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(54) **A CAKY CHROMATOGRAPHIC COLUMN AND THE METHOD FOR PRODUCING IT AND ITS APPLICATIONS**

KUCHENFÖRMIGE CHROMATOGRAPHIESÄULE, VERFAHREN ZU IHRER HERSTELLUNG UND IHRE ANWENDUNGEN

COLONNE CHROMATOGRAPHIQUE REMPLIE D'UNE SUBSTANCE TASSEE, SON PROCEDE DE PRODUCTION ET SES APPLICATIONS

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(73) Proprietor: **Northwest University**
Xi'an,
Shanxi 710069 (CN)

(72) Inventors:
- **GENG, Xindu**
  Institute Of Modern Separation Science
  Xi'an
  Shanxi 710069 (CN)
- **ZHANG, Y.,**
  Institute Of Modern Separation Science
  Xi'an,
  Shanxi 710069 (CN)

(74) Representative: **Debay, Yves**
Cabinet Debay
126, Elysée 2
78170 La Celle Saint Cloud (FR)

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- **TONG LIU, XIN DU GENG: "The separation efficiency of biopolymers with short column in liquid chromatography" CHINESE CHEMICAL LETTERS, vol. 10, no. 3, 1999, pages 219-222, XP009058843**

EP 1 396 721 B1

Description

Technical Field

[0001] This invention relates to a kind of chromatographic cake assembly and its manufacturing method and application, especially to a kind of chromatographic cake assembly and its manufacturing method and application which is applicable to biopolymer separation or renaturation with simultaneous purification.

Background of the Invention

[0002] Chromatography and capillary electrophoretic methods are two means used frequently for the separation of biopolymers. Because of the limitation of the quantity of the employed mobile phase in capillary electrophoretic method, however, such methods can generally only be used on an analytical scale. Chromatography is now the most important and the most effective separation method for the separation, renaturation and purification of biopolymers. It would be desirable to be able to use such technique not only for analytical scale, but also for production scale.

[0003] When chromatography is used for separation, it is commonly accepted that the separating effect is proportional to the number of column plates, e.g., column length. If the column is too long, however, it is too expensive and it leads to a higher column pressure, which must be controlled with a high performance liquid chromatograph. It is reported in the literature that evidence can be obtained showing effective biopolymer separation with a short column using the stoichiometric displacement model for solute-in-liquid chromatography; and, a packed column of 2 mm in length using a slice of membrane having a thickness of about 2 mm that is cut off from a continuous rod is used to make biopolymer separations with good results when used on an analytical scale. It is still unknown, however, whether such a short column with stable effects can be utilized for industrialized production, as well as to what degree such a column can be shortened and still be effective.

[0004] At present, a column for high performance liquid chromatography employed at a production scale level is generally packed with particles having a diameter of about 20-30 μm. It is ideal that the ratio of column length to diameter be about 10 or less to avoid a too-high column pressure under conditions of high flow rate, thereby causing decreasing separation effects. In order to ameliorate the fact that the volume of the column bed always becomes effectively smaller under the pressure accumulated by the soft matrix over an increased column length, Pharmacia Co., which is famous around the world for its production of chronographic media adapted for use at low and middle pressure conditions, has offered a cake-shaped chromatographic column with shorter thickness (but having a length of at least 10 cm) and of greater diameter. When in use, several cake-shaped chromatographic columns of this type would typically be connected together in series. Therefore, the sum of the lengths of these serially connected columns is still many times greater than the diameter of an individual cake-shaped column, so the flow rate through this series of chromatographic cakes still cannot be too high when it is applied.

[0005] Many proteins expressed with *E. coli* in biotechnology exist in the form of inclusion body in *E. coli* because of its high hydrophobicity. Although the primary structures of such proteins may be correct, their third or fourth structures are basically wrong. As the inclusion body generally has the property of high hydrophobicity, it should be dissolved with a denaturant at high concentration, such as 7.0 mol/L guanidine hydrochloride (GuHCl) or 8.0 mol/L urea. Then the renaturation of the proteins can be processed.. In the current technology, the dialysis method and the dilution method are commonly used for protein renaturation. Nevertheless, these two methods not only have a low renaturation effect (on the order of about 5% - 20% generally), but they also require too much time resulting in failure to realize the objective of separating impure proteins. One alternative technology has been developed in which a denatured protein is renatured and simultaneously purified by high performance hydrophobic interaction chromatography (HPHIC). However, if some precipitates of proteins occur by sample injection, the chromatographic column in this technique will be blocked or damaged. Thus, the denatured facilities currently used for protein renaturation and purification and the multifunctional protein-purifying unit as described above have major limitations which reduce their utility and effectiveness.

[0006] It can be concluded that the following major problems currently exist in the separation and purification of proteins in various biotechnology processes:

1. Separation and purification of biopolymers made in a glass column, a plastic column or a stainless steel column, packed by soft based media, and having large diameters. The shortcomings of these processes include low column efficiency, need for large volumes of media, high consumption of mobile phase, and long production periods.

2. When small solutes are separated with a chromatographic column, the resolution should be proportional to the column length. The ratio of column diameter to column length is normally about 1:10. The column length has a small effect on the resolution of biopolymers. In general, a column with a length of about 5 cm is selected. When such a column is packed with small particles, the chromatographic system through pressure is obviously increased. Such

a column should therefore be used with a high-pressure liquid chromatograph, but this results in increased production costs. Such increased costs counterbalance some of the advantages of using a column packed by small particles, namely high efficiency, large volume and good reproducibility.

3. For the usual chromatographic columns, it is preferred that samples having a high viscosity not enter the columns, and that no more than a small quantity of precipitates from samples are allowed to settle in the column head. In practice, however, the inlet and outlet conditions of the mobile phase cannot readily be changed, so such control is not convenient for the operation.

4. In a usual separation and purification process, relatively pure products can be obtained only through the sequential steps of renaturation, removal of denaturants, coarse purification, and multistep fine purification. These steps represent a long and cumbersome processing technology usually involving great loss of mass and bioactivity and, thus, with a relatively low recovery (generally no higher than about 5-20%).

5. Common renaturation methods include a dilution method and a dialysis method. With the dilution method, many dilution steps should be taken to gradually decrease the concentration of the denaturing agent employed. Thus, such a process brings many handling difficulties for the later separation and purification steps if, at each stage, samples are diluted tens, or even hundreds, of times. The dialysis method, on the other hand, takes too much time (e.g., 24 hours for only one dialysis step generally), and, furthermore, the dialysis agent should be changed many times. In addition, with the above two renaturation methods, the subject proteins are easily aggregated and precipitated resulting in a long renaturation time during the renaturation process.

6. There are many steps involved in the current separation and purification methods. In the course of such separation and processing steps, the volume of solution containing the subject proteins is always increasing. Also, each step of these methods requires substantial associated processing equipment. Therefore, current separation and purification techniques require a relatively large investment in equipment with correspondingly high production costs.

[0007] At present, for packing and manufacturing a chromatographic column, satisfactory column efficiency can be obtained when chromatographic packing material is packed using familiar axial and radial pressurization techniques. But these two methods are only applicable to the packing process for a relatively long chromatographic column wherein the ratio R of column length to diameter is greater than unity (i.e., $R > 1$). There are no ideal packing and manufacturing methods currently available, however, for the process of packing a chromatographic "cake" wherein the ratio R of cake thickness to cake diameter is smaller than or equal to unity (i.e., $R \leq 1$). If the traditional technology were used to pack a chromatographic cake wherein $R \leq 1$, the following problems would likely occur:

1. In the traditional process to pack a chromatographic column, liquid goes through the column inlet and column outlet in the axial direction (i.e., along the axis of the column). In general, the traditional packing processes are only applicable to a chromatographic column wherein the ratio R of column length to column diameter is greater than unity.

2. Because the technique of packing columns properly requires a high level of skill, if an operator is lacking the necessary skills, circumstances may occur in which a column is not packed properly, for example having a tight outlet and/or a loose inlet. An improperly packed column has low reproducibility of results because of the defect in the column packing.

3. In a conventional chromatographic column packing process, the inlet end of the column can be made even and smooth with a blade after the column has been packed. For a chromatographic cake, however, there are no easy and effective methods to make such a large surface area of the packing, such as the inlet end of the column, even and smooth after packing the cake.

4. In general, a chromatographic column needs to be repacked after long use at high pressure. Before a column is repacked, the sunken inlet end of a chromatographic column is typically repaired to prolong column life. In order for the sunken inlet end of a chromatographic column to be repaired, however, the column head must be dismantled to remove the frit. For a column with a relatively small diameter, it is generally easy to remove the frit because of the small surface area of the frit. But, the surface area of the inlet end of a "chromatographic cake " is generally more than one up to 100 times or more greater than that of a comparable conventional chromatographic column. Also because the distributor is tabled tightly with the frit, and is normally also relatively tightly pressed in the groove of the column body, it is difficult to remove it. If it is removed with force, the distributor could often be damaged.

