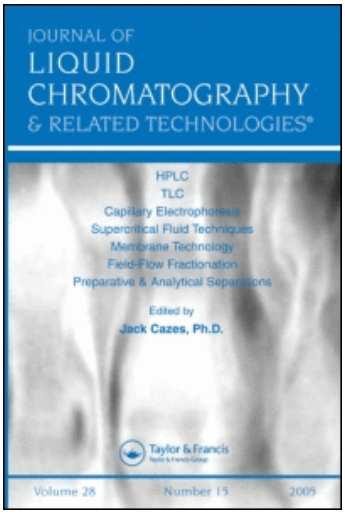

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Stationary Phase Retention in CCC: Modelling the J-Type Centrifuge as a Constant Pressure Drop Pump

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ABSTRACT

To be able to design a J-type centrifuge for a given need, a method of being able to predict peak elution is required. Predicting peak elution will also allow the user to optimise the process parameters for his or her needs. Such predictions require an accurate knowledge of the volume of the stationary phase retained in the coil for a given set of operating conditions. This paper builds upon an experimental relationship in that the stationary phase retention decreases proportionally to the square root of the mobile phase flow rate. Combining this experimental relationship with the hypothesis that the pressure drop across a coil is independent of

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mobile phase flow rate, and assuming that the mobile phase flow is laminar, the equation below is derived:

$$S_f = 100 - \frac{800}{{d_C}^2}\sqrt{\frac{2\mu_m L}{\pi \Delta P}}\sqrt{F}$$

Experimental evidence is presented supporting the above equation. The experimental evidence was gained using, a heptane–ethyl acetate–methanol–water (1.4 : 0.1 : 0.5 : 1) v/v phase system, in normal phase mode using three helical stainless steel coils. These stationary phase retention studies allowed the above equation to be tested under conditions of different rotational speeds and tubing internal diameter. The derived stationary phase retention characteristics from each retention study allowed pressure drop and Reynolds number data to be calculated. The pressure drop data shows that the pressure drop across a coil is constant and independent of the mobile phase flow rate.

Key Words: Countercurrent chromatography; Theory; J-type centrifuge; Liquid stationary phase retention; Constant pressure drop pump; Archimedean forces; Density difference.

INTRODUCTION

Large-scale production versions of J-type centrifuges will be expensive to design and construct. In order to obtain the required level of capital expenditure, it will be necessary to show that such a machine will retain stationary phase for a number of phase systems in both normal and reverse phase modes. It will also be necessary to predict the stationary phase retention for a given flow rate and other operating parameters before such expenditure can begin. To make such a prediction, requires a quantitative understanding of how the stationary phase is retained in the coil. Sutherland et al.[1] gave a qualitative understanding of retention. This paper used a head and tail study to show that a J-type centrifuge will pump the upper phase towards the head end of a coil and the lower phase towards the tail for spiral wound coils. If the upper phase is the mobile phase, then the mobile phase is pumped from the tail end of the coil towards the head end. If the lower phase is the mobile phase, then the mobile phase is pumped from the head end of the coil towards the tail end. Du et al.[2] have shown that the stationary phase retention decreases proportionally to the square root of the mobile phase flow rate.

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This paper builds on these observations and develops a hypothesis that the pressure drop across a coil is constant, regardless of the mobile phase flow rate for normal phase mode (NM).

To predict the retention of stationary phase for a particular centrifuge-coil configuration and set of operating conditions requires an understanding of how retention is affected by each variable. These variables are: rotor radius, coil β-value, helical pitch, spiral pitch, tubing bore, length of coil, coil volume, rotational speed, mobile phase viscosity, and density difference between the phases. Three stainless steel helical coils with the same β-value, helical pitch, and coil length, but different tubing bores, were used to study the effect of tubing bore on retention.

THEORY

One hypothesis and two assumptions are used to develop an understanding of the controlling parameters for retention of the stationary phase. The hypothesis is: that the total pressure drop across a coil is independent of flow rate for a given set of operational parameters.

The assumptions are: (i) that the mobile phase flow is laminar as proposed by Sutherland et al.[3] and (ii) that the interface between the stratified layers of mobile and stationary phases forms one surface of the conduit through which the mobile phase flows.

Mathematically it is possible to develop the empirical equation[2] for retention $S_f = A - B\sqrt{F}$, using the Hagen-Poiseuille equation for laminar flow applied with the hypothesis. The hypothesis may seem to break physical laws governing the flow of fluids, however, it must be remembered that as the mobile phase flow rate increases, the amount of stationary phase in a coil reduces, allowing the mobile phase to flow through a greater cross-sectional area of the coil, thus keeping the same pressure drop. One use of the Hagen-Poiseuille formula is to determine the viscosity of a liquid by applying a known pressure to one end of a glass capillary tube of known length and bore and then measuring the flow rate obtained. Imagine a number of capillary tubes of the same length but different bore diameters; if the same pressure were applied to each capillary tube independently the measured flow rate would increase with bore diameter.

Conway[4] observed the stratified layering of the mobile and stationary phases. The interfacial surface formed between the stratified layers forms the boundary that separates the flow of each phase. Each phase flows through a different conduit. The internal wall of the tubing and the interfacial surface forms the boundary of each conduit. The Hagen-Poiseuille equation for laminar flow was developed for a single phase flowing through straight lengths

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of circular bore tubing. To use the Hagen-Poiseuille equation in this derivation the following additional assumptions are made:

1. The interface between the mobile and stationary phases can be treated as a solid but moveable boundary.
2. The cross-sectional area occupied by the mobile phase can be treated as circular, although in practice it is more like a segment of a circle.
3. The mean cross-sectional area occupied by the mobile phase is constant through out the coil.
4. That the mixing waves observed by Conway[4] do not interfere significantly with the retention of stationary phase.
5. Helically or spirally wound tubing can be treated as straight tubing given that the bend radius is much greater than the radius of the tubing bore.

The Hagen-Poiseuille equation for laminar flow is as follows:

$$F = \frac{\pi {r_m}^4 \Delta P}{8 \mu_m L} \quad \text{(Hagen-Poiseuille)}$$

where F is the volumetric flow rate of mobile phase, r_m is the mean radius of the cross-sectional area occupied by the mobile phase, ΔP the total pressure drop across the coil (measured pressure drop plus pressure drop due to Archimedean action), μ_m the dynamic viscosity of the mobile phase, and L the length of the column/coil.

