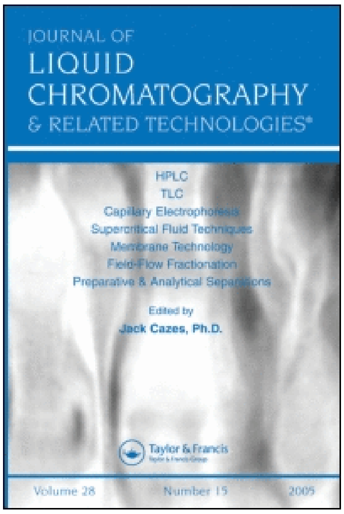

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Determination of J-Type Centrifuge Extra-Coil Volume Using Stationary Phase Retentions at Differing Flow Rates

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ABSTRACT

An alternative method for determining the extra-coil volume (V_{ext}) of a J-type centrifuge and its ancillary equipment is given based upon displaced volumes of stationary phase. The extra-coil volume (V_{ext}) is determined by plotting the volume of stationary phase displaced from the coil and extra-coil volume combined (V_E), against the square root of the mobile phase flow rate. The extra-coil volume is the intercept on the V_E axis for a zero flow rate of mobile phase. This extra-coil volume is then used to produce a stationary phase retention characteristic where the intercept on the retention axis is 100% for a zero flow rate of mobile phase. Experimental evidence was gained for 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. The internal diameters of these coils were 3.73 mm, 5.33 mm, and 7.73 mm. Retention studies

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were conducted at rotational speeds between 600 and 1200 rpm. The same phase system was also tested in a multi-layer PTFE coil in reverse phase mode at a rotational speed of 800 rpm. The internal diameter of the tubing for the PTFE coil was 1.6 mm. All of the coils used were fitted to a J-type centrifuge with a rotor radius of 110 mm. These retention studies allowed the extra-coil volume to be tested under conditions of different rotational speeds and coil tubing internal diameters. It was also tested using different tubing material, and in both normal and reverse phase modes.

Key Words: Countercurrent chromatography; Extra-column volume; Fundamentals; Liquid stationary phase retention.

INTRODUCTION

Conway[1] used the term dead volume to describe the combined volume of inlet (V_{in}) and outlet (V_{out}) flying leads. In classic chromatography, this volume is known as the extra-column volume of a chromatography system.[3] In CCC, the terms coil and column are interchangeable, so, it is proposed to call the combined volume of the inlet and outlet flying leads, the extra-coil or extra-column volume, (V_{ext}) in this paper.

Du et al.[2] showed that the retention of the stationary phase decreases proportionally with the square root of the mobile phase flow rate. This means that the volume of stationary phase displaced from a coil is proportional to the square root of the mobile phase flow rate. If the cross-sectional area of the inlet and outlet leads is small, the stationary phase will be completely replaced by the mobile phase in these leads once the mobile phase begins to flow. It does not matter if the displaced volume of stationary phase from the extra-coil volume is plotted against the flow rate of the mobile phase or the square root of the mobile phase flow rate or this flow rate raised to any other power, as it will always be the extra-coil volume. The total volume of displaced stationary phase (V_E) from a coil and its associated plumbing is the addition of the extra-coil volume and the stationary phase displaced from the coil. Therefore, if V_E is plotted against the square root of the mobile phase flow rate, a straight-line characteristic will be produced. The intercept on the vertical axis, when the mobile phase flow rate is zero, will be the extra-coil volume, as shown in Fig. 1.

THEORETICAL APPROACH

The following derivation is the mathematical proof of the statements made in the previous paragraph. The assumption, that the intercept on the vertical