5. When packing having a small diameter is to be packed into a chromatographic cake using the high pressure slurry method, possible leakage must be tested before the cake can be packed because the diameter of a chromatographic cake is relatively great and, accordingly, it is difficult to seal it off if there is a leak. After the chromatographic packing is packed, if there is still a leakage problem, it can create great difficulties for repairing the apparatus.

6. For a relatively large chromatographic cake, a relatively large slurry tank and associated devices are required which increase the production costs.

[0008] Document EP-A-0 021 606 is directed to an apparatus for treating a liquid material having a permeable element which is rotatable about an axis. The permeable element is in the shape of an annulus with a diameter of 80 cm and an axial thickness of 20 cm.

[0009] Document "The separation efficiency of biopolymers with short column in liquid chromatography" by Tong Liu and Xin Du Geng, Chinese Chemical Letters, vol. 10, no 3, 1999, pages 219-222 discloses a theoretical and experimental study of the separation of biopolymer. In particular, the separation of recombinant human granulocyte colony-simulating factor (rhG-CSF) produced in biotechnology is studied.

Summary of the Invention

[0010] The principal object of this invention is to provide a kind of improved chromatographic cake applicable to the separation, or renaturation with simultaneous purification of biopolymers with good results.

[0011] The present invention is directed to a type of chromatographic cake assembly, which comprises a chromatographic packing cake or shell defining an inner region or cavity, and having a mobile phase inlet and a mobile phase outlet, the chromatographic packing cake being packed in the inner cavity thereof with a suitable chromatographic packing material, wherein the ratio R of the thickness of the inner cavity to the diameter of the inner cavity of the chromatographic packing cake is smaller than or equal to 1 ($R \leq 1$).

[0012] In a preferred embodiment of this invention, the preferable dimensions of the inner cavity of the chromatographic packing cake range from about 0.2 -50 mm in thickness and from about 5.0 -1000 mm in diameter.

[0013] A chromatographic packing cake assembly in accordance with this invention includes upper and lower clamp plates sandwiching a cake body element, each clamp plate having either the said mobile phase inlet or outlet respectively at opposite ends (clamp plates) of the apparatus, and also, preferably, wherein the cake body includes at least one lateral hole or aperture.

[0014] In order to avoid the loss of chromatographic packing from the inner cavity, frits are assembled or located at the lateral sides of the upper and lower clamp plates respectively of the chromatographic packing cake assembly and near to the inner cavity of the chromatographic packing cake assembly. The diameters of the frit holes in the frits should be greater than the typical size of the biopolymers in the mobile phase but also less than the size of the chromatographic packing.

[0015] In order to obtain generally even distributions of mobile phase and solute through the cake, and to enhance the separation effect, in another preferred embodiment of the invention distributor elements are assembled or located between the upper and lower clamp plates of the chromatographic packing cake assembly.

[0016] Such a distributor element comprises one generally flat plate having substantially the same profile as the cross section of the inner cavity of the chromatographic packing cake. On the surface of at least one lateral side of each distributor element are radiating and concentric circular blast grooves. Distributing holes are located at the junctions of the radiating and circular guide grooves.

[0017] The preferred cross section of the guide grooves in the distributor elements is generally triangular and cambered.

[0018] The diameters of the distributing holes in the distributor elements may increase gradually as they move radially outward from the center of the distributor element.

[0019] In another embodiment of chromatographic packing cakes in accordance with this invention, a seal ring may be placed between the upper and lower clamp plates and the cake body.

[0020] Chromatographic cake assemblies in accordance with this invention may be advantageously used in the separation and purification of biopolymers prepared in biotechnology processes, thereby realizing advantages such as high efficiency,processing of large volumes and good reproducibility of results. The packed chromatographic cake assemblies of this invention are equally useful under process conditions of either low or middle pressures. The assemblies of this invention can decrease costs and enhance output. With the chromatographic cake assemblies of this invention, the processes of separation and purification, removal of denaturants during a renaturation process, coarse purification, and multiple-step purifications can be combined and accomplished in only one step. The chromatographic cake assemblies of this invention can work under pressure conditions ranging from about 1-200 kg/cm^2.

[0021] A second objective of this invention is to provide new and improved manufacturing methods for preparing chromatographic cake assemblies in accordance with this invention, such methods being characterized by high speed,

low cost and high efficiency.

[0022] In order achieve this objective, this invention uses the following technical outline of a manufacturing method for chromatographic cake assemblies, the method including the following steps:

1. Manufacturing the chromatographic packing cake assembly. The chromatographic packing cake assembly includes the upper and lower clamp plates, having respectively either a mobile phase inlet or outlet, assembled together to the cake body, which has at least one lateral hole. The ratio of the thickness to the diameter of the inner cavity of the chromatographic packing cake is smaller than or equal to 1.

2. A suitable chromatographic packing is packed into the assembled cake assembly using the lateral hole(s) of the chromatographic packing cake assembly to insert the packing material into the inner cavity of the chromatographic packing cake.

[0023] The lateral hole(s) of the chromatographic packing cake assembly can be directly connected to the slurry tank of a high-pressure slurry packing machine for packing the chromatographic cake.

[0024] When a chromatographic cake with a diameter of less than about 50 mm is manufactured, the chromatographic cake can be directly placed on the high-pressure slurry packing machine. According to the usual method for packing a column, the chromatographic packing can be directly added into the inner cavity of the chromatographic packing cake assembly.

[0025] When a chromatographic cake having a diameter greater than about 50 mm is manufactured, however, the cake should preferably be at least partially packed using a suction method in order to insure that the chromatographic packing are packed tightly. Using the suction method, the chromatographic cake is placed on the high-pressure slurry-packing machine, and the chromatographic packing is added through the lateral hole(s) of the chromatographic packing cake into assembly the inner cavity of the chromatographic packing cake assembly.

[0026] The method of adding the chromatographic packing through the lateral hole of the chromatographic packing cake assembly helps to reduce or eliminate the "dead" volume in the chromatographic cake, which in turn improves the separation effect. Dead volume tends to increase by the loss of chromatographic packing after the chromatographic cake has been in use for some time. The method of adding additional chromatographic packing is similar to the original cake packing method.

[0027] In order to eliminate accumulated impurities on the distributor elements, the frits and the biopolymers that do not enter the packed chromatographic cake, the chromatographic cake should be periodically washed through the lateral hole of the chromatographic packing cake assembly. Because these accumulations can increase the pressure on the chromatographic system, they can result in contaminating follow-up separated samples.A preferred washing method for the chromatographic cakes of this invention is to let buffer solution or water enter the lateral hole, which acts as an inlet, and go out from the inlet and outlet respectively in the upper and lower clamp plates.

[0028] Another embodiment of this invention is to periodically replace deteriorated chromatographic packing through the lateral hole of the chromatographic packing cake assembly and to remove deteriorated chromatographic packing which has a low column efficiency and which makes the chromatographic system pressure increase. The removed packing is then replaced with new packing. One such method is as follows: let the upper and lower clamp plates act as inlets and use the lateral hole as the outlet; purge the deteriorated chromatographic packing with water, and repack the new chromatographic packing into the hollow interior of the cake assembly.

[0029] The operations for the cake packing and packing replacement in accordance with this invention are very simple and easy, and they can save time and workload. As the clamp plates on both ends of the chromatographic cake assembly would not be opened after a leakage check, and the cake can be packed directly, this design can insure a chromatographic cake assembly free from liquid leakage and having an even surface of packing, which is favorable to enhancing the column efficiency. When a bigger chromatographic cake is packed, it does not need a larger slurry tank and other accessory equipment, which means that production costs are also reduced.

[0030] Further explanations of this invention in relation to the following figures and concrete examples appear below.

Brief Description of the Drawings

[0031]

Fig. 1 is a schematic side sectional view of a chromatographic cake assembly in accordance with this invention.

Fig. 2 is a sectional view of the chromatographic cake assembly of Fig. 1 taken along the line A-A'.

Fig. 3 is a schematic top view of a distributor element for use with a chromatographic cake assembly in accordance

with this invention.

Fig. 4 is a sectional view of the distributor element of Fig. 3 taken along the line B-B'.

Fig. 5 is a chromatogram of a chromatographic cake assembly in accordance with this invention which has been radially packed.

Fig. 6 is a chromatogram of a chromatographic cake assembly in accordance with this invention which has been axially packed.

Fig. 7 is a chromatogram for a separation carried out in accordance with Example 5 below using a chromatographic cake assembly in accordance with this invention.

Fig. 8 is a chromatogram for a separation carried out in accordance with Example 5 below using a chromatographic cake assembly that has been washed and repacked with additional packing in accordance with this invention.

