies and are not heat-gellable, the dimensions and strength of individual beads being adequate enough to ensure a sufficient flow rate when used as column packings.

With the use of these activated gel beads, carrier-ligand complexes may also be manufactured. A process for this purpose comprises reacting the activated gel beads with a substance containing a primary or secondary amino group, e.g. an enzyme, protein, peptide, amino acid, coenzyme, enzyme substrate or inhibitor, antigen, antibody, hormone or the like. Preferably in a weakly alkaline aqueous solution and at an optional temperature in the range of from 0°C to 50°C, inclusive.

The following reference and working examples are intended merely to illustrate presently preferred embodiments of the present invention and not to restrict the scope of this invention.

Reference Example 1

The dyeability of the PS-1 gel beads obtained in Examples 2 (Neutralization method) and 19 (Heat method) was investigated using water-soluble dyes according to the method of Nakanishi *et al* [Carbohydrate Research *32* 47-52 (1974)].

The results are set forth in Table 1 as follows:

TABLE 1 *Dyability Test*

Sample / Dye	PS-1 beads (Neutralization method)	PS-1 beads (Heat method)
Congo red	+	+
Trypan blue	−	+
Aniline blue	+	+
Soluble blue	+	+
Fuchsine	+	−
Brilliant blue	−	−
Methylene blue	−	−
Toluidine blue 0	−	−

Reference Example 2

(1) The applicability of the PS-1 gel beads according to Example 1, given hereinafter, as a carrier for immobilized enzyme or affinity chromatography was investigated.

(1)-(i) The above gel beads were packed into a column, 21.1 mm in inside diameter, to prepare a bed with a height of 50 mm. Then, water was passed through the bed by means of a volumetric pump and the relation of flow rate to pressure drop was investigated.

The results are set forth in Table 2 as follows:

TABLE 2

Flow rate (ml/hr.)	Pressure drop (mm H_2O)
48.9	25
71.6	50
112	150

The results given in Table 2 show clearly that the gel beds according to this invention have excellent flow rate properties which make them highly suited for use as a chromatographic carrier.

(1)-(ii) In a procedure for activating gel beads, 100 ml of distilled water were added to 133 ml of gel beads (corresponding to 5 g of PS-1 powder) to obtain a suspension. On the other hand, 5 g of BrCN were dissolved in 100 ml of distilled water. The suspension and solution were combined and the pH was gradually increased by the addition of 5N-NaOH until pH 11 was reached. The system was maintained at pH 11 for 15 minutes, whereby an activated gel of PS-1 was obtained. The gel was filtered through a glass filter and the cake was rinsed with 500 ml of distilled water.

For the purpose of investigating the capability of the above product to retain high flow rates under experimental conditions of column-chromatography, the pressure drops were determined as follows. Thus, the product was packed into a chromatographic column, 19.5 mm in diameter and 229 mm high, and water was passed through this column at varying flow rates by means of a volumetric pump to determine the changes in the heights of bed and pressure drops. The results are set forth in Table 3 as follows:

TABLE 3

Flow rate (ml/hr.)	Pressure drop (mmHg)	Height of bed (mm)
405	14	229
455	20	228
800	54	218

It will be seen that the beads had a high rigidity and non-compressibility and that, as shown in Figure 1 of the accompanying drawings (the pressure drop is expressed with the height of the bed of beads being assumed as 1 meter), the flow rate properties of the beads are superior to the properties of agarose gel which is most commonly employed today, thus making the present beads better suited for use as a chromatographic carrier.

(1)-(iii) The following example is pertinent to the production of an immobilized enzyme preparation using PS-1 gel beads. 126 ml of the above activated PS-1 gel beads (corresponding to 5 g of PS-1 powder) were combined with a solution in 50 ml distilled water of 50.9 mg of the α-amino acid ester hydrolase of *Xanthomonas citri* (IFO 3835) (Japanese Patent Application Laid Open No. 25388/1972) (CMC-treated, lyophilizate). Following the addition of 50 ml of 0.2M-phosphate buffer (pH 8.0) and 24 ml of distilled water, the mixture was stirred at 5°C and pH 8 for 20 hours. The reaction mixture was filtered and the cake was washed with 0.2M-glycine solution, 0.5M-aqueous sodium chloride and distilled water in that order to prepare an immobilized enzyme preparation.

