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Short communication

Evaluation of different tubing geometries for high-speed counter-current chromatography

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Abstract

Different tubing geometries were evaluated for use in high-speed counter-current chromatography. Standard round tubing was compared to rectangular and twisted rectangular tubing. The number of theoretical plates for a standard anthocyanin mixture from black currant was determined for each experiment. The results of twisted rectangular tubing were superior to a standard setup.

Keywords: Counter-current chromatography; Column geometry; Partition efficiency; Efficiency; Instrumentation; Anthocyanins

1. Introduction

High-speed counter-current chromatography (HSCCC), a support-free chromatographic technique, uses round PTFE tubing wound in multiple layers around a spool. The so-called ''multi-layer coil'' is then rotated in a planetary fashion (type-J planetary motion, cf. [1]). The length of the tubing, the diameter of the tubing, the volume of stationary phase, as well as other parameters like sample loading, revolution speed, flow-rate and orientation of the coil influence the separation characteristics and the peak resolution. These factors have been the subject of many studies on counter-current chromatography [1–9]. The planetary motion of the coil creates an heterogeneous force field which results in an efficient mixing of the two phases in such a way that about 50 000 partition steps per hour of the analytes in the solvent system can be achieved. The shape of the tubing is expected to influence the fluid dynamics that occur inside the coil and therefore may have a direct impact on the partition efficiency of the solvent system. Different tubing geometries were investigated in detail in this study.

2. Experimental

2.1. HSCCC

A high-speed countercurrent chromatograph manufactured by P.C. (Potomac, MD, USA) was equipped with one coil and a balanced counterweight. The separations were run at a revolution speed of 800 rpm. The mobile phase was delivered

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by a Biotronik HPLC pump BT 3020 (Jasco, Gross-Umstadt, Germany). A standard anthocyanin mixture was isolated from black currant according to a procedure described in Ref. [2]. The lyophilisate was dissolved in an 1:1 mixture of light and heavy phase. The amount of sample injected was 2 ml of a solution with a concentration of 1.6 mg/ml. Elution was monitored with a Knauer UV–Vis detector (Berlin, Germany) at 520 nm. The solvent system used for the separation of the anthocyanins was previously described [2] and consists of *tert*.-butyl methyl ether (TBME)–*n*-butanol–acetonitrile–water (2:2:1:5; less dense layer as stationary phase, both phases were acidified with 0.1% trifluoroacetic acid; flow-rate: 0.6 ml/min; all chemicals were analytical grade and obtained from local suppliers). The length of the tubing was calculated to give a final volume of 59 ml for all separations of the present study. PTFE tubing SW 12 (standard size) of 2 mm I.D. and rectangular tubing (2×1.5 mm) were obtained from Zeus Industrial Products (Raritan, NJ, USA). Rectangular tubing was either wound with the long side down or the short side down or twisted to produce a defined number of helical turns per 10 cm. In this manner 0.6 and 1.8 helical turns per 10 cm of tubing length were achieved. Round SW 12 tubing was squashed between hard surfaces to deform its round shape and give it an oval shape. This tubing was subsequently twisted to produce a defined number of helical turns similarly to the rectangular tubing.

2.2. Separation procedure

The column was first filled entirely with the upper non-aqueous stationary phase. After injection of the sample, the column was rotated at 800 rpm while the mobile phase was pumped into the head of the column at 0.6 ml/min. After elution of the anthocyanins the rotation was stopped, the coil was pumped out and the retention of stationary phase was measured by determination of the volume ratio of displaced stationary phase to total coil volume.

3. Results and discussion

The separation of anthocyanins using HSCCC was previously described [2]. XAD-7 extracts from black currant contain glucosides of cyanidin and delphinidin as well as the respective rutinosides. HSCCC separation with a stationary phase volume of 850 ml (solvent system TBME–*n*-butanol–acetonitrile–water, 2:2:1:5; less dense layer as stationary phase) resulted in a baseline separation of the first eluting rutinosides. The anthocyanin mixture from black currant was used in the present study to test the influence of different tubing geometries on separation efficiency in HSCCC. For this purpose, a number of coils was wound with different tubing geometries. All other parameters, i.e., flow-rate, sample loading and revolution speed were kept constant.

The insert in Fig. 1 shows the different tubing geometries tested. All results were compared to round standard SW 12 tubing (cf. Fig. 1, left side). Rectangular PTFE tubing was tested in different orientations because it is expected that this might affect the partition efficiency (cf. Fig. 1).

