SPECIFICATION

ADSORBENT AND ADSORPTION APPARATUS

FIELD OF THE INVENTION

[0001] The present invention relates to a column-use adsorbent and an adsorption apparatus.

BACKGROUND OF THE INVENTION

[0002] Hydroxyapatite has high biocompatibility, high safety and the like. For these reasons, in recent years, hydroxyapatite has been widely used, for example, as a material for stationary phase in chromatography, that is, an adsorbent, which is used when biopharmaceutical products, etc. such as antibodies and vaccines are purified and isolated.

[0003] As described above, hydroxyapatite (HAP) used as a material for stationary phase in chromatography is produced, for example, as follows.

[0004] A first liquid containing calcium hydroxide are reacted with a second liquid containing phosphoric acid while being stirred to obtain hydroxyapatite primary particles, and a slurry containing the primary particles and their aggregates is dried and granulated into hydroxyapatite secondary particles (powder).

[0005] The powder is then baked to obtain sintered powder, and the unbaked powder or the sintered powder is filled in a column (adsorption apparatus), etc., as a material for stationary phase (adsorbent) (for example, see Patent Reference 1).

[0006] The hydroxyapatite adsorbent described in Patent Reference 1 is, for example, prepared by pulverizing hydroxyapatite primary particles with a wet-type jet mill, granulating them into secondary particles, and then classifying the secondary particles, which thereby has high compressive strength, resulting in

[0010] This object is achieved by the present invention described in the following (1) to (4).

[0011] [1]

A column-use adsorbent made of powder of a porous particle group of hydroxyapatite or fluoroapatite formed by replacing at least part of a hydroxyl group of hydroxyapatite with fluorine atom,

the porous particle group being a group of a plurality of porous particles having different particle sizes,

the porous particle group meeting the condition of $D_{Av} \times 45/100 \leq D_{10} \leq D_{Av} \times 75/100$, in which D_{Av} (μm) is an average particle size, and D_{10} (μm) is a particle size at which a cumulative volume of the porous particles from the small size side based on a particle size distribution is 10%.

[2]

A column-use adsorbent made of powder of a porous particle group of hydroxyapatite or fluoroapatite formed by replacing at least part of a hydroxyl group of hydroxyapatite with fluorine atom,

the porous particle group being a group of a plurality of porous particles having different particle sizes,

the porous particle group meeting the condition of $D_{Av} \times 135/100 \leq D_{90} \leq D_{Av} \times 165/100$, in which D_{Av} (μm) is an average particle size, and D_{90} (μm) is a particle size at which a cumulative volume of the porous particles from the small size side based on a particle size distribution is 90%.

[3]

A column-use adsorbent made of powder of a porous particle group of hydroxyapatite or fluoroapatite formed by replacing at least part of a hydroxyl group of hydroxyapatite with fluorine atom,

the porous particle group being a group of a plurality of porous particles having different particle sizes,

[0027] The column body 21 equipped with the lid members 22, 23 secures liquid tightness therebetween.

[0028] The lid member 22 (first port) and the lid member 23 (second port) have a cap 28 and a cap 29, an inlet pipe 24 (first flow path) and an outlet pipe 25 (second flow path), and a lid 26 and a lid 27, respectively.

[0029] With the inlet-side end (one end) turned vertically upward and the outlet-side end (the other end) turned vertically downward, the caps 28, 29 are screwed in the inlet-side end and the outlet-side end of the column body 21, respectively, so that the caps 28, 29 and the column body 21 secure liquid tightness therebetween.

[0030] The inlet pipe 24 and the outlet pipe 25 are configured by a pipe body through which the liquid flows, and are liquid-tightly fastened (fixed) to almost the centers of the caps 28, 29, respectively. The lids 26, 27 are arranged between the filter members 4, 5 and the caps 28, 29, and have flow paths 41, 51 communicating with the inlet pipe 24 and the outlet pipe 25, respectively. The liquid is supplied to the adsorbent 3 through the inlet pipe 24, the lid 26 and the filter member 4. The sample liquid supplied to the adsorbent 3 passes through spaces (gaps) between particles of the adsorbent 3, and then flows out of the column 2 through the filter member 5, the lid 27 and the outlet pipe 25. At this time, the material to be isolated (material to be adsorbed) and the contaminants other than the material in the sample liquid (sample) are separated on the basis of the difference of adsorptivity to the adsorbent 3 and the difference of affinity to the eluent.