By the above procedure, 95% of the protein was immobilized. The enzyme activity of this preparation was determined based on the rate of hydrolysis of D-phenyl-glycine methyl ester. According to this assay, 90% of the activity was found to have been immobilized.

Reference Example 3

The applicability of beads of PS-1 gel to gel filtration was investigated.

A glass column with an inside diameter of 26 mm was packed with the beads of PS-1 gel

according to Example 4 to a height of 415 mm and washed with 0.05M-Tris-HCl buffer (pH 7.5) containing 0.1M of KCl overnight at a flow rate of 30 ml/hr.

One ml of the same buffer solution, to which 3 mg of one of the samples mentioned in Table 4 had been added, was applied to the column and, thereafter, development was carried out with the same buffer solution at a flow rate of 30 ml/hr. The effluent was collected in 3 ml-fractions and their absorbencies at 280 mμ were determined, except that the phenol-sulphuric acid method was used for dextran. The results, given in Table 4 below, show that the samples emerged from the columns in the order of molecular weights, thus demonstrating the effectiveness of the beads as a molecular sieve. The relation of molecular weight to distribution coefficient was such at, as shown in Figure 2 of the accompanying drawings, for the above PS-1 gel, the exclusion limit in the molecular weight range for globular proteins was 1.1×10^6.

TABLE 4

Sample	Mol. wt.	Effluent (ml)	Distribution coefficient
Dextran 2000	2,000,000	69	
Cytochrome C	12,400	215	0.96
Bovine albumin	67,000	172	0.68
γ-Globulin	160,000	152	0.55
Glutamate dehydrogenase	350,000	126	0.37

Reference Example 4

The applicability of the PS-1 gel beads according to Example 19, given hereinafter, as a carrier for immobilized enzyme was investigated.

In a procedure for activating gel beads, 100 ml of distilled water were added to 115 ml of gel beads (corresponding to 5 g of PS-1 powder) to obtain a suspension. On the other hand, 5 g of BrCN were dissolved in 100 ml of distilled water. The suspension and solution were combined and the pH was gradually increased by the addition of 5N-NaOH until pH 11 was reached. The system was maintained at pH 11 for 15 minutes, whereby an activated gel of PS-1 obtained. The gel was filtered through a glass filter and the cake was rinsed with 500 ml of distilled water. 126 ml of the above activated PS-1 gel beads (corresponding to 5 g of PS-1 powder) were combined with a solution in 50 ml distilled water of 50.9 mg of the α-amino acid ester hydrolase of *Xanthomonas citri* (IFO 3835) (Japanese Patent Application As Laid Open No. 25388(1972) (CMC-treated, lyophilizate). Following the addition of 50 ml of 0.2M-phosphate buffer (pH 8.0) and 24 ml of distilled water, the mixture was stirred at 5°C and at pH 8 for 20 hours. The reaction mixture was filtered and the cake was washed with 0.2M-glycine solution, 0.5M-aqueous sodium chloride and distilled water in that order to prepare an immobilized enzyme preparation.

By the above procedure, 92.9% of the protein was immobilized. The enzyme activity of this preparation was determined relative to the rate of hydrolysis of D-phenyl-glycine methyl ester. According to this assay, 90% of the activity was found to have been immobilized.

Reference Example 5

The applicability of beads of PS-1 gel obtained in Example 19 to gel filtration was then investigated.

A glass column with an inside diameter of 15mm was packed with the beads of PS-1 gel according to Example 19 to a height of 215 mm, and washed with a 0.05M-Tris-HCl buffer (pH 7.5) containing 0.1M of KCl overnight at a flow rate of 21 ml/hr.

One ml of the same buffer solution, to which 3 mg of one of the samples mentioned in Table 5 had been added, was applied to the column and, thereafter, development was carried out with the same buffer solution at a flow rate of 21 ml/hr. The effluent was collected in 3ml-fractions and their absorbencies at 280 mμ were determined, except that the phenol-sulphuric acid method was used for dextran. The results, given in Table 5, show that the samples emerged from the columns in the order of molecular weights, thus demonstrating the effectiveness of the beads as a molecular sieve. The relationship of molecular weight to distribution coefficient was such that the exclusion limit in the molecular weight range for globular proteins was 7.0×10^5.

