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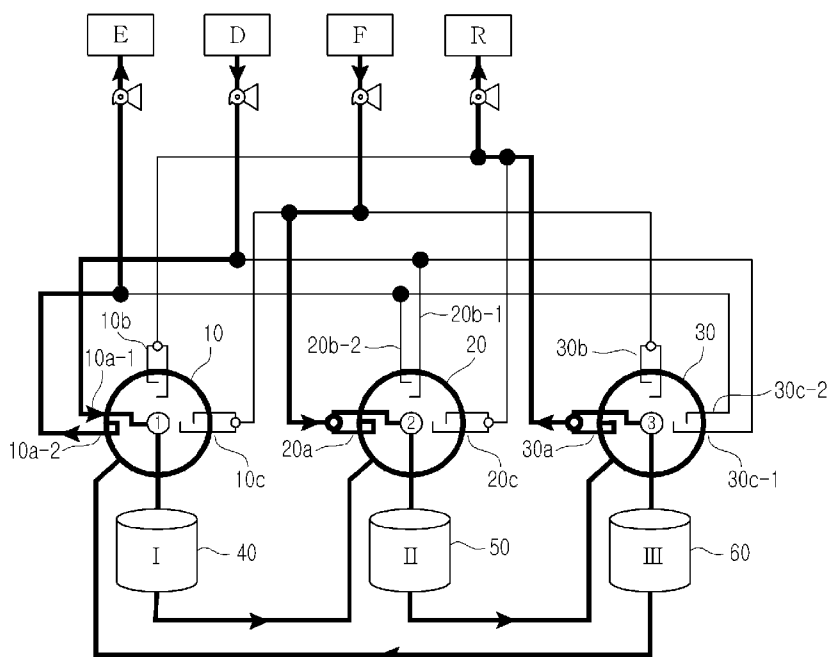(54) Title: AN APPARATUS FOR CONTINUOUS SEPARATION OF VALINE AND A METHOD FOR CONTINUOUS SEPAR-
ATION OF VALINE USING THE SAME

(57) Abstract: The present invention relates
to an apparatus for continuous separation of
valine from the mixture comprising amino
acids such as leucine, isoleucine, etc. and a
method for continuous separation of valine by
using the same, and the present invention can
continuously separate valine from the mixture
comprising amino acids such as leucine, iso-
leucine, etc. in a high purity and yield.

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Description

Title of Invention: AN APPARATUS FOR CONTINUOUS SEPARATION OF VALINE AND A METHOD FOR CONTINUOUS SEPARATION OF VALINE USING THE SAME

Technical Field

[1]   The present invention relates to an apparatus for continuous separation of valine and a method for continuous separation of valine using the same, and more specifically to an apparatus for continuous separation of valine from a mixture comprising amino acids such as leucine, isoleucine, etc. and to a method for continuous separation of valine using the same.

Background Art

[2]   Valine is an L-amino acid having a formula of $HO_2CCH(NH_2)CH(CH_3)_2$ and used as one of the main raw materials in medicine and in cosmetics. It is also one of the important ingredients utilized for animal feed. For this reason, there is a daily increase of interest within commercial markets focusing on the applications of valine.

[3]   The production of valine is generally achieved by a procedure of fermentation of *Corynebacterium*. In this regard, a matter of note is that impurities are also obtained along with valine during the fermentation procedure. These impurities include salt, alanine, leucine, isoleucine, etc., and all of these impurities are required to be separated from valine.

[4]   Separation methods for valine used in the prior art include an ion exclusion-chromatography method (Japanese Laid-open Patent Publication No. 1987-255453), a crystallization method using a precipitant (Japanese Laid-open Patent Publication No. 1996-333312, Japanese Laid-open Patent Publication No. 1998-237030, and U.S. Patent No. 6,072,083), a chemical reaction method (U.S. Patent No. 4,263,450), and the like. However, among the separation methods, the ion exclusion-chromatography method has disadvantages in that the separation of amino acids such as leucine and isoleucine is difficult, a lot of waste water is generated, and a post-treatment process such as crystallization, etc. is additionally needed. Also, since the crystallization method using the precipitant needs a process for removing the precipitant, it has the disadvantage that the process becomes complex and that a post-treatment process for purification is additionally needed. Further, since the chemical reaction method needs a concentration and hydrolysis process, it has the disadvantage that the process is complex and utilizes lot of solvents, and thus a number of labs are needed in the post-treatment process for recovering it, and the costs thereof in the purification process are accordingly increased.

[5]   Meanwhile, the chromatographic separation process is a separation process based on an adsorbent and is widely used in processes for the separation and purification of a plurality of bioproducts, and according to the application is broadly divided into a batch chromatography process and a continuous Simulated Moving Bed (SMB) chromatography process. The SMB process was initially developed for the separation of petrochemicals by UOP, USA in 1961, and it has been reported that its performance and separation efficiency is far superior to that of the batch process. Due to the superiority of the SMB process, it has been expanded to the separation and purification of high-value added products such as sugar materials, chiral compounds, bioproducts, medicines, etc. as well as the separation and purification of petrochemicals.

[6]   Most conventional SMB processes (four-zone SMB) are composed of a structure having four zones (columns) and four ports, as shown in Fig. 1, and each of the mixtures to be separated and the solvent (Desorbent) flow into a port (Feed) for the mixture to be separated and a desorption port (Desorbent) among the four ports. In addition, materials having weak adsorptive power and strong adsorptive power are separated and then obtained via Raffinate port (Raffinate) and Extract port (Extract). In order for the processes corresponding to the input of the mixture and the solvent to be separated and the recovery of the two components above to be continuously performed, the four ports are moved along the direction in which the solvent flows at a constant rate of switching.

[7]   However, the four-zone SMB process needs at least four columns. Generally, since both the valves of each column and the adsorbent are expensive, it is preferable to minimize the number of the columns if possible.

[8]   For this reason, a SMB process having three chromatography zones (three-zone SMB) as shown in Fig. 2 has been introduced. Since it uses three zones, the minimum number of columns can be reduced from four to three. In addition, the positions of the four ports are disposed in a manner identical in terms of structure relative to that of the conventional SMB structure having four chromatography zones.

[9]   However, the low-affinity component having weak adsorptive power and thus moving fast, such as valine in the above three-zone SMB process, is recovered via the Raffinate port as shown in Fig. 2 but, since there is no enrichment zone for the raffinate as in Fig. 2, excessive dilution is unavoidable. That is, since the concentration of valine recovered is lower than the valine concentration in the mixture to be separated, a side effect occurs in that operation cost for the post-treatment process following the SMB separation process is increased. Therefore, the application of the three-zone SMB process found in the new system is needed, in that it is able to overcome the side effect.

[10]   Accordingly, during research in consideration of the above-mentioned matters, the

present inventors have ascertained that valine can be continuously and efficiently separated from a mixture comprising amino acids such as leucine, isoleucine, etc., through an apparatus using a simulated moving bed chromatography process, thereby completing the present invention.

Disclosure of Invention

Technical Problem

[11]   An object of the present invention is to provide the apparatus for continuous separation of valine from the mixture comprising amino acids such as leucine, isoleucine, etc. and the method for continuous separation of valine using the same.

Solution to Problem

[12]   In order to resolve the above problem, the apparatus used for the separation of valine in the present invention includes a Desorbent port (D), a Feed port (F), a Raffinate port (R), an Extract port (E), three rotary valves (10, 20, 30), and three chromatography zones (40, 50, 60) connected to each of three rotary valves (10, 20, 30), as shown in Figs. 4a - 4c.

[13]   Three rotary valves (10, 20, 30) are equipped with three connection ports (10a, 10b, 10c)(20a, 20b, 20c)(30a, 30b, 30c), respectively, and only any one connection port of each rotary valve (10, 20, 30) is opened along with the rotation of the rotary valves (10, 20, 30), and is in fluid communication with the Desorbent port (D), the Feed port (F), the Raffinate port (R) and the Extract port (E).

[14]   That is, the flow passage connected to the Desorbent port (D), the Feed port (F), the Raffinate port (R) and the Extract port (E) have three branches, respectively, and thus, these are all connected to three rotary valves (10, 20, 30), and afterward are connected to a certain rotary valve concurrent with the opening of any one of the connection ports.

[15]   Hereinafter, the specific mode of operation is explained.

[16]   Fig. 3 is a schematic diagram for a constitution of the simulated moving bed process (three-zone SMB) having three chromatography zones used in the present invention. As can be seen from Fig. 3, since the displacement order of the ports is the Desorbent port, the Feed port, the Raffinate port and the Extract port, respectively, it is possible to operate the enrichment zone in such a way as to prevent the dilution of the Raffinate concentration. As a result, it has an advantage in that the degradation of the Raffinate concentration, which is a problem of the conventional three-zone SMB, can be prevented. In addition, since it has the structure wherein the Desorbent port and the Feed port are connected through one column, there is an advantage in that the amount of the solvent used can be reduced.

[17]   Further, the present invention provides the apparatus in which the process as

disclosed in Fig. 4 is continuously in operation. Fig. 4a depicts the apparatus in the first position, Fig. 4b depicts the apparatus in the second position, and Fig. 4c depicts the apparatus in the third position. The first, second and third positions are rotated between continuously. That is, the apparatus according to the present invention has a mode of operation wherein the chronological consists of the first position, the second position, the third position, and then a return to the first position again.

[18]   A setting to a certain position is made by the rotation of rotary valves (10, 20, 30). That is, the first connection ports (10a, 20a, 30a) of rotary valves (10, 20, 30) are opened and are thus set to the first position, the second connection ports (10b, 20, 30b) are opened by rotating the rotary valves (10, 20, 30) and thus are set to the second position, and the third connection ports (10c, 20c, 30c) are opened by rotating the rotary valves (10, 20, 30) again and are set to the third position. If the rotary valves (10, 20, 30) are rotated again, they are set to the first position again.

[19]   Meanwhile, hereinafter, some connection ports (10a, 20b, 30c) are separately shown with regard to inlet ports (10a-1, 20b-1, 30c-1) and outlet ports (10a-2, 20b-2, 30c-2) and explained for purposes of comprehension.

[20]   In the first position, as shown in Fig. 4a, only the first connection ports (10a, 20a, 30a) of the rotary valves (10, 20, 30) are opened, and the second connection ports (10b, 20b, 30b) and the third connection ports (10c, 20c, 30c) are closed.

[21]   In the first position, the desorbent port (D) is connected to the first rotary valve (10), the Feed port (F) is connected to the second rotary valve (20), the Raffinate port (R) is connected to the third rotary valve (30), and the Extract port (E) is connected to the first rotary valve (10).

[22]   Accordingly, the desorbent flowed from the Desorbent port (D) passes through the first rotary valve (10) and the first chromatography zone (40) and then flows in to the second rotary valve (20).

[23]   The mixture to be separated that is flowed from the Feed port (F) is flowed to the second rotary valve (20) together with the desorbent passed through the first chromatography zone (40), and then is passed through the second chromatography zone (50).

[24]   The mixture to be separated in the present invention comprises valine, and additionally comprises salt, alanine, leucine, isoleucine, etc. as impurities therein. The separation of the constituents of the mixture to be separated is achieved by a difference in flow rates after passing through the second chromatography zone (50). Valine is a low-affinity component that has a weaker adsorptive power than that of other impurities, and thereby moves faster. Therefore, the mixture to be separated is then separated into valine and other materials, and each of them flows into the third rotary valve (30) with the time difference.

[25]   Valine intended to be separated flows out via the Raffinate port (R) but, other materials are flowed out to the Extract port (E) by passing through the third chromatography zone (60) and then the first rotary valve (10).

[26]   After the set time (i.e., the rotational time interval of the rotary valve) has elapsed, the rotary valves (10, 20, 30) are rotated and then changed to the second position as shown in Fig 4b. A criterion of the set time is described below.

[27]   In the second position, shown in Fig. 4b, only the second connection ports (10b, 20b, 30b) of rotary valves (10, 20, 30) are opened, and the first connection ports (10a, 20a, 30a) and the third connection ports (10c, 20c, 30c) are closed.