[0031] Namely, the separation of the material to be isolated is carried out by setting the adsorption apparatus 1 to the state in which the lid member 22 is positioned vertically above the lid member 23, supplying the liquid into the adsorbent filling space 20 through the inlet pipe 24 so that the material to be isolated is adsorbed on the adsorbent 3 filled in the adsorbent filling space 20,

and then separating the material to be isolated.

[0032] Each filter member 4, 5 has a function of preventing the adsorbent 3 from flowing out of the adsorbent filling space 20, that is, a function of holding the adsorbent 3 in the adsorbent filling space 20. Each filter member 4, 5 is formed by, for example, a polypropylene mesh filter, a polyethylene particle-sintered filter, a stainless steel mesh filter, and a stainless steel particle-sintered filter.

[0033] In the adsorption apparatus 1, the adsorbent 3 is made of sintered powder, which is obtained by sintering an unbaked powder (unsintered powder) including hydroxyapatite primary particles and secondary particles, as powder of a group of fine particles having different particle sizes, and thus has an adsorption capacity with respect to the material to be isolated (material to be adsorbed) in the sample liquid (sample).

[0034] Herein, the unbaked powder (unsintered powder) including hydroxyapatite primary particles and secondary particles is called "hydroxyapatite powder (or simply called "unsintered powder"), and the baked hydroxyapatite powder is called "sintered hydroxyapatite powder" (or simply called "sintered powder"). In addition, hydroxyapatite powder obtained by mixing a plurality of hydroxyapatite powder groups having different physical properties is called "mixed hydroxyapatite powder" (or simply called "mixed powder").

[0035] The sintered hydroxyapatite powder will be described in detail below.

[0036] The sintered hydroxyapatite powder is preferably obtained by sintering the hydroxyapatite powder at temperatures of 300°C or higher and 900°C or lower. The hydroxyapatite powder is a group of porous particles with fine pores, which is mainly composed of the secondary particles and further includes the primary particles and multiple-order particles.

[0037] The secondary particles of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), that is,

hydroxyapatite powder, are porous bodies mainly composed of hydroxyapatite, which are obtained by drying a slurry containing the primary particles and their aggregates so as to granulate them. Hydroxyapatite has a chemically stable apatite structure. It is intended that an atomic ratio Ca/P of the hydroxyapatite is about 1.64 or more and 1.70 or less. The hydroxyapatite secondary particle may be constituted by a secondary particle of fluoroapatite [$Ca_{10}(PO_4)_6(OH)_{2-2x}F_{2x}$, wherein x is $0 < x \leq 1$] formed by replacing at least part of a hydroxyl group on its surface with fluorine atom. Herein, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and fluoroapatite [$Ca_{10}(PO_4)_6(OH)_{2-2x}F_{2x}$, wherein x is $0 < x \leq 1$] formed by replacing at least part of its hydroxyl group with fluorine atom are collectively called “hydroxyapatite” as described above.

[0038] (D_{10} value)

In one embodiment of the present invention, the hydroxyapatite porous particle group preferably meets the condition of $D_{Av} \times 45/100 \leq D_{10} \leq D_{Av} \times 75/100$, in which D_{Av} (μm) is an average particle size, and D_{10} (μm) is a particle size at which a cumulative volume of the porous particles from the small size side based on a particle size distribution is 10%. The hydroxyapatite porous particle group more preferably meets the condition of $D_{Av} \times 55/100 \leq D_{10}$, and more preferably meets the condition of $D_{10} \leq D_{Av} \times 70/100$.

[0039] (D_{90} value)

In one embodiment of the present invention, the hydroxyapatite porous particle group preferably meets the condition of $D_{Av} \times 135/100 \leq D_{90} \leq D_{Av} \times 165/100$, in which D_{Av} (μm) is an average particle size, and D_{90} (μm) is a particle size at which a cumulative volume of the porous particles from the small size side based on a particle size distribution is 90%. The hydroxyapatite porous particle group more preferably meets the condition of $D_{Av} \times 145/100 \leq D_{90}$.

[0040] In one embodiment of the present invention, the hydroxyapatite powder having the above preferred particle size distribution may be obtained by

In one embodiment of the present invention, the hydroxyapatite porous particle group preferably meets both the above preferred D_{10} value and the above preferred D_{90} value. In one embodiment of the present invention, the hydroxyapatite porous particle group more preferably meets the above preferred back pressure value, as well as the above preferred D_{10} value and the above preferred D_{90} value.