TABLE 5

Sample	Mol. wt.	Effluent (ml)	Distribution coefficient
Dextran 2000	2,000,000	15.8	
Cytochrome C	12,400	39.5	1.08
Bovine albumin	67,000	29.0	0.60
Fibrinogen (ox)	340,000	20.2	0.20

Reference Example 6

To 125 milliliters of PS-1 in the shape of beads (particle diameter 50-200 microns; equivalent to about 5 g of PS-1 in dry weight) obtained in Example 9 were added 100 ml of water and 100 ml of 5% (W/V) cyanogen bromide.

At 25°C and under stirring, 2*N* sodium hydroxide solution was added dropwise by means of an automatic titrator in such amounts as to increase the pH gradually at a rate of about 0.5 pH per minute until a final pH of 11 was established. The system was held at this pH of 11 for about 15 minutes, whereby the reaction was carried to completion. Following this reaction, the solid matter was recovered by filtration and rinsed with 500 ml of distilled water. The above procedure provided an activated bead-shaped PS-1 (dry weight, 5.1 grams).

One gram of the above activated PS-1 gel beads (as dry weight) was suspended in 50 ml of 0.05M-phosphate buffer (pH 8.0) to prepare a suspension.

To this suspension were added 20 ml of the phosphate buffer solution in which 200 mg of ε-aminocaproyl-D-tryptophan methyl ester as a ligand had been dissolved. The mixture was reacted at 5°C under gentle stirring for 20 hours.

Thereafter, the solid matter was recovered by means of a glass filter and washed with 500 ml of 0.05M-phosphate buffer (pH.8.0) containing 1M NaCl.

Based on the absorbence of 280 mμ of the above washings, the percentage immobilization of the ligand was calculated to be 22%.

A glass column with a diameter of 20 mm was packed with the affinity matrix obtained by the above procedure to a height of 100 mm and equilibrated with a 0.05M-Tris-HCl buffer (pH 8.0).

Two ml of the above buffer solution in which 10 mg of α-chymotrypsin (manufactured by Worthington Biochemical Co., U.S.A.) had been dissolved were applied to the column and, thereafter, washing was carried out with 100 ml of the same buffer solution.

By the above procedure, 8% of the original protein (quantified by the absorbence at 280mμ) was emerged. The activity of α-chymotrypsin determined as the esterase activity using benzoyl-L-tyrosine ethyl ester as the substrate was not encountered.

Further, elution was carried out with 100 ml of 0.1M-acetic acid. By the elution, 90% of the original protein and 96% of the activity were recovered.

Example 1

To 9 g of PS-1 (see above) powder were added 270 ml of purified water, followed by stirring to prepare a slurry. To this slurry were added 30 ml of 1N-NaOH, whereupon the PS-1 was dissolved to yield an aqueous solution of PS-1 and sodium hydroxide. A beaker of 2-liter capacity was filled with 1200 ml of toluene and 6 g of Emalex (trade mark) HC-30 (Nihon Emulsion Co. Ltd., Japan, polyoxyethylene hydrogenated caster oil derivative surfactant) and, under agitation with a screw-type stirrer at 800 r.p.m., the above aqueous solution of PS-1 and sodium hydroxide was added dropwise at room temperature. The resulting PS-1 dispersion was added to a mixture of 2000 ml of toluene and 100 ml acetic acid under stirring at 800 r.p.m., followed by stirring for about one additional hour. The resulting mixture was allowed to stand for about 3 hours, by the end of which time the product gel had settled. The solvent was removed by decantation and the residual sediment was rinsed 5 times with 2 l portions of purified water to bring the pH to neutral, the organic solvent being thereby completely removed. 240 ml of PS-1 gel were obtained by the above procedure.

This gel was found to have the following diameters.

30 - 50 μ	4.0%
50 - 100 μ	42.8%
100 - 200 μ	36.8%
200 - 300 μ	16.4%

Each particle was found to have a bead-like shape. A photo-micrograph of these gel beads

is shown in Figure 3 of the accompanying drawings (8 graduations on the scale are equal to 100 μ).

Example 2

By a procedure similar to that described in Example 1, an aqueous solution containing sodium hydroxide and PS-1, prepared from 10 g of PS-1 powder, 140 ml of purified water and 60 ml of 5N-NaOH, was dispersed in 1200 ml of toluene in the presence of 6.7 g of Emalex (trade mark) HC-30 by stirring at 800 r.p.m. The dispersion was added to a mixture of 2000 ml toluene and 100 ml acetic acid. By the above procedure, there were obtained 206 ml of gel beads from 50 to 150 μ in diameter. This product was suited for use as a matrix.

