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Short communication

Multidimensional counter-current chromatographic system and its application

Fuquan Yang[a], Jiang Quan[a], Tian You Zhang[a], Yoichiro Ito[b,*]

[a]*Beijing Institute of New Technology Application, Beijing 100035, China*

[b]*Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA*

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Abstract

A multidimensional counter-current chromatographic system was set up for the first time with two sets of high-speed counter-current chromatography instruments. This system was successfully applied to the preparative separation of isorhamnetin, kaempferol and quercetin from crude flavone aglycones of *Ginkgo biloba* L. and *Hippophae rhamnoides* L. with a two-phase solvent system composed of chloroform–methanol–water (4:3:2, v/v/v). © 1998 Elsevier Science B.V.

Keywords: Counter-current chromatography; Multidimensional separation; *Ginkgo biloba*; *Hippophae rhamnoides*; Isorhamnetin; Kaempferol; Quercetin; Flavone aglycones; Aglycones

1. Introduction

Multidimensional chromatography [1] is a frontier of contemporary chromatographic science. Its contents are abundant and varied, involving all chromatographic branches. Multidimensional chromatography styles so far developed include gas chromatography (GC)–GC, liquid chromatography (LC)–GC, LC–LC, supercritical fluid chromatography (SFC)–SFC, SFC–GC, LC–SFC, LC–capillary zone electrophoresis (CZE), free flow fractionation (FFF)–FFF, et al.. According to the theory of Poisson statistics, overlapping of the components on a complex chromatogram is more serious than was thought. Consequently, increasing the peak capacity is the best strategy to improve the resolution and this is readily achieved by a multidimensional separation [2].

High-speed counter-current chromatography (HSCCC), being a special liquid–liquid partition chromatography using no solid support matrix, eliminates tailing of solute peaks and sample loss due to irreversible adsorption [3]. Consequently the method is ideal for the preparative separation of natural products such as flavonoids. In the past HSCCC was applied to the analytical and preparative separations of isorhamnetin, kaempferol and quercetin that are important standard samples used for controlling the quality of the *Ginkgo* extracts and its preparations [4–7].

This paper describes our multidimensional counter-current chromatography (MDCCC) system and its application to the preparative separation of iso-

*Corresponding author.

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rhamnetin, kaempferol and quercetin from crude samples.

2. Experimental

2.1. Apparatus

A MDCCC system (Fig. 1) is set up with two Model GS10A2 multilayer coil planet centrifuges (HSCCC 1 with a 260 ml capacity column and HSCCC 2 with a 240 ml capacity column; Beijing Institute of New Technology Application, Beijing, China), two constant flow pumps (Model NS-1007; Beijing Institute of New Technology Application), two UV monitors (Model 8823A-UV; Beijing Institute of New Technology Application), two portable recorders (Yokogawa Model 3057; Sichuan Instrument Factory, Chongqin, China), two manual six-port valves, one with a 30 ml loop used as the injection valve and the other without loop used as the switching valve (Tianjin High-New Science and Technology Application, Beijing, China). Also used

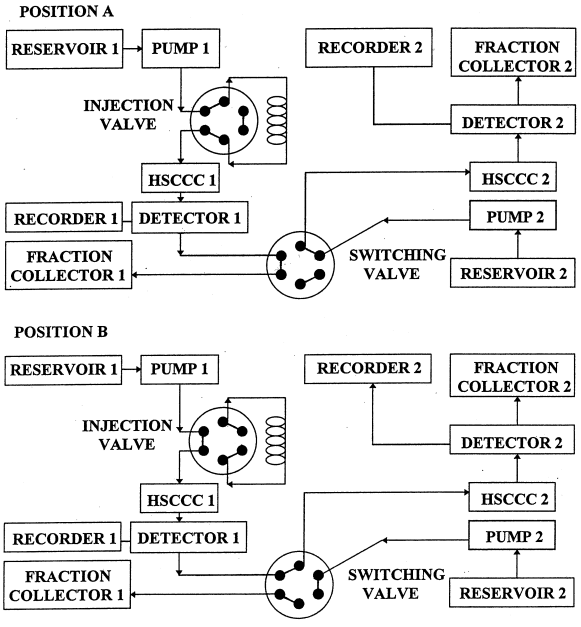

Fig. 1. Schematic diagram of our multidimensional counter-current chromatography (MDCCC) system with two sets of high-speed counter-current chromatography (HSCCC) systems, a six-port injection valve and a six-port switching valve.