[28]   Upon comparison with the first position, the rotary valves (10, 20, 30) connected to ports (D, F, R, E) are moved sequentially one by one in the second position.

[29]   That is, in the second position, the desorbent port (D) is connected to the second rotary valve (20), the Feed port (F) is connected to the third rotary valve (30), the Raffinate port (R) is connected to the first rotary valve (40), and the Extract port (E) is connected to the second rotary valve (20).

[30]   Accordingly, the desorbent flowed in from the desorbent port (D) is flowed in to the third rotary valve (30) after passing through the second rotary valve (20) and the second chromatography zone (50).

[31]   The mixture to be separated that is flowed in from the Feed port (F) is flowed in to the third rotary valve (30, together with the desorbent passed through the second chromatography zone (50), and is passed through the third chromatography zone (60).

[32]   However, in this case, the remaining materials of the mixture to be separated that have flowed in from the first position as shown in Fig. 4a have not yet been flowed into the third rotary valve (30), due to the difference in flow rates. In order to accomplish this, the rotation time interval mentioned above is controlled.

[33]   Therefore, the mixture to be separated that is flowed into the third rotary valve (30) at the second position passes through the third chromatography zone (60) and then the separation of the mixture to be separated is made due to the difference of the flow rates and is then separated into valine and other materials, wherein the valine intended to be separated is flowed out via the first rotary valve (10) and the Raffinate port (R) while remaining materials are combined with other materials that were separated in the first position but were not yet flowed into the third rotary valve (30), to then pass through the second rotary valve (20) more effectively and are then flowed into the Extract port (E).

[34]   After a lapse of the predetermined time period, the rotary valves (10, 20, 30) are rotated and changed to the third position, as shown in Fig. 4c.

[35]   In the third position, shown in Fig. 4c, only the third connection ports (10c, 20c, 30c) of the rotary valves (10, 20, 30) are opened, and the first connection ports (10a, 20a,

6

30a) and the second connection ports (10b, 20b, 30b) are closed.

[36] Upon comparison with the second position, the rotary valves (10, 20, 30) connected to ports (D, F, R, E) are sequentially moved one by one in the third position.

[37] Identical to the explanation of the first and second positions, the mixture to be separated are flowed in from the Feed port (F) and then are flowed into the first rotary valve (10) together with the desorbent passed through the third chromatography zone (60), and then passed through the first chromatography zone (40), wherein, since in this case the remaining materials of the mixture to be separated are flowed in at the second position as shown in Fig 4b and are not yet flowed into the second rotary valve (20) due to the difference of flow rates, they are also combined together and flowed into the Extract port (E) after passing through the third rotary valve (30).

[38] Valine intended to be separated in the mixture to be separated is separated to the Raffinate port (R) after passing through the second rotary valve (20).

[39] After the predetermined time period, the rotary valves (10, 20, 30) are rotated and changed to the first position as shown in Fig. 4a, and the procedure is continuously repeated.

[40] As the adsorbent used in the first, second and third chromatography zones in the present invention, a porous polymer resin can be used, and preferably, the resin consisting of an insoluble polystyrene divinylbenzene polymer material can be used. Since the above resin has a broad surface area, a unique pore size and volume distribution, it can be preferably applied for the purification of various materials, and in particular pharmaceutical compounds. Amberchrom CG161 (Rohm & haas) or Chromalite PCG Series (Purolite), etc. can be used as a specific example. The above commercially available resin has a diameter of a particle of 10 - 300 μm, a surface area of 700 - 900 m²/g, a pore size of 100 - 200 Å, a pore size and volume distribution of 0.7 - 1.5 ml/g, and a uniformity coefficient of less than 2.

[41] The mixture to be separated is a mixture comprising valine, isoleucine or leucine being included in the branched amino acids together with valine, and preferably may be valine fermentation liquor obtained through the fermentation of a microorganism. The specific examples of the present invention utilize the mixture in which the purified valine, isoleucine and leucine are artificially mixed, and the valine fermentation liquor obtained by the microorganism fermentation. This is because there is an infrequent case in which only valine is produced in the fermentation of the microorganism. In most cases, isoleucine and leucine are additionally produced together.

[42] By the continuous separation apparatus according to the present invention, since valine intended to be separated in the mixture to be separated is flowed out via the Raffinate port (R), and the remaining materials are more effectively separated by the rotation of rotary valves (10, 20, 30) as well as the difference of the flow rate and are

flowed out via the Extract port (E), thereby having valine be continuously separated.

[43]   Additionally, through control of the continuous separation apparatus of valine of the present invention, it can be controlled by regulating the flow rates of the desorbent port (D), the Feed port (F) and the Raffinate port (R), and also by regulating the rotation velocity (switching time) of the rotary valve. The flow rate of each port can be regulated in consideration of the intrinsic parameters of the column (adsorption co-efficient, mass transfer coefficient, porosity, material environment, etc).

[44]   In addition, the present invention provides the continuous separation method of valine characterized in that it comprises the steps of flowing the desorbent using the desorbent port, flowing the mixture comprising valine into the Feed port, and re-covering valine from the Raffinate port, by way of the method for the continuous separation of valine by using the continuous separation apparatus of the valine. The desorbent is preferably water. In addition, it is characterized in that the purity of valine recovered by the method is 90% to 99%.

[45]   According to one example of the present invention, as a result of the separation of the mixture to be separated comprising valine, leucine and isoleucine, by using the continuous separation apparatus of valine according to the present invention, the yield of the recovered valine was about 98% or more, the purity was about 98% or more, and thus, it was verified that valine could be effectively separated from the mixture.

Advantageous Effects of Invention

[46]   The present invention can continuously separate valine from the mixture comprising amino acids such as leucine, isoleucine, etc. while having high purity and yield, by way of the apparatus using the simulated moving bed chromatography process.

Brief Description of Drawings

[47]   Fig. 1 depicts the schematic diagram of the Simulated Moving Bed (four-zone SMB) chromatographic process having the conventional four chromatography zones.

[48]   Fig. 2 is the schematic diagram of the Simulated Moving Bed (three-zone SMB) chromatographic process having the conventional three chromatography zones.

[49]   Fig. 3 is the schematic diagram of the Simulated Moving Bed chromatographic process having three chromatography zones (three-zone SMB) used in the present invention and the process is developed based on the port disposition mode which is dif-ferentiated from the conventional three-zone SMB.

[50]   Fig. 4 shows a mimetic diagram of the apparatus for the three-zone SMB process used in the present invention. Figs. 4a, 4b and 4c show the connections of each zone according to the rotation of rotary valves, respectively.

[51]   Fig. 5 shows the pulse test results for selecting the adsorbent. Wherein, (a) represents the result for Amberchrom CG161C, (b) represents the result for Amberlite CG71C,

(c) represents the result for DIAION SK1B, and (d) represents the result for Amberlite XAD-7HP.

[52]   Fig. 6 shows the result of a stepwise frontal test for Amberchrom CG161C adsorbent. Wherein, (a) represents the result for isoleucine, (b) represents the result for leucine, and (c) represents the result for valine.

[53]   Fig. 7 shows the adsorptive equilibrium data (q vs. C) on Amberchrom CG161C adsorbent. Wherein, (a) represents the data for valine, (b) represents the data for leucine, and (c) represents the data for isoleucine.

[54]   Fig. 8 shows a comparison between the frontal test result of the mixture and the simulation result. Wherein, (a) represents the comparison of the result for leucine, (b) represents the comparison of the result for isoleucine, and (c) represents the comparison of the result for valine.

[55]   Fig. 9 shows the result of HPLC concentration analysis for outflow solution discharged through the SMB desorbent port, as SMB test result. Wherein, (a) represents the result for Raffinate concentration analysis, and (b) represents the result for the extract concentration analysis.

[56]   Fig. 10 shows a HPLC raw data chromatogram for the sample obtained from SMB test. Wherein, (a) represents the chromatogram of the mixture to be separated, (b) represents that of raffinate, and (c) represents that of the extract.

[57]   Fig. 11 shows a column profile (60.5 step), as a SMB test result. Wherein the black line represents the simulated result of valine, the gray line represents the simulated result of isoleucine, the black dotted line represents the simulated result of leucine, the circle represents the test result of valine, the quadrangle represents the test result of isoleucine, and the diamond represents the test result of leucine.

[58]   Fig. 12 shows the HPLC concentration analysis result of outflow solutions discharging via the Raffinate port, as the SMB test result conducted based on Chromalite PCG-600 adsorbent.

[59]   Fig. 13 shows the HPLC concentration analysis result for outflow solutions discharged via the Extract port, as the SMB test result conducted based on Chromalite PCG-600 adsorbent.

[60]   Fig. 14 shows a column profile (44.5 step), as SMB test result conducted based on Chromalite PCG-600 adsorbent. Wherein, the black line represents the simulated result of valine, the gray line represents the simulated result of leucine, the circle represents the test result of valine, and the triangle represents the test result of leucine.

Mode for the Invention

[61]   Hereinafter, it is intended to explain the constitution and effects of the present invention in detail via Examples, but these Examples are intended only to illustrate the

description of the present invention and the scope and spirit of the present invention is not intended to be limited by these Examples.

[62]

[63]   **Approach: Investigation for the chromatographic application of Valine purification**

[64]   1) Model-based design approach

[65]   There are two matters that are initially considered upon developing a continuous process for a new separation system. The first is the minimization of the cost and the period required for development. The second is the conditions providing the best output productivity and the best separation efficiency, by maintaining the optimum state of the process to be developed. In order to satisfy both conditions as above, one should precisely grasp the adsorption and material-transferring phenomenon based on the detailed model, and also obtain various parameters related thereto. The method of process design based on this detailed model and parameters is referred to as a "model-based design approach". Also, the present invention has developed a SMB process in carrying out the continuous separation of valine according to the approach.

[66]

[67]   2) Computer Simulation

[68]   One of the key stages of model-based design approach is a computer simulation. This refers to the procedure of obtaining the solution by solving the detailed model equation for the adsorption and material-transferring phenomenon of each component in a column by way of a numerical analytic method. This numerical analytic method is carried out by using a computer, since it requires a vast number of calculations.

[69]   There are many kinds of column model equations to be used in a simulation, and among them the lumped mass-transfer model (Kang, S.H. et al., Process Biochem., 2010, 45, 1468-1476) is determined as the simulation model of the present invention in consideration of accuracy and efficiency. The computer simulation based on lumped mass-transfer model was utilized in the assessment of separation efficiency of the SMB process as well as the measurement and assessment of base parameter of each amino acid component. Furthermore, this model equation is utilized in the manufacture of the SMB optimization computer tool.

[70]

[71]   3) SMB Optimization Tool

[72]   Another key role in the model-based design approach that is utilized after the computer simulation is played by the SMB optimized computer tool. This tool is used in obtaining the optimal operating conditions of SMB process to be developed. The matter first necessary for manufacturing this optimized tool is an optimization algorithm. Conventionally, it is known that a gene algorithm based on stochastic theory

is the most efficient in the complex form of the process optimization such as SMB (Lee, K.B. et al., AIChE J., 2008, 54, 2852-2871).

[73]   Also, the present invention has established a SMB optimized computer program based on a gene algorithm for optimization of continuous separation of valine. The gene algorithm itself has been developed several times in the interim, and the NSGA-II-JG algorithm (Lee, K.B. et al., AIChE J., 2008, 54, 2852-2871), which can be said to be the latest gene algorithm in the manufacturing stage of the optimization tool of the present invention, is adopted as the basic algorithm.

[74]   The method for preparing a SMB optimized tool codes the optimized algorithm by using visual basic application (VBA) language installed in Microsoft Excel software, and allows for the calculations of the detailed model equation and the NSGA-II-JG algorithm to be simultaneously carried out therein.

[75]

[76]   **Preparation of Experiment**

[77]   1) Materials

[78]   Valine and leucine, among three amino acid components constituting the mixture to be separated were purchased from Fluka, and isoleucine was purchased from Sigma. Water used to dissolve amino acid was tertiary Distilled Deionized Water (DDW) and obtained through the Milli-Q system (Millipore). Adsorbent used in the experiment was Amberchrom CG161C (Rohm & haas), Amberlite CG71C (Sigma Aldrich), DIAION SK1B (Mitsubishi Chemical), Amberlite XAD-7HP (Sigma Aldrich). Methanol used in HPLC concentration analysis was purchased from Burdick & Jackson Co. (Muskegon, MI).

