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### A New Small Coil-Volume CCC Instrument for Direct Interfacing with MS

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## A New Small Coil-Volume CCC Instrument for Direct Interfacing with MS

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### ABSTRACT

One of the major factors restricting the use of CCC as an analytical tool is the speed at which a separation may be conducted. This paper describes the phased development of a new low volume capacity Milli-CCC device, which is as rapid as HPLC, achieving high resolutions in minutes as opposed to hours, with the capability of linking with a mass spectrometer (CCC/MS). The Milli-CCC J-type apparatus has gears enclosed in a lubricated case to minimize noise. Its volume with one coil mounted in a cantilever style is 4.6 mL with 2.5 m of 0.76 mm bore tubing. It can rotate at a maximum speed of 2100 rpm. Stationary phase retention factor higher than 60% could be obtained with 1500 rpm and 1 mL/min producing separation of compounds with  $K_D$  distribution coefficient of 1 in less than 5 min. The connection to MS was straightforward.

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*Key Words:* Small volume CCC machine; J-type coil planet centrifuge; Mass spectrometry coupling.

## INTRODUCTION

The first successful commercial production of a CCC device was in the mid 1980s by PC Inc in the USA. This was based on research performed by Ito at NIH. He performed exhaustive tests looking at the variation of retention with speed for both mobile phases for a range of different phase systems varying in polarity.<sup>[1]</sup> However, the whole field has been limited by the equipment available, which has been Ito's laboratory test rigs or PC Inc's commercial devices, or the clones manufactured by other companies that subsequently followed. Bousquet et al.<sup>[2]</sup> were some of the first to systematically look at the resolution and efficiency of the process as flow varied, reporting quite surprising results. They found (for the PC Inc device they were using) that efficiency, as determined by the number of theoretical plates, increased significantly at low flow, reached a minimum at intermediate flows, and increased again at higher flows. But, perhaps more significantly, they found that resolution, while it also increased at low flows (almost double that of intermediate flows), only slightly reduced at higher flows. At Brunel, this has been confirmed, not just for 1.6 mm diameter tubing but for 3.7, 5.3, and 7.7 mm bore tubing as well.<sup>[3]</sup>

With current technology, using Bousquet et al. purifications<sup>[2]</sup> as an example, they demonstrated 500 theoretical plate purifications with 1.66 mm bore tubing, 66 m long (152 turns) with a capacity of 143 mL. They found that the efficiency did not significantly change as flow was increased from 0.2 mL/min (separation time 12 hours) through 0.4 mL/min, 4 mL/min and 9 mL/min (separation time 16 min).

Brunel's modelling work (confirmed by tests) has shown that resolution increased with the square root of the coil length. In addition, experiments have shown that resolution increases linearly with speed of rotation.

The strategy used in this development was to shorten the length of coil tubing by a factor of 16, which reduced the resolution by a factor of 4, then compensated for this reduction by increasing rotational speed by a factor of 4. These two changes return the resolution back to its previous level whilst dramatically reducing the separation time. Applying these two changes to 1.66 mm bore tubing, will reduce the capacity to about 10 mL with the same high-resolution separations possible in just one minute.

Scaling down even further would be possible using 0.8 mm tubing. While separation times would remain the same (2 min), the capacity would reduce still further to about 2.5 mL.





### APPROACH STUDY

The fundamental requirements of an analytical CCC instrument that can directly interface with an MS detector are: (1) it can perform a separation in minutes; (2) it provides good sample resolution; (3) it has a low back pressure to preserve the PTFE flying leads. In addition to these operating requirements, it was important to produce a device that is; (4) easy to use; (5) quiet to operate; (6) of a size that would easily fit into a typical chromatography stack; (7) reliable and safe.

By addressing these criteria in iterative steps it was possible to specify the instrument, based on the established knowledge that separations are possible on machines furnished with 0.8 mm bore tubing.<sup>[4-6]</sup> This development was achieved in two phases: (1) the prototype/feasibility stage to assess, (a) whether large or small radius rotors should be used and (b) whether spiral or helical coils should be used; and (2) the development phase, where a pre-competitive prototype could be produced for extensive user trials.

### RESULTS AND DISCUSSION

#### Phase 1—Prototype/Feasibility Stage

##### Objective

To investigate the trade-offs between (1) large and small rotor radius and (2) spiral and helical coils.

##### Methodology

##### *Large Radius vs. Small Radius Rotor*

To compare different rotor radii a prototype analytical rotor was assembled onto a Brunel CCC housing and control system,<sup>[7]</sup> capable of rotating up to 1400 rpm and was called a high speed analytical experimental (HSACCC) rig. This was assembled with two identical bobbins, each with 0.8 mm bore tubing connected in series to provide a total coil volume of 25 mL with a  $\beta$  value of 0.88.