Fig. 9 is a chromatogram for a separation carried out in accordance with Example 6 below using a chromatographic cake assembly in accordance with this invention.

Fig. 10 is a chromatogram for a separation carried out in accordance with Example 6 but using a conventional chromatographic column instead of a chromatographic cake assembly in accordance with this invention.

Fig. 11 is a chromatogram resulting from use of a 10×50 mm I.D. chromatographic cake assembly in accordance with this invention to separate seven standard proteins.

Fig. 12 is a chromatogram resulting from use of a 10×200 mm I.D. chromatographic cake assembly in accordance with this invention to separate five standard proteins.

Fig. 13 is a chromatogram resulting from the use of a 10×300 mm I.D. chromatographic cake assembly in accordance with this invention to separate five standard proteins.

Fig. 14 is a chromatogram resulting from renaturation carried out with simultaneous purification of rhINF-γ using a 10×50 mm I.D. chromatographic cake assembly in accordance with this invention.

Fig. 15 schematically illustrates the multifunctional utility of chromatographic cakes in accordance with this invention.

Detailed Description of the Invention

Example 1

Structure of a chromatographic cake in accordance with this invention

[0032] As shown in Figs. 1 and 2, a chromatographic cake assembly in accordance with this invention preferably includes a stainless steel chromatographic packing cake, as seen in Fig. 1, filled with a suitable chromatographic packing 10 packed into the inner cavity of the chromatographic packing cake assembly. The cross section of the inner cavity of the packing cake is preferably generally round in shape, with a preferred thickness of about 10 mm and having a diameter of about 100 mm. The degree of roughness on the surface of the inner cavity of the chromatographic packing cake is preferably smaller than about 1.6 μm so that it can be easily sealed and can reduce the irreversible absorption of biopolymers.

[0033] A chromatographic packing cake assembly in accordance with this invention comprises a pair of upper and lower clamp plates, 3 and 4 respectively in Fig. 1, with a mobile phase inlet 1 in one such clamp plate and a mobile phase outlet 2 in the other such clamp plate, together with a cake body 5 having at least one lateral hole or aperture 9 extending from an external region into the inner cavity of the cake. The upper and lower clamp plates 3 and 4 and the cake body 5 define the inner cavity of the chromatographic packing cake assembly. The lateral hole 9 through cake body 5 is designed to be blocked and sealed with an end cap (not shown) after the chromatographic packing 10 has been packed into the inner cavity. In order to prevent leakage, the seal rings 8, preferably made of corrosion-resistant engineering plastic, are installed between the upper and lower clamp plates 3 and 4 respectively and cake body 5. Stainless steel frit elements 6 with holes (not seen in Fig. 1 because they are too small) are installed separately on the

inner sides of the upper and lower clamp plates 3 and 4 adjacent the inner cavity of the chromatographic packing cake assembly. The diameter of the frit 6 is greater than the diameter of the inner cavity of the chromatographic packing cake assembly. The diameter of the frit holes is selected to be a size that is smaller than the diameter of the particles of chromatographic packing 10 but larger than the average size of typical biopolymers being processed.

[0034] As shown in Figs. 3 and 4, the distributor elements 7 used in a preferred embodiment of this invention are preferably made of engineering plastic, which is resistant to acid and alkali. Such distributor elements are installed between the upper and lower clamp plates 3 and 4, respectively, and the associated frit 6. A distributor element 7 comprises a plate member that has the same general shape as the shape of the cross section of the inner cavity of the chromatographic packing cake. On the surfaces of one or both sides of the plate are both radiating and concentric circular blast grooves 11 (same reference numeral used to identify both types of grooves). The cross section of the blast grooves 11 is preferably of a generally triangular shape. In the junctions between the radiating and circular blast grooves 11, are generally round distribution holes 12. In one embodiment of this invention, the diameters of the distribution holes may increase gradually as they are located further radially outward from the center of the distribution element. Such increasing size of the distribution element holes 12 can be seen in Fig. 3.

[0035] In practice, the cross section of the inner cavity of a chromatographic packing cake assembly in accordance with this invention may have many different shapes, for example, it can be round, polygonal, elliptic and so on. But, in each such design, the ratio of the thickness to the diameter (or corresponding dimension) of the inner cavity of the chromatographic packing cake assembly should be smaller than or equal to 1. An optimal cake thickness has been found to be about 0.2 - 50 mm, with the corresponding diametral dimension ranging from about 5.0 - 1000 mm. The chromatographic packing cake assembly can be made from many materials such as resistant-to-acid and alkali stainless steel, titanium alloy, and many kinds of engineering plastics. There are also many kinds of chromatographic packing suitable for various types of chromatographic separations which are useful with the chromatographic cake assembly of this invention. The number of lateral holes 9on the cake body 5 of the chromatographic packing cake assembly may be one or more than one, which can be decided in accordance with each actual situation.

Example 2

Packing and manufacturing a medium-larger sized chromatographic cake with a diameter of more than 50 mm using the radial column packing method

[0036]

1. A chromatographic cake was made in accordance with the method described above in Example 1 and as illustrated in Figs. 1 - 4;

2. The cake was at least partially packed using a usual suction packing method; and,

3. The lateral hole(s) 9 of the chromatographic packing cake assembly was (were) connected directly with the slurry tank on a high-pressure slurry-packing machine, and additional chromatographic packing was added through the lateral hole(s) of the chromatographic packing cake into the inner cavity of the chromatographic packing cake assembly to complete the packing step.

Example 3

Packing and manufacturing a smaller-sized chromatographic cake with a diameter of less than 50 mm using the radial column packing method

[0037]

1. A chromatographic packing cake was made according to the method described above in Example 1 and as illustrated in Figs. 1 - 4; and,

2. The lateral hole(s) 9 of the chromatographic packing cake was (were) connected directly with the slurry tank on a high-pressure slurry-packing machine, and the chromatographic packing was added through the lateral hole(s) of the chromatographic packing cake into the inner cavity of the chromatographic packing cake to fill the inner cavity.

Example 4

Performance comparison between two methods of packing a chromatographic cake

[0038] Under the same cake packing conditions (40 MPa column/cake packing pressure and 30 min. column packing time), small particles (diameter of about 5 μm of a hydrophobic chromatographic packing were packed separately into two identical 10×50 mm I.D. chromatographic cakes first using the usual axial column packing method and, second, using the radial column packing method of this invention. Under the same chromatographic conditions (flow rate of 5.0 mL/min and gradient of 0-100% B for 25 min.), five proteins (cytochrome C, ribonuclease A, lysozyme, α-amylase and insulin) were separated using the two packed cakes. The above operation was repeated five times for each cake. The chromatograms showing the results of the two packing methods are respectively shown in Figs. 5 and 6. In the Figures, peak 1 = cytochromoid C, peak 2 = ribonuclease A, peak 3 = lysozyme, peak 4 = α-amylase and peak 5 = insulin. Comparing Figs. 5 and 6, it can be seen from the results in the Figures that good, substantially similar separation results are obtained with both of the column packing methods for the standard protein separation. One difference found with this Example was that the radial packing method had somewhat better reproducibility of results than the axial column packing method.

Example 5

Separation performance after radially discharging and repacking a chromatographic cake

[0039] A hydrophobic chromatographic packing was packed through the lateral hole of a chromatographic cake using the radial high pressure slurry method, after which the lateral hole was sealed with an end cap. Under the conditions of a flow rate of 5m L/min and a gradient of 100% A - 100% B for a period of 25mins., cytochrome C, myoglobin, lysozyme and α-amylase were separated. The results are shown in Fig. 7. In Fig. 7, peak 1 = cytochrome C, peak 2 = ribonuclease A, peak 3 = lysozyme, and peak 4 = α-amylase. Following this separation, the chromatographic cake was washed thoroughly. The lateral hole was used as a wash fluid outlet, and the inlet and outlet of the clamp plates of the chromatographic cake were used as wash fluid inlets, using water as the mobile phase. A chromatographic pump was turned on to assist with purging the used packing. Packing was added to replace deteriorated packing and any lost during the wash step. After the packing was further processed through degassing with up sonic and evenly slurried, the chromatographic cake was repacked using the same column packing method as previously. Under the same chromatographic conditions used previously, the four proteins were again successfully separated using the washed and repacked chromatographic cake. The results are shown in Fig. 8, in which peak 1 = cytochrome C, peak 2 = ribonuclease A, peak 3 = lysozyme, and peak 4 = α-amylase. From a comparison of Fig. 7 and 8, no significant difference was seen comparing the original separation with the separation carried out after the original packing had been discharged from the lateral hole of the chromatographic cake, washed, treated and repacked in the chromatographic cake again without removal of the upper and lower clamp plates. This Example shows that it is very easy to discharge used, deteriorated packing through the lateral hole of the chromatographic cake, and then also easy to repack the cake with washed or new packing or to add additional packing.

