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Foam counter-current chromatography of bacitracin

II. Continuous removal and concentration of hydrophobic components with nitrogen gas and distilled water free of surfactants or other additives

HISAO OKA[a]
Laboratory of Technical Development, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892 (U.S.A.)
KEN-ICHI HARADA and MAKOTO SUZUKI
Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468 (Japan)
HIROYUKI NAKAZAWA
Institute of Public Health, Tokyo 108 (Japan)
and
YOICHIRO ITO*
Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Building 10, Room 7N-322, Bethesda, MD 20892 (U.S.A.)

ABSTRACT

Foam counter-current chromatography has been successfully applied to continuous removal and concentration of hydrophobic bacitracin (BC) components from a large volume solution using nitrogen gas and distilled water free of surfactants or other additives. The experiment was initiated by introducing nitrogen at the head inlet of the coil rotated at 500 rpm. Then, a 2.5-l volume of the sample solution containing BC at 50 ppm was continuously introduced into the middle portion of the coil at 1.5 ml/min. The hydrophobic components produced a thick foam which was carried with the gas phase and collected from the tail end of the coil while other components stayed in the liquid stream and eluted from the head outlet of the coil. High-performance liquid chromatographic analysis of the foam fraction revealed that the degree of enrichment increased with hydrophobicity of the BC components. BC-A and -F were enriched 1400 and 2260 times, respectively, compared with the original concentration in the sample solution. These results clearly indicate that the present method will be quite effective for detection and/or isolation of a small amount of natural products present in a large volume of aqueous solution.

INTRODUCTION

Commercial bacitracin (BC) is known to contain the major component (BC-A), its degradation product (BC-F) and over 20 UV-absorbing minor components [1].

[a] Visiting scientist from the Aichi Prefectural Institute of Public Health, Nagoya 462, Japan.

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Recently, efficient separations of these components have been performed with analytical high-performance liquid chromatography (HPLC) [1] and high-speed countercurrent chromatography (CCC) [2].

Our previous studies [3] have shown that the surfactant-free foam CCC method can separate the bacitracin components according to their hydrophobicity. The results encouraged us to conduct preparative-scale enrichment of the BC components in continuous sample feeding. Because the method exclusively utilizes inert nitrogen gas and distilled water, the harvested materials in the foam fraction can be easily recovered in a pure state without a risk of decomposition or deactivation which might occur in the process of eliminating the surfactants or other additives. Consequently, the method may be invaluable in detection and/or isolation of a minute amount of surfactant bioactive compounds present in a bulk of the biological fluids such as urine, blood and blood dialysate, tissue culture medium, fermentation mixture, etc.

EXPERIMENTAL

Reagents

The BC sample was purchased from Sigma (St. Louis, MO, U.S.A.). Glass-distilled chromatographic-grade methanol was obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Reagent-grade disodium hydrogenphosphate was obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.).

Apparatus

The design of the apparatus used in the present study having been described in detail in Part I [3] is briefly described here. The foam CCC centrifuge holds a column holder and a counterweight holder symmetrically on the rotary frame at a distance of 20 cm from the central axis of the centrifuge. The column holder revolves around the central axis of the centrifuge and simultaneously rotates about its own axis at the same angular velocity in the same direction.

The separation column was prepared from approximately 10 m of 2.6 mm I.D. PTFE (polytetrafluoroethylene) tubing (Zeus, Raritan, N.J., U.S.A.) by winding it directly on the column holder hub of 15 cm diameter in double layers between a pair of flanges spaced 5 cm apart. The column design is schematically illustrated in Fig. 1.

The separation column is equipped with five flow channels: The liquid feed line and the foam collection line are connected at the tail end of the coil and the gas feed line and the liquid collection line at the head, while the sample feed line opens at the

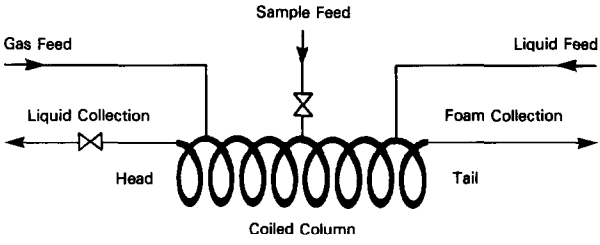

Fig. 1. Column design for foam CCC.

middle portion of the column. The liquid collection line and the sample feed line are each equipped with a needle valve (Washington Valve Co., Rockville, MD, U.S.A.) while the foam collection line is left open to the air. Nitrogen gas was fed through the gas feed line at 80 p.s.i. directly from a nitrogen cylinder. The sample solution was introduced through the sample feed line from a poly(vinyl chloride)-coated glass bottle pressured at 40 p.s.i. through another nitrogen cylinder. The liquid feed line was closed and not used in the present study.

