Journal of Chromatography A, 1217 (2010) 6898–6904

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

An automatic system for multidimensional integrated protein chromatography

Yingjun Kong[a,b], Xiunan Li[a,*], Gaoying Bai[c], Guanghui Ma[a], Zhiguo Su[a,*]

[a] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China
[b] Graduate University of Chinese Academy of Science, Beijing 100049, China
[c] School of Life Science, Lanzhou University, Lanzhou 730000, China

ARTICLE INFO

Article history:
Received 15 May 2010
Received in revised form 23 August 2010
Accepted 24 August 2010
Available online 17 September 2010

Keywords:
Multidimensional
Biorefinery
Chromatography
Protein purification

ABSTRACT

An automatic system for multidimensional integrated protein chromatography was designed for simultaneous separation of multiple proteins from complex mixtures, such as human plasma and tissue lysates. This computer-controlled system integrates several chromatographic columns that work independently or cooperatively with one another to achieve efficient high throughputs. The pipelines can be automatically switched either to another column or to a collection container for each UV-detected elution fraction. Environmental contamination is avoided due to the closed fluid paths and elimination of manual column change. This novel system was successfully used for simultaneous preparation of five proteins from the precipitate of human plasma fraction IV (fraction IV). The system involved gel filtration, ion exchange, hydrophobic interaction, and heparin affinity chromatography. Human serum albumin (HSA), transferrin (Tf), antithrombin-III (AT-III), alpha 1-antitrypsin (α1-AT), and haptoglobin (Hp) were purified within 3 h. The following recovery and purity were achieved: 95% (RSD, 2.8%) and 95% for HSA, 80% (RSD, 2.0%) and 99% for Tf, 70% (RSD, 2.1%) and 99% for AT-III, 65% (RSD, 2.0%) and 94% for α1-AT, and 50% (RSD, 1.0%) and 90% for Hp. The results demonstrate that this novel multidimensional integrated chromatography system is capable of simultaneously separating multiple protein products from the same raw material with high yield and purity and it has the potential for a wide range of multi-step chromatography separation processes.

1. Introduction

Liquid chromatography (LC) has made a great contribution to the development of modern biotechnology [1,2]. Numerous proteins have been successfully purified with different LC techniques such as ion exchange, hydrophobic, affinity and gel filtration [3–5]. However, LC can be time consuming and laborious. It is a general practice for the workers to connect, disconnect and re-connect LC columns by hands for purification of a specific protein. This is because a single LC column, which is based on one separation mechanism, may be inadequate or impossible to achieve satisfactory purification. Although automatic chromatography workstations have been developed, these are mostly designed for single column basis, also requiring manual change of columns. The change of columns by hands may result in contamination either to the environment, or to the protein product. Errors or faults may be generated such as misconnecting a column or leaking of liquid due to connection failure.

Another shortcoming of the current chromatography workstation is the difficulty in achieving simultaneous separation of several proteins from one mixture such as plasma, cell lysate or culture broth. For example, the precipitate from plasma fraction IV, which is a byproduct from classical plasma protein purification by cold ethanol precipitation, contains some therapeutic proteins, such as human serum albumin (HSA), transferrin (Tf), antithrombin-III (AT-III), alpha 1-antitrypsin (α1-AT), and haptoglobin (Hp) [6,7]. The purification of these products (some characteristics of the five target proteins see Supplementary Table S1) from plasma fraction IV must be achieved typically either by parallel or by sequential application of various LC columns [8–12]. In such a system, the fraction eluted from an earlier LC column is generally used as an intermediate product that is reloaded onto the next column for further purification until the target product is obtained with high purity and activity.

Multidimensional liquid chromatography (MDLC) has been developed for high throughput protein separation [13,14]. Some automatic analytical systems for MDLC have been in the market, which can combine 2 or 3 chromatographic steps and integrated with mass spectrometry for trace analysis. These products have been used in proteomics research for unknown protein identification [15–17]. However, they are not designed for preparative protein separation where active proteins are required in large quantities. An automatic preparative chromatography system was reported by Bhikhabhai et al. [18]. This system was for purification

* Corresponding authors. Tel.: +86 10 62561817.
E-mail addresses: xnli@home.ipe.ac.cn (X. Li), zgsu@home.ipe.ac.cn (Z. Su).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.08.067

of recombinant proteins with affinity tags and was capable of producing milligrams of highly purified proteins [18–20]. However, it was not designed for a complex chromatography process involving multiple products. An ideal multidimensional chromatography system should be able to replace all manual operations, such as switching of columns and pipelines, and be intelligent enough to perform a systematic separation/purification of several products from one starting materials. Furthermore, it should use minimal time, reagents, man power, and ensure that the entire purification process is carried out in a closed system.