The HSACCC machine was compared with a PharmaTech CCC3000 (Pharma-Tech Research Corporation, Baltimore, MD), which has a smaller rotor radius ( $R = 38$  mm) and three 11.5 mL multilayer coils connected in series, (total coil volume of 34.5 mL), with a  $\beta$  range of 0.56–0.77. The Pharma-Tech has a top speed of 3000 rpm. All comparative tests were performed using a heptane : ethyl acetate : methanol : water (1.4 : 0.6 : 1.0 : 1.0)





phase system (4B). Resolution tests were performed using a model sample system of benzyl alcohol and phenyl ethanol. These tests were operated at speeds which gave comparable “g” levels, as indicated in Table 1.

### *Spiral vs. Helix*

Another pair of prototype bobbins (Fig. 1) was constructed, which was also fitted to the HSACCC. Each bobbin had three stainless steel coils with 10 loops that were approximately 5.7 m long. The first coil (2B-2.5 mL) was wound helically with a  $\beta$  value of 0.79, the second (2C-2.7 mL), wound helically with a  $\beta$  value of 0.85, and the third, wound spirally (2D-2.5 mL) with a mean  $\beta$  value of 0.79 and a range of 0.74–0.85. The bobbins were identical, with the exception of the tubing bore, which was 0.76 mm on one and 0.5 mm on the other, after Oka.<sup>[6]</sup> The rationale behind being able to run the machine at this high rotational speed, and hence, higher “g” level for these bobbins, was to achieve a high stationary phase retention and maintain the resolution by increasing the number of mixing and settling cycles, with respect to time. Both of these factors were aimed at compensating for shortening the length of coil tubing. If the rationale proved successful, a high percentage stationary phase retention would provide a high mobile phase linear flow rate, hence, speed of separation, while an increase in the number of mixing and settling cycles would provide a good quality separation.

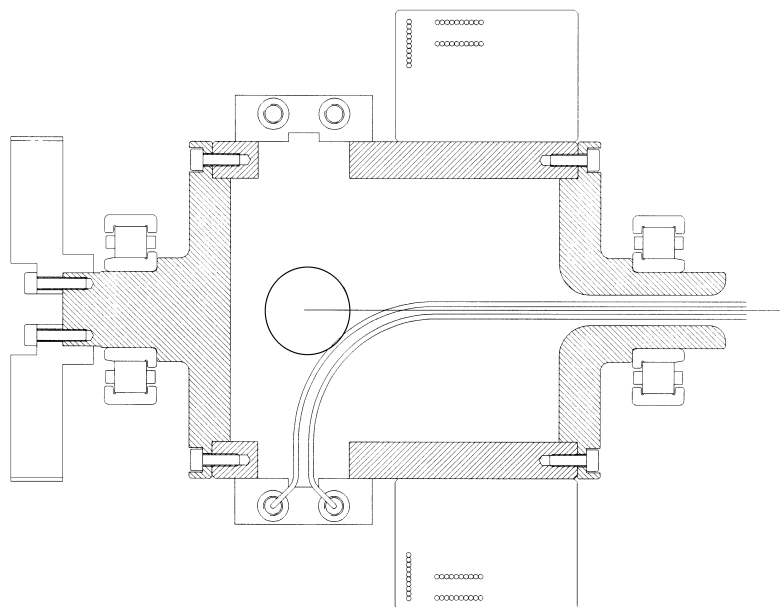
### Results

When comparing similar coils operating at the same “g” field, but at different rotor radii, the results showed no significant difference. It was concluded, that the final choice on whether to build a large or small radius device should rest on engineering, rather than chromatographical factors.

**Table 1.** Comparative rotational speeds to give the same “g” level in the Brunel HSACCC and the PharmaTech CCC3000.

Tangential acceleration	PharmaTech CCC3000	Brunel HSACCC
“g” Level	Speed (rpm)	
78.70	1,360	800
122.96	1,700	1,000
177.07	2,040	1,200
241.01	2,380	1,400





**Figure 1.** Schematic cross-section of prototype bobbin containing three experimental coils.

The results comparing a helical with a spiral coil (Fig. 2) confirmed it was possible to conduct separations in short coils, but only at high “g” levels, and also, showed there was little difference between spiral and helical coils. Lastly, tests using 0.5 mm bore tubing gave poor retention for the “g” fields used and so 0.8 mm tubing was selected.