From the resolution between the second and third peaks, it is clear that the twisted rectangular tubing produced the highest partition efficiency. The deep rectangular coil made by winding its shorter side down gave a slightly higher efficiency than that of the standard coil, while the flat rectangular coil made by winding the same tubing in the longer side down yielded the lowest efficiency among all coils examined. From these chromatograms the partition efficiency in terms of theoretical plates (N) may be computed from the second and the fourth peaks according to the conventional formula:

$$N = 16 \cdot \left(\frac{t_R}{W}\right)^2$$

where t_R is the retention time of the peak maximum and W is the peak width. W of the second peak is calculated as twice that of the first half since the other half is overlapped with the third peak. This was done under the assumption that the peak shape is symmetrical. The results are summarized in Table 1. Higher retention of the stationary phase as well as higher partition efficiency may have contributed to the improved peak resolution observed in peak 2 (Fig. 1) and therefore the twisted rectangular column set-up yielded 15–30% higher efficiency than that of the standard coil.

Although the hydrodynamic mechanism which

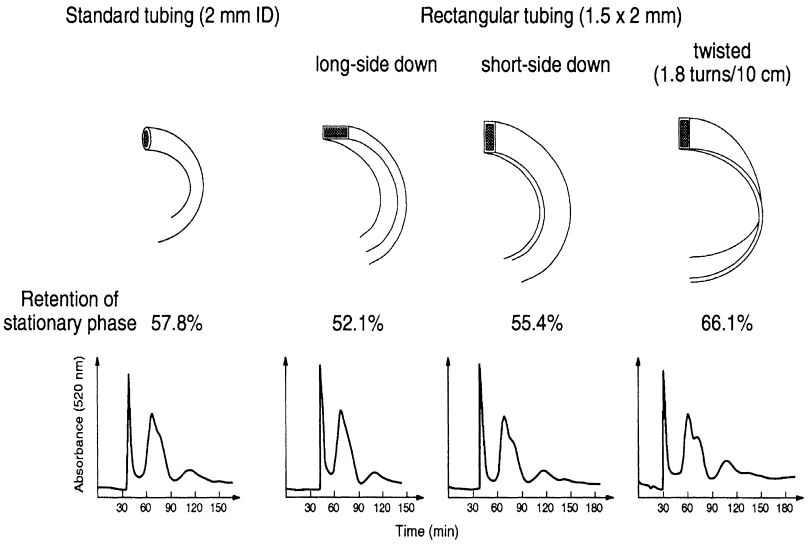

Fig. 1. Effects of tubing geometry on partition efficiency in high-speed counter-current chromatography.

improved the partition efficiency of the twisted rectangular coil is not fully understood, the following possibilities may be considered: in the coiled tube subjected to the type-J planetary motion, two phases are distributed in such a way that they are mixed near the center of the centrifuge (where the centrifugal force is minimum) and separated into two layers at the opposite location (where the centrifugal force is maximum) [1,3]. Between these two locations of the coil, the two phases violently countercurrent to each other back and forth causing longitudinal solute band broadening to reduce the partition efficiency. The twisted rectangular coil will deter this violent sloshing of the two phases to minimize this undesirable band broadening effect. The second possibility is that each phase is forced to change its shape as it flows through the twisted rectangular coil. This will result in constant lateral mixing within each phase (opposed to longitudinal mixing which causes solute band broadening) which contributes to the improved partition efficiency of the twisted rectangular coil. Finally the third possibility is an enhanced retention of the stationary phase in the deep portion of the twisted rectangular column while the shallow portion acts as a barrier to form multiple partition segments along the flow path.

A similar rectangular coil twisted 0.6 turns per 10 cm yielded slightly lower efficiency than that with 1.8 turns per 10 cm described above. The standard tubing flattened and then twisted to form 0.5 turns

Table 1
Partition efficiency and % retention of stationary phase for four types of coiled columns tested by HSCCC

	Peak 2, delphinidin-3-rutinoside (TP)	Peak 4, delphinidin-3-glucoside (TP)	Retention of stationary phase (%)
Standard coil	188	105	57.8
Flat rectangular coil	156	115	52.1
Deep rectangular coil	204	134	55.4
Twisted rectangular coil (1.8 turns/10 cm)	215	140	66.1

TP = Theoretical plates.

per 10 cm failed to improve the results obtained by the standard coil (chromatograms not shown).

4. Conclusion

The overall results of our studies indicate that the partition efficiency of HSCCC can be substantially improved with a twisted rectangular coil as described above. The efficiency may be further improved by selecting the optimum dimensions of the rectangular tubing. It is also interesting to examine the performance of twisted triangular tubing.

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