[79]   An Omnifit glass column used in the adsorbent selection experiment and the measurement experiment of the basic parameter of each material was purchased from Biochemical Fluidics Co. (Boonton, NJ), and in the former experiment the column having 11.6 cm of length and 1.5 cm of diameter was used, and in the latter experiment the column having 21.7 cm of length and 2.5 cm of diameter was used.

[80]

[81]   2) Equipment

[82]   - Single column experimental equipment

[83]   Young-Lin SP930D pump and Young-Lin UV730D detector were used in the adsorbent selection experiment. The control and data processing of these two pieces of equipment was made by Autochro-3000 software. An injector used in injecting the amino acid pulse into the column each filled with adsorbent was the Rheodyne 7725i injector, and the injection volume was 100 $\mu\ell$.

[84]   In the measurement experiment of the basic parameter of each amino acid conducted after completing the adsorbent selection, FPLC P-920 pump, Waters 486 UV detector,

and Amersham FPLC collector (Frac-900) were used. The control and data collection of each of the detailed devices were made through Unicorn 5.1 software.

[85]

[86]   - Three-zone SMB apparatus for continuous separation of valine

[87]   The apparatus as shown in Fig. 4 was self-assembled and used. The flow rate of the desorbent of the completed SMB experimental apparatus and the flow rate of the mixture to be separated were controlled by using the Young-Lin SP930D pump and the raffinate flow rate were controlled by the Ismatec MCP-CPF ISM 919 pump. In order to generate the effect wherein four ports are periodically moved, a ST Valco rotary valve (VICI, Houston, TX) was used. The mimetic diagrams of the ST valve are represented in Figs. 4a-4c. A Valco rotary valve used in the experiment was automatically controlled through Labview 8.0 software.

[88]

[89]   - HPLC concentration analysis device (analyzer)

[90]   In order to measure the concentration of the material collected through SMB experiment, a HPLC concentration analyzer was used. As the column for the analysis, Waters Symmetry C-18 column (250 × 4.6 mm ID, particle size 5 μm) was used. The collection of all data related to HPLC concentration analysis was treated by using Waters Millennium software. The flow rate of the mobile phase was controlled by using the Waters 515 HPLC pump. The sample for the concentration analysis was injected into the column for analysis by using a Rheodyne 9725i injector, and the injection volume was 20 $\mu\ell$. The detection of a sample was conducted by using a Waters 996 PDA detector.

[91]   In the concentration analysis of the sample obtained from a frontal test of valine, a Young-Lin SP930D pump and a Young-Lin UV730D detector were used. The control and data processing of the Young-Lin pump and detector were conducted by Autochro-3000 software. The sample obtained from the experiment was injected into the column for the analysis by using a Rheodyne 7725i injector, and the injection volume was 20 $\mu\ell$.

[92]

[93]   **Example 1: Selection of an adsorbent suitable for separation of valine**

[94]   1) Experimental Method

[95]   The experiment to select the most suitable adsorbent was conducted by using four kinds of conventional adsorbents that have the possibility of separating leucine and isoleucine from valine, such as Amberchrom CG161C (Rohm & haas), Amberlite CG71C (Sigma Aldrich), DIAION SK1B (Mitsubishi Chemical), Amberlite XAD-7HP (Sigma Aldrich), through the manner of a series of pulse tests.

[96]

[97]   The conditions for the pulse test experiments were as follows: Each of the concentrations of three amino acids was 2 g/L, and the injection volume was 100 $\mu\ell$. The flow rate of the mobile phase was 2 ml/min.

[98]

[99]   2) Experimental Results

[100]   The results are represented in Fig. 5. As represented in Fig. 5, it could be ascertained that in case of other adsorbents other than the Amberchrom CG161C adsorbent, the selective separation of valine is difficult. It is ascertained from the above results that the Amberchrom CG161C adsorbent is most suitable for establishing SMB process for separating valine.

[101]

[102]   **Example 2: Measurement of porosity of Amberchrom CG161C column**

[103]   1) Experimental method

[104]   The porosity of the Amberchrom-CG161C adsorbent as selected in Example 1 was measured. At first, the experiment that fills the column with the adsorbent, and then measures an inter-particle porosity (e_b) was conducted. Although it is conducted in the same manner as Example 1, in this instance a tracer molecule suitable for measuring the inter-particle porosity was injected into the column instead of the material to be separated, unlike as in Example 1. The tracer molecule used was blue dextran, and the injection concentration was 1 g/L, the flow rate was 4 ml/min and the injection volume was 500 $\mu\ell$.

[105]

[106]   2) Experimental results

[107]   From the experimental results, the value of the inter-particle porosity of the column was 0.391. The intra-particle porosity, which is one of the important information used the value which is reported in the reference, was 0.737 (Nam, H.G. et al., Process Biochem., 2011, 46, 2044-2053).

[108]

[109]   **Example 3: Measurement of the basic parameter of valine, leucine, isoleucine on Amberchrom CG161C**

[110]   **- Measurement of adsorption coefficient**

[111]   1) Experimental method

[112]   A multiple frontal test was performed in order to measure the basic parameters of valine, leucine, isoleucine that correspond to each component of the mixture which is a subject of the separation. The multiple frontal test is the experiment that is carried out to obtain the adsorption equilibrium data for each material to be separated, and to define the adsorption model equation and determine the relevant adsorption parameters based on the obtained data.

[113]   Specifically, a multiple frontal test used two pumps, and one of the pumps was filled with DDW, and another pump was filled with valine, leucine or isoleucine solution. Then, valine, leucine or isoleucine solution was continuously injected into the column until the equilibrium between the adsorbent phase and mobile phase in the column was achieved. In the equilibrium state, since all of the concentrations between the adsorbent particle and the particle as well as the concentration inside the adsorbent are maintained similar to the concentration of valine, leucine, or isoleucine solution injected, the adsorbent concentration on the adsorbent can be immediately investigated by establishing a simple Mass Balance Equation. After the concentration inside the column reaches an equilibrium state, a new equilibrium state is allowed to be maintained by increasing the ratio of the mixture solution which is the subject of the separation, compared to that of the previous stage. Again, the adsorption concentration on the adsorbent is calculated by establishing a Mass Balance Equation for this equilibrium state. While progressing the multiple frontal test, a step time for each amino acid component is set as 2-3 times the residence time obtained from the results of the pulse test.

[114]   Also, the multiple frontal test for each amino acid was conducted as a total of five steps, and the step time is set as 40 min for valine, 70 min for leucine, and 70 min for isoleucine. In addition, the concentration of all samples was 5 g/L, and the flow rate was constantly maintained as 4 ml/min. The experiments for leucine and isoleucine were conducted in waves of 205 nm, and 218 nm at UV detector. On the other hand, in the case of valine, a direct HPLC analysis manner (the manner sampling the column effluents one by one and then analyzing the concentrations of them by using HPLC device), which is not an on-line monitoring manner as used in the leucine and isoleucine experiments was adopted. The HPLC analyzing conditions are that 10% aqueous methanol solution was used as the mobile phase, the injection volume was 20 $\mu\ell$, and the flow rate was 0.5 ml/min. Before the analysis, a self calibration curve was secured by using a standard solution. The results are represented in Fig. 6, the adsorption equilibrium data is calculated based on these results, and the calculated results are represented on Fig. 7.

[115]

[116]   2) Experimental Results

[117]   As shown in Figure 7, all of valine, leucine and isoleucine represent the linear adsorption relationship on Amberchrom CG161C. Therefore, each of the amino acid components is modeled by the linear adsorption equation, and the linear adsorption coefficient was determined therefrom. The values of the determined adsorption coefficients are represented in Table 1, as below.

[118]

[119]   **- Determination of a mass-transfer coefficient**

[120]   1) Experimental method

[121]   A mass transfer coefficient which can be referred to as another important basic parameter together with the adsorption coefficient was determined as per the following method.

[122]   At first, an axial dispersion coefficient and film mass-transfer coefficient were respectively expected by using Chung & Wen correlation (Chung, S. F. et al., AIChE J., 1968, 14, 857-866) and Wilson & Geankopolis correlation (Wilson, E. J. et al., Ind. Eng. Chem. Fundam., 1966, 5, 9-14), and a molecular diffusivity was calculated by using Wilke & Chang correlation (Wilke, C.R. et al., AIChE J.. 1955, 1, 264-270). Also, intra-particle diffusivity was determined while fitting the frontal experimental data and the simulation results based on the lumped mass-transfer model each other.

[123]

[124]   2) Experimental results

[125]   The values of the molecular diffusivity and the intra-particle diffusivity determined above are represented in Table 1 as below:

[126]   Table 1

[Table 1]

	Linear adsorption coefficient	Intra-particle diffusivity(cm^2/min)	Molecular diffusivity(cm^2/min)
leucine	5.527	5.40×10^{-5}	5.40×10^{-4}
isoleucine	4.361	3.00×10^{-4}	4.35×10^{-4}
valine	0.926	3.00×10^{-4}	5.10×10^{-4}

[127]

[128]   In order to ascertain the suitability of the values of the adsorption and mass transfer coefficient as determined above, the results of model simulation and frontal experimental results substituting the values were compared as shown in Figure 6.

[129]   As shown in Figure 6, it can be ascertained that the simulation results and the experimental data coincide well. Therefore, the value of the basic parameter as determined above can be sufficiently utilized for the optimization of SMB process, as conducted later.

[130]

[131]   **Example 4: Investigation of the basic parameter by a frontal experiment of the mixture**

[132]   1) Experimental method

[133]   A frontal experiment of the mixture was carried out in order to investigate the basic

parameter value determined in Example 3. In this experiment, the frontal experiment was conducted by using the mixture solution comprising all of three amino acids as the mixture to be separated, unlike as in Example 3.

[134]   Also, the model simulation was conducted on grounds similar to those of the basic parameter values determined in Example 3 and the mixture frontal experimental conditions. Additionally, the obtained simulation results were compared to the frontal experimental data of the mixture, and the results are represented in Figure 8.

[135]   2) Experimental results

[136]   As shown in Figure 8, it can be ascertained that the experimental data coincided well with the simulation results. This means that the basic parameter values determined in Example 3 can well explain the behavior of each amino acid in the column in the state of being in the mixture as well as in the state of being a separate component. Furthermore, it means that interaction between each of the amino acids is rarely present.

[137]

[138]   **Example 5: Optimization of the process for continuous separation of valine**

[139]   The matter focused upon in the optimization procedure is to maximize the productivity of valine while assuring the high purity and high yield of valine that is the target of the separation. Wherein the productivity of valine is defined as per the following equation:

[140]   - Productivity of valine = $Q_{raf} * C_{raf,valine}$

[141]   (wherein, Q_{raf} is the flow rate in the Raffinate port and, $C_{ref,valine}$ means the concentration of valine in the Raffinate port.)

[142]   The productivity of valine was established as an objective function and the purity and yield of valine were established as a constraint, to conduct the optimization procedures. The concrete optimization procedures are summarized as follows:

[143]   Max J = Productivity [Q_{feed}, Q_{raf}, t_{sw}]

[144]   Subject to Valine purity = 98%

[145]   Valine yield = 98%

[146]   Fixed variables Q_{des} = 5 mL/min

[147] C_{feed} for each component = 5 g/L

[148] L_C = 21.7 cm, d_C = 2.5 cm

[149]   (wherein, Q_{feed} and Q_{des} independently refer to the flow rate of the mixture to be separated and the flow rate of the desorbent, respectively, and t_{sw} refers to the switching time.)

[150]

[151]   In order to optimize the separation process of valine using the three-zone SMB device of the present invention according to the equation, the optimized computer program based on the NSGA-II-JG algorism was self-coded, and the computer

program was connected to an Aspen simulator to optimize the relevant process. The results are represented in Table 2 as below.