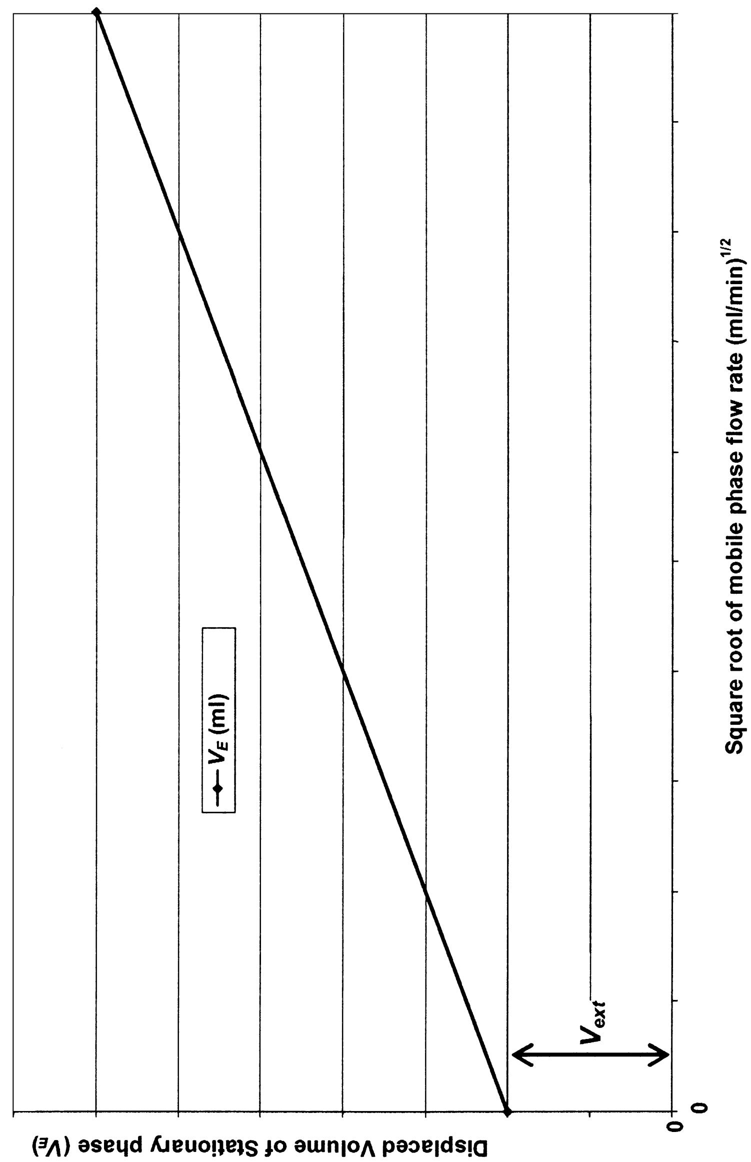

Figure 1. The theoretical relation between V_E and the square root of the mobile phase flow rate. Note the intercept on the vertical axis is the extra-coil volume (V_{ext}).

axis of a stationary phase retention plot is 100% retention of the stationary phase in the coil (or column) when the mobile phase flow rate is zero, is used to derive an equation that allows the extra-coil volume to be determined from the displaced volumes of stationary phase from a coil and its flying leads.

The extra-coil volume (V_{ext}) of a CCC centrifuge is the system volume (V_{SYS}) minus the coil volume (V_C), hence:

$$V_{ext} = V_{SYS} - V_C \tag{1}$$

Traditionally the extra-coil volume has been determined by measuring the length of the inlet and outlet flying leads, and then calculating the extra-coil volume by multiplying the total length by the cross-sectional area of the flying lead, hence:

$$V_{ext} = V_{in} + V_{out} \tag{2}$$

This traditional method does not take into account the volume of the tubing from the ends of the flying leads to the centre or periphery of the coil. These short lengths of tubing are called delivery tubes and are usually made from the same tubing as the coil. During CCC operation, when both stationary and mobile phase can be present in the coil, the delivery tubes will be filled with mobile phase, thus increasing the extra-coil volume above that determined by measuring the length of the flying leads, hence:

$$V_{ext} = V_{in} + V_{out} + V_{DT} \tag{3}$$

where:

V_{in} = input volume upstream of the coil.
V_{out} = output volume downstream of the coil.
V_{DT} = extra-coil volume in the delivery tubes, both in and out.

It is not always easy to measure the length of the delivery tubes and, hence, determine the extra-coil volume contained within these tubes, particularly as some stationary phase can be retained in these tubes. The following method can be used to determine the total extra-coil volume of the CCC system without measuring the lengths of the delivery tubes or the flying leads.