To approach the aforementioned ideal system, we have constructed a new preparative workstation for simultaneous separation and purification of a variety of proteins from a complex mixture. Network of pipelines and valves is controlled by a computer software automatically switching the pipelines and valves according to the designed program. Various LC columns can be set up in the system without the need of manual change. These columns can work sequentially, parallelly or in mixed mode to enable simultaneous separation. The purpose is to realize that one raw material comes in the system, several purified products going out of it, without any worry and hassle of man interruption. To test the possibility, we used a real protein mixture, the precipitate from plasma fraction IV, as the starting material, to purify five proteins, including HSA, Tf, AT-III, α1-AT, and Hp simultaneously. The chromatographic system involved gel filtration, ion exchange, hydrophobic interaction, and heparin affinity chromatography.

2. Experimental

2.1. Instrumentation

Pumps (High pressure infusion pump, P3000) and detectors (UV detector, UV3000) were purchased from Tong Heng Innovation Technology Co. (Beijing, China). Valves (rocker solenoid valve, type: 6128) were purchased from Christian Bürkert GmbH & Co. KG (Ingelfingen, Germany).

As shown in Fig. 1, the automatic and multidimensional chromatography system used in this study consisted of three units connected by a main pipeline. Each unit within the system contained 4 LC columns, and in total, the system integrated 12 LC columns, which were automatically managed by a computer-controlled system.

2.1.1. The flow of each unit

Each unit can perform an intact, single run of a multi-step chromatographic process. Pump A and Pump B provide 4–6 types of solutions by switching the solvent selection valve. These solutions supplies the solvent for equilibration, wash, gradient elution, regeneration of different LC columns, and the on-line clean up of the system. Column switching is performed through pre- and post-column selection, and the fractions eluted from the column are monitored by the UV detector and then loaded into a collection container using the outlet valves (see Supplementary Fig. S1).

2.1.2. The connection of two columns in one unit

To connect two columns in one unit sequentially, a two-dimensional switching valve was used as the modulator. The UV-detected elution fraction from the first column in the series was loaded onto the second column through the two-dimensional switching valve of the first column and the pre-column selection valve of the second (see Supplementary Fig. S2). Thus, one to four steps of the chromatographic purification process could be performed within a single unit. Wash and elution steps were initiated immediately in the second column after each column switching procedure, and the regeneration of all of the columns in one unit was carried out in sequence when all of the steps of elution were completed.

2.1.3. The connection of the columns between two units

A main pipeline was designed to connect columns between two units (see Supplementary Fig. S3). The elution fraction from the column in the first unit was loaded into the main pipeline by switching the inlet valve. Next, the fraction was passed through the main pipeline outlet valve, which was opened at the same time the elution reached one of the columns in the second unit. Each unit ran independently after this switching procedure. Using this method, the system detected the integration and automatic switching of independent and/or cooperative LC columns.

2.1.4. System control

We also developed the chromatography workstation software (high-throughput protein separation workstation, HTPS version 1.1) used to control the entire system. All of the steps, including selection of the buffers, sample loading, column switching in one unit or between two units, purified product collection, etc., was programmed by this software and automatically executed. This optimized design and procedure for multi-step protein purification is rapid and ready for implementation.

2.2. Materials

All LC columns and media were purchased from GE Healthcare, USA. All chemicals were analytical grade reagents. The precipitate from plasma fraction IV was provided by Hualan Biological Engineering Inc., China. All solutions were made using Mill-Q grade water (Millipore, USA).

2.3. Pretreatment of the precipitate from plasma fraction IV

The precipitate from plasma fraction IV was stored frozen at −20 °C before use. For purification, the precipitate was dissolved in physiological saline solution (0.9% NaCl) at a ratio of 1:3.5 (weight:volume, w/v), filtrated and centrifuged. The 15% (w/v) PEG4000 was dissolved in the suspension. After 15 min, the suspension was centrifuged (10,000 rpm, 30 min). The supernatant was kept for chromatographic purification.

2.4. Chromatographic purification methods

2.4.1. The octyl Sepharose 4 fast flow chromatography (Octyl) purification of the supernatant

The supernatant was loaded onto a Sephadex G25 chromatography (G25) column to change the buffer for the Octyl column. The UV-detected fraction was loaded onto an Octyl column. The Octyl column was equilibrated and washed with 20 mM sodium phosphate buffer (PB), pH 7.0, 1.6 M $(NH_4)_2SO_4$, and eluted with a step gradient of 20%, 50%, 100% 20 mM PB, pH 7.0.

Using the Octyl column, the supernatant was divided into three fractions, the main components of which were Tf, α1-AT, Hp, HSA and AT-III.

2.4.2. Purification of the Tf

The fraction eluted from the Octyl column with the gradient of 20% 20 mM PB was loaded onto a DEAE Sepharose Fast Flow chromatography (DEAE) column after a G25 column. This DEAE column was equilibrated with 20 mM Tris–HCl buffer, pH 7.5 and eluted with the gradient of 24% 20 mM Tris–HCl buffer, pH 7.5, 0.5 M NaCl. The fraction from the DEAE column was loaded onto a Superdex 75 gel filtration chromatography (Superdex 75) column to obtain Tf.

