Radial-Flow Disk Chromatography for Continuous Separation

Radial-Flow Disk Chromatography for Continuous Separation

Tsuyoshi Shishibori[1)] *, Yasuhiko Ohta[2)]

[1)] *Department of Liberal Arts and Sciences and*
[2)] *Department of Medical Technology, Faculty of Health Sciences,*
Kagawa Prefectural College of Health Sciences

Abstract

A radial flow disk chromatograph was designed and created for the continuous separation of mixture solution. The chromatographic disk was packed with silica gel-C_{18} under the influence of a relative centrifugal field at 400×g (1200 rpm). The continuous separation of di-2-ethylhexyl phthalate and dibutyl phthalate was achieved by rotating this chromatographic disk at 2 rph, at a flow rate of 8 ml/min using a methanol-water (19: 1) mixture as eluent. Continuous separations of the phthalates on a silica gel disk and two kinds of amino acids on a hydrophilic polyvinyl gel disk were verified, respectively.

Key Words: chromatography, continuous separation, phthalates, amino acids

*Correspondence to: Tsuyoshi Shishibori, Department of Liberal Arts and Sciences, Faculty of Health Sciences, Kagawa Prefectural College of Health Sciences, 281-1 Murecho-hara, Takamatsu, Kagawa 761-0123 Japan

INTRODUCTION

Separation of mixtures by column chromatography in preparative scale is time consuming, and is inefficient because of the use of the packing materials. Therefore, a continuous chromatographic separation of mixtures such as biomolecules and synthetic compounds has long been desired for both laboratory and industrial processes.

The principle of continuous chromatographic separation was presented by Martin[1] as early as 1949, and thereafter, considerable efforts were made to design efficient systems that separate a continuous mixture feed[2-8]. Recently, a continuous annular chromatography for the isolation of recombinant protein drugs has been reported[9]. However, development of continuous chromatography has been difficult, particularly in applications in which an organic solvent is used as eluent. The reason for this may be the inability of the materials to meet the quality requirements of the stationary phase for continuous chromatography.

A uniform flow rate throughout the column is critical for a continuous chromatography. Advances in the art and the technique of stationary phase material production enabled uniform packing with higher resolving power, leading to the development of high performance liquid chromatography(HPLC). Modern packing materials are useful for the continuous chromato graphic separation of mixtures. The improvement in the packing process of stationary phase materials is also important to achieve a uniform flow.

A radial-flow disk chromatographic device, packed with a fixed phase material under centrifugal force field, has been developed in this research. The present paper describes the principle, design, and operation of the disk chromatography for continuous separation. Advantages of the apparatus were verified by separating phthalates mixture solution or amino acids mixture solution.

1. PRINCIPLE

The principle of the continuous disk chromato graphy is illustrated in Fig. 1. The chromato graphic bed (fixed phase media) of the stationary phase is packed between the opposing parallel disks and therefore is a disk in shape. The disk rotates counterclockwise as indicated by an arrow, and the eluting solvent is supplied to the center of the disk by constant flow rate. Then a mixture solution containing components (a) and (b) to be separated are supplied continuously from the supply orifice at the fixed position as shown in Fig. 1.