[152]   Table 2

[Table 2]

Inlet and outlet flow rates (mL/min)	Q_{feed}	2.42
	Q_{raf}	2.71
	Q_{ext}	4.71
	Q_{des}	5.00
Zone flow rates (mL/min)	Q_1	5.00
	Q_2	7.42
	Q_3	4.71
Switching time (min)		21.33
Valine Purity (%)		98.0
Valine yield (%)		98.1

[153]

[154]   **Example 6: SMB Experiment**

[155]   By using the date obtained above, valine was separated by the continuous separation device of valine according to the present invention, and the yield and purity thereof were measured as follows:

[156]

[157]   1) Experimental method

[158]   As shown in Fig. 4, a SMB experiment was carried out by using the optimized conditions derived from above Example 5. Amberchrom CG161C was used as the column.

[159]   To begin, the column was connected prior to the start of the experiment. At this time, the flow rate of desorbent (water) was maintained as 2 ml/min, and the operation of the other pump was halted. Each of the valve and column were connected, while at this time care was taken such that no air is entered inside the column. After completing all the connections of the columns, the flow rate of the desorbent was increased up to the target value. Since the start of the SMB experiment is for the time that the mixture solution to be separated is injected, the pump of the mixture to be separated was operated concurrent to the operation of Labview 8.0 software. The switching and switching time of the valve were simultaneously controlled via Labview 8.0.

[160]   The SMB experiment was progressed until it sufficiently reached a cyclic steady state. It was ascertained that the steady state was achieved after the 25th step.

Therefore, the SMB experiment progressed up to the 60th step, which is a value greatly in excess of that of the steady state. Throughout the SMB experiment, at every step, the concentration of the solution eluted from each outlet port was analyzed by using the HPLC device. Also, in order to secure the column profile, the relevant sample was taken at the last step. For the purpose of this, all driving pumps were stopped when the SMB experiment reached the 60.5 step. Additionally, the solution exiting from the column by opening the lower part of the column connected to the valve was collected, and the concentration of the solution was analyzed. HPLC analysis conditions were that: 10% aqueous methanol solution was used as the mobile phase, the injection volume was 20 $\mu\ell$, and the flow rate was 0.5 ml/min. Before the analysis, the self-calibration curve was secured by using the standard solution. The results are represented in Figure 9. In Figure 10, (a) is the analysis result of the raffinate concentration, and (b) is the analysis result of the extract concentration.

[161]

[162]   2) Experiment results

[163]   As shown in Figure 9a, it could be ascertained that most of the components in the raffinate solution were valine. Leucine and isoleucine, belonging to the impurities, were rarely present. This means that the purity of valine being maintained is very high. Also, as shown in Figure 9b from the analysis result of the extract concentration, most of the components were leucine and isoleucine, and the detected amount of valine was of a negligible quantity. This means that the loss of valine via the outlet port is minimized.

[164]   Also, the computer simulation for the relevant SMB process was carried out simultaneously with the progress of the SMB experiment, and the result was directly compared with the HPLC concentration analysis data of the raffinate and the extract. As shown in Figure 9, it can be seen that the simulation result and SMB experimental data correlated well.

[165]   In order to make a quantitative measure of the final purity and yield of valine separated by SMB experiment as mentioned above, the effluent solutions obtained during the last six steps were mixed in the same ratio and HPLC concentration analysis for the solution was then conducted. Based on the analyzed concentration data, the purity and yield of valine were calculated, and the results were represented in Table 3 as below.

[166]   Table 3

[Table 3]

	Experiment	Simulation
Yield (%)	98.25	98.01
Purity (%)	97.50	98.03

[167]

[168]   From the results of Table 3 and Fig. 9, it could be ascertained that the SMB process using Amberchrom CG161C of the present invention is superior in assuring the continuous separation of valine and maintaining high purity and high yield throughout. As the experimental evidence data for this, a raw chromatogram of HPLC concentration analysis (HPLC raw data) was represented in Figure 10.

[169]   At first, in HPLC raw data (Fig. 10a), for the mixture solution to be separated all of three amino acid components represented large peaks. In HPLC raw data (Fig. 10b) for the Raffinate port effluent that belongs to the production port of valine, only the valine component clearly showed a large peak while the peak of isoleucine showed a very small peak, and further the peak of leucine was rarely found. In HPLC raw data (Fig. 10c) for the Extract port effluent which is established so as to obtain only data corresponding to the impurity, the peak of the valine component is negligible, while the two remaining amino acid components showed a large peak. Through this series of HPLC raw data, it could be ascertained again that the SMB experiment for the continuous separation of valine carried out as in the present invention was successfully conducted.

[170]   In addition to the concentration graphs of the raffinate and extract as mentioned above, the column profile data is also important SMB experimental data. For this reason, the samples required for securing the column profile data were taken during the halfway mark of the final switching period, that is, at step 60.5. After analyzing the sample concentration, the result was represented in Fig. 12. As can be seen from Fig. 11, it can be ascertained that the column profile data also correlates well with the simulation result. This discloses that the column profile proves the SMB experiment was conducted well. That is to say, it can be said that the facts as experimentally verified that solute waves of each of the amino acids in the SMB column were distributed so that they are fully advantageous to a high purity and yield.

[171]   Therefore, from all of the SMB experimental data (the concentration graph at the outlet port, column profile) mentioned above, it could be seen that the SMB process of the present invention using Amberchrom CG161C is sufficiently applicable to the continuous separation process of valine on an industrial scale.

[172]   The above mentioned SMB process was optimized based on Amberchrom CG161C adsorbent, and also verified experimentally. In addition to this adsorbent, SMB process

based on Chromalite PCG-600 wherein the effect on the valine separation was verified was also conducted for optimization, and the experiment in this regard was also conducted. As a result, it was ascertained that Chromalite PCG-600 adsorbent was also sufficiently utilizable for the continuous separation of valine upon being applied to the SMB process.

[173]

[174]   **Example 7: Experiment in the actual fermentation mixture**

[175]   According to a method similar to that of the previous experiment, an experiment was carried out by using the actual fermentation mixture.

[176]   In order to measure the basic parameter (adsorption and mass transfer coefficient) of valine and leucine components in the actual fermentation mixture, a mixture frontal experiment was conducted wherein the solution of the fermentation mixture was injected into a single column filled with Chromalite PCG600C resin while the concentration of column effluent was measured over the time.

[177]   The basic parameters of valine and leucine were determined by using the concentration profile data obtained from the experiment and the inverse method, and the results are given in Table 4.

[178]   Table 4

[Table 4]

	Linear isotherm parameter	Intra-particle diffusivity(cm^2/min)	Molecular diffusivity(cm^2/min)
valine	0.6065	1.20×10^{-5}	5.10×10^{-4}
leucine	3.925	1.75×10^{-5}	5.40×10^{-4}

[179]

[180]   On the basis of the basic parameters given in the above Table 4, the PCG600C-SMB process continuously separating valine and leucine from the actual fermentation mixture was optimally designed. This SMB process was also based on the new port disposition order of the present invention, and the column configuration adapted the three-zone structure as shown in Fig. 3.

[181]   The matter being focused upon in the optimization procedure of PCG600C-SMB process was the maximization of the productivity of valine while assuring a high yield of valine and a high removal efficiency of leucine. In order to achieve this optimization purpose, the productivity of valine was established as an objective function, and the yield of valine and the removal rates of leucine were established as the constraint to the conduct of the optimization procedure. The concrete optimization procedures are summarized below. Qdes is the value which is controlled within the scope that the

purity and yield of valine can be increased.

[182]   Max J = Productivity [Q_{feed}, Q_{raf}, t_{sw}]

[183]   Subject to Valine purity = 97%

[184]   Leucine removal efficiency = 90%

[185]   Fixed variables Q_{des} = 6 mL/min

[186] L_C = 21.7 cm, d_C = 2.5 cm

[187]   Column configuration = 1 - 1 - 2

[188]   (wherein, Q_{feed} and Q_{des} refer to the flow rate of the mixture to be separated and the flow rate of the desorbent, respectively, and t_{sw} refers to the switching time.)

[189]   In order to conduct the optimization of PCG600C-SMB process according to the above equation, the optimized computer program based on NSGA-II-JG algorithm was self-coded, and the computer program was connected to the Aspen simulator to obtain the optimization of the relevant process. The optimization results are given in Table 5.

[190]   Table 5

[Table 5]

Inlet and outlet flow rates (mL/min)	Q_{feed}	3.27
	Q_{raf}	3.93
	Q_{ext}	5.34
	Q_{des}	6.00
Zone flow rates (mL/min)	Q_1	6.00
	Q_2	9.27
	Q_3	5.34
Switching time (min)		17.85

[191]

[192]   In order to experimentally verify the optimization results of Table 5, PCG600C-SMB process device was self-assembled. The mimetic diagram of the assembled process device is given in Fig. 4.

[193]   The SMB experiment for separating valine wherein the actual fermentation mixture is the subject was conducted by using the optimization result of Table 2 and the process device of Fig. 4. The concentrations of valine and leucine in the actual fermentation mixture used in this experiment were 71.8 g/L and 0.742 g/L, respectively.

[194]   The results of the SMB experiment for separating valine, wherein the actual fermentation mixture was the subject are represented in Figs. 12-14. As shown in the result of the raffinate effluent history in Fig. 12, the high concentration recovery of valine was made in accordance with the level being expected by the simulation, and

the effluent concentration level of leucine was low enough to the extent of being considered a negligible quantity. As shown in the result of the extract effluent history of Fig. 13, it could be ascertained that leucine is removed in accordance with the level expected by the simulation. Also, it could be ascertained that the loss of valine via an Extract port was minimized. These experimental results mean that a high yield of valine and a high removal rate of leucine can be sufficiently assured by the continuous separation of valine and leucine by the PCG600C-SMB process.

[195]   Further to the effluent history results of Figs. 12 and 13, the column profile result of Fig. 14 also comprehensively shows that the continuous separation of valine and leucine was successfully conducted. As shown in Fig. 14, it can be ascertained that the concentration distribution of valine and leucine in each column is made to be very advantageous in assuring a high yield recovery of valine and a high removal rate of leucine. Due to these results, 99.7 % of valine in the actual fermentation mixture could be recovered via the Raffinate port, and at the same time 98.0 % of leucine could be removed via the Extract port.

Claims

[Claim 1] An apparatus for continuous separation of valine, which comprising:

a Desorbent port (D);

a Feed port (F);

a Raffinate port (R);

an Extract port (E);

a number of rotary valves (10, 20, 30) each selectively connected to the ports (D, F, R, E); and

a number of chromatography zones (40, 50, 60) each equipped with every a number of rotary valves (10, 20, 30),

wherein the number of rotary valves (10, 20, 30) are connected to each other,

wherein the number of rotary valves (10, 20, 30) are equipped with the number of connection ports (10a, 10b, 10c) (20a, 20b, 20c) (30a, 30b, 30c), respectively, and

wherein only any one of the number of connection ports (10a, 10b, 10c) (20a, 20b, 20c) (30a, 30b, 30c) is opened, along with the rotation of the number of rotary valves (10, 20, 30), and subsequently any one of the rotary valves (10, 20, 30) selectively connected to each of the ports (D, F, R, E) are changed.

[Claim 2] The apparatus for continuous separation of valine according to claim 1, wherein:

the number of rotary valves (10, 20, 30) rotate after a short period of time, and

the rotary valves (10, 20, 30) connected to the ports (D, F, R, E) are changed, along with the rotation of the number of rotary valves (10, 20, 30).

[Claim 3] The apparatus for continuous separation of valine according to claim 2, wherein:

the number of rotary valves (10, 20, 30) are continuously changed so as to rotate among the first, second and third positions along with the rotation after the short period of time,

the first connection ports (10a, 20a, 30a) are opened at the first position,

the second connection ports (10b, 20b, 30b) are opened at the second position, and

the third connection ports (10c, 20c, 30c) are opened at the third

position.

[Claim 4]   The apparatus for continuous separation of valine according to claim 3, wherein, at the first position,

the Desorbent port (D) is fluid-communicated with the first chromatography zone (40) through the first connection port (10a) of the first rotary valve (10),

the Feed port (F) is fluid-communicated with the second chromatography zone (50) through the second connection port (20a) of the second rotary valve (20),

the second chromatography zone (50) is fluid-communicated with the Raffinate port (R) through the third connection port (30a) of the third rotary valve (30), and

the Extract port (E) is fluid-communicated with the third chromatography zone (60) through the first connection port (10a) of the first rotary valve (10).