Example 3

The procedure of Example 1 was repeated, except that 6g of Emalex (trade mark) HC-40 (Nihon Emulsion Co. Ltd., Japan) was used as the surfactant, to yield 265 ml of gel beads of from 50 to 250 μ in diameter. The beads were suited for use as a matrix.

Example 4

The procedure of Example 1 was repeated with 6 g of Emalex (trade mark) H-20 (Nihon Emulsion Co. Ltd., Japan) to obtain 280 ml of gel beads of from 30 to 150 μ in diameter.

Example 5

The procedure of Example 1 was repeated with 6 g of Emalex (trade makr) C-40 (Nihon Emuilsion Co. Ltd. Japan, polyoxyethylene-castor oil derivative) to obtain 312 ml of gel beads from 15 to 100 μ in diameter. These, beads too, were suitable for use as a matrix.

Example 6

The procedure of Example 1 was repeated with 6 g of Tween (trade mark) 60 as the surfactant, to obtain gel beads of from 15 to 200 μ in diameter. The beads were suited for use as a matrix.

Example 7

The procedure of Example 1 was repeated with benzene in lieu of toluene as the dispersion vehicle to yield 240 ml of gel beads with diameters of from 100 to 300 μ. These beads were suited for use as matrix.

Example 8

The procedure of Example 1 was repeated with stirring at 1200 r.p.m. to yield 223 ml of gel beads of from 20 to 100 μ in diameter. These beads were useful as a matrix.

Example 9

The procedure of Example 1 was repeated except that formic acid was used in lieu of acetic acid as the neutralizer to obtain 250 ml of gel beads of from 40 to 200 μ in diameter, which were suited for use as a matrix.

Example 10

To 3 g of pachyman powder there were added 90 ml of purified water, followed by stirring to obtain a slurry. Upon the addition of 10 ml of 5N-NaOH, the pachyman was dissolved. A beaker having a capacity of 1 liter was filled with 500 ml of toluene and 2 g of Emalex (trade mark) HC-30 and, under stirring 800 r.p.m., the above aqueous solution of pachyman and sodium hydroxide was added dropwise at room temperature.

The resulting pachyman dispersion was added to a mixture of 2000 ml toluene and 100 ml acetic acid, followed by stirring for about an hour. The solvent was removed by decantation and the sedimented gel was rinsed with water. By the above procedure there were obtained 80 ml of a pachyman gel of from 50 to 300 μ in diameter. This product was suited for use as a matrix.

Example 11

Except that the stirring was effected at 2100 r.p.m., the procedure of Example 1 was repeated to yield 280 ml of gel beads of from 5 to 50 μ in diameter. The beads were suited for use as a matrix.

Example 12

Except that the stirring was effected at 400 r.p.m., the procedure of Example 1 was repeated to yield 200 ml of gel beads of from 200 to 1000 μ in diameter. The beads were of use as a matrix.

Example 13

3 g of PS-1 powder were dissolved by the addition of 144 ml of purified water and 16 ml of 1N-NaOH. To this solution were added 9 g of SHIRASU (trade mark) beads (40-80 mesh (J.I.S.), Sanki Engineering Co. Ltd., Japan,) followed by stirring. Under stirring at 360 r.p.m., this mixture was added dropwise to a solution of 600 ml toluene and 2 g. Emalex (trade mark) HC-30. To this dispersion were added 15 ml of acetic acid to cause gelation. The reaction mixture was filtered through a nylon cloth and the cake was rinsed with water. As will be seen from Figure 4 of the accompanying drawings (8 graduations on the scale are equal to 100 μ), a microscopic examination of the cake showed that there had been produced 20 to 40-mesh (J.I.S.) spherical microcapsules each comprising a SHIRASU bead core and a PS-1 shell.

Example 14

The procedure of Example 13 was repeated except that dichloroethane was used instead of toluene to obtain the microcapsules.

Example 15

The procedure of Example 13 was repeated except that spherically moulded alumina (Mizusawa Industrial Chemicals Ltd., Japan, Neo Bead C, 14-60 mesh (J.I.S.)) was used instead of SHIRASU beads to obtain microcapsules. The microcapsules had diameters of 500-2794μ.