The Hagen-Poiseuille equation can be reorganised as follows:

$$\Delta P = \frac{8 \mu_m L}{\pi} \frac{F}{{r_m}^4} \tag{1}$$

Applying the hypothesis to the above equation shows that:

$$\Delta P = \frac{8 \mu_m L}{\pi} \frac{F}{{r_m}^4} = \text{constant} \tag{2}$$

The mean cross-section area occupied by the mobile phase is $A_m = \pi {r_m}^2$ and $V_m = A_m L$, therefore, $V_m = \pi {r_m}^2 L$. Rearranging the equation for ${r_m}^2$ gives:

$${r_m}^2 = \frac{V_m}{\pi L} \Rightarrow {r_m}^4 = \frac{{V_m}^2}{\pi^2 L^2}$$

Substituting into Eq. (2) for ${r_m}^4$ gives:

$$\Delta P = 8 \pi \mu_m L^3 \frac{F}{{V_m}^2} = \text{constant} \tag{3}$$

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For the hypothesis to be true the $F/V_m{}^2$ must also be a constant since the μ_m and L terms of Eq. (3) are constants. Rearranging Eq. (3) as follows:

$$V_m{}^2 = \frac{8\pi\mu_m L^3}{\Delta P} F \tag{4}$$

Equation (4) shows that plotting $V_m{}^2$ against F will produce a straight-line characteristic that passes through the origin if the hypothesis is correct and the gradient would be $(8\pi\mu_m L^3)/(\Delta P)$.

Rearranging Eq. (4) for V_m gives:

$$V_m = \sqrt{\frac{8\pi\mu_m L^3}{\Delta P}}\sqrt{F} \tag{5}$$

Also, the coil volume (V_C) equals the volume of stationary phase (V_S) in the coil plus the mobile phase (V_m) in the coil i.e.:

$$V_C = V_S + V_m$$

Therefore,

$$V_S = V_C - V_m \tag{6}$$

And multiplying both sides by $100/V_C$ gives:

$$\frac{100V_S}{V_C} = 100 - \frac{100V_m}{V_C} \tag{7}$$

Now by definition:

$$S_f = \frac{100V_S}{V_C} \tag{8}$$

Substituting Eq. (8) into Eq. (7) gives:

$$S_f = 100 - \frac{100V_m}{V_C} \tag{9}$$

Substituting for V_m from Eq. (5) into Eq. (9) gives:

$$S_f = 100 - \frac{100}{V_C}\sqrt{\frac{8\pi\mu_m L^3}{\Delta P}}\sqrt{F} \tag{10}$$

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Equation (10) can be compared to the stationary phase retention characteristic

$$S_f = A - B\sqrt{F},$$

where the constant $A = 100$ and the gradient B is as shown below:

$$B = \frac{100}{V_C}\sqrt{\frac{8\pi\mu_m L^3}{\Delta P}} \tag{11}$$

Now $V_C = A_C L$ and $A_C = (\pi {d_C}^2)/4$, hence, $V_C = (\pi {d_C}^2 L)/4$, therefore, Eq. (10) can be written as:

$$S_f = 100 - \frac{800}{{d_C}^2}\sqrt{\frac{2\mu_m L}{\pi\Delta P}}\sqrt{F} \tag{12}$$

Comparing $S_f = A - B\sqrt{F}$ and Eq. (12) shows that the formula for the gradient B is also as shown below:

$$B = \frac{800}{{d_C}^2}\sqrt{\frac{2\mu_m L}{\pi\Delta P}} \tag{13}$$

Each term in Eq. (12) is dimensionless, hence, the gradient B should have the fundamental dimensions $[TL^{-3}]^{1/2}$, and dimensional analysis of Eq. (13) shows this to be the case.

The gradient B of a given stationary phase retention characteristic is constant; therefore, Eq. (13) must produce a constant result. In this equation the only likely variable is the ΔP term, all of the other symbols represent constants for the given stationary phase retention characteristic, therefore, the ΔP term must also be a constant for the given stationary phase retention characteristic. This means that the pressure drop across the coil must be independent of the flow rate of the mobile phase, which is consistent with the hypothesis.

EXPERIMENTAL

A heptane : ethyl acetate : methanol : water phase system (1.0 : 0.1 : 0.5 : 1.0 v/v/v/v) abbreviated as 4A was used. Details of the physical properties are given in Wood et al.,[5] Tables 1 and 2. The upper (organic) phase was pumped from the tail to the head end of each helical coil, i.e., NM. The J-type centrifuges used in these experiments are based upon the Brunel CCC

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described by Sutherland and Wood[6] and originally called the "Quattro." These J-types had a rotor radius of 110 mm and were modified to rotate at any speed between 200 and 1400 rpm. Each machine used has two bobbins with each bobbin having a maximum possible β-value of 0.95.

A traditional approach to determine experimental accuracy, would be to repeat each test a minimum of three times. This would have required three times as much testing, hence, three times as much experimental time or a reduction in the number of parameters tested. A reduction in the parameters tested would have reduced the thoroughness of the testing of the hypothesis and theories contained in this paper and in Ref.[7] Previous normal and reverse phase mode retention studies by Sutherland et al.[8] have shown the importance of accuracy and the author realised that the procedures then in use would not produce the required accuracy. The procedures given in Chapter 2 of Ref.[9] were written to systematically produce accurate results that are self-checking to detect random errors. The improvements to increase accuracy are described in the following paragraphs.

A stationary phase collector was designed for NM. This collector stops the systematic errors in measuring the volume of stationary phase collected each time the flow from the J-type centrifuge is switched from a full measuring cylinder to an empty measuring cylinder, see Experimental Retention Tests of Ref.[3] These errors would increase at the higher flow rates used in the tests described in this paper. The design of the stationary phase collector was also based upon a 100 mL burette. The smallest volume division on a 100 mL burette is 0.2 mL allowing the smallest measurable reading to be 0.1 mL compared to 0.5 mL on a 100 mL measuring cylinder, thus increasing the accuracy of the volume measurement by a factor of five.

The stationary phase collector was designed to trap the stationary phase as it is displaced from the coil and flying leads and allow the mobile phase to flow through the collector, see Fig. 1. This stationary phase collector is used when the lower phase is the stationary phase and the upper phase is the mobile phase. The output from the coil flows into the top of the stationary phase collector. The stationary phase sinks to the bottom, while the mobile phase flows out of the top of the collector. The stationary phase collector relies upon the acceleration due to gravity to separate the mobile and stationary phases.