[Claim 5]   The apparatus for continuous separation of valine according to claim 4, wherein:

the mixture to be separated flowed in via the Feed port (F) at the first position is separated into valine and the remaining materials, along with passing through the second chromatography zone (50),

the valine separated from the first position flows in to the third rotary valve (30) and then flows out via the Raffinate port (R), and

the remaining materials separated from the first position flow into the first rotary valve (10) and then flow out via the Extract port (E).

[Claim 6]   The apparatus for continuous separation of valine according to claim 5, wherein:

the short period of time is the time that the valine separated from the mixture to be separated is flowed into the third rotary valve (30), but the remaining materials are not flowed into the third rotary valve (30).

[Claim 7]   The apparatus for continuous separation of valine according to claim 6, wherein:

when the rotary valves (10, 20, 30) rotate from the first position to the second position, the mixture to be separated flowed in via the Feed port (F) is separated to valine and the remaining materials, along with passing through the third chromatography zone (60),

the valine separated at the second position is flowed into the first rotary valve (10) and then flowed out via the Raffinate port (R), and

the remaining materials separated from the second position are flowed

into the second rotary valve (20), along with the remaining materials separated during the first position and then flowed out via the Extract port (E).

[Claim 8]   The apparatus for continuous separation of valine according to claim 1, wherein:

the number of rotary valves (10, 20, 30) are alternatively changed at the first, second and third positions by the continuous rotation, and

the rotary valves to which the ports (D, F, R, E) are connected at the first, second and third positions are different from each other.

[Claim 9]   The apparatus for continuous separation of valine according to claim 8, wherein:

the mixture to be separated flowed in via the Feed port (F) is separated to the valine and the remaining materials, and

the rotation interval in which the number of rotary valves (10, 20, 30) change their positions is the time that the valine moves any one of the rotary valves to another rotary valve but the remaining materials do not move.

[Claim 10]   A method for continuously separating valine by using the apparatus for continuous separation of valine as defined in any one of claims 1 to 9, comprising:

flowing a desorbent into the Desorbent port,

flowing a mixture comprising valine into the Feed port, and

recovering valine from the Raffinate port.

[Claim 11]   The method for continuously separating valine according to claim 10, wherein the desorbent is water.