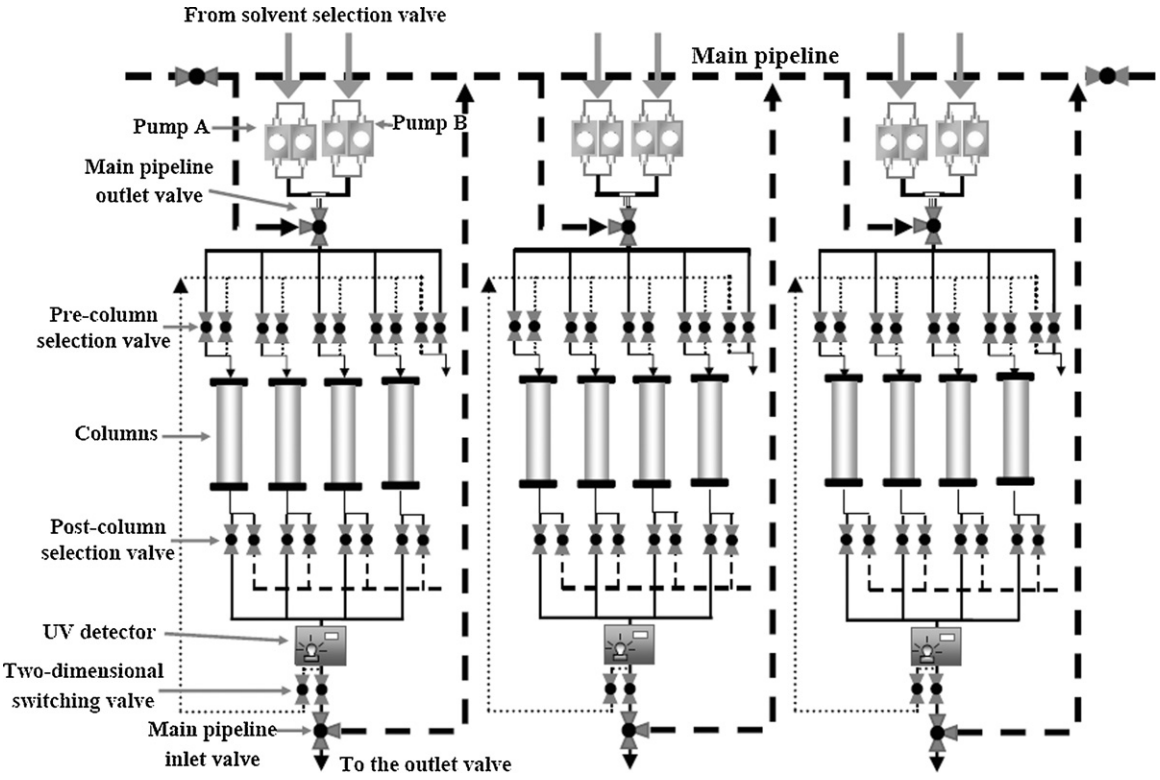

Fig. 1. The whole flow chart of the system.

2.4.3. Purification of the α1-AT and Hp

The fraction eluted from the Octyl column with the gradient of 50% 20 mM PB was loaded onto a DEAE column after a G25 column. This DEAE column was equilibrated with 20 mM Tris–HCl buffer, pH 7.5 and eluted with the gradient of 22% and 50% 20 mM Tris–HCl buffer, pH 7.5, 0.5 M NaCl. The fraction eluted with the gradient of 22% was α1-AT. The fraction eluted with the gradient of 50% was loaded onto a Superdex 75 column to obtain Hp.

2.4.4. Purification of the HSA and AT-III

The fraction eluted from the Octyl column with the gradient of 100% 20 mM PB was loaded onto a Heparin Sepharose 6 Fast Flow affinity chromatography (Heparin) column after a G25 column. This Heparin column was equilibrated with 20 mM Tris–HCl buffer, pH 8.0 and eluted with the gradient of 32.5% and 100% 20 mM Tris–HCl buffer, pH 8.0, 2 M NaCl. The HSA flowed through the Heparin column, the fraction eluted with the gradient of 32.5% comprised low affinity impurities, and the AT-III was eluted with the gradient of 100%.

In the manual purification process, the chromatographic conditions, such as flow velocity, pH of buffers and inlet amount of each column, were optimized. The volume of one column (CV) was defined in terms of how much proteins were loaded from the front column. The inlet amount for manual operation was 20 mL of supernatant and for the automatic system was 70 mL. The parameters of the automatic system were defined as the residence time of the same fraction being equal. Both of two purification processes were carried out five times for the recovery assay. All buffers and columns used in the experiment are listed in Table 1, and the purification process is outlined in Fig. 2.

2.5. Analysis by SDS page

Samples containing 0.5–2 μg protein were heated to 100 °C for 5 min in sample buffer containing 2% 2-mercaptoethanol and applied to 15% homogeneous polyacrylamide gels containing SDS as described by Laemmli [21]. Afterwards, the gels were silver stained.

2.6. Determination of HSA, Tf, AT-III, α1-AT and Hp

HSA was confirmed using bromocresol green solution, because it has been shown that bromocresol green changes color from yellow to green when coupled to HSA at pH 4.0 [22], and the intensity of the green color is proportional to the albumin concentration.