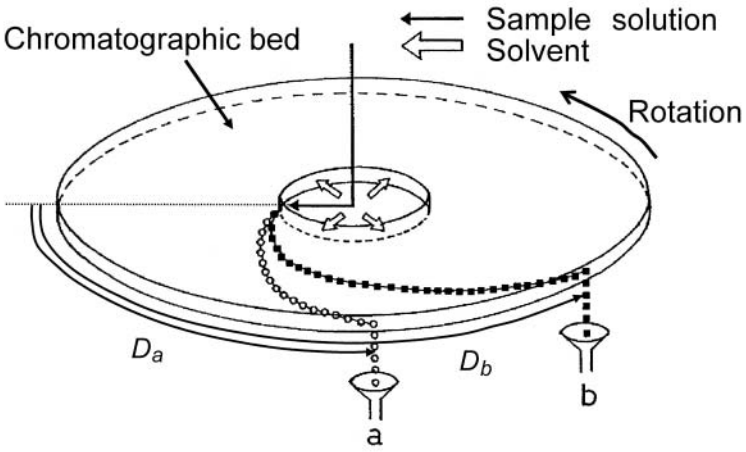


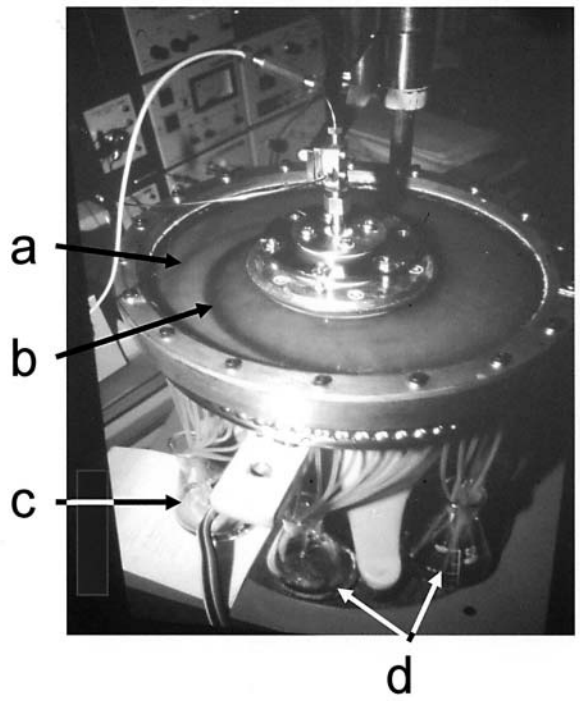
Figure 1. Principle of continuous radial-flow disk chromatography. Continuous separation of mixture of two components. ○, fast moving component (a); ■, slow moving component (b).

Components (a) and (b) move radially outward through the chromatographic bed. Component (a) (circles○) moves faster than component (b) (squares■) does. Because the chromatographic disk rotates at a constant speed, the disk moves distance D_a along the circumference by the time component (a) goes out of the disk,

$D_a = (\text{rpm}) \times \pi d t_a$

where (rpm) is the revolutions per minute of the disk, d is the diameter of the disk, and t_a is the time (min) that component (a) is in the disk. This formula also holds true for component (b) . Since $t_b > t_a$, it implies that $D_b > D_a$, ; in other words, component (b) comes out of the disk at a distance D_b-D_a farther along the circumference than component (a) does. Since the mixture solution is applied to the disk continuously, components (a and b) are eluted continuously at D_a and D_b, respectively.

On the basis of the principle of continuous separation, the actual separation of two kinds of the colored substances is shown with Photo. 1.



Photograph1. The radial-flow disk chromato graphy for the continuous separation.
(a) Yellow band for butter yellow. (b) Red band for sudan red G. (c) Collected eluates of butter yellow. (d) Collected eluates of sudan red G.

2. APPARATUS AND OPERATION

1) Chromatographic disk

The diagrams of the continuous chromatograph are shown in Fig. 2. In this apparatus, the chromatographic bed (1) is formed between the upper flat glass disk (2) having inner (3) and outer (4) stainless steel rings, and a lower stainless steel disk (5). A ring of poly(tetrafluoroethylene) (PTFE) sheet is clamped between the outer ring (4) of the glass disk (2) and shoulder of the stainless steel disk (5), to prevent leakage of sample solution and solvent. The ring (4) and the shoulder determine the depth of the space for chromatographic packing materials (1). Therefore, the bed of the fixed phase takes the shape of a disk (2mm thick, 270mm diameter). The inner rings (3) of stainless steel (80mm I.D.) are fixed in the central hole of the glass disk (2) by four screws using PTFE film (0.2mm thick) as a sealing material. A ring of glass fiber filter (78mm I.D., 86mm O.D., #GA200, Toyo Roshi, Tokyo, Japan) (6) is sandwiched between the inner ring (3) and disk-shaped block (78 mm O.D.) (7) with a brim using three screws, this results in the formation of an annulus gap (1 mm width) between them. The sample solution and solvent flow through this gap into the glass fiber filter layer and then into the chromatographic bed (1). There are 36 exit orifices (8), drilled equidistantly along the circumference of the lower stainless steel disk (5). The semicircular glass fiber filters (9) are set on each orifice to support the chromatographic bed (1). The eluate from each orifice is transported to the individual vial of the fraction collector described below.