Example 6

Comparison of separations carried out using a chromatographic column and a chromatographic cake

[0040] A chromatographic cake with the specification of 5x50 mm I.D. and a chromatographic column with the specification of 200x7.9 mm I.D. were selected for the Example. The volumes of the packing cavities in both cases was 9.9±0.2 mL. Under the same 40 MPa pressure conditions, both chromatographic apparatuses were packed using the same batch of HPHIC packing. Under the conditions of same sample size and flow rate of 4.0 mL/min., six standard proteins were separated using the two chromatographic devices. The results are shown in Figs. 9 and 10, respectively, in which peak 1 = cytochrome C, peak 2 = myoglobin, peak 3 = ribonuclease A, peak 4 = lysozyme, peak 5 = α-amylase, and peak 6 = insulin. It can be seen from comparing Figs. 9 and 10 that the chromatographic cake and the chromatographic column produce generally comparable resolutions for the six proteins. But, the advantage of the chromatographic cake according to this invention is that the thickness of the chromatographic cake is only 1/40 the length of the chromatographic column with substantially the same geometric volumes of the respective packed beds. This Example thus shows that satisfactory chromatographic resolution is achieved with the chromatographic cakes of this invention even though they are configured with a relatively larger diameter and relatively short column length, but with the same geometric volume as a conventional chromatographic column.

Example 7

Separations using different types of chromatographic cakes

[0041] In this Example, the standard proteins were separated using three sizes of chromatographic cakes manufactured in accordance with this invention, namely a 10x50 mm I.D. cake, a 10x200 mm I.D. cake and a 10x300 mm I.D. cake. The results are shown respectively in Figs. 11, 12 and 13. As shown in Fig. 11 wherein peak 1 = cytochrome C, peak 2 = myoglobin, peak 3 = ribonuclease A, peak 4 = lysozyme, peak 5 = α-chmotropsen, peak 6 = α-amylase, and peak 7 = insulin, the proteins are separated under the chromatographic conditions of 5.0 mL/min. flow rate, 0.08 AUFS, and a gradient ranging from 100% A to 100% B for a period of 40 mins. As shown in Fig. 12, wherein peak 1 = cytochrome C, peak 2 = myoglobin, peak 3 = lysozyme, peak 4 =α -amylase, and peak 5 = insulin, the proteins are separated under the chromatographic conditions of 100.0 ml/min. flow rate, 0.05 AUFS, and a gradient ranging from 100% A to 100% B for a period of 40 mins. As shown in Fig. 13, wherein peak 1 = cytochrome C, peak 2 = myoglobin, peak 3 = ribonuclease A, peak 4 = lysozyme, and peak 5 = α-amylase, the proteins are separated under the chromatographic conditions of 120.0 ml/min. flow rate, 0.1 AUFS, and a gradient ranging from 100% A to 100% B for a period of 60 mins. It can be seen in the Figures that all of these chromatographic cakes with different specifications result in satisfactory resolution.

Example 8

Renaturation efficiency of denatured lysozyme with urea and guanidinine hydrochloride using a chromatographic cake

[0042] In this Example, under two different chromatographic conditions, sample injections were made of denatured lysozyme into solutions of urea and guanidinine hydrochloride (GuHCl), after the 5×50 mm I.D. chromatographic cakes in accordance with this invention were equilibrated with solution A. Then the renatured components coming from the chromatographic cakes were collected. The bioactivity recovery of these effluent streams was measured as shown in Table 1 below. The gradient used was changed from 100% A to 100% B. It can be seen from the results in Table 1 that the chromatographic cake has a substantial renaturation effect on the denatured lysozyme by urea and GuHCl.

Table 1: Bioactivity recovery of Lysozyme under different chromatographic conditions

Sample	Flow rate, 4 mL/min. 25 min. linear gradient time	Flow rate, 2.mL/min. 50 min. linear gradient time
Denatured Lysozyme by urea	102.9%	104.7%
Denatured Lysozyme by GuHCl	103.7%	104.0%

Example 9

Renaturation with simultaneous purification of the recombined human interferon rhINF-γ using a chromatographic cake

[0043] Fig.14 shows the chromatographic results of this Example in which renaturation is carried out simultaneously with purification of rhINF-γ in a 10×50 mm I.D. chromatographic cake in accordance with this invention. The sample used was an rhINF-γ solution extracted from the cellular cataclastic solution of *E. coli* with 7.0 mol/L GuHCl solution. The bioactivity measuring method of rhINF-γ was the restraint method of cellular pathologic change. The operating conditions were as follows: the 7.0 mol/L GuHCl solution containing rhINF-γof 1mL was injected into the chromatographic cake equilibrated with mobile phase A from the extracting solution of *E. coli* at a flow rate of about 3.0 - 7.0 mL/min. over an interval of 25-40 mins., wherein the composition of the mobile phase was changed gradually from 100% A to 100% B. The fractions were collected and their bioactivities were measured separately. It can be seen in the results that the bioactivity recovery was 1774.57%, i.e., 17 times greater than the results achieved using the usual methods.

Industrial applications

[0044] Chromatographic cake assemblies in accordance with this invention can be used successfully at a pressure of more than 20 Mpa. Such assemblies do not deform during use, which helps to insure the surface evenness of the chromatographic packing at the end of a sample injection which, in turn, enhances the resolution. Using the assemblies of this invention, separation is fast even with a low system pressure (e.g., lower than 5.0 Mpa generally). From the

standpoint of good resolution under high flow rate conditions, the performance of chromatography cake assemblies accordance with this invention is comparable to that of perfusion chromatography and, therefore, has superior industrial applicability. The chromatographic cake assemblies of this invention also allow for sample injections having a relatively high viscosity and a little precipitation. When the chromatographic separation is completed with the assemblies of this invention, no fixed inlet and outlet are needed. The assemblies of this invention show only a small irreversible absorption on objective products, which enhances the recovery of such objective products. With the chromatographic cake assemblies of this invention, the separation, renaturation, and purification processes can be performed in one step, which makes it at least three times simpler than the normal renaturation and purification technology. With the chromatographic cake assemblies of this invention, the relevant production period can also be shortened by at least several times compared with comparable conventional processes and chromatographic devices. The investment for equipment is also reduced significantly. At the same time, denaturants can also be recovered when using chromatographic cakes in accordance with this invention. Not only can such recovered denaturants be reused, but also the environmental pollution caused by disposing of such denaturants can be reduced. Fig. 15 shows that a chromatographic cake assembly in accordance with this invention can play a roll of "killing four birds with one stone", (that is, quick and complete elimination of denaturants, easy recycling of denaturants, protein renaturation, and separation of impure proteins). Using only the same packing volume as a conventional chromatographic column, in comparison with normal columns, more packing material can be packed into the inner cavity of a chromatographic cake packed in accordance with this invention. Relatively greater mass loading and volume loading is therefore possible with the chromatographic cake assemblies of this invention.

[0045] Thus, the chromatographic cake assemblies of this invention will find a wide range of applications in the separation, renaturation and purification of biopolymers.

Claims

1. A chromatographic packing cake assembly, comprising a pair of upper and lower clamp plates (3, 4) forming an inner cavity, the upper clamp plate (3) having a mobile phase inlet (1) and the lower clamp plate (4) having a mobile phase outlet (2), a chromatographic packing (5) being packed in the inner cavity, and a lateral hole or aperture (9) extending from an external region into the inner cavity of the cake, wherein:

    - the ratio of the thickness to the radial length of the inner cavity is smaller than or equal to 1,
    - the thickness of the inner cavity is 0.2 - 50mm and the radial length is 5.0-1000 mm.

2. The said chromatographic cake assembly of Claim 1, comprising frits (6) that are installed respectively on the sides of the inner cavity of the cake near the upper and lower clamp plates (3, 4) of the chromatographic packing cake assembly.

3. The said chromatographic cake assembly of Claim 2, comprising distributors (7) that are installed respectively between the upper and lower clamp plates (3, 4) and the frit (6).

4. The said chromatographic cake assembly of Claim 3, **characterized in that** the said distributor (7) is a plate that has the same shape just as the shape of the cross section of the inner cavity of the cake, at least on one surface side of the said distributor, the radiating and cyclic blast grooves (11) are set, in the junction of the radiating and cyclic blast grooves, a distributing hole (12) is made.

5. The said chromatographic cake assembly of Claim 4, **characterized in that** the cross section of the said blast grooves (11) is triangular or cambered in shape.

6. The said chromatographic cake assembly of Claim 4, **characterized in that** the hole diameter of the said distributing hole (12) is increased gradually at the time when the center distance of the distributor (7) is increased.

7. The said chromatographic cake assembly of Claim 4, **characterized in that** a seal ring (8) is installed between the upper and lower clamp plates (3, 4) and the chromatographic packing (5).