[Claim 12]   The method for continuously separating valine according to claim 10, wherein the purity of the valine recovered is 90% to 99%.
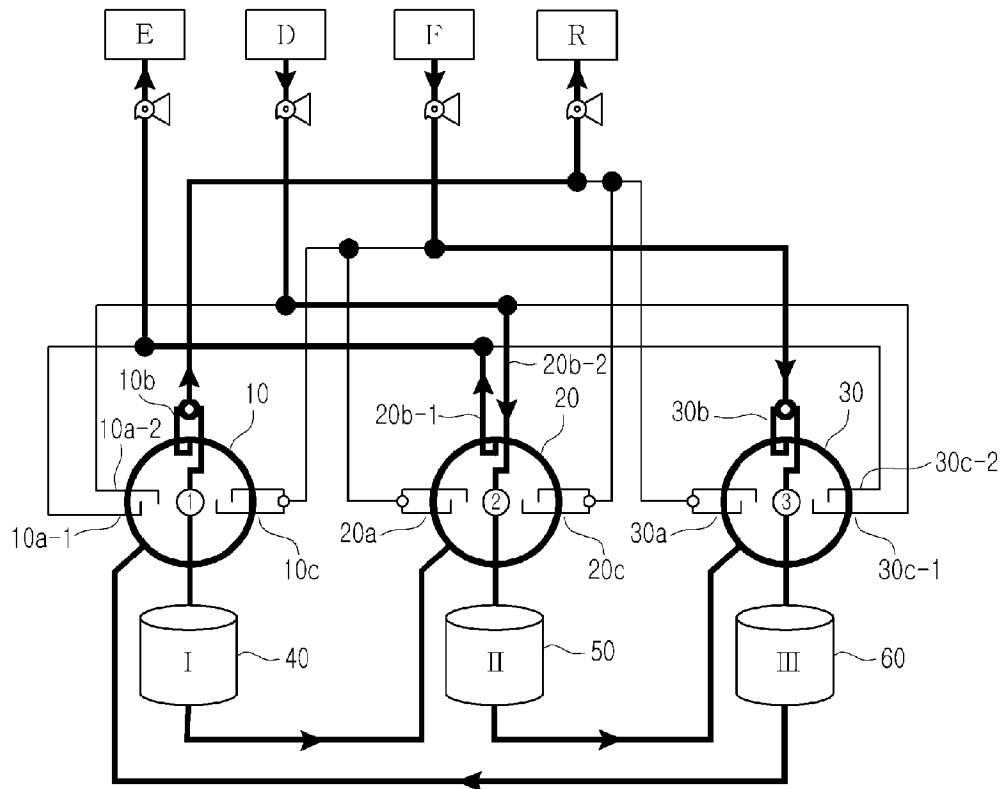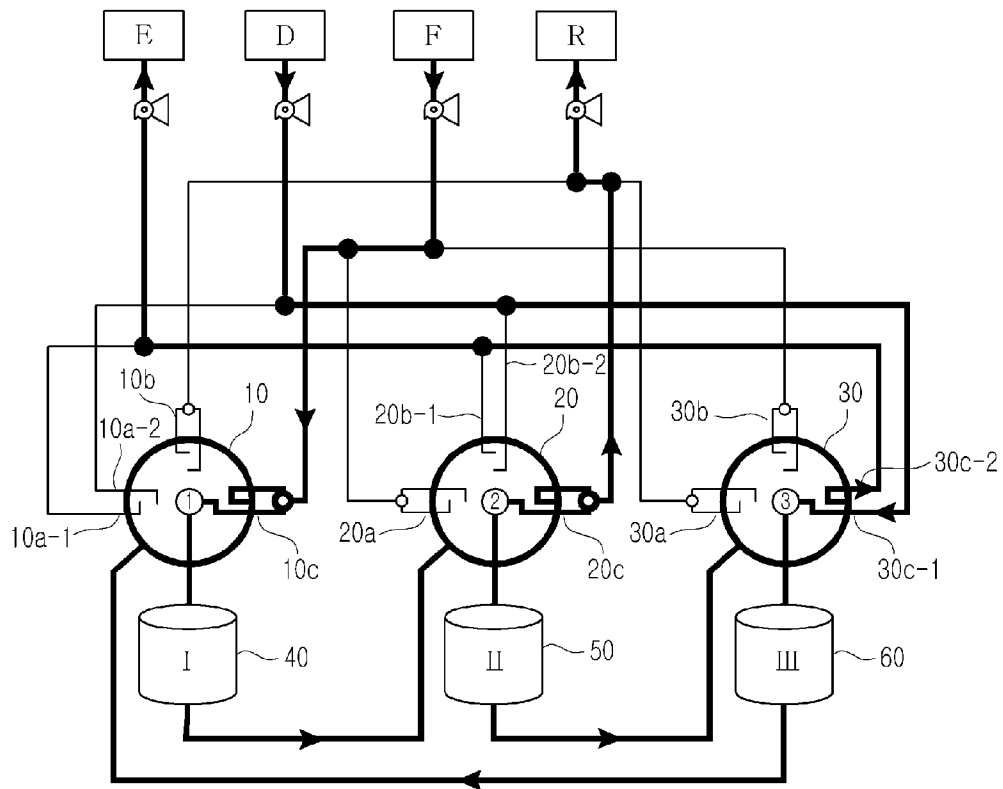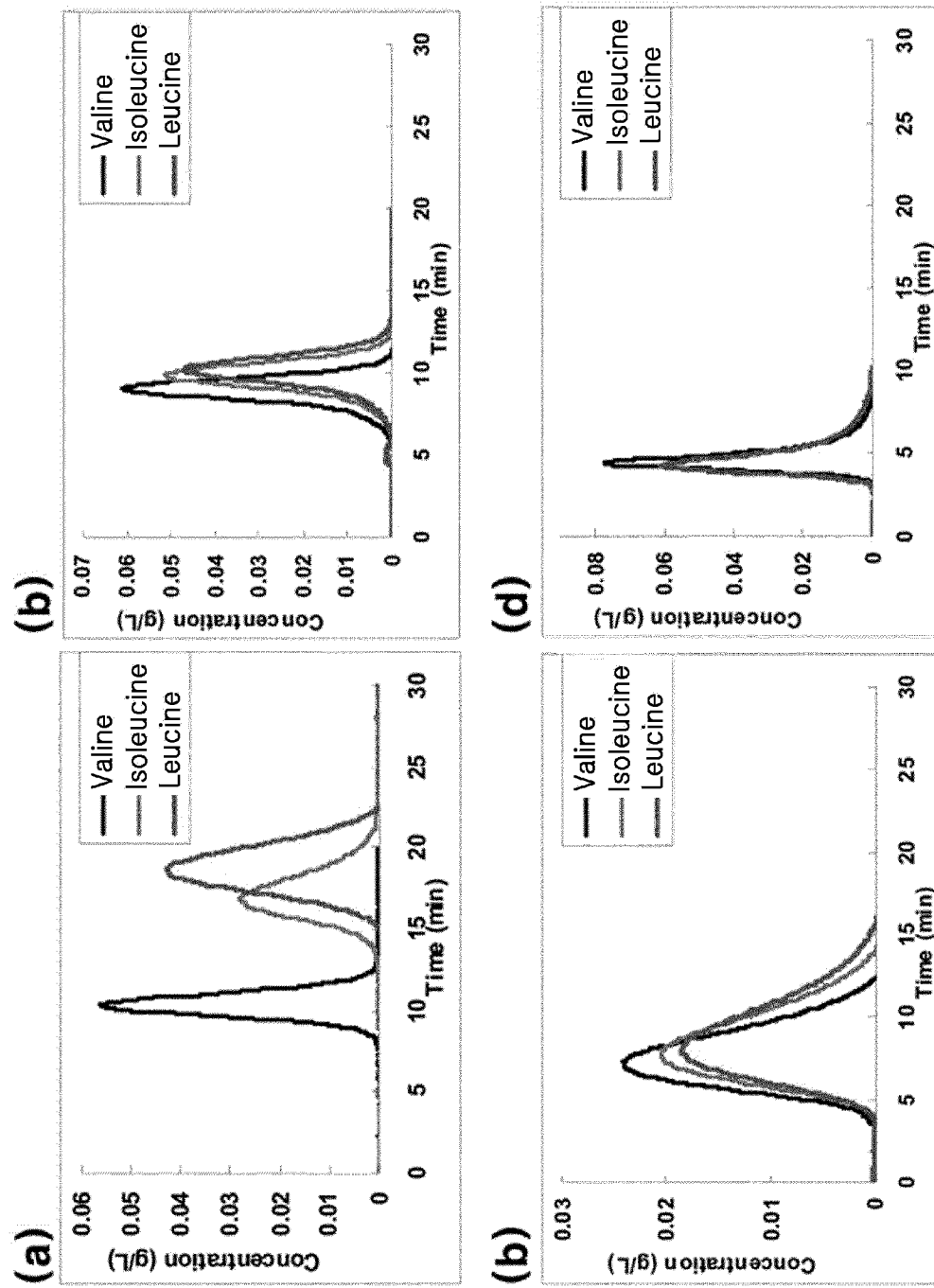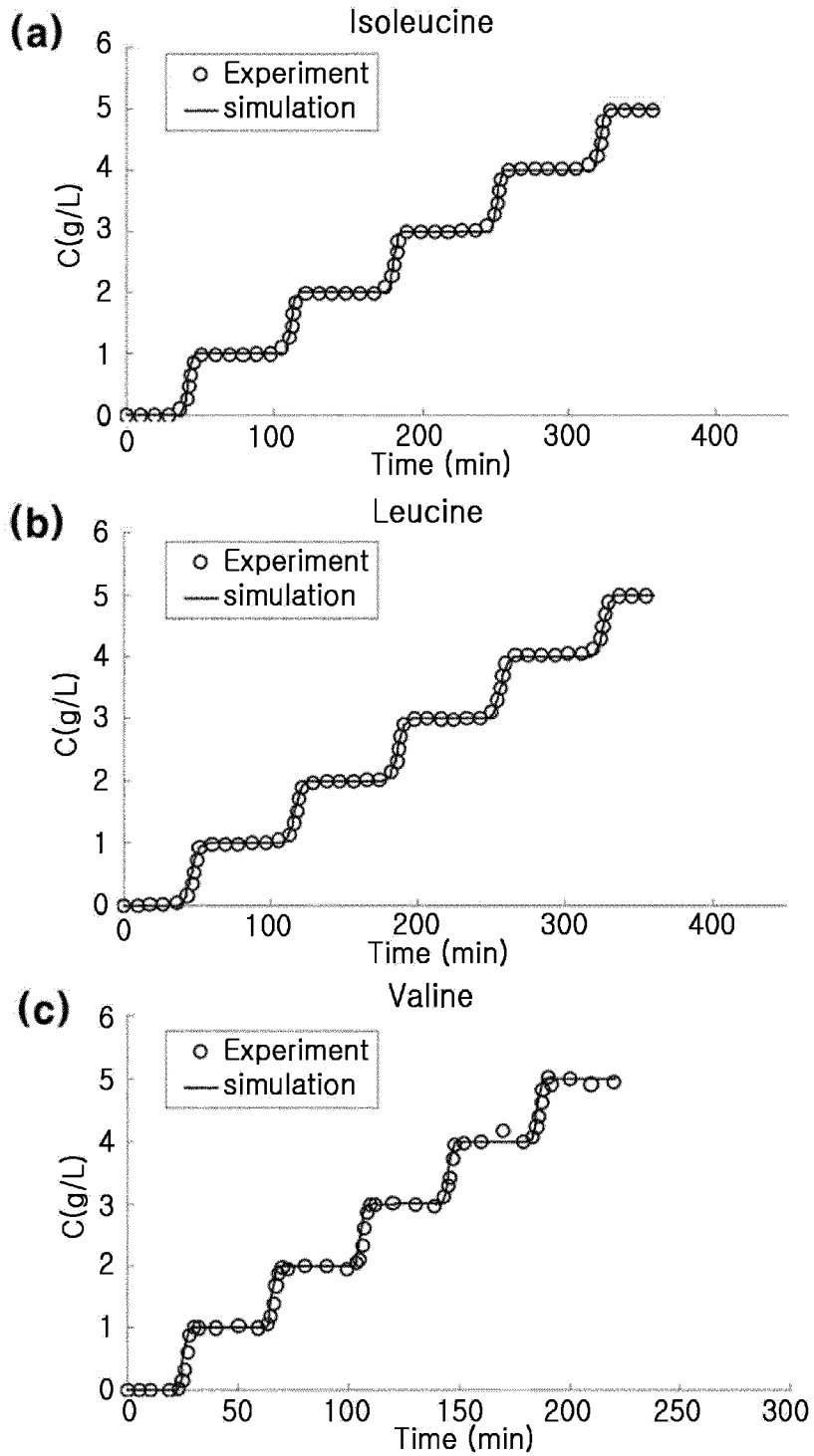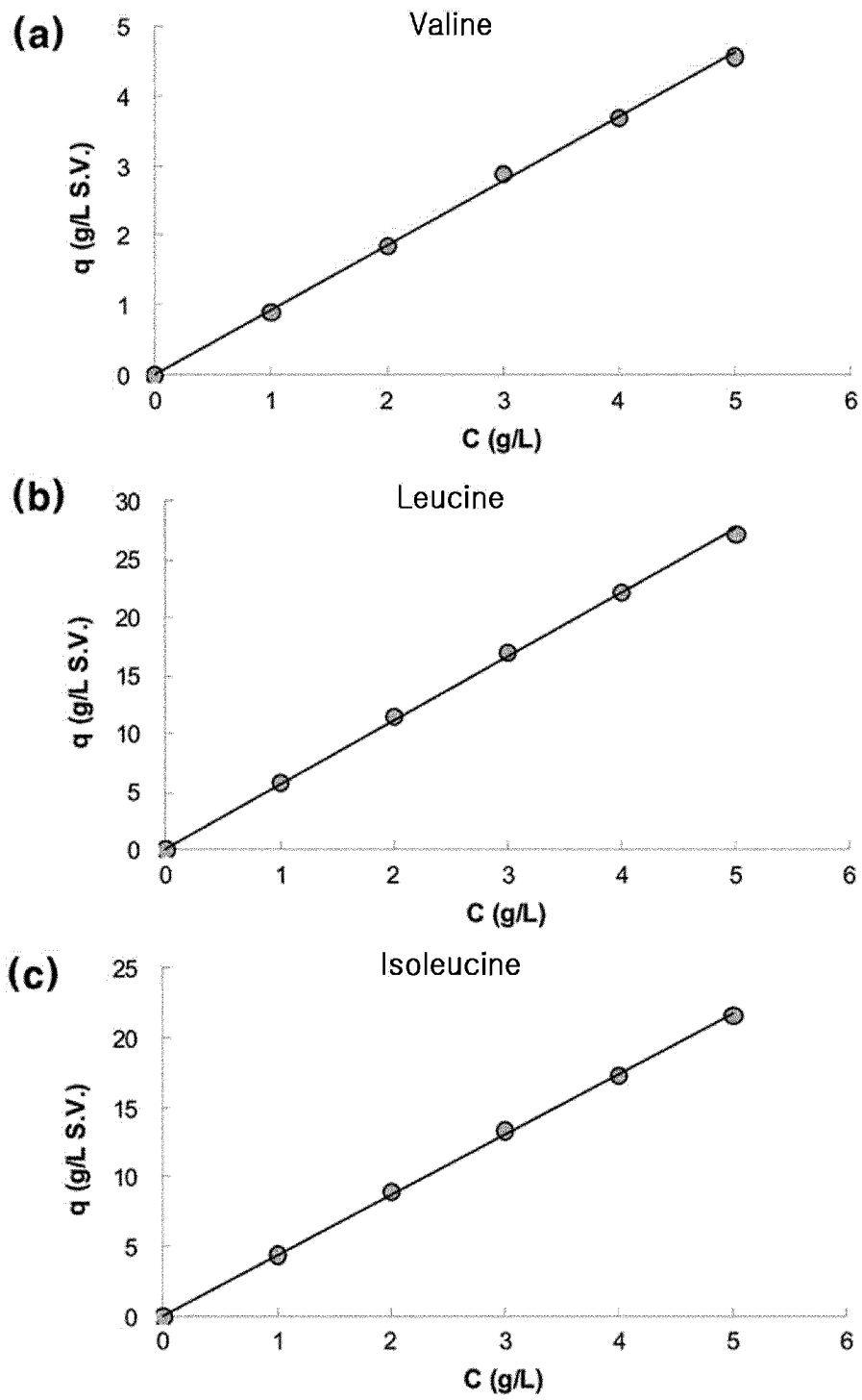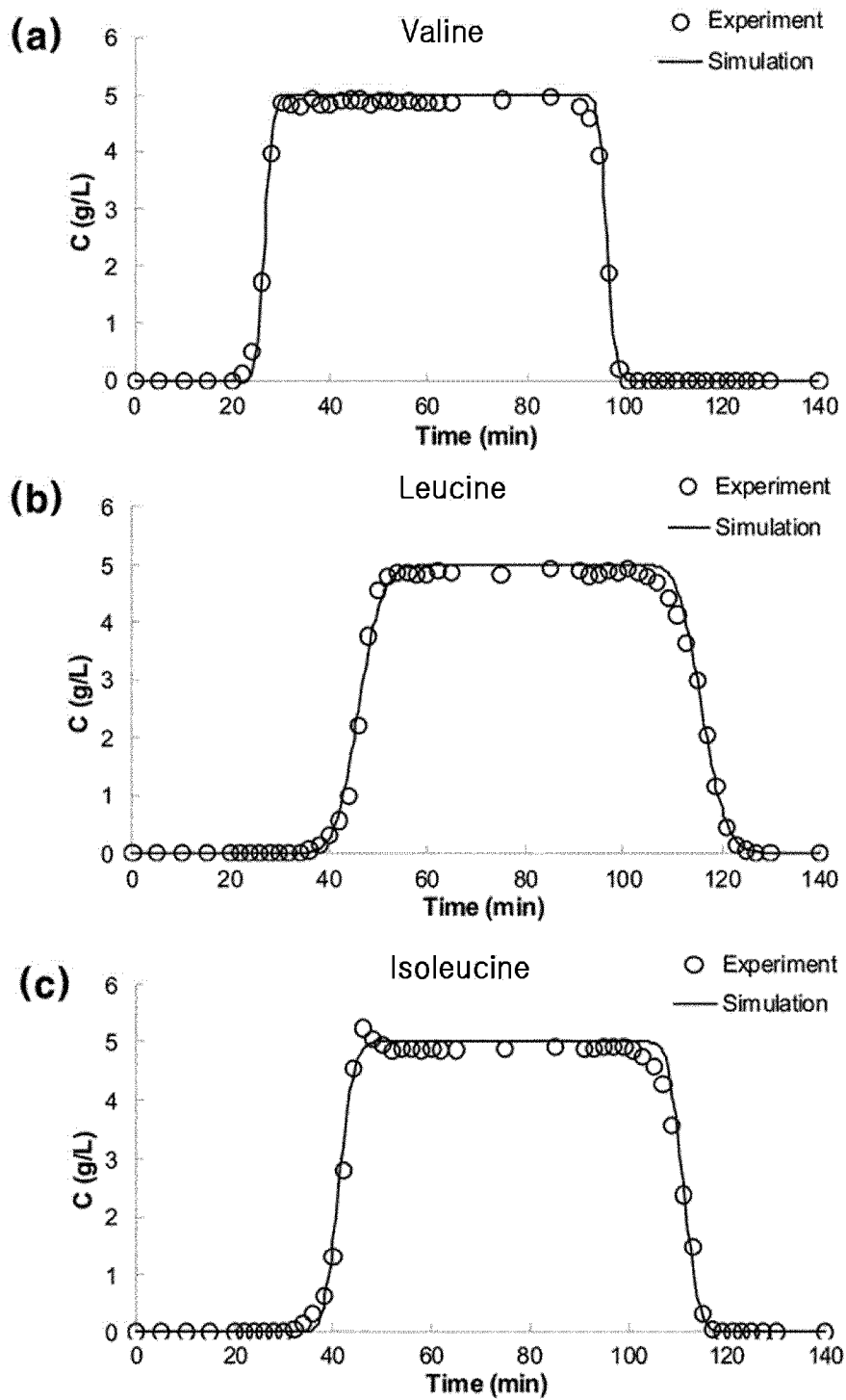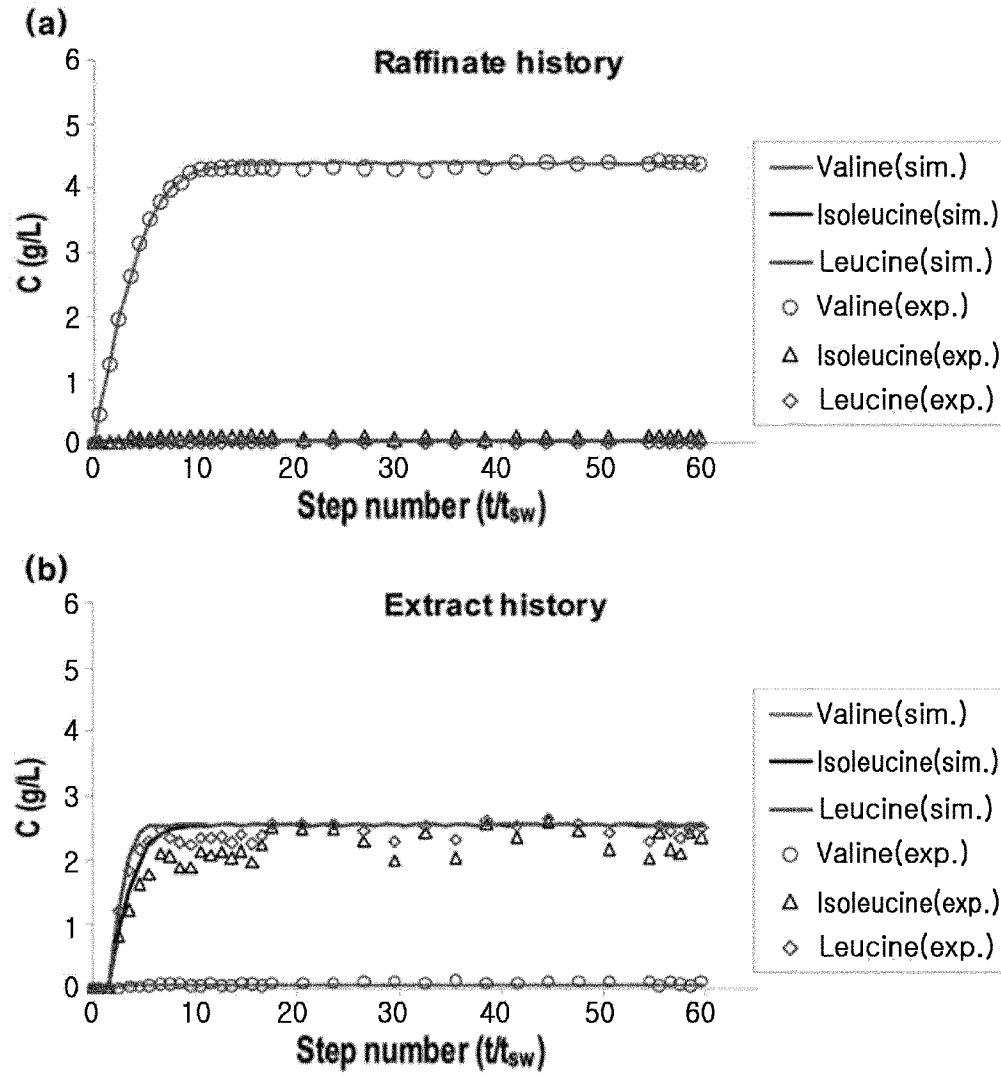
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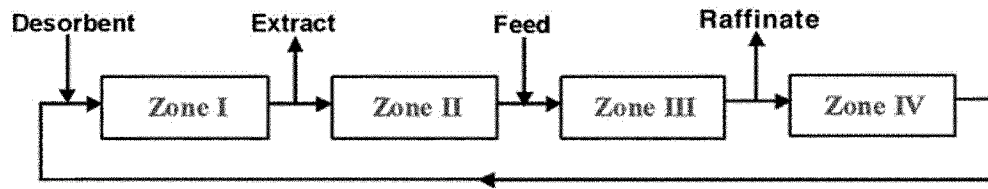
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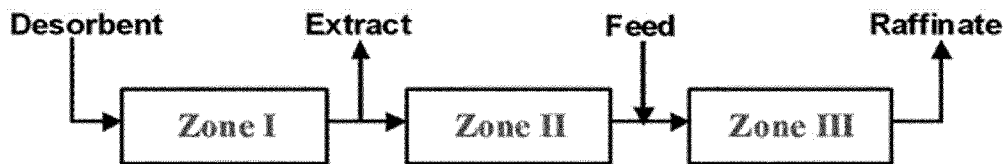
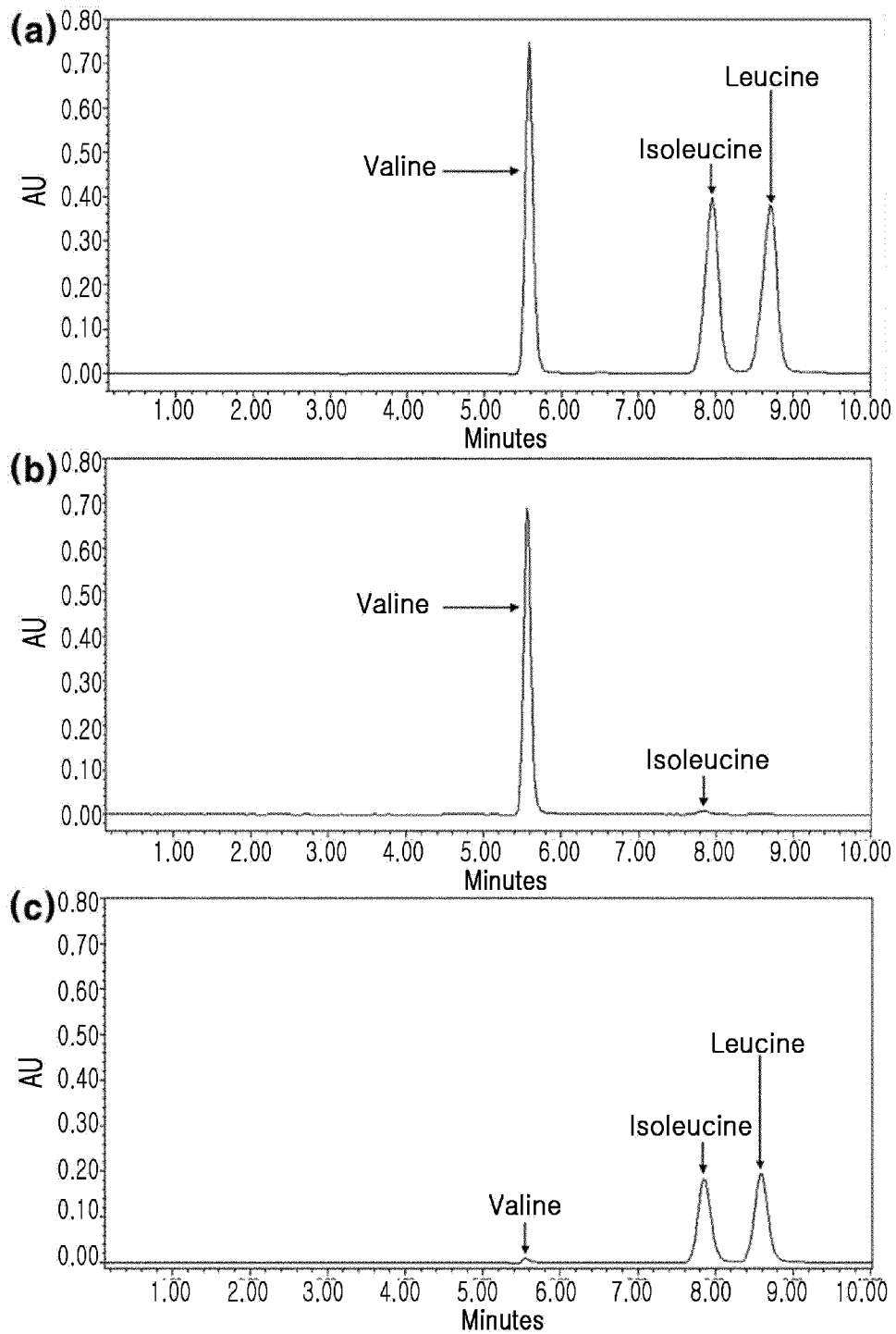
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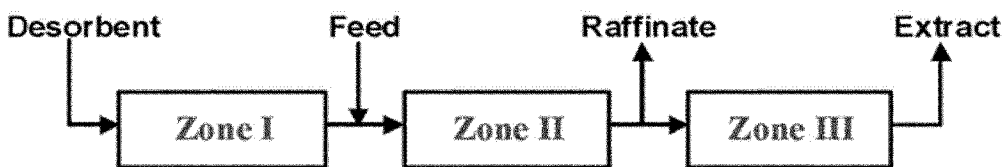
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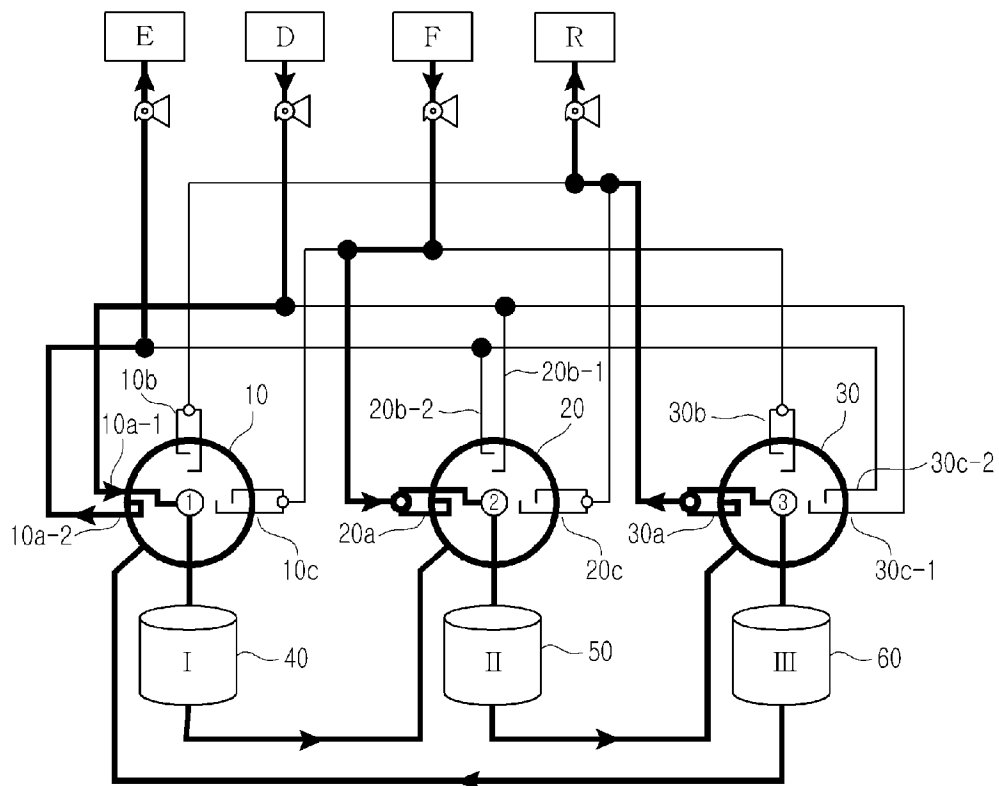
[Fig. 4b]