The Dynamax SD-1 pump used to pump the mobile phase in these retention studies produces an accurate steady non-pulsatile flow at all flow rates.[10] The Dynamax SD-1 pump is combined with a 1.7 bar backpressure valve placed downstream of the coils to ensure accurate operation of the pump's non-return values and to stop siphoning of either phase through the coil. These measures ensure that the actual flow rate is the same as that set.

A steady flow of the mobile phase appears to be necessary to obtain linear stationary phase retention characteristics in NM when the flow of mobile phase is less stable than in reverse phase mode.[11] This is confirmed by work

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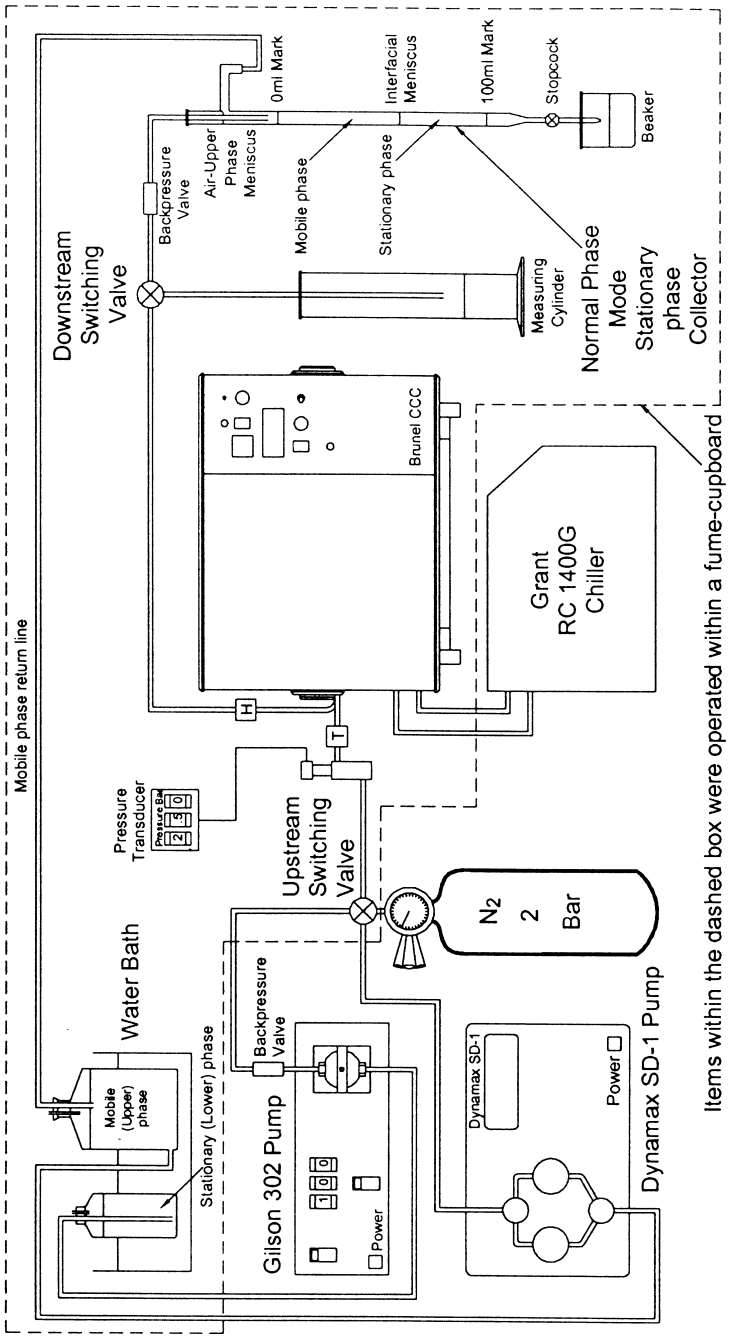

Figure 1. Shows the experimental set up for normal phase mode retention measurements taken from Ref.[9]

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conducted by Berthod and Billardello[12] using a heptane–methanol–water phase system in NM with the upper phase mobile. Berthod used a Shimadzu LC10-AS pump, these Shimadzu pumps are known for pulse-free constant flow delivery[13] unlike the pulsatile flow produced by the Gilson 302 HPLC pump used by Sutherland et al.[8] For the 4A phase system used, the upper phase is less viscous than the lower phase, see Table 2 of Ref.[5] Without the use of the pulse-free Dynamax SD-1 pump the results produced in this chapter would have had significant errors similar to those shown in our previous results.[8]

The mobile and stationary phase reservoirs were placed above the Dynamax SD-1 and Gilson pumps to gravity feed the pumps to ensure that bubbles did not form between the reservoirs and the pumps during the filling stroke of one of the pistons belonging to either pump. Bubbles in the feed lines to the pumps can cause the actual flow rate of the pumps to be lower than that set because the pump's cylinder is not completely filled during the filling stroke. The fed line between the mobile phase reservoir and the Dynamax pump had a 4.8 mm (3/16 inch) bore to stop the air bubbles forming at the high mobile phase flow rates used. The phase systems were also degassed using a Jones Chromatography 7600 series vacuum de-gasser before each experiment. The use of the 1.7 bar backpressure valve also minimises the formation of bubbles between the pumps and the backpressure valve, i.e., in the coil and flying leads, see Fig. 1. The formation of bubbles in this region can cause errors in volume measurements of the mobile and stationary phase and the flow rate of the mobile phase.

The Brunel CCC machine is temperature controlled. The mobile and stationary phase reservoirs are also temperature controlled, being placed in a water bath, as shown in Fig. 1. The operating temperature of the Brunel CCC machine and the water bath was set at 30°C. This ensures that the physical properties of the phase system, while under test, are the same as those listed in Table 2 of Ref.[5] Retention test results were rejected if the temperature varied more than ±1°C, i.e., outside of the range of 29–31°C.

The last volume of displaced stationary phase (i.e., the greatest V_E, see Ref.[7] for the highest flow rate of mobile phase) was subtracted from the volume of the mobile phase collected when emptying the coil after a retention test. If the result of the subtraction was more than 3 mL, the results were rejected and the retention test was repeated.[a] If the difference between these volumes was less than 3 mL, the extra-coil volume would be determined as described in Ref.[7] and the stationary phase retention characteristic plotted.

[a]An accuracy of 3 mL is used as it allows for small inaccuracies caused by evaporation, spillages, and wetting of surfaces above the meniscus in measuring cylinders.

