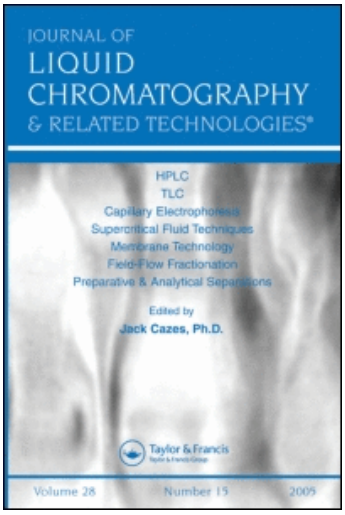

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EXTRACTIONS AND PURIFICATIONS

AN EVALUATION OF THE PERFORMANCE OF A PREPARATIVE CCC MACHINE FOR THE SEPARATION OF AN ACTIVE PHARMACEUTICAL INGREDIENT

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ABSTRACT

A laboratory-scale CCC (LCCC) of approximately 80 mL capacity was used to investigate the purification of an active pharmaceutical intermediate (API) using polar liquid-liquid systems based around water / n-butanol. Variation of pH and the polarity of the mobile phase by the use of a gradient pumping system, lead to the development of an isocratic system (where the upper organic phase was mobile). The method was then transferred to a larger (preparative PCCC) unit of 930 mL capacity. An estimated output from the large scale machine was made and compared with the production rate to current methods of purification, i.e., HPLC.

The capacity of the PCCC has been estimated to be equivalent to a 30 cm × 7.5 cm i.d. dynamic axially compressed (DAC) LC column; it is anticipated to be of comparable capital cost. The PCCC machine failed to purify the API to the product specification using the current eluent system. The potential output was

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comparable and the solvent consumption only 25 % of the HPLC system. Weepage or stripping of stationary phase (SP) was observed on runs with a relatively high mobile phase flow rate. From a process robustness and, hence, validation point of view, weepage is unacceptable.

INTRODUCTION

The separation of a multi-component mixture into pure entities can be achieved in a number of ways. Where possible, chemists and process engineers in the Chemical Industry will try to develop purification using crystallization or distillation. If a satisfactory quality cannot be achieved other techniques can be employed, such as liquid-liquid extraction (LLE) or preparative liquid chromatography (LC). In the former example, LLE components are partitioned between two liquid phases; separation is a function of the partition coefficient, P. In general, this technique is only capable of purifying 'simple systems' or P, for the component of interest must be high with respect to the rest of the mixture. The equipment can be a box type contactor, vertical column, or centrifugal, but all have similar features, e.g., they have regions of mixing and settling, operate on a batch wise, a semi-continuous, or continuous basis. The latter technique, LC, has the ability to provide the greatest resolving power, values of >6000 theoretical plates per meter are quoted when using a high performance system (HPLC).

This technology has a number of drawbacks, such as relatively high capital cost, high production costs because of the solvent usage and stationary phases, and its limit of scale. The use of simulated moving bed (SMB) systems is now being considered for more general purification, and has the potential to deliver tonnage quantities for binary mixtures.

Countercurrent chromatography (CCC) is a simple process, which has the ability to capture most of the advantages demonstrated by the techniques discussed above, whilst avoiding many of their inherent problems.[1,2] It is a liquid-liquid process based on partition and is devoid of adverse interactions with the solvents used (unlike LC where side reactions can take place on the surface of acidic silica gel). All of the product (plus impurities if required) can be recovered. It operates at a low pressure drop and potentially requires lower capital costs when compared with HPLC. The CCC machine used for this experimental work is based on coils rotating in a planetary motion.[3] The stationary phase is first allowed to fill the coils, and once the speed of rotation has been achieved mobile phase (MP) is pumped in at a controlled rate. Displacement of some of the stationary phase takes place (the amount is characteristic of the operating parameters), the sample can then be loaded into the mobile phase and separation effected.

Depending on the partition coefficient, it is normal to have the phase mobile which will carry the component of interest out of the machine. Therefore, operation could be normal phase (upper phase mobile) or reversed phase (lower phase mobile); rotation should be in the direction of the coil winding. In normal phase mode, the lighter (mobile) phase is introduced tail (periphery) flowing to the head (center) or visa versa for reversed phase. The head-to-tail rule for operation has been reported by Sutherland.[4]

Three experiments were performed at this scale with the following objectives: to compare performance (resolution) of 1.6 mm, i.e., PTFE tubing with 3.68 mm, i.e., stainless steel; prove if data gathered on smaller machines will enable prediction of elution times at increased scales of operation (cf. scale-up of chromatography on solid supports at a constant linear velocity of eluent); investigate the effect of flow rate (5 mL/min -v- 20 mL/min) on the larger machine; similarly investigate operation at 800 rpm -v- 1200 rpm at a high flow rate (20 mL/min) of mobile phase.