The chromatographic disk (5) is fastened to the turntable (10), which is set on a vertical shaft (12). The disk (5) is driven by two types of electronically controlled variable speed motors (Models IHT 8 S 15 and IHT 6 P 3 with an 1 : 1500 gear reducer, Japan Servo, Tokyo, Japan) that provide rotation speeds ranging from 70 to 1400 rpm or from 1 to 20 rph, respectively. The higher speed motor is used for preparation of the gel bed and the lower speed motor for continuous chromatographic separation.

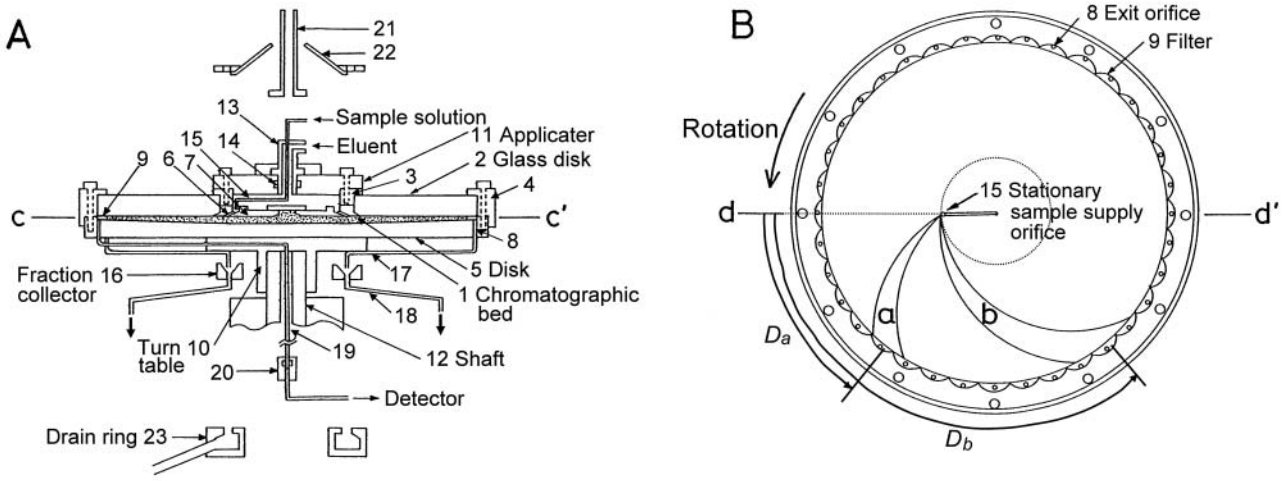


Figure 2. Continuous radial-flow disk chromatography.

(A) A vertical section of the chromatographic disk taken from a plane of d-d′ of Fig. 2 (B). A chromatographic bed (1, stippled) is formed between the upper glass disk (2) and the lower stainless steel disk (5). Funnels (21 and 22) and a drain ring (23) are shown at the top and bottom of Fig. 2 (A), respectively.

(3) Inner stainless steel rings, (4) outer ring, (5) lower stainless steel disk, (6) glass fiber filter, (7) disk-shaped block, (8) exit orifice, (9) semicircular glass fiber filter, (13) inlet plug, (14) rotary seal, (15) sample supply orifice, (17) drip tip, (18) PTFE tube, (19) tube to a detector, (20) rotary sealed joint.

(B) Horizontal section of the disk taken from a plane of c-c′ of Fig. 2 (A), showing the central sample supply orifice (15), and phenomenal paths of a mixture of components (a) and (b). The disk rotates counterclockwise as indicated by an arrow, and the sample supply orifice (15) keeps the fixed position.