The determination of antithrombin III (AT-III) activity was performed by microassay with chromogenic tripetide substrate (S2238, Kabivitrum, Sweden) [23].

The determination of alpha 1-antitrypsin (α1-AT) was conducted by microassay with chromogenic tripetide substrate (BAPNA, Fluka, Switzerland) [24].

The determination of transferrin (Tf) and haptoglobin (Hp) was performed using the enzyme-linked immunosorbent assay (ELISA). The ELISA kit for Tf was manufactured by Roche Diagnostics GmbH, Germany. The ELISA kit for Hp was manufactured by R&D Systems Inc., USA.

3. Results and discussion

3.1. Selection and integration of the chromatographic steps

There have been many reports on the purification and separation of human plasma fraction IV proteins [25–29]. For example, to purify α1-AT, Kumpalume et al. used a three-step chromatographic process, including a copper-charged chelating Sepharose column, a Ni Sepharose 6 fast flow column, and an anion exchange column. The α1-AT was purified, providing at least 80% pure and 100% active. Such purification processes included two or more kinds of chromatography, but only one or two products could be obtained. In the present study, we designed a fully chromatographic process

Table 1
The parameters of the columns.

Columns	Chromatographic media	Buffer A	Buffer B	Column size (i.d. × L, cm)	
				Manual operation	Using the automatic system
Column 1	Sephadex G 25	20 mM pH 7.0 PB + 1.6 M $(NH_4)_2SO_4$		2.6 × 23.5	5.0 × 17.8
Column 2	Octyl Sepharose 4FF	20 mM pH 7.0 PB + 1.6 M $(NH_4)_2SO_4$	20 mM pH 7.0 PB	1.6 × 26.0	2.6 × 24.5
Column 3	Sephadex G 25	20 mM pH 8.0 Tris–HCl		2.6 × 23.5	5.0 × 17.9
Column 4	Heparin Sepharose 6FF	20 mM pH 8.0 Tris–HCl	20 mM pH 8.0 Tris–HCl + 2 M NaCl	1.6 × 21.0	2.6 × 7.0
Column 5	Sephadex G 25	20 mM pH 7.5 Tris–HCl		2.6 × 23.5	5.0 × 17.5
Column 6	DEAE Sepharose FF	20 mM pH 7.5 Tris–HCl	20 mM pH 7.5 Tris–HCl + 0.5 M NaCl	1.6 × 12.9	2.6 × 11.9
Column 7	Superdex 75	20 mM pH 7.0 PB + 0.2 M Na_2SO_4		1.0 × 30	2.6 × 56.6
Column 8	Sephadex G 25	20 mM pH 7.5 Tris–HCl		2.6 × 23.5	5.0 × 18.0
Column 9	DEAE Sepharose FF	20 mM pH 7.5 Tris–HCl	20 mM pH 7.5 Tris–HCl + 0.5 M NaCl	1.6 × 12.9	2.6 × 11.3
Column 10	Superdex 75	20 mM pH 7.0 PB + 0.2 M Na_2SO_4		1.0 × 30	2.6 × 47.0

for the simultaneous purification of AT-III, Tf, α1-AT, Hp and HSA from plasma fraction IV.

The purification process began by removing PEG and exchanging the buffer using a G25 column, followed by a fractionation step using an Octyl column, which separated fraction IV into three fractions with respect to the different hydrophobicities of the five proteins. The first fraction contained mainly Tf, the second fraction contained α1-AT and Hp, and the third fraction contained HSA and AT-III. In this way, the Octyl column greatly facilitated the subsequent purification procedure.

Further purification was carried out by loading the three fractions onto the next LC columns. The Tf was easily purified from the first part eluted from the Octyl column via the removal of major impurities with the DEAE column, which was followed by the Superdex 75 column for polishing. The α1-AT was purified from the second fraction by the DEAE column using the charge difference between α1-AT and Hp at pH 7.5, and the product of Hp was obtained by the Superdex 75 column. It is well known that AT-III can form a complex with heparin through specific affinity, and using the Heparin column, AT-III and HSA were effectively purified from the third fraction. In this way, a process for the simultaneous purification of the five proteins was established, as shown in Fig. 2. In this process, the G25 column was used to exchange the buffers for the different chromatography steps. To evaluate the efficiency of buffer exchange by G25 column, the conductivity of the fraction eluted from G25 column was detected. For example, the first G25 column was used for the buffer exchange of Octyl column. The difference between the conductivity of the fraction eluted from this column (166.26 ms/cm) and that of the equilibrating buffer of Octyl column (165.71 ms/cm) is about 0.3%. Therefore, the G25 column can effectively exchange buffers. Compared with ultrafiltration and dialysis [30,31], the use of the G25 column reduced the operation time and facilitated the follow-up auto-chromatographic purification process.