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Three Stainless Steel Coils

These coils were used to study the affect of bore on retention. Three helical stainless steel coils, each with different bores, were wound. For clarity, these coils will be known as the IMI (EPSRC—Innovative Manufacturing Initiative) coils, which acknowledges the funding source for these coils. Each IMI coil has been made from the same length of tubing (5.656 m) and has been wound at the same β-value, 0.82, with the same helical pitch, 11.5 mm (and same helix angle), that gives each coil 10 loops. The bore of the first coil is 3.73 mm, the second is 5.33 mm, and the third is 7.73 mm. This means that the volume of each coil is different. The first coil has a measured volume of 59.1 mL, the second 120.5 mL, and the third 259.5 mL.

Stationary phase retention tests were conducted at rotational speeds of 600, 800, 1000, and 1200 rpm, using the 4A phase system. These experiments show the effect of bore on retention.

The following flow rates were used for each coil: 3.73 mm bore coil 5, 10, 20, 40, and 50 mL/min; 5.33 mm bore coil 10, 20, 40, 60, and 80 mL/min, and 7.73 mm bore coil 20, 50, 80, 110, and 140 mL/min. These flow rates were used to produce straight stationary phase retention characteristics. At higher flow rates, the stationary phase retention characteristic becomes non-linear, i.e., no longer produces a straight-line characteristic.

These stationary phase retention characteristics were produced using an accurate extra-coil volume determined for each test. The system extra-coil volume was estimated from flying lead volumes and other associated tubing and then confirmed accurately by volume measurement of displaced stationary phase as described in Ref.[7] The test data for estimating the extra-coil volume is obtained during the retention tests. The raw data for the results of this paper are given in Appendix 4 of Ref.[9]

The 3.73 mm bore coil had the NM retention test repeated four times to determine the repeatable accuracy (precision) of the retention test at 1200 rpm.

RESULTS

Mobile Phase Flow Rate vs. Pressure Drop

Figures 2–4 plot $V_m{}^2$ against the mobile phase flow rate (F) for each of the three IMI stainless steel coils. Each characteristic on a particular graph represents a different rotational speed. In each figure, the linear regression is displayed for the two extreme values of rotational speed. In Fig. 2, the 1200 rpm characteristic was repeated four times to give an indication of reproducibility. The extra-coil volume determined by measuring the lengths of

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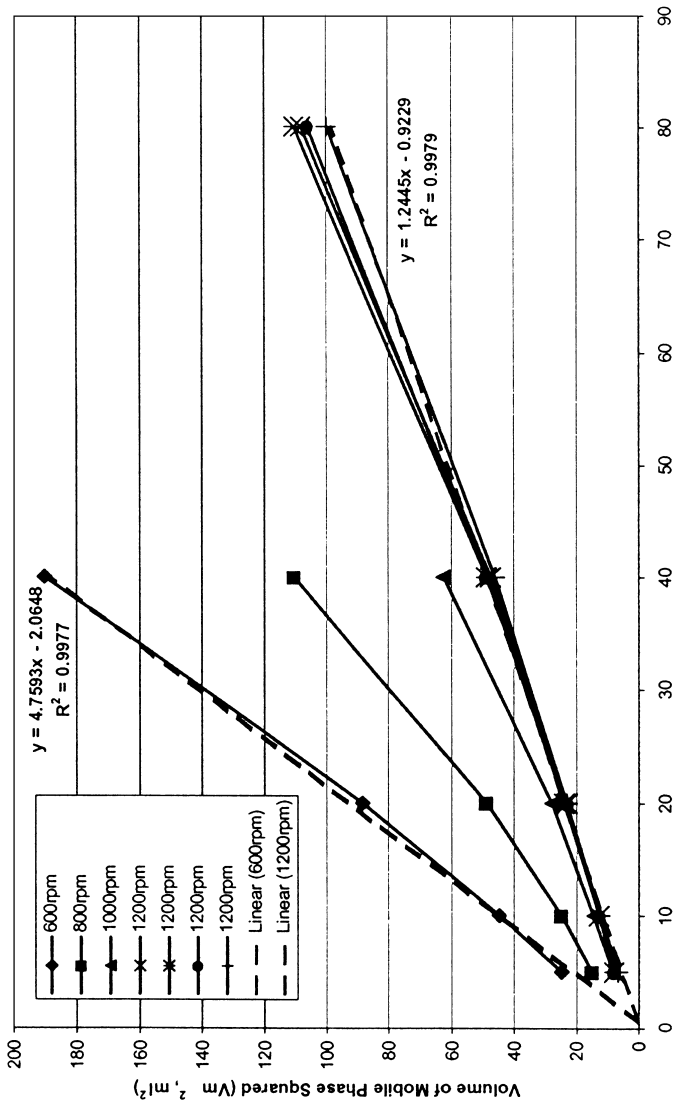

Figure 2. Shows the volume of mobile phase in the column (V_m) squared plotted against the mobile phase flow rate (F) for the 3.73 mm bore (60 mL) IMI stainless steel helical coil.

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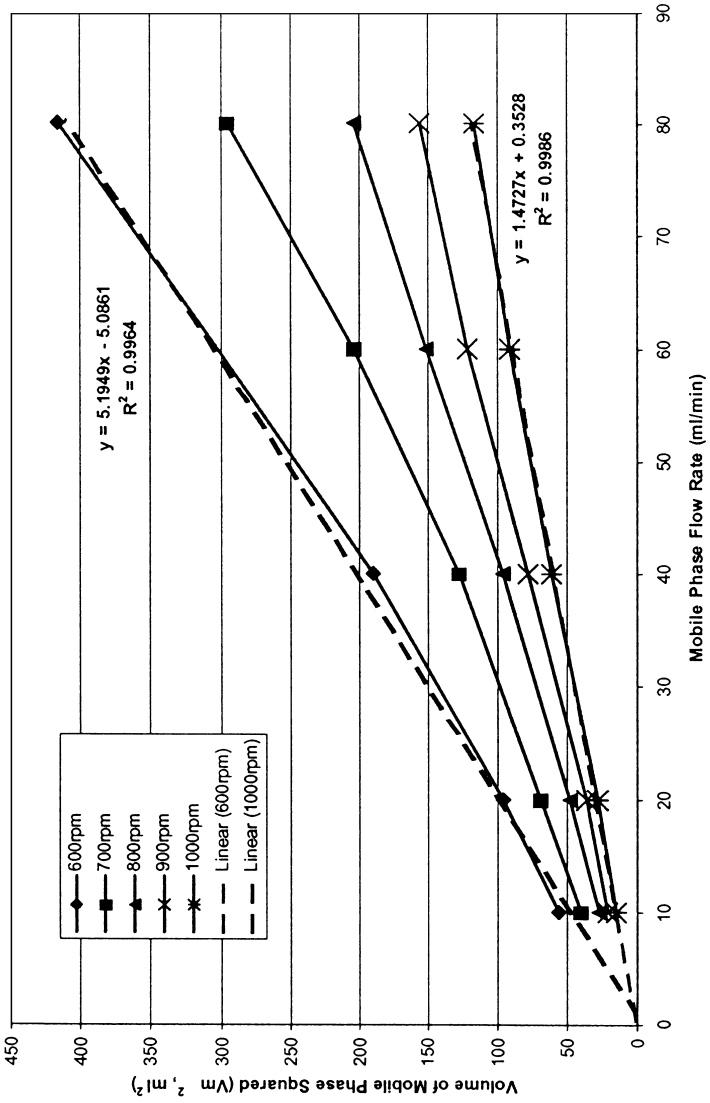