2) Sample applicator

In order to apply the sample solution and eluent to the rotating disk continuously, a sample applicator (11) consisting of an inlet plug (13) and a flange is mounted on the center of the chromatographic disk,as shown in Fig. 2 A. The plug (13) is set in the flange with a rotary seal (14) (10 mm I.D.) and is held stationary by an arm to prevent rotation. A sample supply orifice (15) (0.5 mm I.D.) with a 22 gauge hypodermic needle is set in through the hole of the stationary inlet plug (13) as shown in Fig. 2 A. The eluent is pumped into the sample applicator (11) through the inlet plug (13), and then through the filter layer into the chromatographic bed (1). The sample solution is continuously applied to the bed through the stationary sample supply orifice (15) as shown in Fig. 2 B.

3) Fraction collector

The fraction collector (16) is a cylindrical stainless steel block with 36 collecting positions (Fig. 2 A). The collecting positions are conical dent drilled with the same radius as a half size of the interval of the drip tips (17). The tips (17) carry the eluate from the exit orifices (8) of the chromatographic disk to the dent of the fraction collector (16). At the bottom of the conical dents, PTFE tubes (18) are connected through holes. The fractions dripping into the conical dents are collected in the appropriate vessels; flasks, bottles, and etc.

4) Flow through detection

In order to monitor the continuous separation of the mixtures, the eluate from an exit orifice is carried by stainless steel tubing (19) (0.5 mm I. D.) to a UV spectrophotometric detector (Model UVDEC-100-III, Japan Spectroscopic, Hachioji, Tokyo, Japan) *via* a rotary sealed joint (20), as

shown in Fig. 2A. The flow rate of the eluate into the detector was maintained at one thirty-sixth of that of the eluent, by adjusting the level of the outlet tube of the detector.

5) Preparing gel bed

To prepare the stationary phase bed (1), special funnels are used instead of the sample applicator (11) described above (section 2). The top of Fig. 2A shows a stainless steel pipe (21) (10 mm I.D. ×8 cm) having a flange and a conical polyethylene funnel (22) without the stem. The former (21) was mounted on the center of the disk-shaped block (7) of chromatographic disk and the latter (22) on the inner stainless steel ring (3) of the glass disk (2). A drain ring (23) was placed under the dip tips (17) of the chromatographic disk (5) to collect the effluent (at the bottom of Fig. 2A), instead of the fraction collector (16) described above.

The disk apparatus was rotated at 100 rpm and first the space for chromatographic bed was filled with an eluent. The gel suspended in the eluent was poured into the central funnel (21) with increasing rotational speed of the disk up to 1200 rpm for approximately 10 min. After the disk was packed with gel, the eluent was passed for another 10 min.

6) Continuous separation

In continuous separation, the sample applicator (11) was mounted on the inner ring (3), and the drain ring (23) was replaced by the fraction collector (16). The rotational speed of the disk was maintained at approximately 2rph. The sample solution and eluent were continuously pumped into the disk through the sample applicator (11), employing two constant flow-rate pumps (Model TWINCLE, Japan Spectroscopic, Hachioji, Tokyo, Japan). The separation of the sample was monitored by the flow-through UV detector. Each fraction from the dip tips (17) of the disk was fractionated into 36 receivers by the fraction collector (16), and subjected to HPLC analysis using a TWINCLE solvent delivery system and a UVIDEC-100-III detector with a Chromatopac C-R 3A Integrator (Shimadzu, Kyoto, Japan) on a silica gel-C_{18} column (Develosil-ODS, 3㎛ mean particle size, 50×4.6 mm I.D., Nomura Chemical, Seto, Aichi, Japan) at a flow rate of 1 ml/min using a methanol-water (19: 1) mixture.

The apparatus of a radial flow disk chromato graphy designed and created for the continuous separation in this research is shown in Photo. 1.