3.2. Purification results by manual operation

The chromatographic profiles of the octyl column, heparin column, and two DEAE IEC columns, which played the major purification role in the whole process, are shown in Supplementary Figs. S4–S7. The profiles of the Superdex 75 columns and the G25 columns are not shown. The SDS-PAGE results for the five proteins obtained by manual operation are shown in Fig. 3, and the purities and recovery yields are shown in Table 2. These results indicated that the five proteins could be purified from fraction IV with high purity (>90%) by the full-chromatographic purification method. It was easy to maintain the natural activity of the protein and to obtain the product with high purity because of the mild conditions and high resolution of the process. In particular, this full-chromatographic purification process is easy to scale up and automatic control can be achieved, using the automatic multidimensional chromatography system.

3.3. Establishment of the automatic multidimensional chromatography method

In the designed system, two columns in one unit are connected in series by the two-dimensional switching valve. Two columns between two units are connected in series by a main pipeline, and the different modes of chromatographic operation can be carried

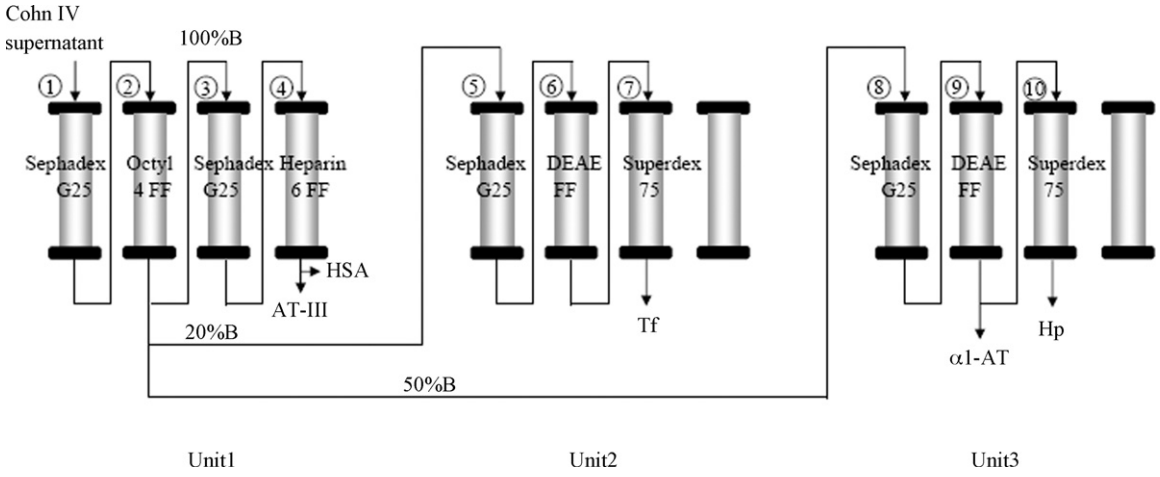

Fig. 2. The flow chart of the purification of plasma fraction IV.

Table 2
The purities and recovery yields of the proteins purified by manual operation and using the automatic system.

Proteins	By manual operation		Using the automatic system	
	Purity (%)	Recovery yield (%), ave. (RSD)	Purity (%)	Recovery yield (%), ave. (RSD)
HSA	93	87(4.4)	95	95(2.8)
AT-III	99	65(6.8)	99	70(2.1)
Tf	99	74(7.8)	99	80(2.0)
α1-AT	92	62(5.3)	94	65(2.0)
Hp	90	40(6.0)	90	50(1.0)

out in the three units independently at the same time. As depicted in Section 3.1, all of the three fractions eluted from the Octyl column need to be loaded onto the next LC column for further purification. To save operation time, the first two fractions eluted from the Octyl column are respectively fed into the G25 columns prior to DEAE column in the second and third unit of the system, and the third fraction is fed into the G25 column prior to Heparin column in the first unit. Therefore, to purify HSA and AT-III, a G25 column, an Octyl column, a G25 column and a Heparin column were located in the first unit. To purify Tf, a G25 column, a DEAE column and a Superdex 75 were located in the second unit. To purify α1-AT and Hp a G25 column, a DEAE column and a Superdex 75 were located in the third unit. Using this method, the three fractions can be purified at the same time. Thus, the automatic multidimensional chromatography method was established (as described in Section 2) and could be carried out for the purification of five proteins after placing the buffer into the proper inlet and locating the columns. During the first run, the system saved all of the parameters and settings used in this process, such as the flow velocity, gradient, and valve switching time, as a method file. All of the operations, including when collecting the eluted fraction should be started, finished and transferred to the second column, also were programmed by the software. By loading the methods file, the purification process can be subsequently carried out automatically. In the automatic process, the RSD of the peak retention time in each column was lower than 1%.

