

## Note

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### Increasing the speed of droplet counter-current chromatography separations

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Droplet counter-current chromatography (DCCC) has been shown to be a very efficient method of separation. It has found most use in the preparative separation of very polar compounds<sup>1</sup>. Other methods of chromatography suitable for very polar compounds, such as ODS-silica adsorption chromatography, suffer from an inherently low sample capacity and a high cost of adsorbent. DCCC has an inherently large sample capacity, of up to several grams<sup>2</sup>, and since the separation is based upon solvent partitioning it requires no costly adsorbent. Several excellent reviews have been written on the technique<sup>3,4</sup>.

Typical separations take from 1 to 3 days. The system operates at low flow-rates and pressures and does not require attention during the elution period. However, if other laboratory operations can not be performed during the elution period, the time required for separation becomes a severe limitation. We report an inexpensive modification to the commercial instrument\* that allows a substantial decrease in the time required between injections.

The period of separation, from injection to the following injection, can be subdivided into periods of washing, loading and elution. Washing the solvent path is necessary to collect former sample that has remained in the stationary phase. Loading of a new stationary phase is necessary to prevent contamination from previous samples and to ensure a proper composition of the stationary phase.

The maximum speed of elution is limited by droplet formation and is characteristic of a particular solvent system. Little can be done to hasten the elution time. However, the speed of washing and loading can be increased. In a current commercial design (Eyla DCC-300, Tokyo Rikakikai), the maximum flow-rate in washing and loading is limited by solvent back pressure and the pressure limitations of the system's coupling system (300–450 p.s.i.). The back pressure at high flow-rates is caused by the long path length of PTFE capillary tubing (160 m × 0.5 mm I.D.) and vertical glass columns (120 m × 2.0 mm I.D.). The stationary phase component solvents

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\* We are aware of two companies marketing DCCC instruments. These instruments are the Eyla DCC-300 and DCC-300G from Tokyo Rikakikai Co., Ltd., and the Buchi model 670 DCCC from Buchi Laboratory Techniques, Ltd. Although this paper discusses only the Eyla model DCC-300, we believe the modification can be applied also to the Buchi model since they are of similar design.

TABLE I

MAXIMUM FLOW-RATE POSSIBLE DURING WASHING AND LOADING PERIODS FOR NORMAL AND IMPROVED SOLVENT PATHS

Solvent	Viscosity (cP)	Flow-rate (ml/min)	
		Normal	Improved
Hexane	0.31	2.4	13.4
Methanol	0.55	2.4	13.4
Chloroform	0.57	2.4	13.4
Water	1.0	1.4	12.0
<i>n</i> -Butanol	2.98	0.4	6.9

have different viscosities (see Table I) and normal maximum washing and loading flow-rates can vary from 0.5 to 2.5 ml/min. A system equipped with 300 vertical glass columns ( $400 \times 2.0$  mm I.D., 400 ml total volume) would then take from 3.5 to 16 h to wash or load, depending upon the stationary phase composition, just to displace the previous solvent. More time may be necessary to counteract incomplete washing and to counter any mixing at the interphase of the new and old solvent systems.

One obvious solution to this flow limitation is to shorten the solvent path and reduce the amount of back pressure. This was done by the incorporation of five inert valves. These valves allow the operator to alternate between the normal solvent path of 300 head-to-tail vertical columns attached in a series and a modified solvent path where four groups of 75 head-to-tail vertical columns are attached in parallel (see Fig. 1). To place the instrument into the wash mode, each valve is rotated 90°. In this position, the solvent is diverted from the pump directly into each of the four groups, and at the same time is diverted from each of the four groups directly to the

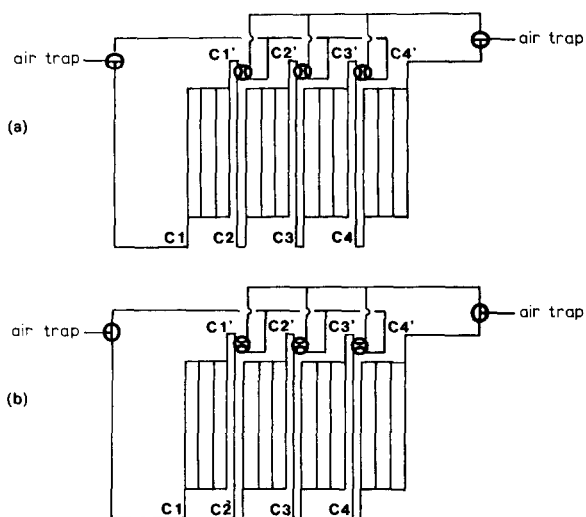


Fig. 1. DCCC solvent path after the installation of valves. (a) Normal solvent path, 300 head-to-tail columns. (b) Wash and Load solvent path, four groups of 75 head-to-tail columns.

exit. The solvent path is now 1/4 the normal length. The associated drop in back pressure allows many times the normal maximum flow-rate (see Table I). Water can now be pumped 8.6 times faster and *n*-butanol seventeen times faster than before the modification. Although chloroform, methanol and hexane could be pumped 5.6 times faster than normal, flow-rates are now limited only by the maximum flow-rates of the instrument's pump, 13.4 ml/min, rather than the systems pressure limitations. Equipping the instrument with a high flow-rate pump would further increase the maximum flow-rate of these three solvents.