General procedure of foam CCC

Each experiment was initiated by introducing nitrogen gas at 80 p.s.i. into the head of the column rotating at 500 rpm. Then, an aqueous sample solution containing bacitracin at a desired concentration was continuously fed into the column through the sample feed line which was connected to the reservoir pressured at 40 p.s.i. The opening of the needle valve on the liquid collection line was adjusted to obtain the desired liquid and foam outputs. Effluents from the foam and liquid collection lines were collected separately and subjected to HPLC analysis.

HPLC analysis

HPLC analyses of the foam and liquid fractions were performed in Shimadzu (Kyoto, Japan) equipment consisting of a Model LC-6A pump, a manual injector, A Model SPD-6A detector, and a Model C-R5A recording data processor using a Capcell Pak C_{18} column, 15 × 0.46 cm I.D. (Shiseido, Tokyo, Japan). The mobile phase, composed of 0.04 *M* disodium hydrogenphosphate (pH 9.4)–methanol (38:62, v/v), was isocratically eluted at a flow-rate of 1 ml/min and the effluent was monitored at 234 nm.

RESULTS AND DISCUSSION

As described elsewhere [1], BC consists of more than twenty components, including the major component BC-A, its oxidation product BC-F, and other minor components of unknown structure. Under the present reversed-phase HPLC conditions, BC components were eluted in the increasing order of hydrophobicity to yield over 15 resolved peaks as shown in Fig. 2A, where the polarity of each component may be judged from its retention time. In the present study, peaks, 3, 7, 11 (BC-A) and 14 (BC-F) are selected to study the degree of enrichment in the foam fraction.

The first series of experiments was conducted to optimize the operational conditions for continuous enrichment of the BC components. These studies were performed under a set of fixed conditions of sample volume (100 ml), sample feed rate (1.5 ml/min at 40 p.s.i.), nitrogen gas feed pressure (80 p.s.i.), and the rotational speed of the column (500 rpm) as indicated in Table I. Liquid was not fed from the liquid feed line, because it was found that liquid feeding dilutes the solutes, resulting in interruption of the steady foam elution. Effects of the needle valve opening at the liquid outlet on the enrichment of the BC components was investigated with a 100-ml sample volume by varying the BC concentration from 10 to 100 ppm. As shown in Table I, the results indicated that opening the needle valve less than 1.0 turn or over 5.0 turns yielded no foam fraction. However, when the needle valve was opened in an optimum range between 1 and 3 turns, peak 3 was enriched by 13 to 77 times; peak 7,

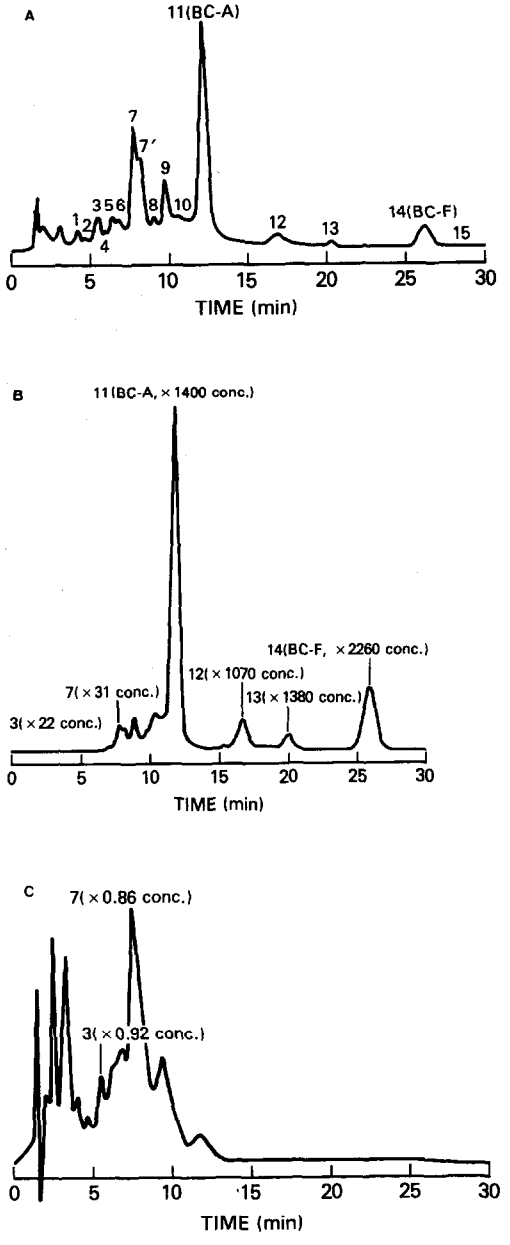

Fig. 2. HPLC analysis of bacitracin. (A) Chromatogram of original sample; (B) chromatogram of foam fraction; (C) chromatogram of liquid fraction. Experimental conditions: solvent: 0.04 *M* disodium hydrogenphosphate–methanol (38:62); flow-rate: 1 ml/min; detection: 234 nm.