8. A method of manufacturing a chromatographic cake assembly, comprising the following steps of:

    a) providing a chromatographic cake assembly comprising a pair of upper and lower clamp plates (3, 4) forming an inner cavity, the upper clamp plate (3) having a mobile phase inlet (1) and the lower clamp plate (4) having a mobile phase outlet (2), and a lateral hole or aperture (9) extending from an external region into the inner

cavity of the cake, wherein

- the ratio of the thickness to the radial length of the inner cavity of the chromatographic packing cake is smaller than or equal to 1,
- the thickness of the inner cavity is 0.2 - 50mm and the radial length is 5.0-1000 mm,

b) introducing a chromatographic packing from the lateral hole (9) of the chromatographic cake assembly into the inner cavity.

9. The method of Claim 8, wherein frits (6) are installed respectively on the sides of the inner cavity of the cake near the upper and lower clamp plates (3, 4) of the chromatographic cake assembly.

10. The method of Claim 8, comprising providing distributors (7) that are installed respectively between the upper and lower clamp plates (3, 4) and the frit (6), each distributor (7) being a plate that has the same shape just as the shape of the cross section of the inner cavity of the cake, at least on one surface side of the said distributor, the radiating and cyclic blast grooves (11) are set, in the junction of the radiating and cyclic blast grooves, a distributing hole (12) is made, the cross section of the said blast grooves (11) is triangular or cambered in shape, the hole diameter of the said distributing hole is increased gradually at the time when the center distance of the distributor is increased.

11. The method of Claim 8, **characterized in that** a seal ring (8) is installed between the upper and lower clamp plates (3, 4) and the chromatographic packing (5).

12. The method of Claims 8, or 9, or 10, **characterized in that** the lateral hole (9) of the said chromatographic cake assembly is directly connected with a slurry tank on the high pressure slurry packing machine for column packing.

13. The method of anyone of Claims 8, or 9, or 10, or 11, **characterized in that** the chromatographic cake assembly is directly installed on a high pressure slurry packing machine when a chromatographic cake with a diameter of more than 50 mm is manufactured, the chromatographic medium solution will be added through the lateral hole (9) on the chromatographic cake assembly in the inner cavity of the cake.

14. The method of anyone of Claims 8, or 9, or 10, or 11, **characterized in that** the column is packed with the suction method, and then the chromatographic cake will be installed on a high pressure slurry packing machine when a chromatographic cake with a diameter of more than 50mm is manufactured, the chromatographic packing cake solution will be added through the lateral hole (9) on the chromatographic cake assembly in the inner cavity of the cake.

15. The method of anyone of Claims 8, or 9, or 10, or 11, which is **characterized in that** the chromatographic packing (5) is added, the chromatographic cake is washed and the deteriorated chromatographic cake is replaced through the lateral hole (9) on the chromatographic cake assembly.

16. Use of a chromatographic cake assembly of any of Claims 1-7 for the separation of biopolymers.

17. Use of a chromatographic cake assembly of any of Claims 1-7 for the renaturation with simultaneous and purification of biopolymers.

Patentansprüche

1. Chromatographische Packkuchenanordnung, aufweisend ein Paar einer oberen und einer unteren Klemmplatte (3,4), die einen inneren Hohlraum bilden, wobei die obere Klemmplatte (3) einen Einlass (1) für eine mobile Phase hat und die untere Klemmplatte (4) einen Auslass (2) für eine mobile Phase hat, eine Chromatographiepackung (5), die in den inneren Hohlraum gepackt ist, und ein laterales Loch oder eine laterale Öffnung (9), welche(s) sich von einer externen Region in den inneren Hohlraum des Kuchens erstreckt, wobei:

- das Verhältnis der Dicke zur radialen Länge des inneren Hohlraums kleiner als oder gleich 1 ist,
- die Dicke des inneren Hohlraums 0,2 - 50 mm beträgt und die radiale Länge 5,0 - 1000 mm beträgt.

2. Chromatographische Packkuchenanordnung nach Anspruch 1, die Fritten (6) aufweist, die jeweils an den Seiten des inneren Hohlraums des Kuchens nahe der oberen und nahe der unteren Klemmplatte (3, 4) der chromatogra-

phischen Packkuchenanordnung angebracht sind.

3. Chromatographische Packkuchenanordnung nach Anspruch 2, die Verteiler (7) aufweist, die zwischen der oberen bzw. der unteren Klemmplatte (3, 4) und der Fritte (6) angebracht sind.

4. Chromatographische Packkuchenanordnung nach Anspruch 3, **dadurch gekennzeichnet, dass** der Verteiler (7) eine Platte ist, die die gleiche Form hat wie die Form des Durchschnitts des inneren Hohlraums des Kuchens, und an mindestens einer Oberflächenseite im Innern des Verteilers sternförmige und kreisförmige Rillen (11) angeordnet sind, wobei am Knotenpunkt der sternförmigen und kreisförmigen Rillen ein Verteilerloch (12) vorhanden ist.

5. Chromatographische Packkuchenanordnung nach Anspruch 4, **dadurch gekennzeichnet, dass** der Querschnitt der Rillen (11) von dreieckiger oder gewölbter Form ist.

6. Chromatographische Packkuchenanordnung nach Anspruch 4, **dadurch gekennzeichnet, dass** der Lochdurchmesser des Verteilerlochs (12) allmählich zunimmt, wenn der Mittenabstand des Verteilers (7) zunimmt.

7. Chromatographische Packkuchenanordnung nach Anspruch 4, **dadurch gekennzeichnet, dass** ein Dichtungsring (8) zwischen der oberen und der unteren Klemmplatte (3, 4) und der Chromatographiepackung (5) angebracht ist.

8. Verfahren zur Herstellung einer chromatographischen Packkuchenanordnung, das die Schritte aufweist:

(a) eine chromatographische Packkuchenanordnung bereitstellen, die ein Paar einer oberen und einer unteren Klemmplatte (3,4) aufweist, die einen inneren Hohlraum bilden, wobei die obere Klemmplatte (3) einen Einlass (1) für eine mobile Phase hat und die untere Klemmplatte (4) einen Auslass (2) für eine mobile Phase hat und ein laterales Loch oder eine laterale Öffnung (9), welche(s) sich von einer externen Region in den inneren Hohlraum des Kuchens erstreckt, wobei

- das Verhältnis der Dicke zur radialen Länge des inneren Hohlraums der chromatographischen Packkuchenanordnung kleiner als oder gleich 1 ist,
- die Dicke des inneren Hohlraums 0,2 - 50 mm beträgt und die radiale Länge 5,0 - 1000 mm beträgt und

(b) eine Chromatographiepackung von der lateralen Öffnung (9) der kuchenförmigen Chromatographie-Plattenanordnung in den inneren Hohlraum einführen.

9. Verfahren nach Anspruch 8, wobei Fritten (6) jeweils an den Seiten des inneren Hohlraums der chromatographischen Packkuchenanordnung nahe der oberen und der unteren Klemmplatte (3, 4) der chromatographischen Packkuchenanordnung angebracht werden.

10. Verfahren nach Anspruch 8, aufweisend: Verteiler (7) bereitstellen, die zwischen der oberen bzw. der unteren Klemmplatte (3, 4) und der Fritte (6) angebracht werden, wobei jeder Verteiler (7) eine Platte ist, die die gleiche Form hat wie die Form des Durchschnitts des inneren Hohlraums des Kuchens, und an mindestens einer Oberfläche im Innern des Verteilers sternförmige und kreisförmige Rillen (11) angeordnet sind, wobei am Knotenpunkt der sternförmigen und kreisförmigen Rillen ein Verteilerloch (12) angelegt wird, wobei der Querschnitt der Rillen (11) von dreieckiger oder gewölbter Form ist und der Lochdurchmesser des Verteilerlochs allmählich zunimmt wenn der Mittenabstand des Verteilers (7) zunimmt.

11. Verfahren nach Anspruch 8, **dadurch gekennzeichnet, dass** ein Dichtungsring (8) zwischen der oberen und der unteren Klemmplatte (3, 4) und der Chromatographiepackung (5) angebracht wird.

12. Verfahren nach einem der Ansprüche 8 oder 9 oder 10, **dadurch gekennzeichnet, dass** das laterale Loch,(9) der kuchenförmigen Chromatographie-Plattenanordnung direkt mit einem Aufschlämmungstank einer Hochdruck-Aufschlämmungspackmaschine zum Packen einer Säule verbunden ist.

13. Verfahren nach einem der Ansprüche 8 oder 9 oder 10 oder 11, **dadurch gekennzeichnet, dass** die Chromatographiekuchenanordnung, wenn ein Chromatographiekuchen mit einem Durchmesser von mehr als 50 mm hergestellt wird, direkt auf einer Hochdruck-Aufschlämmungspackmaschine montiert wird, wobei die Chromatographiemedium-Lösung durch das laterale Loch (9) der Chromatographiekuchen in den inneren Hohlraum gegeben wird.