## EXPERIMENTAL

Two 3-port-3-port and three 4-port-4-port valves (Hamilton panelmount PTFE valves, Nos. 86777 and 86779, VWR Scientific) are mounted on a 2 × 100 × 250 mm metal plate and connected to two 4-way junctions (No. 200-22, Alltech Assoc.) and each other with 1/4 × 28 thread per inch (tpi) fittings (No. 20022, Alltech Assoc.) and 0.5 mm I.D. PTFE tubing as shown in Fig. 2. Care should be taken to place all the valve housings square with respect to the panel edges as shown in Fig. 2. In this way all valve handles can be positioned horizontally for the washing or loading mode or all vertically for the separation mode. Otherwise, the correct position of each valve could be difficult to ascertain. The ascending air trap is then connected to position T1 and the descending airtrap to T2. After arranging the twelve cassettes of 25 vertical columns each into four groups of three cassettes, the groups are labeled 1-4. The bottom of the first tube in group 1 (position C1) is attached to position C1 on valve T1 (see Fig. 2). The top of the last tube in group 1 (position C1') is attached to position C1' on valve V1. The first and last tubes of each of the remaining groups

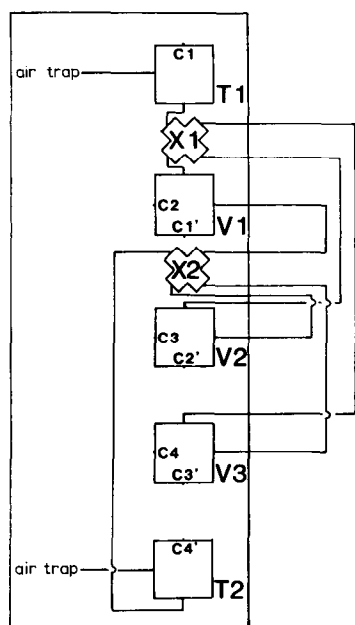


Fig. 2. Valve panel schematic showing the placement and connections of valves.

of columns are connected to their corresponding positions on the valves in a similar manner by following Fig. 2. If the solvent path length, hence back pressure, is not the same for all four groups of columns uneven washing and loading will occur. The approximate lengths of tubing necessary can be divided into four types and are listed in Table II.

To check for leaks, the valve handles should all be positioned in the separation mode (vertical position) and the pump flow-rate should be adjusted to produce a high pressure. After a check of leaks has been made, the panel can be secured to the instrument with the same two screws used to hold the sample loop brackets.

TABLE II  
LENGTH OF PTFE TUBING (0.5 mm I.D.) FOR VALVE CONNECTIONS  
See Fig. 2 for details.

<i>From</i>	<i>To</i>	<i>Length (cm)</i>
T1 or T2	X1 or X2	10
T1 or T2	columns	63
V1, V2 or V3	X1 or X2	23
V1, V2 or V3	columns	30

## RESULTS AND DISCUSSION

The methanol washing of columns and the loading of a water based stationary phase can now be performed in 1 h rather than in 7.5 h. A methanol washing and *n*-butanol based stationary phase loading can now be completed in 1.5 h rather than the normal 19.5 h. It was usual practice to wash the system first and then load the stationary phase overnight, since it is rare that both could be completed within a work period, so the washing and loading would then contribute 24 h to the total separation time if the separation time is considered to be the sum of the actual separation and the washing and loading time. The removal of this time due to the now possible increase in washing and loading flow-rates can give a 30% increase in the overall rate of separation for a 3-day separation.

There is an added benefit of this modification. For simple separations where fewer numbers of columns are needed, it is no longer necessary to undo fittings to shorten the number of columns and hence increase the speed of elution. When only valve T2 and V1, V2 or V3 are placed in the wash position, the 300 columns are reduced to 75, 150 or 225 columns, respectively. Also, if only valves T1, V2 and T2 are placed in the wash position the 300 columns are divided into two separate 150 column units. In this way the instrument uses half the number of columns for the separation, but with twice the overall capacity.

## REFERENCES

- 1 I. Kubo, J. A. Klocke, I. Ganjuian, N. Ichikawa and T. Matsumoto, *J. Chromatogr.*, 257 (1983) 157.
- 2 I. Kubo and A. Matsumoto, *J. Agr. Food Chem.*, 32 (1984) 687.
- 3 K. Hostettmann, *Planta Med.*, 39 (1980) 1.
- 4 K. Hostettmann, C. Appolonia, B. Domon and M. Hostettmann, *J. Liquid Chromatogr.*, 7 (1984) 231.