[Fig. 4c]

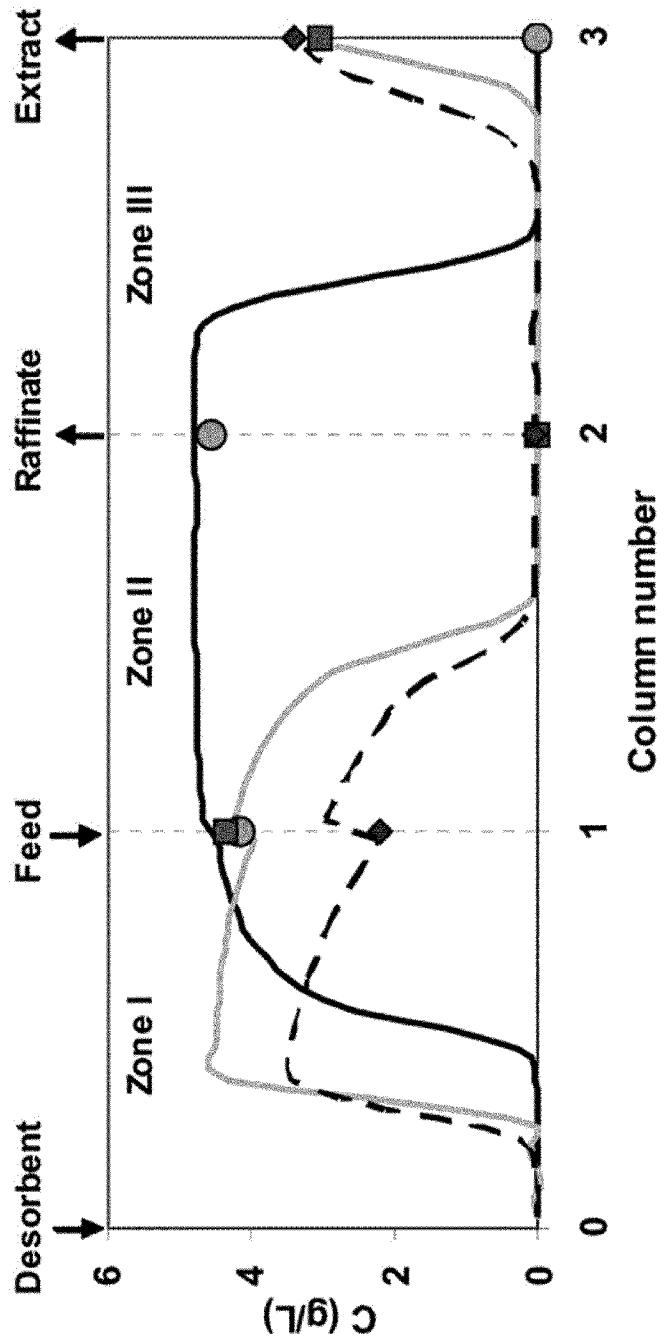[Fig. 5]