Du et al.[2] have shown that there is a linear relationship between the square root of the mobile phase flow rate and the stationary phase retention.

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Equation (4) describes the mathematical relationship of a stationary phase retention characteristic.

$$S_f = \mathrm{A} - \mathrm{B}\sqrt{F} \tag{4}$$

where:

S_f = % stationary phase retention.
F = mobile phase flow rate.
A = the intercept on the vertical axis.
B = gradient of the linear relationship.

It can be assumed, that the intercept A on the vertical axis is 100% when the coil has just been completely filled with stationary phase and before the flow of mobile phase has begun, i.e., the mobile phase flow rate is zero. This assumption allows Eq. (4) to be modified as follows:

$$S_f = 100 - \mathrm{B}\sqrt{F} \tag{5}$$

Now, by definition:

$$S_f = \frac{100V_S}{V_C} \tag{6}$$

Substituting Eq. (6) into Eq. (5) gives:

$$\frac{100V_S}{V_C} = 100 - \mathrm{B}\sqrt{F}$$

$$V_S = V_C - \frac{\mathrm{B}V_C}{100}\sqrt{F} \tag{7}$$

The displaced volume of stationary phase (V_E) from a CCC system equals the amount of mobile phase in the extra-coil volume (V_{ext}) plus the volume of mobile phase in the coil (V_m) i.e.,:

$$V_E = V_m + V_{\mathrm{ext}}$$

Therefore:

$$V_m = V_E - V_{\mathrm{ext}} \tag{8}$$

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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:

$$V_S = V_C - V_m \tag{9}$$

Substituting for V_m in Eq. (9) from Eq. (8) gives:

$$V_S = V_C - V_E + V_{\text{ext}} \tag{10}$$

Substituting in Eq. (7) for V_S from Eq. (10) gives:

$$V_S = V_C - V_E + V_{\text{ext}} = V_C - \frac{\mathrm{B}V_C}{100}\sqrt{F}$$

$$- V_E + V_{\text{ext}} = -\frac{\mathrm{B}V_C}{100}\sqrt{F}$$

$$V_E - V_{\text{ext}} = \frac{\mathrm{B}V_C}{100}\sqrt{F}$$

$$V_E = \frac{\mathrm{B}V_C}{100}\sqrt{F} + V_{\text{ext}} \tag{11}$$

Equation (11) governs the relationship between the displaced volume of stationary phase from the coil and flying leads and the square root of the mobile phase flow rate, and V_{ext} is the intercept on the vertical axis. Plotting the displaced volume of stationary phase against the square root of the mobile phase flow rate, and then fitting a linear relationship to the data points, will allow the extra-coil volume to be determined, as it will be the intercept on the vertical axis, see Fig. 1. A linear trend line is fitted to the plotted points and the equation of the trend line is in the form of Eq. (11).

EXPERIMENTAL

The J-type centrifuges used in these experiments are based upon the Brunel CCC described in Ref.,[5] originally called the "Quattro." These J-type centrifuges had a rotor radius of 110 mm and were modified to rotate at any speed between 200 and 1400 rpm. Each centrifuge used has two bobbins with each bobbin having a maximum possible β-value of 0.95. The following phase system was used for both normal and reverse retention studies: heptane–ethyl acetate–methanol–water (1.4 : 0.1 : 0.5 : 1). In all of the figures, this phase system is abbreviated to the 4A phase system.

Reverse Phase Mode in a PTFE Coil

A reverse phase mode retention study was performed using a syringe and syringe driver instead of a HPLC pump, since the syringe and syringe driver produces a constant non-pulsatile flow of mobile phase. The volume of the PTFE coil used was 95.9 mL[a] and was rotated at 800 rpm, so that the coil was orientated head-centre tail-periphery as this gives the best retention of stationary phase.[4] The lower (aqueous) phase was used as the mobile phase and was pumped from the head-centre to tail-periphery. The mobile phase flow rate was determined by measuring the time taken to fill measuring cylinders.

These reverse phase experiments used a 50 mL glass-measuring cylinder to measure the displaced stationary phase. The smallest volume division on the 50 mL glass-measuring cylinder was 1 mL.