14. Verfahren nach einem der Ansprüche 8 oder 9 oder 10 oder 11, **dadurch gekennzeichnet, dass** die Säule durch das Ansaugverfahren gepackt wird und, wenn eine Chromatographiekuchen mit einem Durchmesser von mehr als 50 mm hergestellt wird, der Chromatographiekuchen auf einer Hochdruck-Aufschlämmungspackmaschine montiert wird, wobei die Chromatographie-Packkuchen-Lösung durch das laterale Loch (9) der Chromatographiekuchenanordnung in den inneren Hohlraum gegeben wird.

15. Verfahren nach einem der Ansprüche 8 oder 9 oder 10 oder 11, **dadurch gekennzeichnet, dass** die Chromatographiepackung (5) zugegeben wird, der Chromatographiekuchen gewaschen wird und der Chromatographiekuchen, der sich verschlechtert hat, durch das laterale Loch (9) der Chromatographiekuchenanordnung ersetzt wird.

16. Verwendung einer Chromatographiekuchenanordnung nach einem der Ansprüche 1-7 zur Trennung von Biopolymeren.

17. Verwendung einer Chromatographiekuchenanordnung nach einem der Ansprüche 1-7 zur Renaturierung und gleichzeitigen Reinigung von Biopolymeren.

Revendications

1. Ensemble formant gâteau de garnissage chromatographique, comprenant une paire de plaques formant pince supérieure et inférieure (3, 4) formant une cavité interne, la plaque formant pince supérieure (3) ayant une entrée de phase mobile (1) et la plaque formant pince inférieure (4) ayant une sortie de phase mobile (2), un garnissage chromatographique (5) étant tassé dans la cavité interne, et un trou ou orifice latéral (9) s'étendant depuis une région externe dans la cavité interne du gâteau, dans lequel :

    - le rapport entre l'épaisseur et la longueur radiale de la cavité interne est inférieur ou égal à 1,
    - l'épaisseur de la cavité interne est comprise entre 0,2 à 50 mm et la longueur radiale est comprise entre 5,0 et 1 000 mm.

2. Ensemble formant gâteau de garnissage chromatographique selon la revendication 1, comprenant des frittes (6) respectivement installées sur des côtés de la cavité interne du gâteau près des plaques formant pince supérieure et inférieure (3, 4) de l'ensemble formant gâteau de garnissage chromatographique.

3. Ensemble formant gâteau de garnissage chromatographique selon la revendication 2, comprenant des distributeurs (7) respectivement installés entre les plaques formant pince supérieure et inférieure (3, 4) et la fritte (6).

4. Ensemble formant gâteau de garnissage chromatographique selon la revendication 3, **caractérisé en ce que** ledit distributeur (7) est une plaque ayant exactement la même forme que la forme de la section transversale de la cavité interne du gâteau, des rainures soufflantes rayonnantes et cycliques (11) étant établies au moins sur un côté superficiel du distributeur, un trou de distribution (12) étant réalisé dans la jonction des rainures soufflantes rayonnantes et cycliques.

5. Ensemble formant gâteau de garnissage chromatographique selon la revendication 4, **caractérisé en ce que** la section transversale desdites rainures soufflantes (11) est de forme triangulaire ou cambrée.

6. Ensemble formant gâteau de garnissage chromatographique selon la revendication 4, **caractérisé en ce que** le diamètre de trou dudit trou de distribution (12) est progressivement accru au moment où la distance centrale du distributeur (7) est accrue.

7. Ensemble formant gâteau de garnissage chromatographique selon la revendication 4, **caractérisé en ce qu'**une bague d'étanchéité (8) est installée entre les plaques formant pince supérieure et inférieure (3, 4) et le garnissage chromatographique (5).

8. Procédé de fabrication d'un ensemble formant gâteau de garnissage chromatographique comprenant les étapes suivantes consistant à :

    a) mettre à disposition un ensemble formant gâteau de garnissage chromatographique comprenant une paire de plaques formant pince supérieure et inférieure (3, 4) formant une cavité interne, la plaque formant pince

supérieure (3) ayant une entrée de phase mobile (1) et la plaque formant pince inférieure (4) ayant une sortie de phase mobile (2), et un trou ou orifice latéral (9) s'étendant depuis une région externe dans la cavité interne du gâteau, dans lequel

- le rapport entre l'épaisseur et la longueur radiale de la cavité interne du gâteau de garnissage chromatographique est inférieur ou égal à 1,
- l'épaisseur de la cavité interne est comprise entre 0,2 et 50 mm et la longueur radiale est comprise entre 5,0 et 1 000 mm,

b) introduire un garnissage chromatographique depuis le trou latéral (9) de l'ensemble formant gâteau chromatographique dans la cavité interne.

9. Procédé selon la revendication 8, dans lequel des frittes (6) sont respectivement installées sur les côtés de la cavité interne du gâteau près des plaques formant pince supérieure et inférieure (3, 4) de l'ensemble formant gâteau chromatographique.

10. Procédé selon la revendication 8, comprenant l'étape consistant à mettre à disposition des distributeurs (7) respectivement installés entre les plaques formant pince supérieure et inférieure (3, 4) et la fritte (6), chaque distributeur (7) étant une plaque de la même forme exactement que la forme de la section transversale de la cavité interne du gâteau, les rainures soufflantes rayonnantes et cycliques (11) étant établies au moins sur un côté superficiel dudit distributeur, un trou de distribution (12) étant réalisé dans la jonction des rainures soufflantes rayonnantes et cycliques, la section transversale desdites rainures soufflantes (11) étant de forme triangulaire ou bombée, le diamètre de trou dudit trou de distribution étant progressivement accru au moment où la distance centrale du distributeur est accrue.

11. Procédé selon la revendication 8, **caractérisé en ce qu'**une bague d'étanchéité (8) est installée entre les plaques formant pince supérieure et inférieure (3, 4) et le garnissage chromatographique (5).

12. Procédé selon les revendications 8, 9 ou 10, **caractérisé en ce que** le trou latéral (9) dudit ensemble formant gâteau de garnissage chromatographique est directement relié à une cuve de bouillie sur la machine de garnissage de bouillie haute pression pour garnissage de colonne.

13. Procédé selon l'une quelconque des revendications 8, 9, 10 ou 11, **caractérisé en ce que** l'ensemble formant gâteau de garnissage chromatographique est directement installé sur une machine de garnissage de bouillie haute pression lorsqu'un gâteau chromatographique d'un diamètre de plus de 50 mm est fabriqué, la solution de milieu chromatographique étant ajoutée par l'intermédiaire du trou latéral (9) sur l'ensemble formant gâteau de garnissage chromatographique dans la cavité interne du gâteau.

14. Procédé selon l'une quelconque des revendications 8, 9, 10 ou 11, **caractérisé en ce que** la colonne est tassée au moyen du procédé d'aspiration, puis le gâteau chromatographique est installé sur une machine de garnissage de bouillie haute pression lorsqu'un gâteau chromatographique d'un diamètre de plus de 50 mm est fabriqué, la solution de gâteau de garnissage chromatographique étant ajoutée par l'intermédiaire du trou latéral (9) sur l'ensemble formant gâteau de garnissage chromatographique dans la cavité interne du gâteau.

15. Procédé selon l'une quelconque des revendications 8, 9, 10 ou 11, **caractérisé en ce que** le garnissage chromatographique (5) est ajouté, le gâteau chromatographique est lavé et le gâteau chromatographique détérioré est remplacé par l'intermédiaire du trou latéral (9) sur l'ensemble formant gâteau de garnissage chromatographique.

16. Utilisation d'un ensemble formant gâteau de garnissage chromatographique selon l'une quelconque des revendications 1 à 7 pour la séparation de biopolymères.

17. Utilisation d'un ensemble formant gâteau de garnissage chromatographique selon l'une quelconque des revendications 1 à 7 pour la renaturation et la purification simultanée de biopolymères.