3.4. Results of the automatic multidimensional chromatography system

The chromatographic profiles for the three units of the automatic multidimensional chromatography system are shown in Figs. 4–6. The SDS-PAGE results for the five proteins obtained by automatic system are shown in Fig. 7. The purities and recovery yields are shown in Table 2. The recoveries of the five proteins obtained by the automatic chromatography system were higher

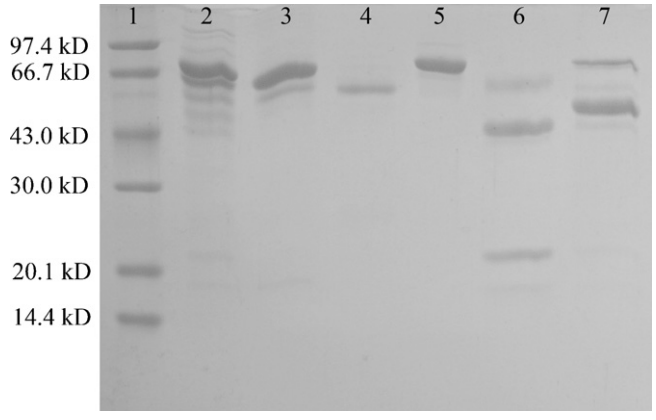

Fig. 3. The SDS-PAGE results of proteins purified by manual operation. 1: Marker, 2: Fraction IV, 3: HSA, 4: AT-III, 5: Transferrin, 6: Haptoglobin, 7: α1-AT.

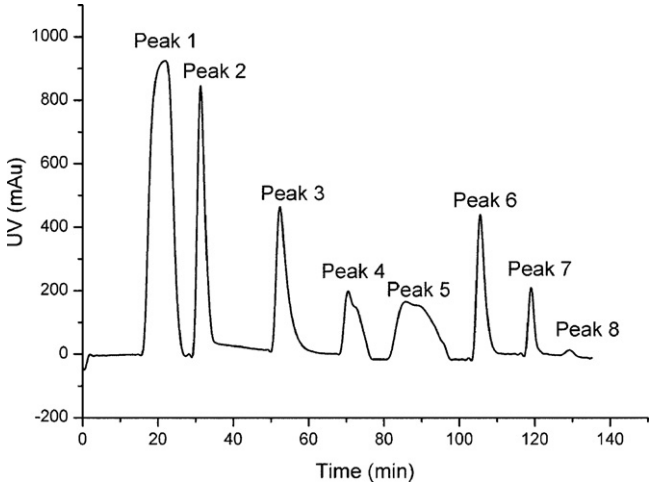

Fig. 4. The chromatography profile of unit 1. Peak 1 was loaded onto column 2, peak 2 was loaded onto column 5, peak 3 was loaded onto column 8, peak 4 was loaded onto column 3, peak 5 was loaded onto column 4, peak 6 was loaded into the collection container of HSA, peak 7 was loaded into the waste container and peak 8 was loaded into the collection container of AT-III.

than those obtained using the manual procedure, whereas the purities were similar. The fluctuations in yield using the automatic chromatography system were lower than 3%, whereas those obtained using the manual procedure were approximately 8%. This finding indicates that the operation stability of the automatic process is better than that of the manual process by avoiding fabricated error, such as erroneous valve switching, fraction collection, etc.

Moreover, as shown in Table 3, the entire purification process lasts only 180 min using the automatic chromatography system. During the manual procedure, chromatographic operation alone

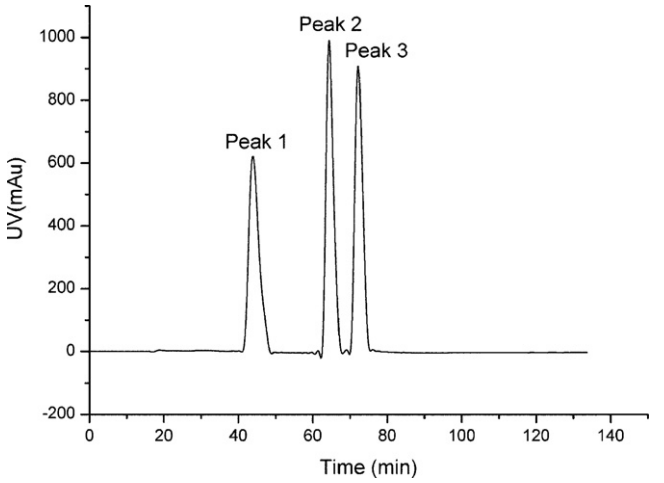

Fig. 5. The chromatography profile of unit 2. Peak 1 was loaded onto column 6, peak 2 was loaded onto column 7 and peak 3 was loaded into the collection container of Tf.

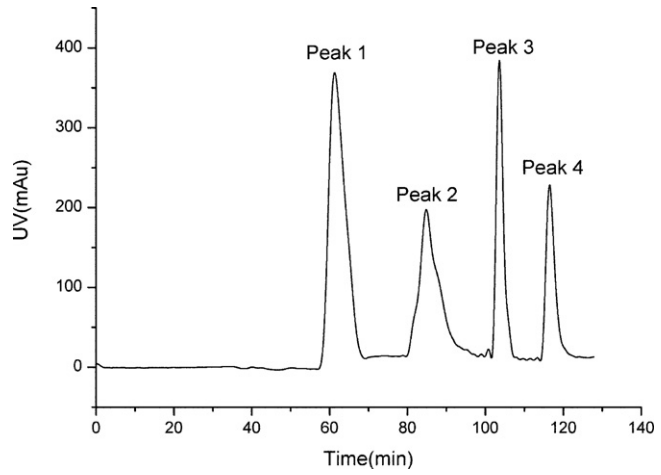

Fig. 6. The chromatography profile of unit 3. Peak 1 was loaded onto column 9, peak 2 was loaded into the collection container of α1-AT, peak 3 was loaded onto column 10 and peak 4 was loaded into the collection container of Hp.