The extra-coil volume was determined by two different methods: the first measured the various lengths of tubing that contribute to the extra-coil volume to then calculate the total volume of this tubing, and the second method used is described in the previous section.[b] For both methods of determining the extra-coil volume, the volume of stationary phase in the coil was calculated using Eq. (10). The percentage of stationary phase retained in the coil (S_f) was then calculated using Eq. (6). S_f was then plotted against the square root of the mobile phase flow rate to produce stationary phase retention characteristics[c] for both methods of determining the extra-coil volume.

Normal Phase Mode in Three Stainless Steel Coils

Three helical stainless steel coils, each with different bores, have been wound. For clarity, these coils will be known as the IMI (EPSRC-Innovative Manufacturing Initiative) coils, which acknowledges the funding source of

[a]The PTFE coil used with the syringe driver had a volume of 95.9 mL, a 1.59 mm bore, a length of approximately 48.5 m and a β-value range 0.816–0.845.

[b]Microsoft Excel was used to plot the results for extra-coil volume, fit linear trend lines and determine the equations of the fitted trend lines.

[c]Microsoft Excel was used to plot the results for retention, fit linear trend lines and determine the equations of the fitted trend lines.

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these coils. Each IMI coil has been made from the same length of tubing (5.656 m) and wound at the same β-value (0.82) with the same helical pitch (11.5 mm), to give the same helix angle, giving 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 only 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.

Retention tests were conducted at rotational speeds of 600, 800, 1000, and 1200 rpm. 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. A twin head Dynamax SD-1 HPLC steady flow pump was used to pump the organic mobile phase. At such high flow rates, the stationary phase was collected in a graduated glass container called a stationary phase collector, which trapped the stationary phase while allowing the mobile phase to flow through.

A helical coil has a constant β-value and, therefore, does not have a centre or periphery in the same sense as a spiral coil. This means that a helical coil cannot be originated head-centre tail-periphery or head-periphery tail-centre. As there is no change in the β-value for helical coil, there is only the Archimedean pumping effect and no pumping effect from the change in β-value, hence, in a head and tail study see Ref.,[4] the upper phase will distribute to the head end of a coil and the lower phase will distribute to the tail end. Hence, the organic (upper) mobile phase was pumped from the tail end of the coil to the head end following the recommendations of Ref.[4] modified for helical coils.

The 3.73 mm bore coil had the normal phase mode retention test repeated four times to determine the repeatable accuracy (precision) of the retention test at 1200 rpm. The results for these retention tests are shown in Table 2.

RESULTS

The raw data for the results from this paper are shown in Ref.[6] The extra-coil volume for each set of tests was different because different flying leads were used for each set of tests. The extra-coil volume for the appropriate test is recorded against the appropriate results.

Reverse Phase Mode in a PTFE Coil

The extra-coil volume determined in the traditional manner by measuring the lengths of the flying leads and the calculating the volume from the cross-sectional area was 6.4 mL. A set of data showing how the displaced volume of

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Table 1. The mobile phase flow rate and displaced volumes of stationary phase (V_E) for the test using a syringe driver and syringe to pump the mobile phase of the phase system in reverse phase mode.

Mobile phase flow rate (mL/min)	Square root of mobile phase flow rate $(mL/min)^{1/2}$	Volume of displaced stationary phase (mL)
0.455	0.675	13.3
0.902	0.950	15.8
1.979	1.407	19.5
2.714	1.647	22.0
4.250	2.062	25.5

stationary phase varies with flow is shown in Table 1. These results were used to plot V_E against the square root of mobile phase flow in Fig. 2, in order to calculate the true extra-coil volume from the intercept, i.e., extra-coil volume was determined as approximately 7.3 mL. Both of these extra-coil volumes were used to plot stationary phase retention against the square root of mobile phase flow and fit linear relationships.

Normal Phase Mode in Three Stainless Steel Coils

The extra-coil volume, determined by measuring the lengths of the flying leads and other associated tubing, was 8.5 mL for the normal phase mode retention studies using the IMI coils. The first column of Table 2 contains: the experiment number, the bore of the coil tubing, and the rotational speed. The second column contains the extra-coil volume determined, as described in the theory section. The fit coefficients shown in the third column are for the trend lines fitted to the extra-coil volume characteristics and the stationary phase retention characteristics.