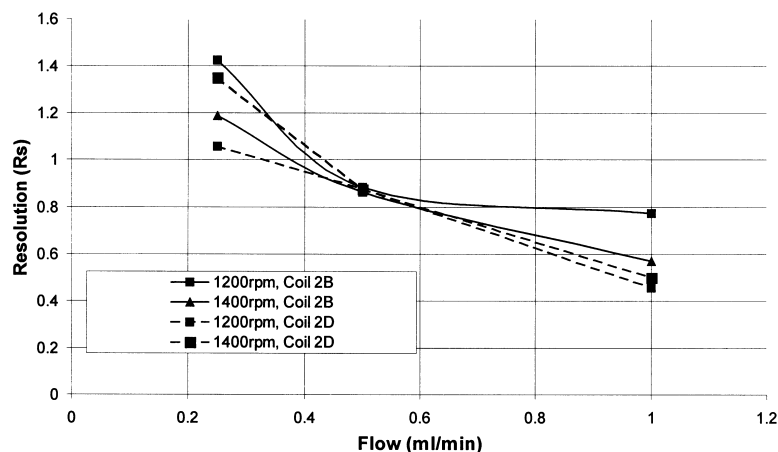
As a general observation, it was noted during the above tests, that although resolution improved by increasing speed, it was not a one to one relationship and could, at very high speed, reach a maximum. It was also noted that the resolutions obtained on the short 2.5 mL coils were related to those obtained using the higher capacity coils by the ratio  $\sqrt{V_{c1}/V_{c2}}$ .

### Phase 2—Prototype/Development Stage

#### Objectives

To design an analytical CCC instrument that would: (1) perform a separation in minutes; (2) provides good sample resolution; (3) have a low back pressure; (4) be easy to use; (5) be quiet to operate; (6) be of a size that





**Figure 2.** The variation of resolution vs. mobile phase flow, comparing a helical coil (2D-2.6 mL) with a spiral coil (2B-2.5 mL) at two different rotational speeds.

would easily fit into a typical chromatography stack; and (7) be reliable and safe.

### Design

Armed with the knowledge that it was possible to conduct separations in relatively short lengths of small-bore tubing, it was important to appreciate the implications of rotating at a fast rpm to provide the high “g” level. The two major considerations are that the planetary bearing life will be considerably reduced and the flying leads will experience a much greater amount of stress. Not only are they subjected to a higher “g” level, but they will also experience a greater number of cycles per minute.

For the instrument (Fig. 3) described in this paper, it was decided to base the design on three key considerations: (1) The least stressful path for the flying leads; (2) An easy to change bobbin design; (3) A size that would fit in with common chromatography practice.

This led to a design featuring a cantilevered rotor, driving a single planet shaft, with the bobbin mounted on the end of the shaft in a cantilever fashion. (A cantilever in this design means that both the bobbin and rotor are mounted on shafts, which are supported from one end only. Unlike traditional CCC machines where they are mounted on shafts between both ends, which are supported.) These decisions allow a bobbin to be removed easily from the end of the shaft; with the flying lead emerging from the centre of the bobbin



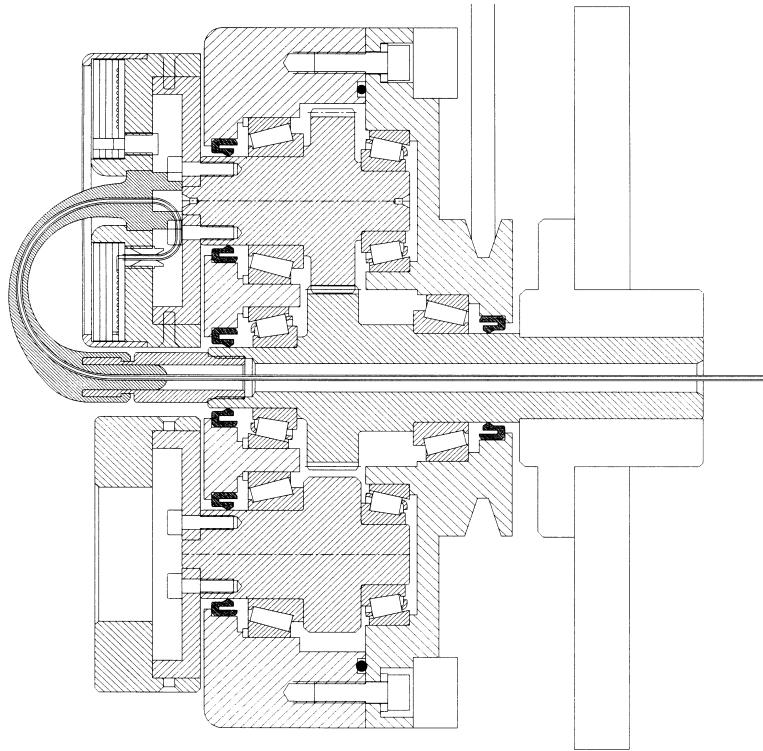


Figure 3. Cross-section of prototype Milli rotor.

passing through a 180° turn to the stationary, sun shaft. The merit of this simple route is the flying lead only experiences the “g” field along its length and is, therefore, less likely to distort.