Table 3
The column parameters of the purification by manual operation and using the automatic system.

Columns	By manual operation		Using the automatic system	
	CV (ml)	Time (min)	CV (ml)	Time (min)
Column 1	125	50	350	180
Column 2	55	120	130	
Column 3	125	50	350	
Column 4	23	80	37	
Column 5	125	50	350	
Column 6	27	60	63	
Column 7	24	130	300	
Column 8	125	50	350	
Column 9	27	110	60	
Column 10	24	130	250	

requires 830 min to complete and involves 14 fraction collections, 10 sample injections, and 9 column exchanges. Including the column exchange time, the manual process takes 3 working days. Therefore, the use of the automatic system can shorten the operation time, enhance the operational efficiency and reduce the manual error.

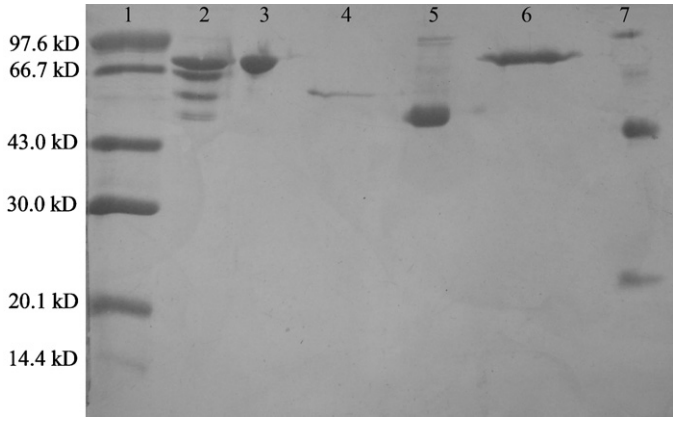

Fig. 7. The SDS-PAGE results of proteins purified by the automatic system. 1: Marker, 2: Fraction IV, 3: HSA, 4: AT-III, 5: α1-AT, 6: Transferrin, 7: Haptoglobin.

3.5. Evaluation of the designed system

Two basic requirements must be met for a multidimensional chromatography system [32]. First, increasing the number of chromatographic steps involved in the system, increases the number of buffers needed to complete the process. In the designed system, the use of the solvent selection valve provided sufficient buffer lines to attach all of the required solvents. Second, there needs to be sufficient column attachment and switching ability. In the designed system, each column can be switched into or out of the flow path, allowing the multidimensional chromatography experiments to be carried out successfully in this system using the custom designed software.

At present, there are two types of multi-column chromatography systems: the parallel multi-column chromatography system and the serial multi-column chromatography system [33,34]. The parallel multi-column chromatography system places many columns in a single parallel system. The system can carry out chromatographic operations in these columns at the same time; however, only the same chromatographic operation can be carried out at the same time. Such systems improve only the flux of the chromatography and are not equipped to handle multi-step separation processes. A series multi-column chromatography system can accommodate a multi-step chromatography process by switching valves. In such a system, the fraction eluted from one column is directly loaded onto another column or deposited into the sample loop prior to being loaded onto another column. All columns must be operated in order; only one column can be operated at a time, and only 2–4 columns can be contained in such a system. Therefore, the available chromatography mode in such a system is limited.

In comparison with existing multi-column systems, the designed automatic chromatography system supplies a variety of column connection modes to carry out different types of chromatography to separate multiple products. In this system, the series and parallel chromatographic processes can be operated in isolation or simultaneously. The described system meets the need of multi-products purification from one raw material. For example, the five-protein purification process described in this study involved 10 LC columns including hydrophobic interaction, ion exchange, affinity and gel filtration chromatography. In this process, when the fraction eluted from the buffer exchange column was loaded onto the octyl column, the series connection of columns in one unit was utilized. After the first fraction eluted from the octyl column was loaded onto the column in the second unit, units 1 and 2 were run simultaneously. After the second fraction eluted from the octyl column was loaded onto the column in the third unit, three units of the automatic chromatography system worked simultaneously (including parallel and series operation) and were controlled by the chromatography workstation software. Using both series and parallel connections among those of 10 LC columns, five proteins were successfully purified from fraction IV.

4. Conclusion

The purification of multiple products from one raw material stems from a bio-refinery concept that attempts to take full advantage of material resources to reduce production costs. This is achieved by the parallel or sequential application of various chromatography units. In this paper, we have shown that the designed automatic, multidimensional chromatography system can be used to produce target proteins with high purity in a multi-product purification process. In this novel system, an automatic switch of pipelines to either another chromatography unit or to a collection container in the closed system ensures the fast and efficient separation of multiple target products. This system can also be used in

purification procedures designed for specific proteins containing a series of column steps to produce the target protein with sufficient quantity and purity. Such multi-step purifications have traditionally been performed as a series of independent procedures. The ability of this system to combine multiple steps into one procedure has obvious benefits in terms of time and labor and, in the case of labile targets, an increase in yield. The chromatographic parameters can be conveniently altered and optimized with the supporting software when the default settings provide undesirable results.

Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (nos. 20636010, 20820102036 and 20976178) and the Major State Basic Research Development Program of China (973 Program, no. 2007CB714305).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.08.067.

References

[1] J.M. Simpson, Protein Purification Process Engineering, Dekker, New York, 1994.
[2] J. Janson, L. Rydén, Protein Purification: Principles, High-Resolution Methods, and Applications, Wiley-VCH, 1998.
[3] R. Hatti-Kaul, B. Mattiasson, Isolation and Purification of Proteins, CRC, 2003.
[4] P. Cutler, Protein Purification Protocols, Humana Pr Inc., 2004.
[5] P. Schoenmakers, Annu. Rev. Anal. Chem. 2 (2009) 333.
[6] E. Cohn, F. Gurd, D. Surgenor, B. Barnes, R. Brown, G. Derouaux, J. Gillespie, F. Kahnt, W. Lever, C. Liu, J. Am. Chem. Soc. 72 (1950) 465.
[7] E. Cohn, L. Strong, W. Hughes, D. Mulford, J. Ashworth, M. Melin, H. Taylor, J. Am. Chem. Soc. 68 (1946) 459.
[8] S. Yueh, Y. Lai, W. Chen, H. Hsu, S. Mao, J. Chromatogr. B 845 (2007) 210.
[9] R. Zhao, J. Luo, D. Shangguan, G. Liu, J. Chromatogr. B 816 (2005) 175.
[10] K.B. McCann, B. Hughes, J. Wu, J. Bertolini, P.T. Gomme, Biotechnol. Appl. Biochem. 42 (2005) 211.
[11] K. Tanaka, E. Shigueoka, E. Sawatani, G. Dias, F. Arashiro, T. Campos, H. Nakao, Braz. J. Med. Biol. Res. 31 (1998) 1383.
[12] G. Finette, B. Baharin, Q. Mao, M. Hearn, Biotechnol. Prog. 14 (1998) 286.
[13] J. Tang, M. Gao, C. Deng, X. Zhang, J. Chromatogr. B 866 (2008) 123.
[14] P. Sajonz, W. Schafer, X. Gong, S. Shultz, T. Rosner, C. Welch, J. Chromatogr. A 1145 (2007) 149.
[15] L. Hu, K. Boos, M. Ye, R. Wu, H. Zou, J. Chromatogr. A 1216 (2009) 5377.
[16] P. Mauri, M. Scigelova, Clin. Chem. Lab. Med.: CCLM/FESCC 47 (2009) 636.
[17] U. Rajcevic, S. Niclou, C. Jimenez, Front. Biosci.: J. Virt. Libr. 14 (2009) 3292.
[18] R. Bhikhabhai, A. Sjöberg, L. Hedkvist, M. Galin, P. Liljedahl, T. Frigård, N. Pettersson, M. Nilsson, J. Sigrell-Simon, C. Markeland-Johansson, J. Chromatogr. A 1080 (2005) 83.
[19] H. Liu, J. Naismith, Protein Expr. Purif. 63 (2009) 102.
[20] C. Ludwig, M. Wear, M. Walkinshaw, Protein Expr. Purif. 71 (2010) 54.
[21] U. Laemmli, Nature 227 (1970) 680.
[22] L. Rodkey, Arch. Biochem. Biophys. 108 (1964) 510.
[23] M. Blombäck, B. Blombäck, P. Olsson, L. Svendsen, Thromb. Res. 5 (1974) 621.
[24] A. Dietz, H. Rubinstein, L. Hodges, Clin. Chem. 20 (1974) 396.
[25] W. Lebing, D. Hammond, J. Wydick III, G. Baumbach, Vox Sang. 67 (1994) 117.
[26] P. Kumpalume, C. Lepage, J. Dalton, Food Bioproducts Process. 86 (2008) 65.
[27] C. Tseng, H. Huang, Y. Yang, S. Mao, Protein Expr. Purif. 33 (2004) 265.
[28] T. Krell, M. Chevalier, L. Lissolo, Protein Expr. Purif. 24 (2002) 323.
[29] L. Zhixin, C. Kongchang, Dyes Pigm. 22 (1993) 27.
[30] A. Ozler, E. Karakus, S. Pekyardimci, Prep. Biochem. Biotechnol. 38 (2008) 358.
[31] G. Dobrev, B. Zhekova, G. Delcheva, L. Koleva, N. Tziporkov, I. Pishtiyski, World J. Microbiol. Biotechnol. 25 (2009) 2095.
[32] R. Watson, T. Nadler, Methods Mol. Biol. - Clifton Then Totowa 244 (2004) 425.
[33] P. Dugo, F. Cacciola, T. Kumm, G. Dugo, L. Mondello, J. Chromatogr. A 1184 (2008) 353.
[34] K. Fujii, T. Nakano, H. Hike, F. Usui, Y. Bando, H. Tojo, T. Nishimura, J. Chromatogr. A 1057 (2004) 107.