Example 16

The procedure of Example 13 was repeated except that SIRASU bead was replaced by glass beads (Nippon Electric Glass Co., Ltd., Japan, BH-W, 150-200 mesh (J.I.S.)) to obtain microcapsules. The microcapsules had diameters of 147-200 μ.

Example 17

Except that pumice (Ishikawaraito Co. Ltd., Japan, 28-40 mesh (J.I.S.)) was used instead of SHIRASU bead, the procedure of Example 13 was repeated to obtain microcapsules. The microcapsules had diameters of 833-1168 μ.

Example 18

To 20 g of PS-1 powder were added 660 ml of distilled water, followed by stirring to prepare a suspension. This suspension was added dropwise to 2 l of corn salad oil heated to 80°C-85°C under agitation with a homomixer at 2500 r.p.m., followed by stirring for 30 additional minutes. After the resulting PS-1 dispersion had been cooled to room temperature, the corn salad oil was removed by decanting and the sedimental gel was then rinsed with 400 ml of toluene. This rinsing was repeated completely to remove the salad oil. The toluene was removed with distilled water by decantation. By the above procedure, 460 ml of PS-1 gel were obtained. This gel was found to have the following diameters:

50 - 100 μ	: 22.5%
100 - 150 μ	: 46 %
150 - 200 μ	: 21 %
200 - 250 μ	: 5 %
250 - 300 μ	: 4 %
300 - 350 μ	: 1.5%

WHAT WE CLAIM IS

1. A carrier for immobilized enzymes, affinity chromatography, gel filtration and ion exchange, in the form of a matrix comprising a water-insoluble β-1, 3-glucan gel which is in the form of beads having diameters within the range of from 5 to 1000 μ, inclusive.
2. A matrix according to claim 1, wherein the matrix is a carrier for immobilized enzyme and affinity chromatography.
3. A matrix according to claim 2, wherein the carrier has a diameter within the range of from 30 to 300 μ, inclusive.
4. A matrix according to claim 2, wherein the carrier comprises a core material coated with a water-insoluble β-1,3-glucan.
5. A matrix according to claim 4, wherein the core material is pumice.
6. A matrix according to claim 4, wherein the core materuial is spherically moulded alumina.
7. A matrix according to claim 4, wherein the core material is glass bead.
8. A matrix according to claim 1, wherein the matrix is a carrier for gel filtration.
9. A matrix according to claim 8, wherein the carrier has a diameter within the range of

from 30 to 150 μ, inclusive.

10. A process for producing a matrix in the shape of heads with diameters within the range of from 5 to 1000 μ, inclusive, which process comprises dispersing an aqueous alkaline solution of a water-insoluble β-1,3-glucan in an organic solvent which is not freely miscible with water, and adding an organic acid to the resulting dispersion.

11. A process according to claim 10, wherein the concentration of the water-insoluble β-1,3-glucan in the aqueous alkaline solution of the water-insoluble β-1,3-glucan is within the range of from 0.5 to 10 percent, inclusive.

12. A process according to claim 10 or 11, wherein the aqueous alkaline solution of water-insoluble β-1, 3-glucan contains a core material.

13. A process according to claim 12, wherein the core material is pumice.

14. A process according to claim 12, wherein the core material is spherically moulded alumina.

15. A process according to claim 12, wherein the core material is glass bead.

16. A process according to any of claims 10 to 15, wherein the pH value of the aqueous alkaline solution of the water-insoluble β-1, 3-glucan is within the range of from 9 to 14, inclusive.

17. A process according to any of claims 10 to 16, wherein the alkali is sodium hydroxide.

18. A process according to any of claims 10 to 17, wherein the organic solvent is an aromatic hydrocarbon.

19. A process according to claim 18, wherein the organic solvent is benzene or toluene.

20. A process according to any of claims 10 to 17, wherein the organic solvent is dichlorethane.

21. A process for producing the matrix according to any of claims 10 to 20, wherein the organic solvent contains a surface-active agent.

22. A process according to claim 21, wherein the concentration of the surface-active agent is within the range of from 0.1 to 29 percent by weight, inclusive, based on the volume of the organic solvent.

23. A process according to claim 21 or 22, wherein the surface-active agent is a non-ionic surfactant.

24. A process according to any of claims 10 to 23, wherein the organic acid is acetic acid.

25. A process according to any of claims 10 to 23, wherein the organic acid is formic acid.

26. A process for producing a matrix substantially as herein described with reference to the accompanying drawings and/or any of the specific examples.