Figure 3. Shows the volume of mobile phase in the column (V_m) squared plotted against the mobile phase flow rate (F) for the 5.33 mm bore (120 mL) IMI stainless steel helical coil.

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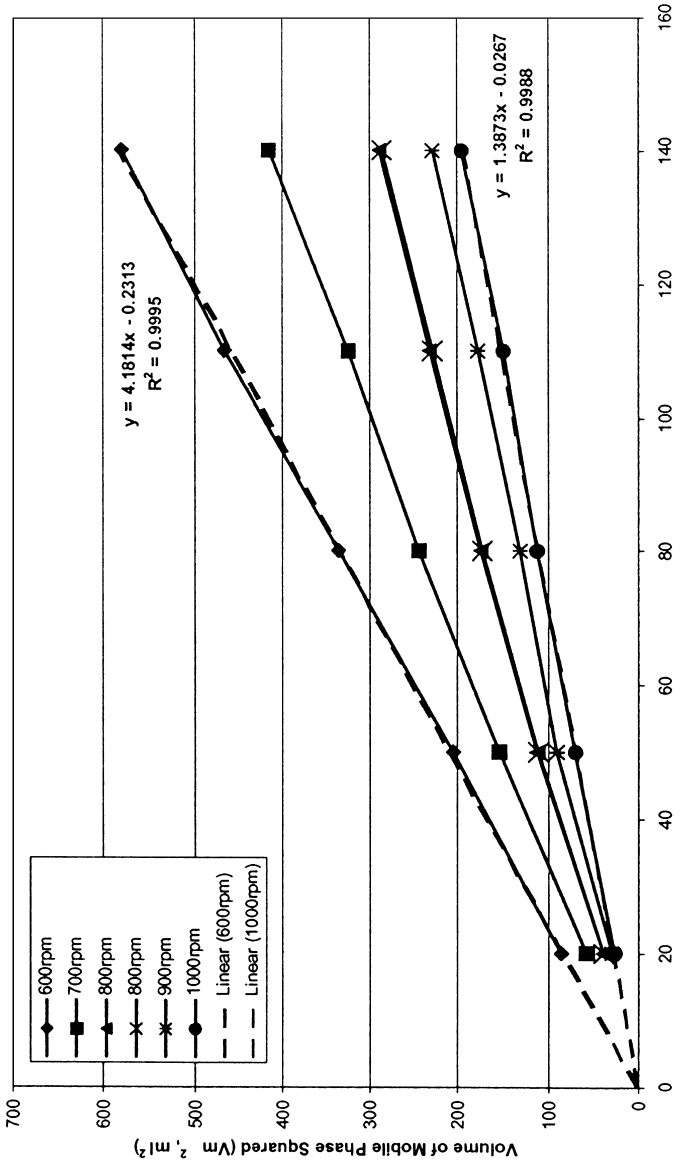

Figure 4. Shows the volume of mobile phase in the column (V_m) squared plotted against the mobile phase flow rate (F) for the 7.73 mm bore (260 mL) IMI stainless steel helical coil.

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the flying leads and other associated tubing, was 8.5 mL for all the results presented here.

Table 1 gives the gradients (column 2), intercepts (column 3), and fit coefficients (column 4) for the results presented in Figs. 2–4. The first column of Table 1 contains: the experiment number, the bore of the coil tubing, the rotational speed, and the phase system used. The raw data for V_m and F can be obtained from Appendix 4 of Ref.[9]

Table 2 shows the volume of mobile phase (V_m) in each IMI coil for the same rotational speed. The first four rows show the same experiment repeated four times on the same coil at the same rotational speed. The values shown in bold indicate approximately equal volumes displaced from different IMI coils at the same rotational speed and flow rate.

Retention vs. Bore

Table 3 gives the B gradients for a range of different tubing bore sizes operating at different rotational speeds in NM. The first column of Table 3

Table 1. Shows the gradients, intercepts, and fit coefficients of the fitted straight-line characteristics for Figs. 2–4.

Experiment	Gradient (mL min)	Intercept $(mL)^2$	Fit coefficient (R^2)
39, 3.73 mm, 600 rpm, 4A	4.7593	−2.0648	0.9977
41, 3.73 mm, 800 rpm, 4A	2.7495	−1.6874	0.9940
42, 3.73 mm, 1,000 rpm, 4A	1.5573	−0.8626	0.9954
33, 3.73 mm, 1,200 rpm, 4A	1.3438	−1.7354	0.9942
34, 3.73 mm, 1,200 rpm, 4A	1.3718	−1.7142	0.9953
35, 3.73 mm, 1,200 rpm, 4A	1.3199	−1.1304	0.9967
44, 3.73 mm, 1,200 rpm, 4A	1.2445	−0.9229	0.9979
47, 5.33 mm, 600 rpm, 4A	5.1949	−5.0861	0.9964
50, 5.33 mm, 700 rpm, 4A	3.6225	−4.8237	0.9906
48, 5.33 mm, 800 rpm, 4A	2.5576	−2.1269	0.9983
51, 5.33 mm, 900 rpm, 4A	1.9791	−0.7529	0.9981
49, 5.33 mm, 1,000 rpm, 4A	1.4727	0.3528	0.9986
56, 7.73 mm, 600 rpm, 4A	4.1814	−0.2313	0.9995
55, 7.73 mm, 700 rpm, 4A	2.9568	2.4640	0.9992
57, 7.73 mm, 800 rpm, 4A	2.0660	3.7360	0.9970
54, 7.73 mm, 800 rpm, 4A	2.0403	3.5573	0.9950
52, 7.73 mm, 900 rpm, 4A	1.6072	2.4960	0.9965
53, 7.73 mm, 1,000 rpm, 4A	1.3873	−0.0267	0.9988

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Table 2. Comparison of the mobile phase volume (V_m, mL) present in each IMI coil at the same flow rate and rotational speed for the 4A phase system in normal phase mode.