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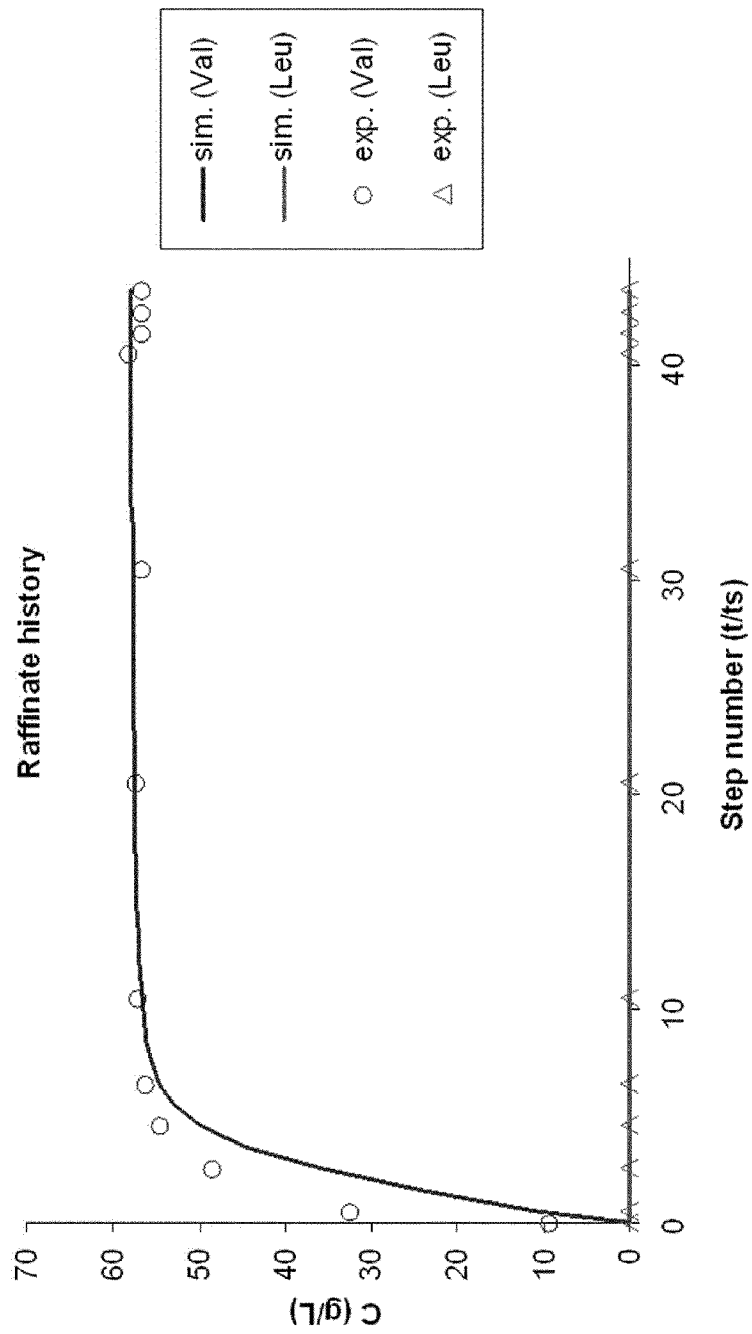
[Fig. 7]

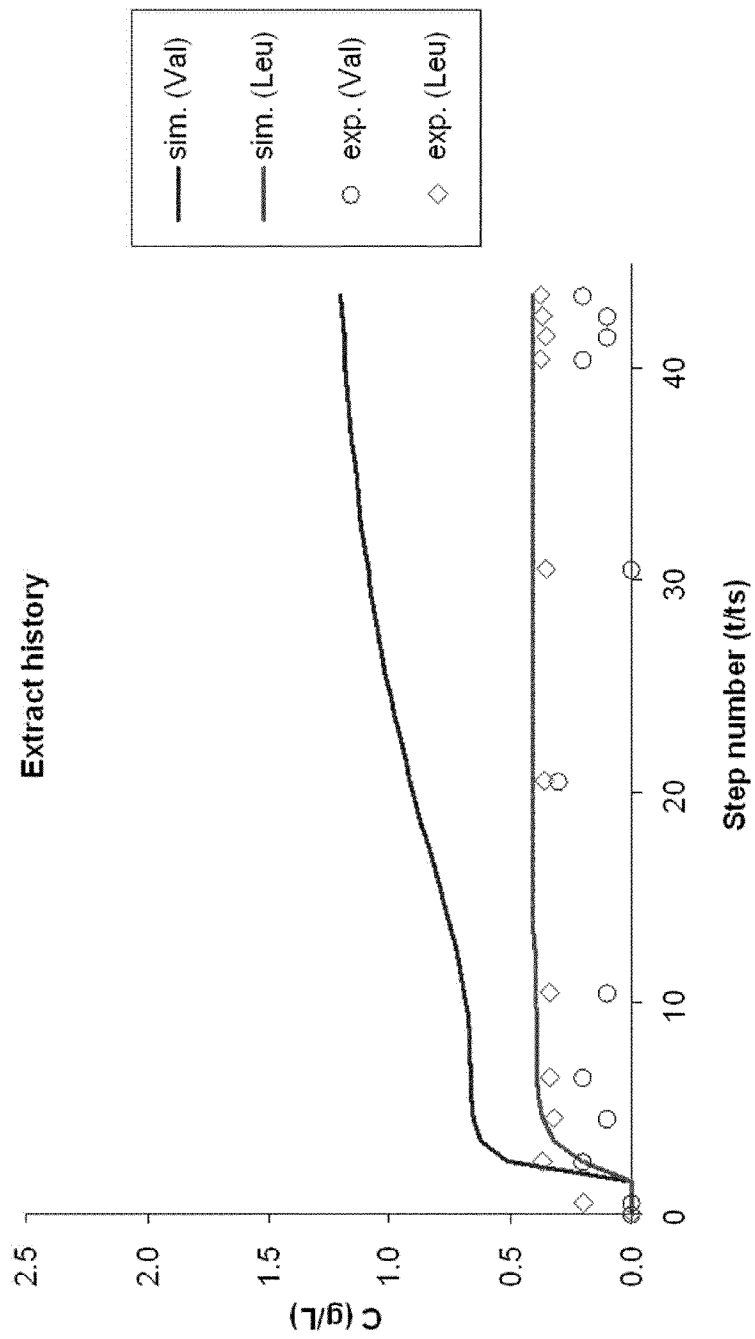[Fig. 8]

[Fig. 9]
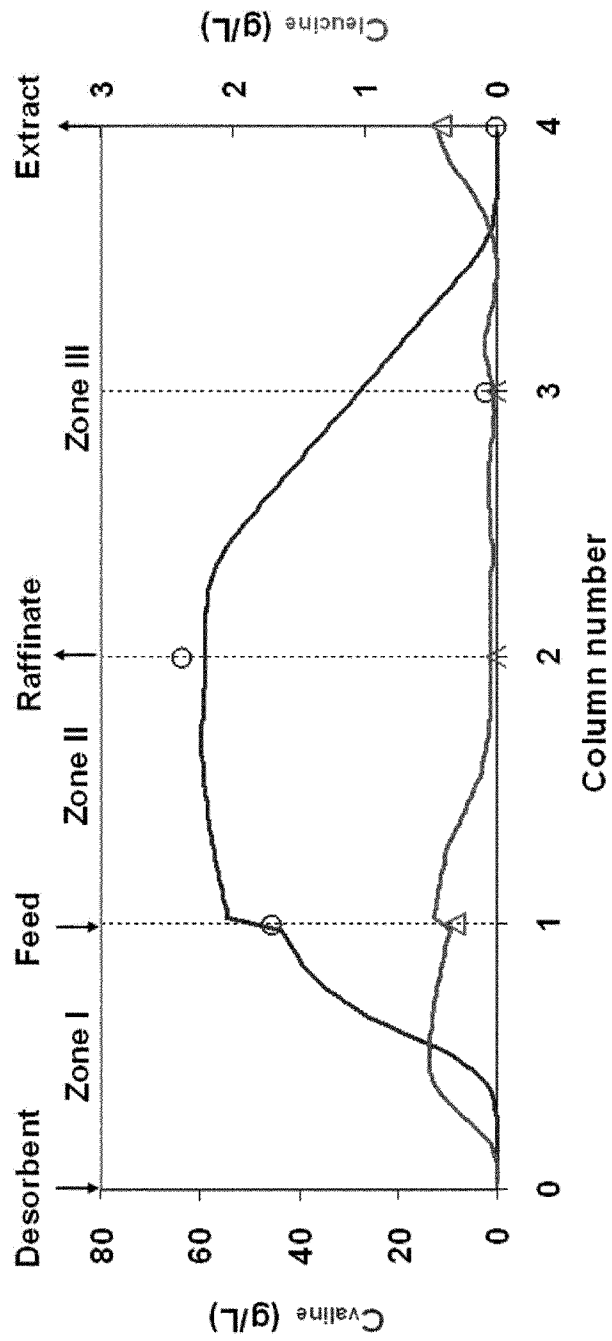
(a)

(b)

[Fig. 10]

[Fig. 11]

[Fig. 12]

[Fig. 13]

[Fig. 14]

| A. | CLASSIFICATION OF SUBJECT MATTER |

C07C 227/40(2006.01)i, B01D 15/08(2006.01)i, C07C 7/12(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

| B. | FIELDS SEARCHED |

Minimum documentation searched (classification system followed by classification symbols)
C07C 227/40; B01D 15/02; C07C 229/08; B01D 9/00; B01D 15/08; B01D 15/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: valine , continous separation, mixture, amino acids

| C. | DOCUMENTS CONSIDERED TO BE RELEVANT |

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SUNGYONG MUN, "Strategy of rearranging the port locations in a three-zone simulated moving bed chromatography for binary separation with linear isotherms", Journal of Chromatography A, Vol. 1230, pp. 100-109 (4 February 2012) See the abstract and Figure 2.	1-12
A	KR 10-2007-0067926 A (SAMSUNG TOTAL PETROCHEMICALS CO., LTD.) 29 June 2007 See claim 1 and Figure 1.	1-12
A	CN 101948399 A (JIANGNAN UNIVERSITY) 19 January 2011 See claim 1 and Figure 1.	1-12
A	KR 10-2012-0009962 A (INDUSTRY-ACADEMIC COOPERATION FOUNDATION, YONSEI UNIVERSITY) 02 February 2012 See claim 1 and Figure 1.	1-12
A	EP 1106602 A1 (ARCHER-DANIELS-MIDLAND COMPANY) 13 June 2001 See claims 1-2 and Figure 1.	1-12

| ☐ Further documents are listed in the continuation of Box C. | ☒ See patent family annex. |

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 2013 (21.06.2013)	**21 June 2013 (21.06.2013)**

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Facsimile No. 82-42-472-7140	Telephone No. 042 481 8657

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KR 10-2007-0067926 A	29.06.2007	US 2008-0149565 A1 US 7649124 B2	26.06.2008 19.01.2010
CN 101948399 A	19.01.2011	None	
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