27. A matrix produced by a process as claimed in any of claims 10 to 26.

28. A matrix according to claim 1, substantially as herein described with reference to the accompanying drawings and/or any of the specific examples.

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1577955 COMPLETE SPECIFICATION
3 SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheet 1

Fig. 1.

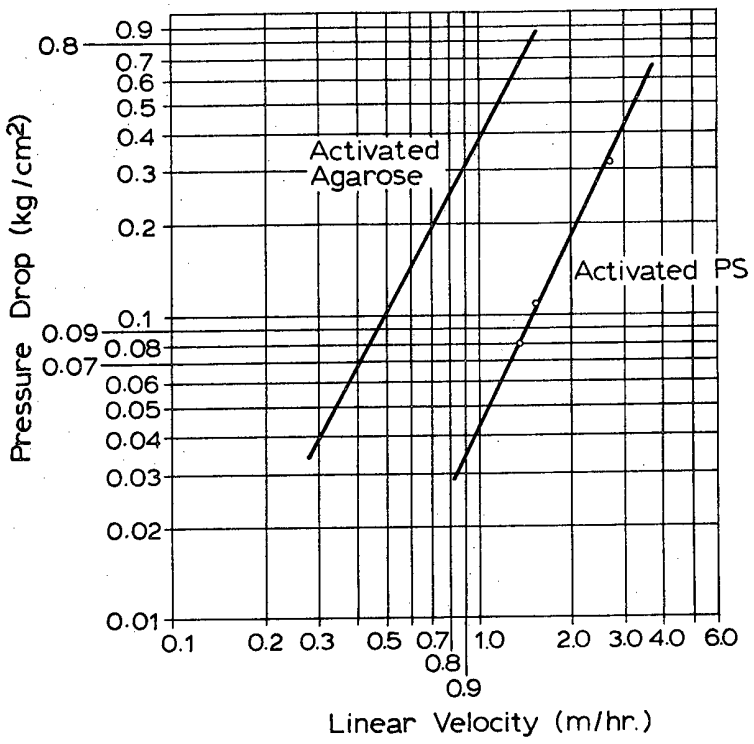
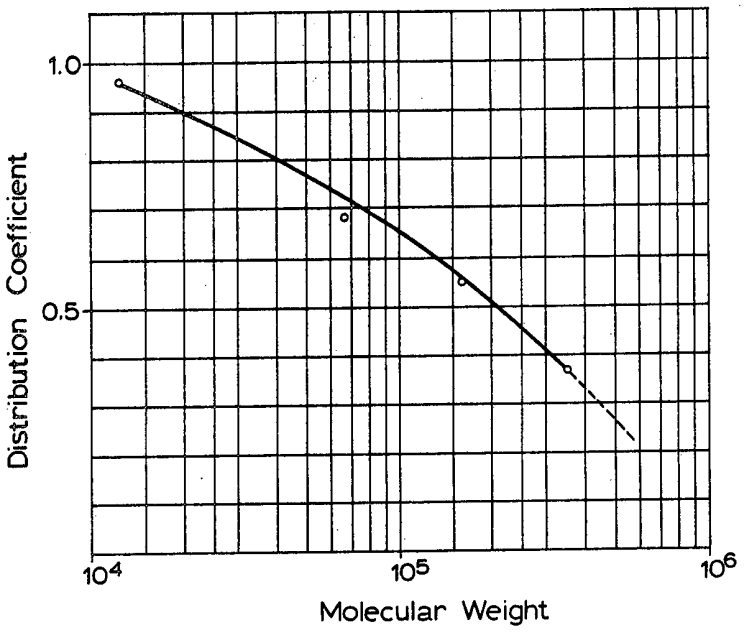


Fig. 2.


1.0
0.5
Distribution Coefficient
10⁴
10⁵
10⁶
Molecular Weight

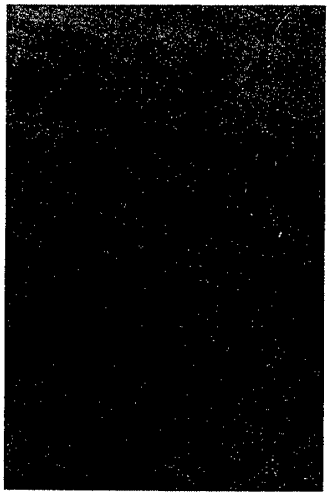


Fig. 3

Fig. 4