3. RESULTS AND DISCUSSION

The chromatograms obtained by the flow-through UV detection of the continuous separations of phthalate mixtures on silica gel or silica gel-C_{18} are represented in Figs. 3A and B. The HPLC analysis of eluate collected in 36 receivers are shown in Figs. 4 A and B. These results indicate complete compositional separation of di-2-ethylhexyl and dibutyl phthalates in a mixture solution. Under the operational conditions, this apparatus was capable of separating the phthalates on silica gel and silica gel-C_{18} at feed rates of 180 and 60 mg/hr, respectively. The results obtained by the flow-through UV detection are similar to those obtained by the HPLC analysis. The HPLC analysis needs much more time and effort. Therefore the adoption of the flow-through UV detection in this apparatus is effective to monitor the continuous separation of the mixtures.

Figure 3C shows a chromatogram obtained by the flow-through UV detection of the continuous separation of the mixture of amino acids, phenylalanine and tryptophan, on the hydrophilic polyvinyl gel, TOYOPEARL, by eluting with water. Adequate separation of the mixture into its components was observed. However, the yield of the separation is unsatisfactorily low for the sample loaded.

In summary, the radial flow disk continuous liquid chromatograph in the present study proved to be suitable for compositional separation of similar as well as different substance in mixture solutions. This disk chromatography is effective in the saving of time and the decrease of the labor by monitoring the continuous separation of the mixtures with the flow-through UV detector. Scale up of the system for the larger volume can

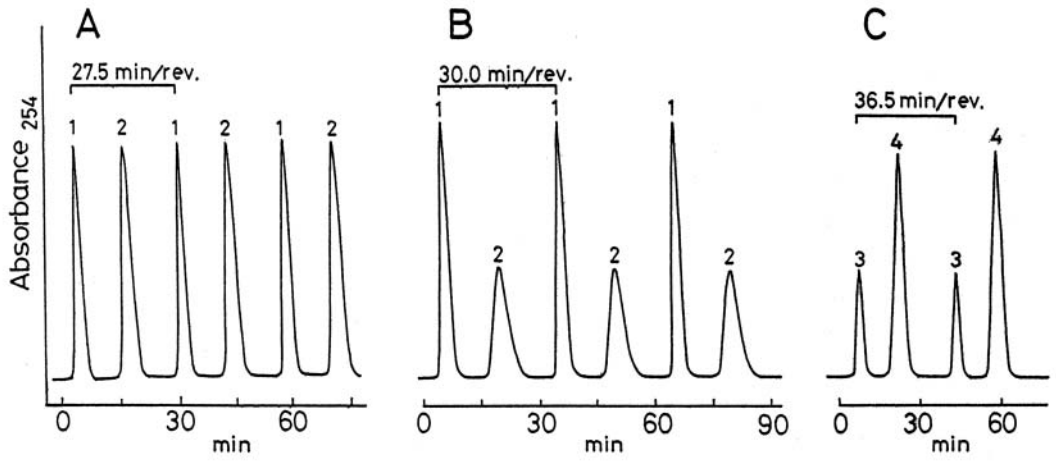


Figure 3. Representative chromatograms obtained in continuous separations.
(A) Separation of di-2-ethylhexyl phthalate (1) and dibutyl phthalate (2) on silica gel using hexane-ethylacetate (19: 1) mixture as an eluent. (B) Separation of di-2-ethylhexyl phthalate (1) and dibutyl phthalate (2) on silica gel-C_{18} using methanol-water (19: 1). (C) Separation of phenylalanine (3) and tryptophan (4) on polyvinyl gel using water as an eluent.