EXPERIMENTAL

Solvent System

The API used for this work is a hydrophilic peptide supplied as a 4% w/w solution in methanol. It elutes from a standard C_{18} analytical HPLC system (25 × 0.46 cm ODS2 5 μm column at 1.0 $mL.min^{-1}$) in 7 minutes using 45% aqueous phosphate buffer in methanol as eluent. This result indicates a solvent system referred to in the work of Brown *et al* as Type A, should be used.[5] Appropriate mixtures within this class which have been used are based on n-butanol / water or n-butyronitrile / water. The former was chosen based on its predicted solubility characteristics[6] (recognizing also the objective of this exercise was not to develop a system capable of achieving >99% separation of the active compound, but one which could be used in the PCCC).

A pre-requisite target for a Manufacturing Facility is to have a 'simple' isocratic solvent system which ideally can be re-cycled and the relative volumes of each saturated phase at separation are equal. Development of a solvent system considered the effect of pH and addition of polar modifiers on the separation; this work, however, will not be discussed in detail here. A single partition experiment (shake test) followed by HPLC analysis of each phase gave an indication of the potential separation to be expected. The next step used the LCCC and studied the effect of continuously varying pH and polarity of the mobile phase with time; this was achieved using a Gilson double pump system with gradient capability. Once again, the choice of acid and base was restricted to aid method development, therefore, acetic acid and pyridine were used to investigate effects of pH.

Solvent modifiers investigated were methanol, butyl acetate, and acetonitrile; of this set acetonitrile was adopted. To increase the surface tension of the final solvent system, the ionic strength of the aqueous phase was adjusted by the addition of sodium chloride. The two phase solvent mixture adopted is listed in Table 1.

The physical properties of the system were estimated using propriety software and data from the Physical Properties Data Service National Engineering Laboratories (PPDSNEL) and Universal Function Activity Coefficients (UNIFAC) for non-ideal systems. They are listed in Tables 2 and 3.

Material

Materials used to evaluate solvent systems were purchased as general purpose grade from Fisher Scientific. The compound under evaluation was used as a 4% w/w active substrate in 10 % methanol/water. The LCCC is a two bobbin, four coil, J-type machine (planet radius (R), 10 cm and a γ value of 0.38 - 0.85) fitted with 1.66 mm i.d. PTFE tubing. Only one coil was used for this experiment. The coil is estimated to be 40.5 m long and holds 80 mL. The PCCC, also a J-type machine, is fitted with two bobbins supporting stainless steel coils of 3.68 mm i.d. and 91 m long. The coils are connected by an internal 'flying lead' giving a total capacity of 928 mL.

The machine was operated at two speeds (800 rpm and 1200 rpm) and two flow rates of MP (5 mL.min^{-1} and 20 mL.min-^{1}). Fractions were collected according to when the product was expected to elute based on theoretical prediction.[7] MP was pumped using a single piston Gilson system operating in isocratic mode (or in tandem with a second unit to give gradient capability, control was via a PC).

Table 1. Solvent System Developed for Trial of the Laboratory CCC (LCCC) and Preparative CCC (PCCC)

Component	Quantity	Weight (g)
n-Butanol	100 mL	80.93
Acetonitrile	5 mL	3.90
Water	100 mL	99.95
Glacial acetic acid	1.0 mL	
Sodium chloride	1.0 g	
Upper phase volume	114 mL	
Lower phase volume	90 mL	

Note: Volumes are observed to contract on mixing.

Table 2. Pure Component Properties at 20°C by PPDSNEL

Component	Density (kg/m^3)	Viscosity (cP)	Surface Tension (dynes/cm)
n-Butanol	809.3	2.945	24.42
Acetonitrile	780.4	0.378	29.29
Water	999.5	0.952	72.74

HPLC analysis was performed using a HP 1050 and a size exclusion column, type TSK gel G2000 supplied by TOSO Haas. The eluent was a 9/1 (v/v) acetonitrile/0.1M perchloric acid buffered to pH 2 with sodium hydroxide. Operating conditions are flow = 1.0 $mL.min^{-1}$; λ = 280 nm; column temp = 30°C. Sample preparation involved evaporating each fraction to a dry residue under vacuum. Dissolving the solid in 1:1 acetonitrile/water (1 mL (LCCC) and 10 mL (PCCC)); 0.2 mL (LCCC), and 0.1mL (PCCC), it was then diluted with 1mL of eluent and analysed.