were a rotary evaporator (Model RE-90; Beijing Institute of New Technology Application) and high-performance liquid chromatography (HPLC) equipment (Model SD-200 HPLC system; Rainin, Woburn, MA, USA).

2.2. Reagents

All organic solvents were of analytical grade and purchased from Beijing Factory (Beijing, China).

2.3. Preparation of two-phase solvent system

In the present study, a two-phase solvent system composed of chloroform–methanol–water at a volume ratio of 4:3:2 was used. The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated shortly before use.

2.4. Preparation of sample solutions

Crude mixtures of flavone aglycones from *Ginkgo biloba* L. and *Hippophae rhamnoides* L. were provided by National Engineering Research Center for Traditional Chinese Medicine (Shanghai, China). The sample solutions were prepared by dissolving the above crude samples each separately in two solvent phases used for the separation.

2.5. MDCCC separation procedure

Fig. 1 shows our MDCCC system in two valve positions, A and B.

The switching valve is initially set in position A, and HSCCC systems 1 and 2 are simultaneously filled with the upper aqueous stationary phase using pumps 1 and 2, respectively. Then, both apparatuses are rotated at 800 rpm, while the lower organic phase is eluted through HSCCC systems 1 and 2 using their respective pumps at a flow-rate of 2.0 ml/min. After the mobile phase front emerges and the two phases have established hydrodynamic equilibrium in each column, the sample solution is injected into HSCCC 1 through the injection valve while pump 2 is stopped. The effluent from the outlet of HSCCC 1 is continuously monitored with UV detector 1 at 254 nm, and collected with fraction collector 1 according

to the chromatogram traced by recorder 1. When the target peak appears, the effluent from HSCCC 1 is cut and introduced into HSCCC 2 by turning the switching valve to position B. After the target peak has eluted from HSCCC 1, the switching valve is returned to position A and the target peak fraction introduced in HSCCC 2 is eluted by restarting pump 2 together with detector 2, recorder 2, and fraction collector 2.

2.6. HPLC analysis

The analysis of each major HSCCC peak fraction containing the target analytes was performed with a Microsorb-MV ODS column (150×4.6 mm I.D., 5 μm; Rainin). The mobile phase composed of methanol–0.04% H_3PO_4 (50:50, v/v) was isocratically eluted at a flow-rate of 1.0 ml/min and the effluent monitored at 254 nm.

3. Results and discussion

Fig. 2 shows the separation of 200 mg of the crude flavone aglycones from *Hippophae rhamnoides* L. by the MDCCC system. Fig. 2A shows the chromatogram obtained from HSCCC 1 and recorder 1. The first peak in Fig. 2A contained unknown lipophilic impurities. The second large peak between the broken lines, containing a large amount of isorhamnetin, a small amount of kaempferol and unknown impurities, was cut and introduced into HSCCC 2. The chromatogram in Fig. 2B was obtained by HSCCC 2 from the cut fraction eluted from HSCCC 1 (the second peak between the broken lines in Fig. 2A). It shows that isorhamnetin was well separated from kaempferol and lipophilic impurities.