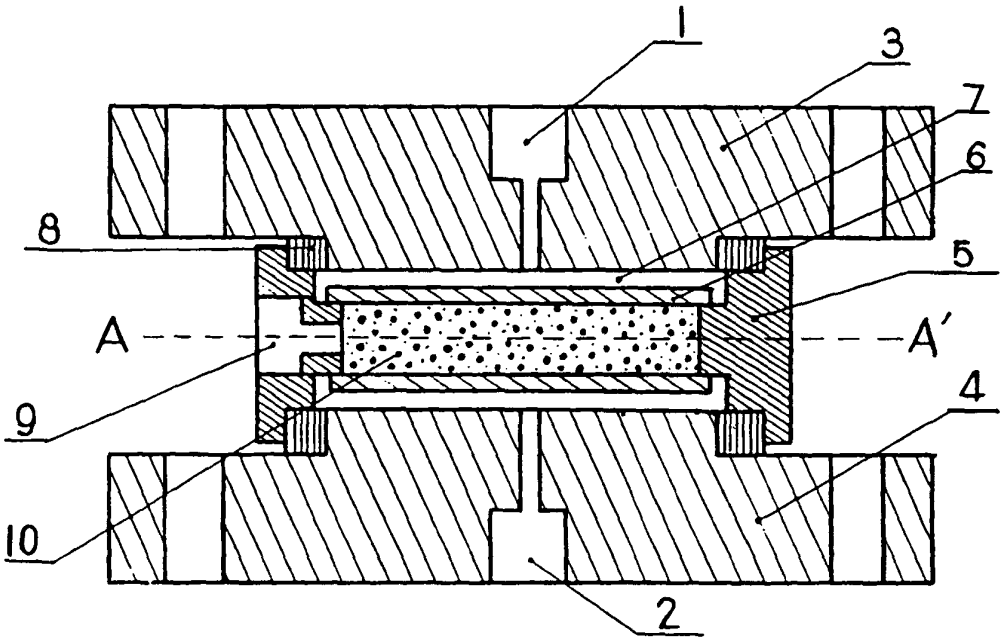

Fig. 1

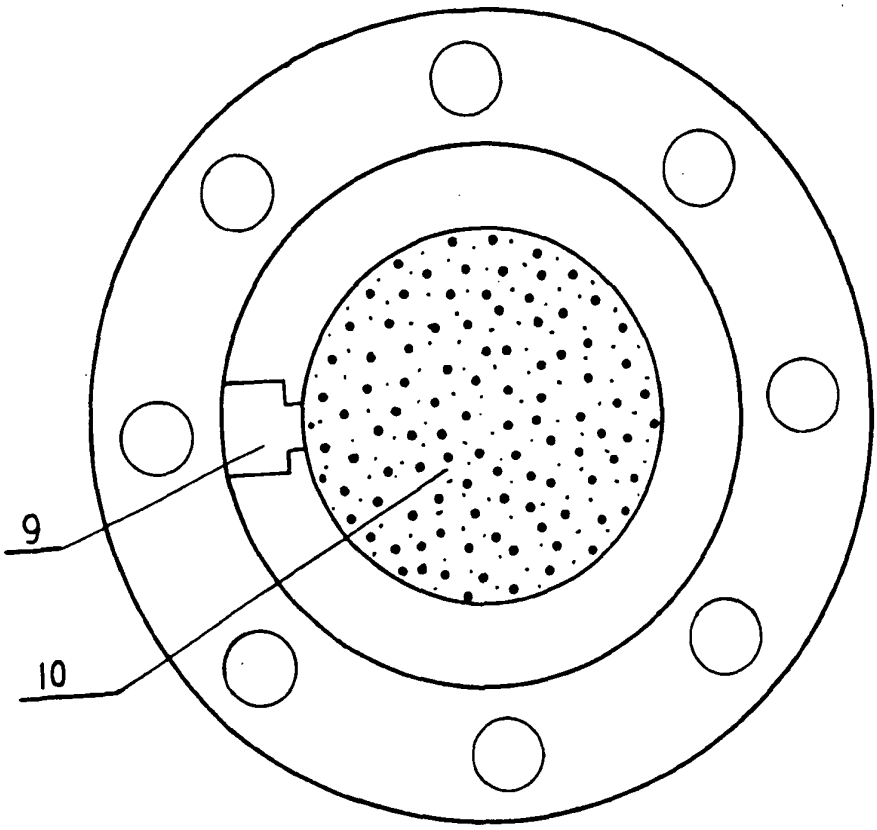

Fig. 2

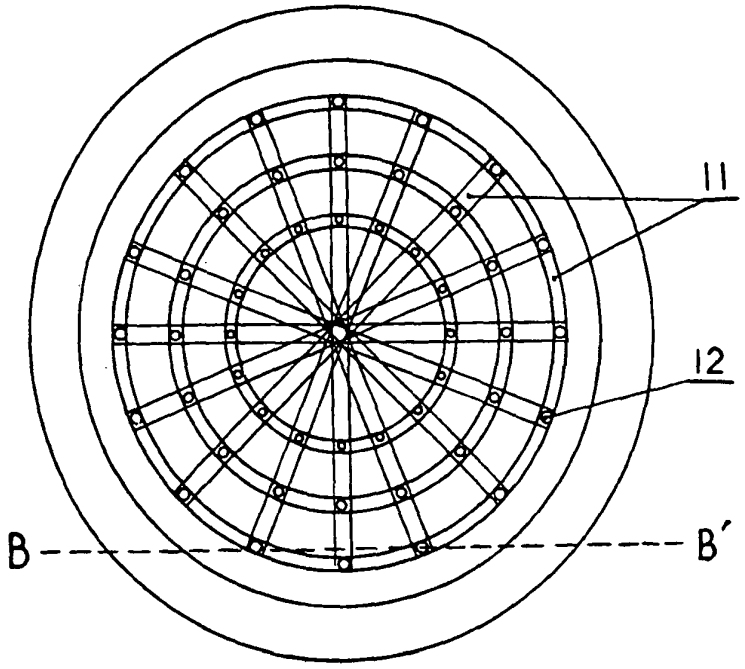

Fig. 3

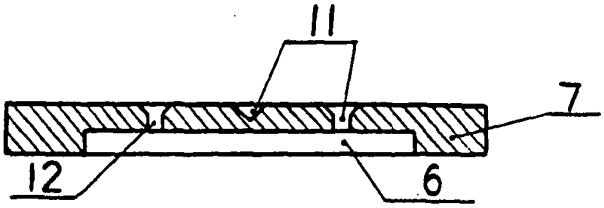

Fig. 4

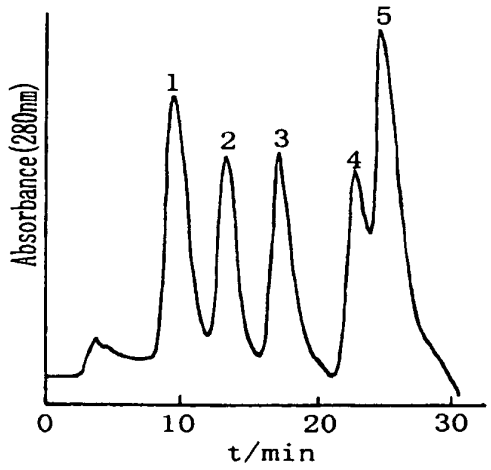
Absorbance (280nm)
1
2
3
4
5
0
10
20
30
t/min

Fig. 5

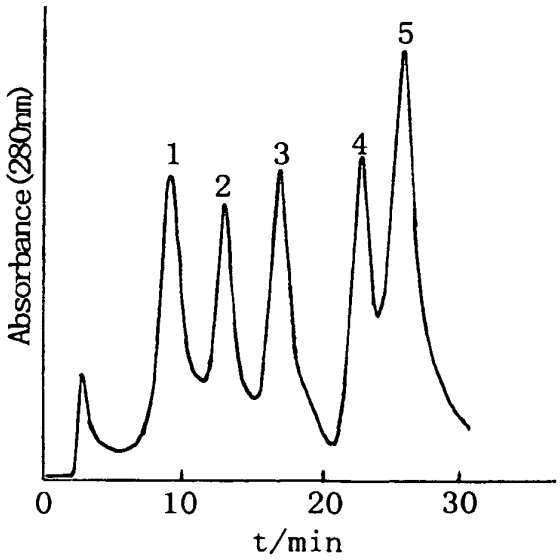
Absorbance (280nm)
1
2
3
4
5
0
10
20
30
t/min

Fig. 6

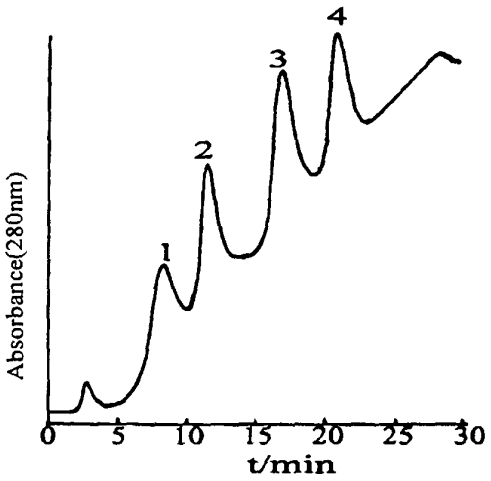
Absorbance(280nm)
1
2
3
4
0
5
10
15
20
25
30
t/min