Run 1: LCCC (800 rpm, 1 mL/min)

The solvent mixture was prepared as per table 1. Lower SP was charged into a single 80 mL coil. The machine was set to rotate in a clockwise direction at 800 rpm and the MP (upper organic) was pumped tail (periphery) to head (center) at 1.0 $mL.min^{-1}$ (ΔP = 2 - 3 bar); fractions of 3 mL were collected. A quantity of SP equal to 16 mL was displaced. One minute later (17 minutes total run time) 1.0mL of sample was injected via a Rheodyne valve. Fractions of 2 x 9 mL and 15 x 3 mL were collected, commencing 16 minutes after injection (33 minutes total run time). Each fraction was analyzed, the data is presented in Appendix 1,

Table 3. Phase Equilibrium at 20°C Determined Using UNIFAC for LLE (Based on a Salt and Acetic Acid Free Basis)

Component	Density (kg/m^3)	Viscosity (cP)	Surface Tension (dynes/cm)
Upper phase (mobile)	842.3	2.30	49.53
Lower phase (stationary)	977.4	1.04	71.44

table 7. Stripping of SP was observed during the initial stage of the run, the degree of SP loss was not quantified.

Run 2: PCCC (800 rpm, 20 mL/min)

The objective of this experiment was to investigate high flow of MP (approximately four times the linear velocity of Run 1) at 800 rpm. Lower SP was charged into the coils over 30 minutes. The machine was set to rotate in a forward clockwise direction at 800 rpm and the MP (upper organic) was pumped tail (periphery) to head (center) at 20.0 $mL.min^{-1}$ (no pressure gauge fitted to determine pressure drop). A quantity of SP equal to 235 mL was displaced (74.7% retention). Once MP had broken through and deemed stable, 13 mL of sample (containing 0.52 g of active component) was injected. After 12 minutes had elapsed, fractions of 3 x 4 minutes and 13 x 1.5 minutes were collected. Stripping of SP was observed throughout the run and is tabulated in Appendix 2, Table 8. The coil was blown clear with 6 psi nitrogen (0.4 kg/cm^2) and comprised: upper phase = 481 mL; lower phase = 437 mL (an additional 26.4% displacement of SP). Each fraction was analyzed, the data is presented in Appendix 1, Table 7.

Run 3: PCCC (800 rpm, 5 mL/min)

The objective of this experiment was to compare the performance of the LCCC to that of the preparative machine. Scale-up is based on linear velocity of the MP, however one should bear in mind the difference in coil length is a factor of 2.24 times in favor of the latter. The machine was set-up as per Run 2. The flow of MP was adjusted to 5 $mL.min^{-1}$ and 110 mL of SP displaced (88.2% retention). When stable, 13 mL of sample was injected and approximately 22 minutes later fractions of 3 x 20 minutes and 16 × 5 minutes were collected. No stripping of SP was observed during this run. The coil was blown clear with 6 psi nitrogen (40 kPa) and comprised: upper phase = 110 mL; lower phase = 810 mL (86% retention of stationary phase). Each fraction was analyzed, the data is presented in Appendix 1, Table 7.

Run 4: PCCC (1200 rpm, 20mL/min)

The objective of this experiment was to compare high flow, i.e., 20 $mL.min^{-1}$ with high rotational speed, i.e., 1200rpm. The machine was set-up as per Run 2. The flow of MP was adjusted to 20 $mL.min^{-1}$ and 115 mL of SP displaced (87.6%

retention). When stable 13 mL of sample was injected and approximately 7 minutes later fractions of 6 × 2.5 minutes and 17 × 1.5 minutes were collected. Stripping of SP was observed during the run and was variable. The coil was blown clear with 6 psi nitrogen (0.4 bar) and comprised: upper phase = 210 mL; lower phase = 690 mL (an additional 10% of SP displaced, refer to Appendix 2). Each fraction was analyzed, the data is presented in Appendix 1, Table 7.

DISCUSSION

A direct comparison between the two machines is not exactly valid due to the different coil lengths, i.e., 40.5 m versus 91 m.