	Flow rate (mL/min)								
Experiment	5	10	20	40	50	60	80	110	140
33, 3.73 mm, 1,200 rpm	2.8	3.6	4.8	6.9			10.4		
34, 3.73 mm, 1,200 rpm	2.8	3.6	4.9	7.0			10.5		
35, 3.73 mm, 1,200 rpm	2.8	3.6	4.8	7.0			10.3		
44, 3.73 mm, 1,200 rpm	2.6	3.5	4.8	6.8			10.0		
39, 3.73 mm, 600 rpm	5.0	**6.7**	**9.4**	**13.8**					
47, 5.33 mm, 600 rpm		**7.5**	**9.8**	**13.8**			**20.4**		
56, 7.73 mm, 600 rpm			**9.2**		14.3		**18.3**	21.6	24.1
50, 5.33 mm, 700 rpm		6.3	**8.3**	11.3		14.3	**17.2**		
55, 7.73 mm, 700 rpm			**7.6**		12.4		**15.6**	18.0	20.4
41, 3.73 mm, 800 rpm	3.9	**5.0**	**7.0**	**10.5**					
48, 5.33 mm, 800 rpm		**5.2**	**6.9**	**9.8**		12.3	**14.3**		
57, 7.73 mm, 800 rpm			**6.2**		10.6		**13.2**	15.2	17.0
54, 7.73 mm, 800 rpm			6.2		10.5		13.1	15.1	16.9
51, 5.33 mm, 900 rpm		4.6	**6.0**	8.8		11.0	**12.5**		
52, 7.73 mm, 900 rpm			**5.5**		9.4		**11.4**	13.3	15.1
42, 3.73 mm, 1,000 rpm	2.9	**3.8**	**5.3**	**7.9**					
49, 5.33 mm, 1,000 rpm		**3.9**	**5.3**	**7.8**		9.5	**10.8**		
53, 7.73 mm, 1,000 rpm			**5.2**		8.4		**10.6**	12.2	14.0

Note: The figures in bold highlight approximately equal mobile phase volumes for different coils tested using the same flow rate and rotational speed.

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Table 3. Shows the retention results for the 4A phase system in normal phase for the three IMI stainless steel coils.

Experiment	Extra-coil vol. (mL)	Pump out vol. —last displaced vol. (mL)	*B* gradient $(\text{min/mL})^{1/2}$	Fit coefficient (R^2)
39, 3.73 mm, 600 rpm, 4A	7.7738	1.4	3.6573	0.9970
41, 3.73 mm, 800 rpm, 4A	8.2064	−0.6	2.7556	0.9917
42, 3.73 mm, 1,000 rpm, 4A	8.6958	−1.3	2.0809	0.9941
33, 3.73 mm, 1,200 rpm, 4A	8.3911	−0.8	1.9212	0.9924
34, 3.73 mm, 1,200 rpm, 4A	8.4693	0.0	1.9488	0.9940
35, 3.73 mm, 1,200 rpm, 4A	8.4372	−1.0	1.9057	0.9946
44, 3.73 mm, 1,200 rpm, 4A	8.9983	−0.8	1.8650	0.9976
47, 5.33 mm, 600 rpm, 4A	6.9008	0.2	1.8659	0.9950
50, 5.33 mm, 700 rpm, 4A	7.4967	−0.7	1.5817	0.9973
48, 5.33 mm, 800 rpm, 4A	8.1579	0.5	1.3189	0.9976
51, 5.33 mm, 900 rpm, 4A	8.4708	0.0	1.1655	0.9971
49, 5.33 mm, 1,000 rpm, 4A	8.8049	0.4	1.0093	0.9986
56, 7.73 mm, 600 rpm, 4A	7.5927	−0.7	0.7880	0.9996
55, 7.73 mm, 700 rpm, 4A	7.8261	−0.2	0.6650	0.9991
57, 7.73 mm, 800 rpm, 4A	8.6726	−2.2	0.5612	0.9961
54, 7.73 mm, 800 rpm, 4A	8.2971	0.8	0.5565	0.9969
52, 7.73 mm, 900 rpm, 4A	8.3837	0.0	0.4931	0.9965
53, 7.73 mm, 1,000 rpm, 4A	8.2685	0.7	0.4551	0.9991

contains: the experiment number, the bore of the coil tubing, the rotational speed, and the phase system used. The second column contains extra-coil volume data determined as described in Ref.[7] The values in the third column are calculated using raw data from Appendix 4 of Ref.[9] The fit coefficients shown in the fifth column are for the trend lines fitted to both the extra-coil volume characteristics and the stationary phase retention characteristics, which are identical for the same experiment.

Figure 5 uses results from Table 3 and plots the *B* gradient against $1/d_C{}^2$ for normal phase operation, at three different rotational speeds. The coil tubing bore d_C was measured in cm.

Reynolds Numbers vs. Bore

Table 4 shows the Reynold's number (*Re*) of the mobile phase flow for three different sizes of tubing bore arranged in groupings of different rotational speeds. The values in Table 4 were calculated using Eq. (16)