Fig. 7

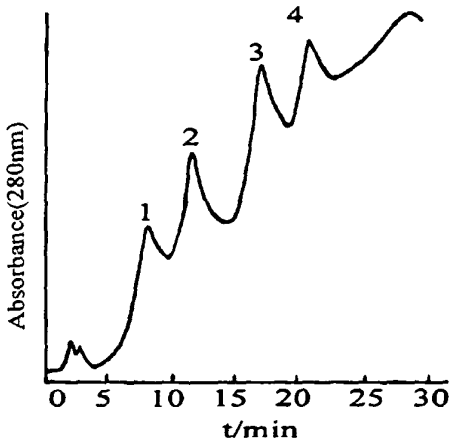
Absorbance(280nm)
1
2
3
4
0
5
10
15
20
25
30
t/min

Fig. 8

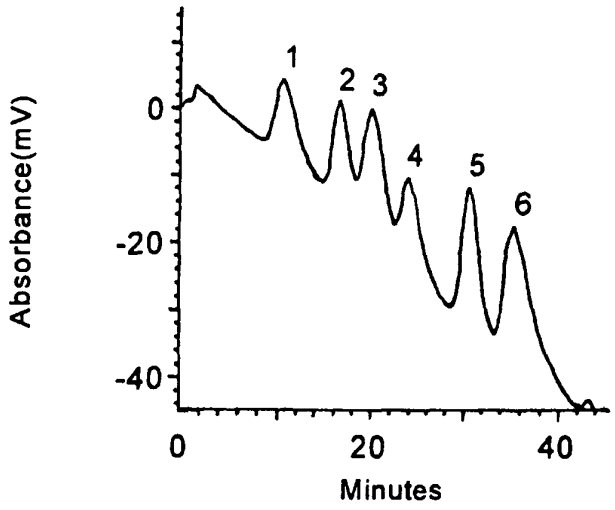
Absorbance(mV)
0
-20
-40
1
2
3
4
5
6
0
20
40
Minutes

Fig. 9

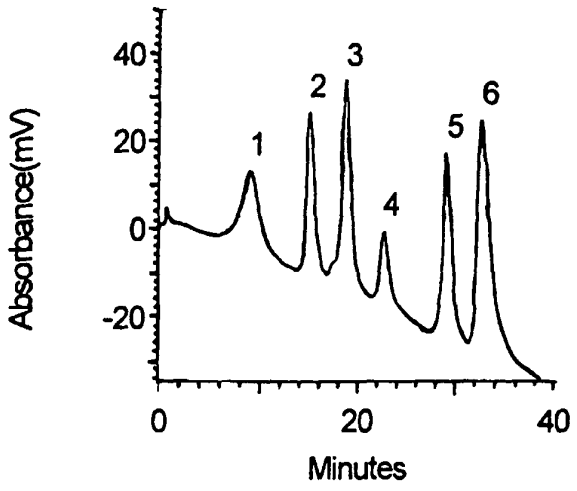
Absorbance(mV)
40
20
0
-20
1
2
3
4
5
6
0
20
40
Minutes

Fig. 10

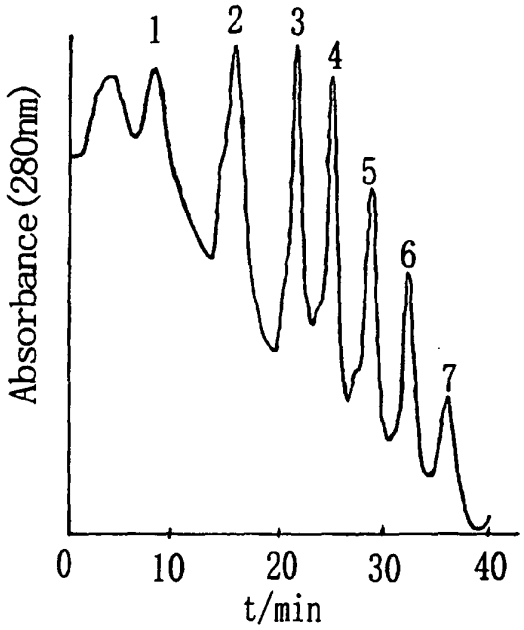
1
2
3
4
5
6
7
Absorbance (280nm)
0
10
20
30
40
t/min

Fig. 11

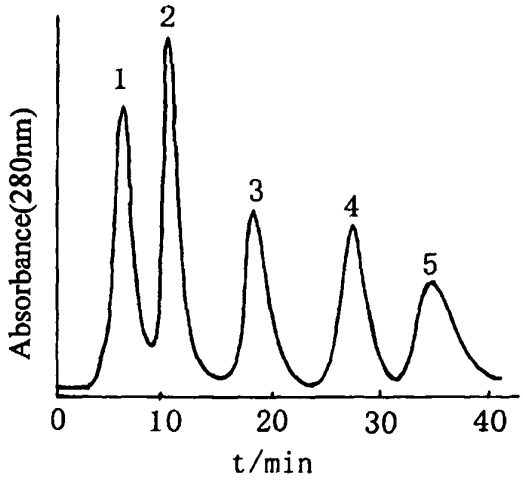
1
2
3
4
5
Absorbance(280nm)
0
10
20
30
40
t/min

Fig. 12

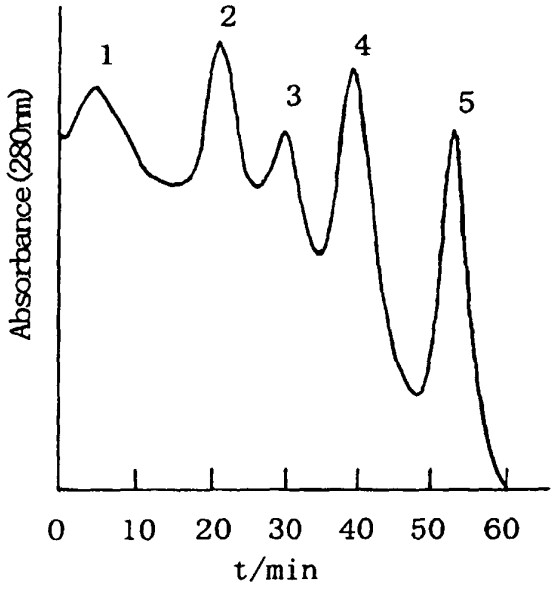
1
2
3
4
5
Absorbance (280nm)
0
10
20
30
40
50
60
t/min

Fig. 13

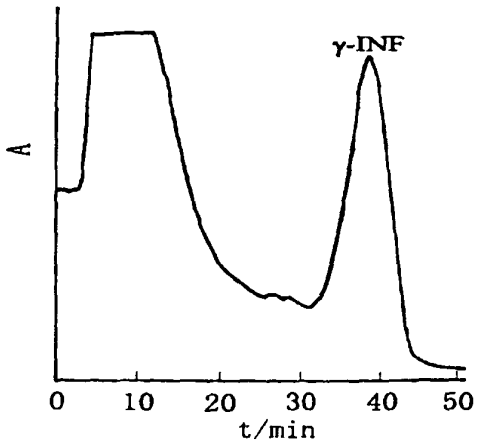
γ-INF
A
0
10
20
30
40
50
t/min

Fig. 14

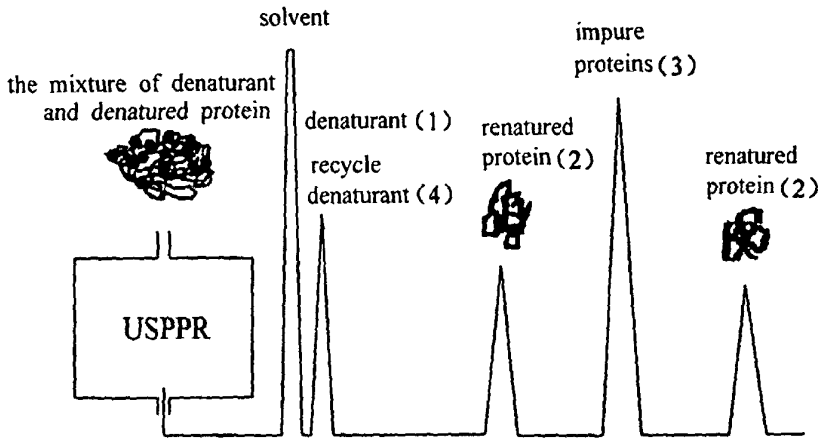
solvent
impure
proteins (3)
the mixture of denaturant
and denatured protein
denaturant (1)
recycle
denaturant (4)
renatured
protein (2)
renatured
protein (2)
USPPR

Fig. 15

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- EP 0021606 A **[0008]**

Non-patent literature cited in the description

- **TONG LIU ; XIN DU GENG.** The separation efficiency of biopolymers with short column in liquid chromatography. *Chinese Chemical Letters,* 1999, vol. 10 (3), 219-222 **[0009]**