Performance Between LCCC and PCCC

Product quality and recovery for Runs 1 to 4 can be found in Table 4 and Appendix 1. Judgment of yield from each run was based on those fractions with a HPLC strength of ≥ 95% by area. For the LCCC 92.1% of the original 40 mg charge of API was found to meet this criteria. Runs 2 - 4 on the PCCC unit gave 83.2%, 82.6% (recovery and 87.2 % if fractions 5 - 10 are combined), 83.8%, respectively, of the initial 0.52 g of API @ 100%. Based on the calculated linear velocities, refer to Appendix 3, experiments employing slower flows gave better resolution, which may suggest the flow inside the coil is laminar (see Table 9), and true mixing of the phases is not taking place. As a consequence of high flow, there is less time for mass transfer to take place and is reflected in the results seen for Runs 2 and 4. A second conclusion, which may be drawn from Run 1, is improved mass transfer caused by additional mixing due to wall effects one would expect as the bore size of the tubing is reduced.

Table 4. Separation Factors for Runs 1 - 4

Run Number	Initial Displacement of Stationary Phase	k' Based on Sutherland's Approach[a]
1	20 %	0.70
2	25.3 %	0.81
3	11.8 %	0.61
4	12.4 %	0.59

[a] The volume taken to determine k' is at a point of maximum concentration for the elution profile.

Performance Between Runs 2-4 on the PCCC

High flow and low rotational speed results in a larger initial displacement of SP, which is higher than theory predicts based on the data of Sutherland *et al*.[7] A second feature is the effect caused by flow of sample through the coils leading to an additional loss of SP equivalent to 25%. There are a number of possible reasons for this observation, such as the change in composition of the MP effecting the interfacial tension and, hence, shear forces acting between the two liquid phases.

The applied gravitational force produced at 800 rpm lies at the extreme edge of the operating window[8] for this particular solvent system (see Tables 2 and 3 for predicted values of interfacial tension, viscosity and density). Increasing the rotational speed improves the retention of SP by approximately 5% per 100 rpm; 1200 has only an additional loss of 10%. By extrapolation, one might expect at 1500 rpm and a flow rate of 20 $mL.min^{-1}$, that no further loss of SP should occur after injection of the sample. If one compares Run 3 with Run 2, a second approach to stem the initial displacement of SP is to reduce the flow rate.

Theoretical and Observed Elution Profiles

Using the method of Sutherland,[7] an attempt was made to predict elution of API when transferring a method from the LCCC to the PCCC machine, refer to Table 4. Using accepted chromatography theory, the retention factor k can only be determined if the initial displacement volumes are the same (cf. Runs 3 and 4) and from the experiments reported here, a link between MP flow rate and rotational speed has been demonstrated. A further complication to the prediction is caused by displacement of SP due to sample effects.

Comparison of Output for LC Versus CCC

The API used in this study has been investigated previously using LC. To try and compare equivalent capacities, the PCCC has a void volume of 930 mL. If one considers a HPLC column containing packing of approximate bulk density 0.49 g/cm^3, this would give a column size of 30 cm x 7.5 cm i.d. based on an equivalent mass of SP. Purification of the API using chromatography on a reversed phase silica and an aqueous based eluent has been carried out previously. From an initial loading of 1 g , 0.587g met the ≥ 95%, i.e., a 58.7% acceptance; it was expected only one run per hour would be performed and required 8.4 l of eluent per hour to support the chromatography. In one month 112 g could be produced.

Table 5. Cycle Time and Volume Requirements for Mobile Phase

Run Number	Service Time (min)	Displ. Time (min)	Elution Time (min)	Cycle Time (min)	Vol. of MP (cm^3)
1	35	16	45	96	96
2	30	12	29	71	1051
3	–	22	100	152	610
4	–	6	26	62	625
LC	20	0	40	60	8400

Similar data can be generated for the two CCC machines. To estimate processing time and output the following is used:

Cycle time = (SP fill time + coil emptying)
+ MP displacement time + elution time of product

This information has been tabulated in Table 5. This data was obtained by first combining those fractions with HPLC strength of ≥ 95% by area. The % recovery was then determined by:

$$\frac{\text{sum of the detector output for the API peak in combined fractions}}{\text{sum of the total detector output for fractions containing API}} \times 100$$

Table 6. Production Data

Run Number	Recovery %	Weight of AI g/h
1	92.0	0.024
2	83.2	0.37
3	82.6 (87.2)[a]	0.179
4	83.8	0.44
LC[b]	58.7	0.58

[a] The recovery is 87.2 % for fractions 5 - 10; fraction 5 is marginally outside the >95 % by area cut off.