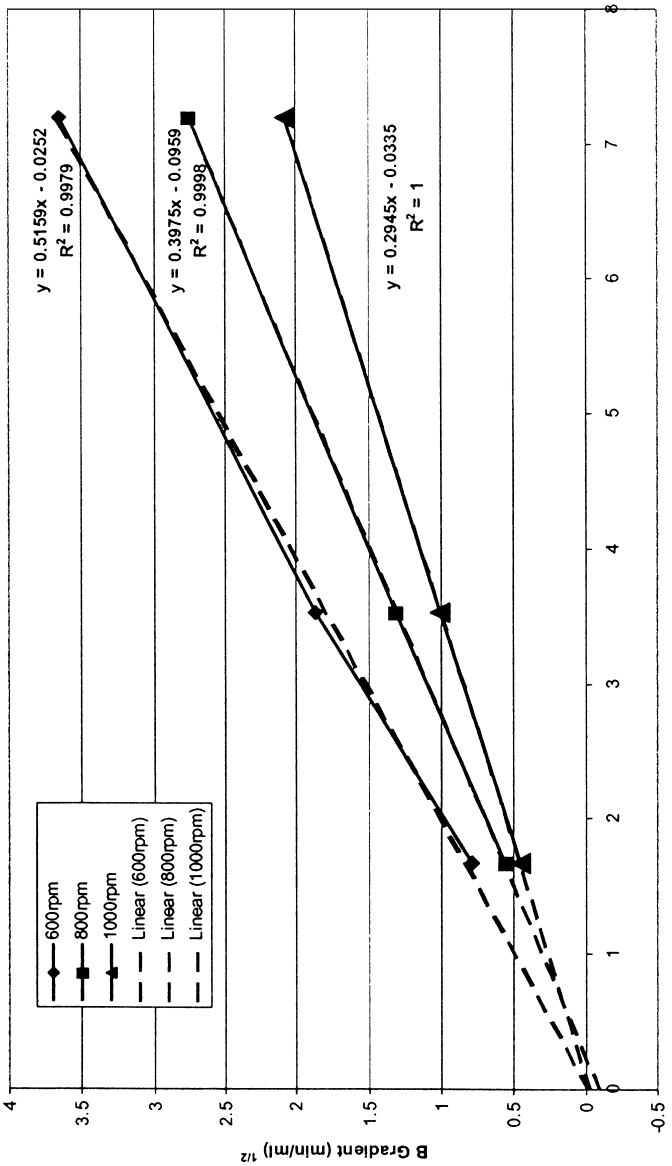

Figure 5. Shows how the B gradient varies with $1/d_C^2$ for the 4A phase system in normal phase mode in the three IMI stainless steel coils.

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Table 4. Shows the Reynolds number results for the three IMI stainless steel coils arranged in terms of rotational speed.

	Flow rate (mL/min)								
Experiment	5	10	20	40	50	60	80	110	140
39, 3.73 mm, 600 rpm	184	**318**	**537**	**886**					
47, 5.33 mm, 600 rpm		**301**	**527**	**888**			**1,460**		
56, 7.73 mm, 600 rpm			**550**		1,103		**1,560**	1,974	2,378
50, 5.33 mm, 700 rpm		328	**572**	960		1,285	**1,590**		
55, 7.73 mm, 700 rpm			**606**		1,186		**1,691**	2,164	2,587
41, 3.73 mm, 800 rpm	209	**369**	**623**	**1,018**					
48, 5.33 mm, 800 rpm		**360**	**626**	**1,051**		1,408	**1,741**		
57, 7.73 mm, 800 rpm			**668**		1,279		**1,834**	2,351	2,830
54, 7.73 mm, 800 rpm			670		1,287		1,843	2,361	2,840
51, 5.33 mm, 900 rpm		383	**671**	1,110		1,489	**1,863**		
52, 7.73 mm, 900 rpm			**710**		1,359		**1,975**	2,514	3,003
42, 3.73 mm, 1,000 rpm	242	**423**	**716**	**1,172**					
49, 5.33 mm, 1,000 rpm		**418**	**716**	**1,181**		1,605	**2,007**		
53, 7.73 mm, 1,000 rpm			**729**		1,436		**2,046**	2,623	3,117
33, 3.73 mm, 1,200 rpm	246	434	752	1,254			2,043		
34, 3.73 mm, 1,200 rpm	245	432	742	1,243			2,032		
35, 3.73 mm, 1,200 rpm	249	437	755	1,249			2,057		
44, 3.73 mm, 1,200 rpm	255	440	752	1,264			2,085		

Note: The figures in bold highlight similar Reynolds numbers obtained in different IMI coils at the same rotational speed and flow rate.

data for the physical properties of the 4A phase system, which were taken from Table 2 of Ref.[5] and data from Appendix 4 of Ref.[9] The values highlighted in bold indicate similar *Re* at the same rotational speed and flow rate but in different bore coils. The 1200 rpm values in the last four rows for the 3.73 mm bore coil give an indication of the repeatable accuracy (precision) for the other results.

DISCUSSION

Mobile Phase Flow Rate vs. Pressure Drop

Equation (4) shows that if the square of the mobile phase volume (V_m^2) is plotted against mobile phase flow (F), the slope will be proportional to the viscosity of the mobile phase (μ_m), the coil length cubed (L^3), and inversely proportional to the pressure drop across the coil (ΔP). As the viscosity of the mobile phase and the length are constant in normal phase, a linear result will indicate that pressure drop (ΔP) is constant.

Table 1 shows that the (R^2) fit coefficient varies from 0.9906 to 0.9995 indicating that a straight-line characteristic is applicable when the volume of mobile phase in the coil squared (V_m^2) is plotted against mobile phase flow rate (F). The intercepts on the vertical (V_m^2) axis vary from -5.09 to 3.74 $(\text{mL})^2$ straddling the origin, and these values are small when compared to the ranges of the vertical axes of Figs. 2–4. If the hypothesis that the pressure drop across a coil is constant and independent of mobile phase flow rate (F) is correct, then plotting (V_m^2) against F as shown in Eqs. (3) and (4) should produce straight-line characteristics that pass through the origin similar to Figs. 2–4. These figures, therefore, indicate that the pressure drop across a coil is constant and independent of mobile phase flow rate for a given coil, phase system, and operational conditions. These results validate the hypothesis that the pressure drop across a coil is independent of the mobile phase flow rate and is constant for a given coil and rotational speed.

Retention vs. Bore

In Table 2, the values shown in bold indicate approximately equal volumes of mobile phase from each IMI coil at the same rotational speed and flow rate. Given that the volumes of the IMI coils approximately double for each increase in tubing bore, these displaced volumes are remarkably similar. They show that the mobile phase occupies the same volume in each coil for the same flow rate and rotational speed. As each of the IMI coils is the

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same length, the mean cross-sectional area occupied by the mobile phase must be the same in each coil. This implies that the pressure drop across each coil is identical for the same flow rate and rotational speed. This demonstrates that the pressure drop is independent of tubing bore.

Examination of Eq. (13) and Fig. 5 shows that the gradient of the stationary phase retention characteristic is inversely proportional to the square of the bore of the coil tubing for coils of the same length. The gradient of Fig. 5 is:

$$\text{Gradient} = \frac{B}{1/d_C{}^2} = Bd_C{}^2 = 800\sqrt{\frac{2\mu_m L}{\pi \Delta P}} \tag{14}$$

The above equation shows that the only possible variable for the gradients of each characteristic shown in Fig. 5 is the pressure drop (ΔP). Figure 5 shows that there are straight-lines, constant gradients; characteristics for each rotational speed that pass through the vertical axis close to the origin. This shows that the pressure drop is constant for a given rotational speed and is independent of the bore of the coil tubing, provided that coils of the same length, β-value and helical pitch are used.