[0045] (Adsorption capacity)

In one embodiment of the present invention, the adsorption capacity of the hydroxyapatite porous particle group can be measured in the following method. The column (φ 4.0 mm × length 100 mm) in which the sintered hydroxyapatite powder is dry-filled as the adsorbent is prepared, and a sample liquid is prepared by dissolving bovine serum albumin (material to be adsorbed) in 10 mM sodium phosphate buffer solution (pH6.5) so that the concentration is 1.0 mg/mL. The prepared sample liquid is supplied into the column at a flow rate of 1.0 ml/min, while measuring the absorbance value X (wavelength: 280 nm) of the sample liquid to be supplied into the column and the absorbance value Y(wavelength: 280 nm) of the eluate eluted from the column, until the absorbance value Y (wavelength: 280 nm) reaches 10% of the absorbance value X (wavelength: 280 nm), and then the quantity of the material adsorbed on the sintered hydroxyapatite powder to the amount of the sample liquid supplied up to that time (mg/mL) is calculated.

[0046] Furthermore, in the measurement test of the antibody adsorption quantity, the adsorption quantity of the material to be adsorbed is preferably 15 mg/mL or more. The adsorption quantity is more preferably 20 mg/mL or more, further preferably 25 mg/mL or more, and most preferably 28 mg/mL or more.

[0047] <Production method of hydroxyapatite powder >

In one embodiment of the present invention, the powder made of

into unsintered powder (dried powder) mainly composed of hydroxyapatite secondary particles, that is, hydroxyapatite powder.

[0062] The method of drying the slurry is not particularly limited, but is preferably a spray-drying method. According to this method, the aggregates are granulated so that powder with a desired particle size can be obtained more reliably and in a shorter time.

[0063] The drying temperature of the slurry is preferably about 75℃ or higher and 250℃ or lower, and more preferably about 95℃ or higher and 220℃ or lower. By setting the drying temperature in this range, secondary particles with a high bulk density and a large specific surface area can be obtained.

[0064] The above steps [P1] to [P2] constitute a first step of preparing hydroxyapatite powder.

[0065] [P3: Classification step of classifying hydroxyapatite powder as necessary]

This step is to classify the dried powder obtained by the steps [P1] to [P2]. In this classification step, for example, the average particle size and particle size distribution of the powder can be controlled by removing particles with smaller particle sizes than a predetermined value and particles with larger particle sizes than a predetermined value. When it is not necessary to control the average particle size and particle size distribution of the powder, the classification step can be omitted.

[0066] [P4: Sintering step of baking hydroxyapatite powder to obtain sintered hydroxyapatite powder]

This step is to bake the powder obtained by the steps [P1] to [P3]. The baking temperature of the powder may be about 300℃ or higher and 900℃ or lower, and is preferably 350℃ or higher. The baking temperature is preferably 850℃ or lower, and more preferably 800℃ or lower.

[0067] The time of holding the baking temperature of the hydroxyapatite

particles with larger particle sizes than the D75 value of the unclassified particles, by using a cyclone classifier (“TC-15” manufactured by NISSHIN ENGINEERING INC.).

[0079] [P4]

The dried powder obtained by the spray drier was baked at 400℃ to obtain sintered powder. This sintered powder was regarded as a “sintered powder 1.”

[0080] (Production of sintered powder 2)

[P1] to [P4]

The dried powder was obtained in the same manner as in the preparation step [P1] and the drying step [P2] of the production of the sintered powder 1. Without performing the classification step [P3], the dried powder was baked at 400℃ to obtain sintered powder [P4]. A stirring step using a wet-type jet mill was not performed also in this production of the sintered powder. The sintered powder obtained in this way was regarded as a “sintered powder 2.”

[0081] [P5]

The sintered powder 1 and the sintered powder 2 were mixed in mass ratios shown in Table 1 below.

[0082] 2. Evaluation of hydroxyapatite powder

2-1. Measurement of back pressure

The resultant mixed powder was dry-filled into a stainless steel column with an inner diameter φ of 4.0 mm and a length of 100 mm, and then a 10 mM sodium phosphate aqueous solution at pH 6.5 was supplied into the column at a flow rate of 1.0 ml/min, while the back pressure of the column was measured. The result is shown in Table 1 below.

[0083] 2-2. Evaluation of antibody adsorption capacity

Regarding the column containing each mixed powder as the

adsorbent, the sample liquid containing the antibody was supplied into the column at a flow rate of 1.0 ml/min, and then the antibody adsorption capacity of the adsorbent was measured in the following means.