[b]The recovery from LC is lower than CCC but it does provide a material which meets the specification, i.e., no single impurity >0.2 % w/w and total imps. <1.5.

A sample loading of 40 mg was used for Run 1, 0.52 g for Runs 2 – 4, and 1.0 g for LC; all charges are corrected to 100% w/w API, therefore, correcting for recovery and operating time, an hourly output can be estimated, refer to Table 6. The solvent usage per run is 2070 mL, i.e., 1140 mL of upper mobile phase and 930 mL of lower stationary phase. On balance, CCC uses 75% less solvent than LC. The potential output per month @ 60% occupation can be expressed by:

PCCC = 72.5 g (Run 3) to 177 g (Run 4)
HPLC = 112.5 g

It is anticipated that a capital cost comparison of a 7.5cm i.d. HPLC (DAC) unit with PCCC is neutral.

CONCLUSION

During the runs, losses of SP were observed with both machines. The degree to which is governed by the difference in interfacial tension of the two phases and the interaction of the sample as it progresses through the coil. It can be countered by either reducing the flow rate of MP or increasing the speed of rotation. If the sample volume is too large or its interaction with the SP is significant, a plug flow within the coils can occur. It will result in a significant displacement of the SP from the machine.

The elution profile of the API from the CCC experiments exhibits some fronting. This observation is attributed to solvent in sample affecting the partition coefficient. The purification of API using this particular system has furthered our understanding of CCC. Prediction and performance of the LCCC and PCCC machines is dependent on the solvent system used, and may become more difficult to interpret for Type A systems exhibiting very close interfacial tension, viscosity, and density. Providing it is recognized, a link exists between flow rate and rotational speed, an experimentalist could predict a scale up factor with a moderate degree of accuracy.

If one refers to Table 4, Run 1 provided material with the highest recovery, i.e., 92% @ > 95% strength by area, as assessed by HPLC. Considering the coil length of the LCCC, which is approximately 40% that of the PCCC machine, as the bore size is increased a loss in efficiency might be expected. More work is necessary to prove this point. When developing a 'normal phase' system, i.e., upper phase mobile using the LCCC, the flow rate should be chosen which leads to minimal loss of stationary phase for a given rotational speed. A cautionary note should be presented here, that when working in 'reversed phase' mode a completely different set of parameters may be operating and before true conclusions can be drawn, more work is necessary.

The flow of mobile phase in the coils is laminar as judged by the Reynolds and Froude numbers quoted in Table 9 (the purpose of which is to apply accepted engineering principles for fluid dynamics to understand performance). Runs 1 and 3 used a low flow and performed slightly better than 2 or 4 in terms of recovery. The separation takes place at the interface, it is a mass transfer process limited by the rate of diffusion from the bulk. Low flow rate of mobile phase will assist this process. A second approach is to increase the area of contact between the two non-miscible phases by having a larger retention of mobile phase, i.e., slow the rotational speed of the PCCC or design a machine whose coils are of an elliptical cross section. Whilst recognizing the potential to increase efficiency, it may result in reduced resolution.[10]

Comparison of HPLC versus CCC for this example, the former can provide material of a higher strength but it creates large volumes of eluate, necessitating long evaporation time and recycling to achieve a satisfactory recovery. Recognizing the efficiency of a HPLC column, it should be stated that the solvent system used for the CCC was not fully optimized. Using CCC the process is simple; solvent requirement is 25% of that for the HPLC. All the API is recovered as a 0.2 % w/v solution; no special steps are necessary prior to commencing the next run. In combination, CCC may complement HPLC; in the example cited here, difficult impurities were readily removed using CCC.

One can conclude: for every solvent system a CCC machine will have an optimum flow rate at a particular speed of rotation. In the example used here for the PCCC, it is > 5 mL/min @800 rpm and < 20 mL/min @1200 rpm.