Reynolds Numbers, Pressure Drop, and Laminar Flow

In the past, the Hagan-Poiseuille equation for laminar flow has not been seriously considered for the analysis of flow within a coil on a J-type centrifuge because of the mixing that occurs adjacent to the proximal key node. Low *Re* indicate laminar flow, and turbulence occurs at higher *Re*. Reynolds number is the ratio of the inertia force to the viscous force, i.e.:

$$Re = \frac{\text{Inertia force}}{\text{Net viscous force}} \tag{15}$$

The inertia force $= \rho u^2 d^2$ and the net viscous force $= \mu u d$, p. 290,[14] which gives the classic equation for *Re* in circular bore tubing:

$$Re = \frac{\rho u d}{\mu} \tag{16}$$

Examination of the equations for inertia force and viscous force shows that neither of these forces is directly affected by the high accelerations experienced by the mobile and stationary phases in a coil of a J-type centrifuge. Therefore *Re* are still valid for the study of fluid motion in the coil of a J-type centrifuge. However, the critical values for the transition from laminar to turbulent flow at high accelerations are not known.

The *Re* obtained for the three stainless steel IMI coils, see Table 4, vary from 184 (Experiment 39) to 3117 (Experiment 53) for the 4A phase system in normal phase mode. Table 4 shows that the *Re* in the 3.73 mm bore coil vary from 184 to 255 at 5 mL/min. At 5 mL/min, it is reasonable to assume that the flow of the mobile phase is laminar. The 1200 rpm results for the 3.73 mm bore coil have a highest flow rate of 80 mL/min when the *Re* reaches a maximum value of 2085. Figure 2 indicates that the pressure drop is the same at both 5 and 80 mL/min; if a transition to turbulent flow had occurred this pressure would have increased significantly. Therefore, it is reasonable to assume that flow of the mobile phase is still laminar at 80 mL/min with a *Re* of 2085 in the 3.73 mm bore coil. The *Re* for the 7.73 mm bore coil vary between 550 and 729 for a flow rate of 20 mL/min, see Table 4. These *Re* are below the 2085 encountered in the 3.73 mm bore coil at a flow rate of 80 mL/min when the flow was laminar. Therefore, it is reasonable to assume that the mobile phase flows in a laminar manner in the 7.73 bore coil at a flow rate of 20 mL/min. Figure 4 shows that the pressure drops are constant across the 7.73 mm bore coil for flows varying from 20 to 140 mL/min for each rotational speed tested. The maximum *Re* in the 7.73 mm bore coil is 3117. This indicates that the flow of the mobile phase can still be considered as laminar at such high *Re*.

Table 4 also shows that similar *Re* are obtained in each IMI coil at the same rotational speed and flow rate. This shows that the *Re* is independent of the coil-tubing bore for these coils. This also indicates that the flow of the mobile phase is similar (laminar) in each of these coils for the same rotational speed and flow rate.

Experimental Accuracy

An analysis of the four 1200 rpm normal phase retention tests for the 3.73 mm (59.1 mL) IMI stainless steel coil shows that the *B* gradients are within ±2.5% of the mean of these four gradients. At 1200 rpm, the *B* gradient is going to be the lowest for this coil, as this is the highest rotational speed at which a retention test was performed. This means that the volume of stationary phase displaced from the coil will be the smallest, and hence, any errors in the volume measurement will be proportionally the greatest. The results from Table 2 show that the same volumes of stationary are displaced from each of the IMI coils at identical rotational speeds and flow rates.[b] This

[b]The stationary phase is displaced from a coil by the mobile phase; therefore the volume of mobile phase in a coil (V_m) equals the volume of stationary phase displaced from a coil.

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means that the retention in the largest bore coil will be the best due to the largest bore coil having the greatest volume. It also means that the largest bore coil will have the lowest *B* gradient. However, the error in the *B* gradient for the 7.73 mm bore IMI coil at 1200 rpm would still be within ±2.5%. This is demonstrated by the following example. Assume that one of the IMI coils has a volume of 10 mL and that another IMI coil has a volume of 100 mL. Both have retention tests performed at 1200 rpm and that 5 mL of stationary phase are displaced at a flow rate of 25 mL/min. The retention in the 10 mL coil at this flow rate will be 50% and the *B* gradient will be 10 $(\text{min/mL})^{1/2}$. The retention in the 100 mL coil will be 95% and the *B* gradient will be 1 $(\text{min/mL})^{1/2}$. Now assume, that the possible error in the volume of displaced stationary phase is +1 mL. The retention in the 10 mL coil would be 40% and the *B* gradient would be 12 $(\text{min/mL})^{1/2}$, giving an error in the *B* gradient of +20%. Assuming the same error for the 100 mL coil, the retention would be 94% and the *B* gradient would be 1.2 $(\text{min/mL})^{1/2}$, also giving an error in the *B* gradient of +20%. Therefore, the percentage of errors in the *B* gradient for each coil will be similar at the same rotational speed.

If the 100 mL coil was rotated at 600 rpm, and assuming that twice as much stationary phase was displaced from the coil, i.e., 10 mL at a flow rate of 25 mL/min. Then the retention would be 90% and the *B* gradient would be 2 $(\text{min/mL})^{1/2}$. Again assuming a possible +1 mL reading error in the volume of displaced stationary phase, the volume of displaced stationary would be 11 mL. The retention would be 89% and the *B* gradient would be 2.2 $(\text{min/mL})^{1/2}$, giving a possible 10% error in the *B* gradient. Compared with the 20% error at 1200 rpm, this means that the percentage of errors in the *B* gradients reduce as rotational speed is reduced, and the errors in the *B* gradients for rotational speeds below 1200 rpm are better than ±2.5% for the IMI coils.

CONCLUSIONS

The *Re* results confirm that the flow of mobile phase is laminar and that the use of the Hagan-Poiseuille equation is justified when deriving an expression for the retention of stationary phase. As the flow of the mobile phase is laminar, it does not directly cause mixing on either side of the proximal key node. The mixing near the proximal key node is caused by an interfacial instability between the mobile and stationary phases at the interface.[9]

The pressure drop across a coil is independent of the mobile phase flow rate and remains constant for a given coil, phase system, and rotational speed.

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The pressure drop is independent of the coil tubing bore, provided coils of the same length, β-value, and helical pitch are used.

The volume of mobile phase and *Re* are both independent of bore size, provided coils of the same length, β-value, and helical pitch are used.

To complete the prediction of the stationary phase retention an equation relating the factors affecting the pressure drop across a coil needs to be developed.

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