In addition to the three key considerations, this machine also offers the opportunity to reduce the noise generated by the Sun and Planet gears of a “J” type CCC centrifuge, by allowing the design to feature an enclosed gearbox. This means, during operation the gears and bearings can be soaked in an oil bath, which in itself will reduce noise, improve heat transfer from the bearings to the gearbox casing, and increase bearing life.

To summarise: the instrument constructed<sup>[8]</sup> is a “J” type CCC centrifuge with an enclosed lubricated gearbox rotor driven around a stationary central shaft. It has two planetary shafts, rotated about a 50 mm radius up to a maximum speed of 2100 rpm. One is driven, upon which a coil is mounted cantilever style, where the shaft has emerged from the enclosed gearbox, and the other, not driven, has a counterweight mounted in exactly the same way as

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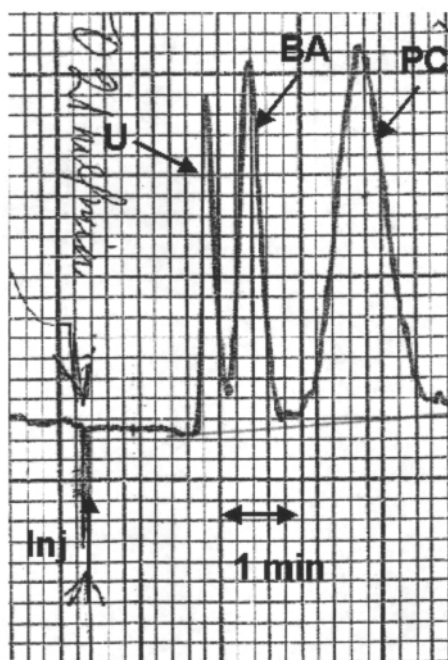


the coil. The coil has a  $\beta$  value range 0.68–0.79, a volume of 4.6 mL using 0.76 mm bore tubing which is 10.15 m long. The coil is serviced by a single pair of 0.5 mm bore PTFE flying leads, that run from the centre of the bobbin to the stationary central shaft, in a simple 180° turn that is aligned with the “g” field.

In use this instrument requires a mobile phase flow of up to 2 mL/min, conducts a separation in 3 to 20 min, depending on the flow rate, and has a low coil back pressure. Therefore, with the PTFE tubing rated at 13 kg/cm<sup>2</sup> it can be directly interfaced to a MS that generates a typical back pressure of 6–10 kg/cm<sup>2</sup>.<sup>[9,10]</sup>

### Results

The chromatogram in Fig. 4 shows a separation of Uracil (U, with a  $K_D=0$  marker), Benzyl Alcohol (BA,  $K_D=0.25$ ) and Paracresol (PC,  $K_D=0.85$ ) at a flow of 1.02 mL/min and speed of rotation of 1500 rpm. An



**Figure 4.** A typical chromatogram illustrating a separation of uracil, benzyl alcohol, and paracresol in a heptane:ethyl acetate:methanol:water (4B-1.4:0.6:1.0:1.0) phase system. The rotational speed was 1500 rpm, flow 1 mL/min and temperature 30°C.



analysis based on Sutherland et al.<sup>[11]</sup> shows that there are 41.5 CCD steps per minute resulting in 74 steps to elution of the solvent front, 103 steps to the elution of the benzyl alcohol peak, and 174 steps to the elution of the paracresol peak. As the prototype tests show, increased resolution can be obtained by decreasing flow or by increasing the length of the coil. However, it is considered that 40 CCD steps per minute is adequate for most fractionation problems. The chromatogram shown took 5 min at a flow of 1 mL/min. This gives separation times ranging from 2.5 to 20 min for flow ranging from 2 mL/min to 0.25 mL/min. Although this "Milli" prototype has yet to be connected to an MS, the HSACCC machine has been coupled to two, both Thermofinnigan machines, one a TSQ 700 and the other an AQA. Considering the "Milli" machine's tubing is the same bore but only 20% of the length of the HSACCC, and hence, lower back pressure, connecting it to an MS should be straight forward.<sup>[9,10]</sup>

### CONCLUSION

This paper presents a "J" type CCC centrifuge that can not only conduct separations in an analytical timescale but also is versatile, with an ability to be connected to a MS detector as a CCC in-line column. The important step to acknowledge is that this CCC centrifuge can complement HPLC in today's modern analytical laboratory, encouraging more CCC applications, where 100% sample recovery or the presence of particulates makes CCC the method of choice.

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