[0084] That is, first, γ-Globulins from bovine blood (bIgG) was dissolved in 10 mM sodium phosphate (NaP) buffer solution at pH6.5 so that the concentration was 1.0 mg/mL, to prepare the sample liquid (bIgG solution). Next, the stainless steel column (inner diameter φ of 4.0 mm × length of 100 mm) in which each mixed powder is dry-filled as the adsorbent was prepared, and then the prepared sample liquid was sent from a chromatographic pump and supplied into the column at a flow rate of 1.0 ml/min. Then, the absorbance value X (wavelength: 280 nm) of the sample liquid to be supplied into the column and the absorbance value Y(wavelength: 280 nm) of the eluate eluted from the column were measured, the sample liquid was sent until the absorbance value Y (wavelength: 280 nm) reaches 10% of the absorbance value X (wavelength: 280 nm).

[0085] 2-3. Evaluation of durability

<A1> The adsorption apparatus in which the mixed powder 1 was filled was mounted on the chromatograph. 400 mM sodium phosphate buffer solution (pH 6.5, temperature: 25℃) was then supplied into the adsorption apparatus at a flow rate of 1.0 mL/min and a supply amount of 10 CV so as to substitute the inside of the adsorption apparatus with 400 mM sodium phosphate buffer solution.

[0086] <A2> Next, 10 mM sodium phosphate buffer solution (pH 6.5, temperature: 25℃) was supplied into the adsorption apparatus at a flow rate of 1.0 mL/min and a supply amount of 15 CV so as to equilibrate the adsorption apparatus.

[0087] <A3> Next, A sodium phosphate buffer solution (pH 6.5, temperature: 25℃) containing 10 mM sodium phosphate and 1.0 M sodium chloride was

particle size distribution has the highest processing capacity (that is, adsorption capacity× durability / unit weight of adsorbent). As D_{10} value of the carrier for chromatography approaches the average particle size, the width of the particle size distribution becomes narrower and the particle sizes become closer to uniform, resulting in expectation that the target material to be separated can be eluted at a more uniform timing. However, the carrier for chromatography in which a large amount of process toward the target material to be separated is required preferably has a wide particle size distribution and a high filling density to some extents, which usefully increasing the protein processing amount of the carrier for chromatography per one column. When the particle size distribution is too wide, however, the filling density becomes too high, thereby increasing the back pressure of the column, resulting in decrease in durability of the column. We have found that the above powder has an excellent balance of adsorption capacity and durability as the carrier for chromatography.

DESCRIPTION OF REFERENCE NUMERALS

[0093]
1: Adsorption apparatus
2: Column
3: Adsorbent
4: Filter member
5: Filter member
20: Adsorbent filling space
21: Column body
22: Lid member
23: Lid member
24: Inlet pipe
25: Outlet pipe
26: Lid

27: Lid

28: Cap

29: Cap

30: Slurry

41: Flow path

51: Flow path

WHAT IS CLAIMED IS:

1. A column-use adsorbent made of powder of a porous particle group of hydroxyapatite or fluoroapatite formed by replacing at least part of a hydroxyl group of hydroxyapatite with fluorine atom,

the porous particle group being a group of a plurality of porous particles having different particle sizes,

the porous particle group meeting the condition of $D_{Av} \times 45/100 \leq D_{10} \leq D_{Av} \times 75/100$, in which D_{Av} (μm) is an average particle size, and D_{10} (μm) is a particle size at which a cumulative volume of the porous particles from the small size side based on a particle size distribution is 10%.

2. A column-use adsorbent made of powder of a porous particle group of hydroxyapatite or fluoroapatite formed by replacing at least part of a hydroxyl group of hydroxyapatite with fluorine atom,

the porous particle group being a group of a plurality of porous particles having different particle sizes,

the porous particle group meeting the condition of $D_{Av} \times 135/100 \leq D_{90} \leq D_{Av} \times 165/100$, in which D_{Av} (μm) is an average particle size, and D_{90} (μm) is a particle size at which a cumulative volume of the porous particles from the small size side based on a particle size distribution is 90%.

3. A column-use adsorbent made of powder of a porous particle group of hydroxyapatite or fluoroapatite formed by replacing at least part of a hydroxyl group of hydroxyapatite with fluorine atom,

the porous particle group being a group of a plurality of porous particles having different particle sizes,

back pressure of a column being 13 to 24 psi when the adsorbent classified so as to have an average particle size of 40 ± 4 μm is dry-filled into a stainless steel column with an inner diameter φ of 4.0 mm and a length of 100 mm, and a 10 mM sodium phosphate aqueous solution at pH 6.5 is supplied into

the column at a flow rate of 1.0 ml/min.

4. An adsorption apparatus comprising the column-use adsorbent according to any one of claims 1 to 3.