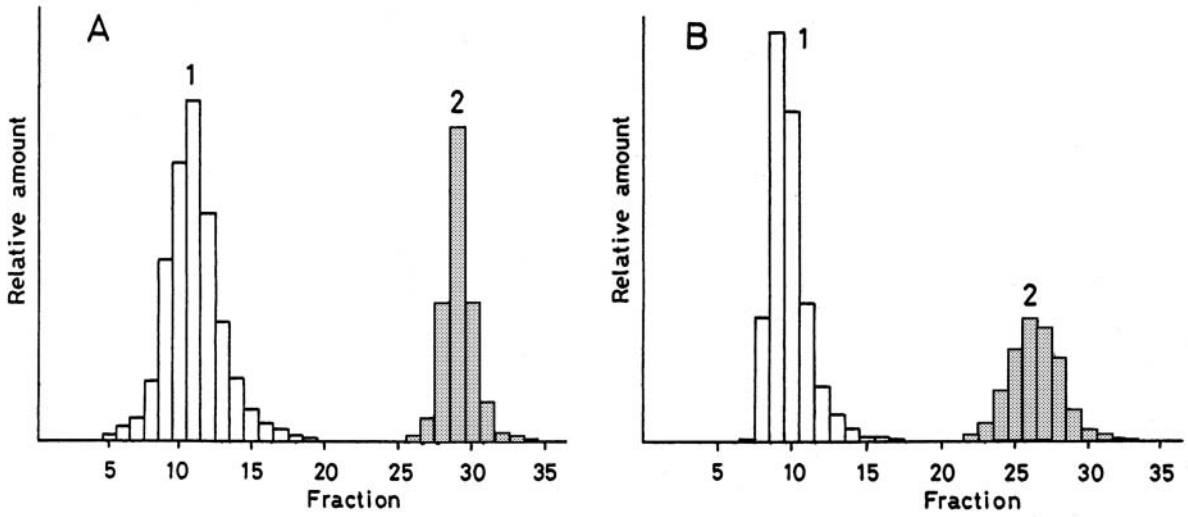


Figure 4. Results obtained from the HPLC analysis of fractions from the continuous chromatography of the mixture of di-2-ethylhexyl phthalate (1) and dibutyl phthalate (2). The mixtures were chromatographed on (A) silica gel using hexane-ethyl acetate (19: 1) and on (B) silica gel-C_{18} using methanol-water (19: 1) as an eluent.

be achieved by improving or modifying the disk chromatographic device designed in the present study.

REFERENCES

1) Martin AJP (1949) Summarizing paper. Discuss. Faraday Soc 7: 332－336.

2) Svensson H, Agrell CE, Dehlen SO, Hagdahl L (1955) An apparatus for continuous chromato graphic separation. Sci Tools 2: 17－21.

3) Solms J (1955) Kontinuierliche papierchro matographie. Helv Chim Acta 38: 1127－1133.

4) Fox JB Jr, Calhoun RC, Eqlinton WJ (1969) Continuous chromatography apparatus. Ⅰ. Construction. J Chromatog 43: 48－54.

5）Fox JB Jr (1969) Continuous chromatography apparatus. Ⅱ. Operation. J Chromatog 43: 55－60.
6）Nicholas RA, Fox JB Jr (1969) Continuous chromatography apparatus. Ⅲ. Application. J Chromatog 43: 61－65.
7）Barker PE, Barker SA, Hatt BW, Somers PJ (1971) Separation by continuous chromatography. Chem Proc Eng 52: 64－66.
8）Hilbrig F, Freitag R (2003) Review: Continuous annular chromatography. J Chromatog B 790: 1－15.
9）Vogel JH, Nguyen H, Pritschet M, Wegen RV, Konstantinov K (2002) Continuous annular chromatography: General characterization and application for the isolation of recombinant protein drugs. Biotechnol Bioeng 80:559－568.

要旨

混合物溶液を連続して分離するために，放射状流ディスククロマトグラフを設計し，作製した．クロマトグラフのディスクは400×g（1200rpm）の相対的な遠心力場の下でシリカゲル-C_{18}を充填することによって作られた．この充填したディスクを2 rphで回転させながら，メタノール－水（19：1）混合物を溶離剤として用い，8 m l /minの流速で流すことにより，フタル酸ジ-2-エチルヘキシルとフタル酸ジブチルの連続分離が達成された．また，シリカゲルディスクを用いてフタル酸エステルの連続分離が，親水性ポリビニールゲルディスクを用いて2種類のアミノ酸の連続分離がそれぞれ確かめられた．

受付日　2007年10月29日
受理日　2008年1月21日