Fig. 3 shows the separation of 200 mg of the crude *Ginkgo* flavone aglycones obtained by MDCCC. Fig. 3A shows a chromatogram obtained from HSCCC 1. The first large peak contained several unknown impurities. The major component of the second peak is isorhamnetin and that of the third peak is kaempferol. The second and the third peaks between the broken lines were cut and introduced together into HSCCC 2 for further separation. Fig. 3B is a chromatogram of the cut fraction by HSCCC 2 showing a good separation between isorhamnetin and kaempferol.

During the separation of the crude flavone aglycones from *Hippophae rhamnoides* L. by HSCCC 1, the initial loss of the stationary phase was about 65 ml. After injection, carryover of the stationary phase

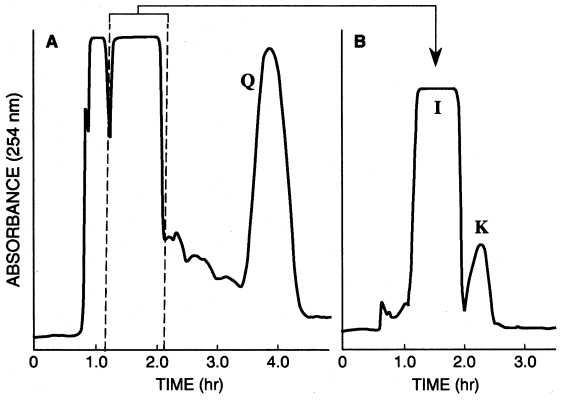

Fig. 2. Multidimensional counter-current chromatograms of the crude flavone aglycones from *Hippophae rhamnoides* L. I, isorhamnetin; K, kaempferol; Q, quercetin. Solvent system, chloroform–methanol–water (4:3:2, v/v/v); mobile phase, lower organic phase; flow-rate, 2.0 ml/min; sample size, 200 mg. (A) Chromatogram obtained by HSCCC 1; (B) chromatogram of the cut fraction (between the broken lines) obtained by HSCCC 2.

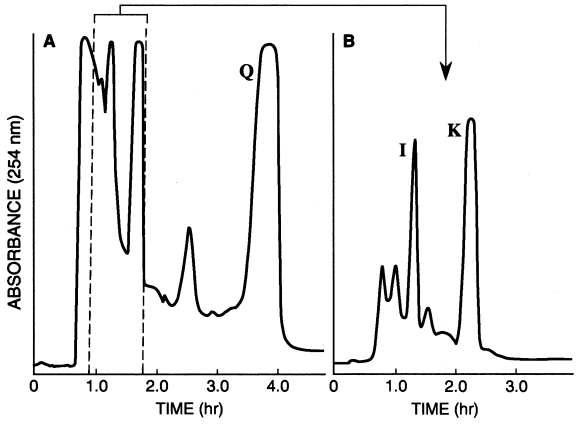

Fig. 3. Multidimensional counter-current chromatograms of the crude *Ginkgo* flavone aglycones. I, isorhamnetin; K, kaempferol; Q, quercetin. Solvent system, chloroform–methanol–water (4:3:2, v/v/v); mobile phase, lower organic phase; flow-rate, 2.0 ml/min; sample size, 200 mg. (A) Chromatogram obtained by HSCCC 1; (B) chromatogram of the cut fraction (between the broken lines) obtained by HSCCC 2.

began and continued until the lipophilic impurities had eluted from the column. This additional loss of the stationary phase was 35–45 ml which may be the main cause of inefficient peak resolution obtained from HSCCC 1. If HSCCC systems 1 and 2 were simply connected in series, the loss of the stationary phase and its adverse influence on the separation would spread to HSCCC 2. Furthermore, a large amount of lipophilic impurities would adversely affect the separation of the target compounds in HSCCC 2. This may also be the case in the separation of the crude *Ginkgo* flavone aglycones by MDCCC.

By using the MDCCC system, the serious loss of the stationary phase caused by lipophilic impurities was limited to HSCCC 1. The peak resolution of the MDCCC system may be improved by the use of the two-phase solvent system with two different volume ratios, one for HSCCC 1 and the other for HSCCC 2.

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