APPENDIX 1

Table 7. Experimental Data for the 4 Runs

RUN 1 – LCCC machine, volume 80 mL, 800 rpm, 1 mL/min

	Relative Retention Time of Impurities						
Fr. No.	0.43	0.59	0.75	0.86	0.93	1.0	1.05
6	0.83	0	0.89	5.6	0.33	92.35	0
7	0	0	0	4.25	0.41	95.34	0
8	0	0	0	3.04	0.40	96.56	0
9	0	0	0	1.91	0.40	97.69	0
10	0.51	0	0	1.26	0.42	97.82	0
11	4.70	0.17	0	0.56	0.38	90.46	3.73

(continued)

Table 7. Continued

RUN 2 – PCCC machine, volume 928 mL, 800 rpm, 20mL/min

	Relative Retention Time of Impurities							
Fr. No.	0.59	0.64	0.75	0.86	0.92	1.0	1.05	1.15
9	0.48	0	0.82	4.27	0.56	93.86	0	0
10	0	0.11	0	3.43	0.29	96.16	0	0
11	0	0.10	0	2.57	0.30	97.03	0	0
12	0	0	0	1.78	0.23	97.98	0	0
13	0.73	0	0	1.22	0.30	97.74	0	0
14	6.16	0.26	0	0.68	0.26	87.48	2.58	2.58

RUN 3 – PCCC machine, volume 928 mL, 800 rpm, 5 mL/min

	Relative Retention Time of Impurities							
Fr. No.	0.59	0.64	0.75	0.86	0.92	1.0	1.05	1.15
4	0.86	0	0	6.94	0	92.21	0	0
5	0	0	0	5.35	0	94.65	0	0
6	0	0	0	4.36	0.17	95.47	0	0
7	0	0	0	3.60	0.27	96.13	0	0
8	0	0	0	2.48	0.26	97.26	0	0
9	0	0	0	1.52	0.27	98.21	0	0
10	0	0	0	0.55	0.25	99.2	0	0
11	3.83	0	0	0.23	0.29	93.39	1.88	0.38

RUN 4 – PCCC machine, volume 928 mL, 1200 rpm, 20 mL/min

	Relative Retention Time of Impurities							
Fr.No.	0.59	0.64	0.75	0.86	0.92	1.0	1.05	1.15
7	0.46	0	0.49	5.13	0.20	93.72	0	0
8	0	0	0	4.28	0.28	95.44	0	0
9	0	0	0	3.31	0.23	96.46	0	0
10	0	0	0	2.55	0.23	97.22	0	0
11	0	0	0	2.02	0.15	97.83	0	0
12	0.12	0	0	1.14	0.30	98.45	0	0
13	4.78	0.28	0	0.54	0.28	94.12	0	0

APPENDIX 2

Table 8. Data for the Stripping of Stationary Phase

	Run 2		Run 4	
Fr. No.	Upper	Lower	Upper	Lower
1	37	38	49	1
2	39	36	49	1
3	37	43	37	13
4	25	5	30	20
5	20	10	38	12
6	20	10	46	4
7	21	9	27	3
8	21	9	23	7
9	18	12	21	9
10	16	14	18	12
11	13	7	17	13
12	20	10	25	5
13	29	1	27	3
14	30	0	28	2
15	30	0	28	2
16	30	0	30	0
17	-	-	30	0
18	-	-	30	0
19	-	-	30	0
20	-	-	30	0

Note:

a) Stripping was observed for Run 1 but not measured.

b) No displacement of stationary phase took place in Run 3.

APPENDIX 3

Table 9. Calculation of the Linear Velocity of the Mobile Phase

Run Number	Mobile Phase Section[a] ($m^2 \times 10^{-6}$ or mm^2)	Flow Rate ($m^3.s^{-1}$)	u ($m.s^{-1}$)
1	0.43	1.7×10^{-7}	0.038
2	2.7 to 5.5	3.3×10^{-7}	0.124 to 0.060
3	1.3	0.83×10^{-7}	0.066
4	1.3 to 2.4	3.3×10^{-7}	0.254 to 0.138

Run Number	Hydraulic Radius[b] (m)	Reynolds Number	Froude Number
1	1.285×10^{-3}	20	0.69
2	3.272×10^{-3}	89	13.88
3	2.035×10^{-3}	45	3.47
4	2.089×10^{-3}	149	31.25

[a] The mobile phase section is calculated as $\pi d^2/4$ (1-Sf), with d=1.66 mm (Run #1) and 3.68 mm (Runs #2-4).

[b] $Re = u\ R_H\ \rho/\upsilon$ with R_H, the mobile phase equivalent hydraulic radius (= 2d x 360/π) and ρ and υ, the mobile phase density and kinematic viscosity, respectively. $Fr = F\omega^2/2\pi\ g\ \upsilon$ with the flow rate, F, and the angular rotation speed, ω.

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