TABLE I

OPTIMIZATION OF OPERATIONAL CONDITIONS FOR ENRICHMENT OF BACITRACIN ON CONTINUOUS SAMPLE FEEDING

Sample size: 100 ml in water; sample feeding-rate: 1.5 ml/min (40 p.s.i.); nitrogen gas pressure: 80 p.s.i.; liquid feeding-rate: 0.

Conditions	Enriched concentration (times)			
	Peak 3	Peak 7	Peak 11	Peak 14
Needle valve				
<1.0 turn open	–[a]	–	–	–
1–*ca.* 3	13–77	52–180	180-4000	220–11 670
>5.0	–	–	–	–
Sample concentration				
<25 ppm	–	–	–	–
25	27–77	52–130	180–350	220–410
50	13–65	87–170	630–2000	1340–7700
100	55–77	94–150	280–4000	570–11 670

[a] No foam eluted.

52 to 180 times; peak 11, 180 to 4000 times; and peak 14, 220 to over 11 000 times. Studies on the sample concentration showed that no foam eluted if the concentration was less than 25 ppm. As the concentration was increased to 25 ppm, the foam began to emerge through the foam collection line with a substantial degree of enrichment of the BC components ranging from 27 to 410 times their concentration in the original sample solution, and further increase of the sample concentration at 50 ppm resulted in the enrichment of peak 11 by 2000 times and that of peak 14 by near 8000 times. Finally, at the 100 ppm sample concentration, peak 11 was enriched by 4000 times and peak 14 over 11 000 times. The above results clearly indicate that the yield of the solute concentration in the foam fraction increases with the hydrophobicity of the BC components and also with the original sample concentration within the applied range.

In the light of the results obtained from the above preliminary studies, large-scale enrichment of the BC components was performed as follows: The experiment was initiated by introducing nitrogen (80 p.s.i.) from the tail of the column rotated at 500 rpm; then, the dilute BC aqueous solution (50 ppm) was continuously fed through the sample feed line at a flow-rate of 1.5 ml/h from the reservoir bottle pressured at 40 p.s.i. The liquid feed line was closed, and the opening of the needle valve on the liquid collection line was adjusted at 2.0 turns to maintain the minimum foam output through the foam collection line. The run was continued for 28 h until the total 2.5 l volume of the sample solution was eluted. Both foam and liquid effluents were each separately pooled in a container and subjected to the HPLC analysis.

The results of the experiments are shown in Fig. 2B and C where the selected peaks are labelled together with the degree of enrichment over the original concentration in the sample solution. In the foam fraction (Fig. 2B), the yielded concentration increases with the hydrophobicity of the BC components as demonstrated in the preliminary studies described earlier: peak 3 was enriched by 22 times; peak 7, 31

times; peak 11 (BC-A), 1400 times; peak 12, 1070 times; peak 13, 1380 times; and peak 14 (BC-F), 2260 times. In the liquid fraction (Fig. 2C), the hydrophobic BC components corresponding to peaks 11 to 14 were undetected, while peaks 3 and 7 were eluted with no enrichment in concentration.

The overall results of the present studies clearly indicate that the foam CCC method is capable of concentrating a small amount of the hydrophobic BC components present in a large volume of the sample solution. The method may be effectively applied to detection and/or isolation of various natural products from a large volume aqueous solution.

REFERENCES

1 H. Oka, Y. Ikai, N. Kawamura, M. Yamada, K.-I. Harada, Y. Yamazaki and M. Suzuki, *J. Chromatogr.*, 462 (1989) 315.
2 K.-I. Harada, Y. Ikai, Y. Yamazaki, H. Oka, M. Suzuki, H. Nakazawa and Y. Ito, *J. Chromatogr.*, 538 (1991) 203.
3 H. Oka, K.-I. Harada, M. Suzuki, H. Nakazawa and Y. Ito, *J. Chromatogr.*, 482 (1989) 197.