DISCUSSION

Reverse Phase Mode in a PTFE Coil

The extra-coil volume, determined in the traditional manner by measuring the lengths of the flying leads and calculating the volume from the cross-sectional area, was 6.4 mL. Figure 2 shows the displaced volume of stationary

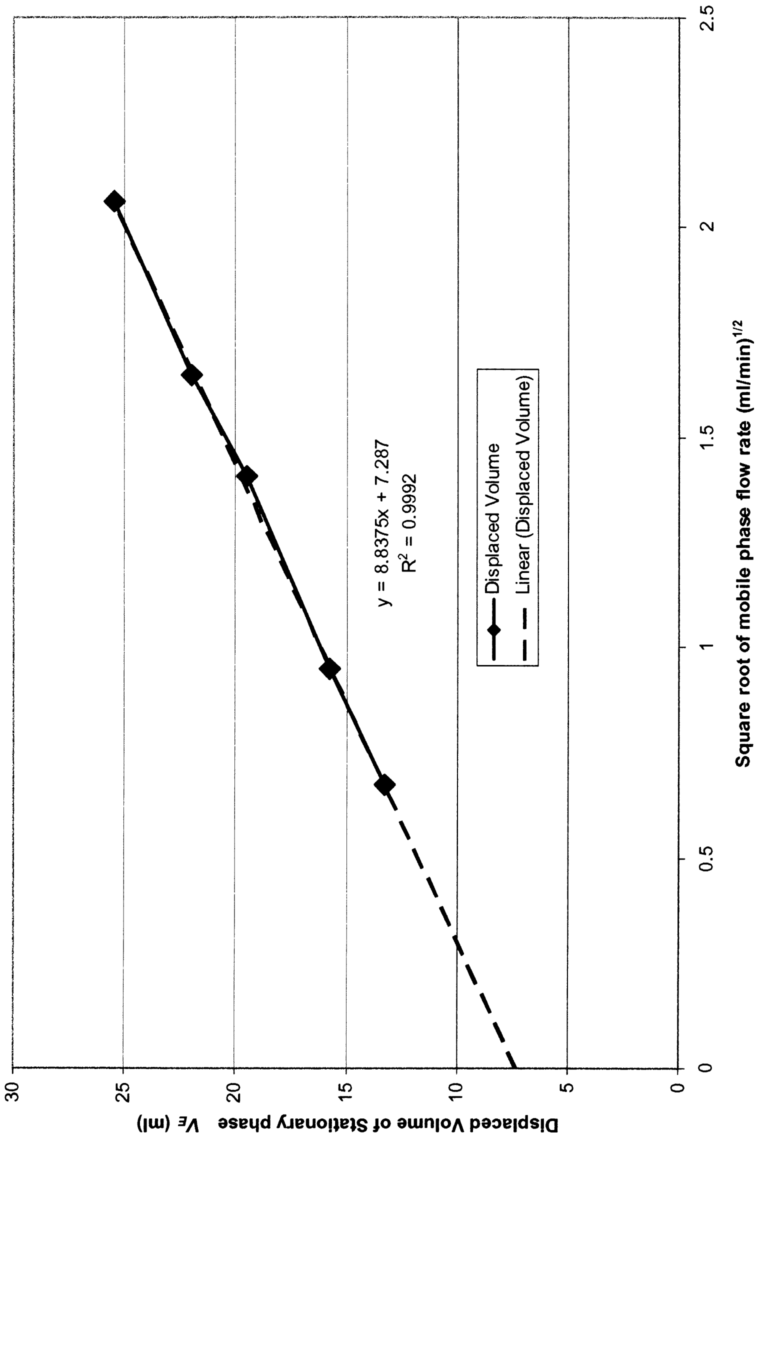

Figure 2. The displaced volume of stationary phase plotted against the square root of mobile phase flow rate in reverse phase mode using a syringe driver.

Table 2. The retention results for the phase system in normal phase for the three IMI stainless steel coils.

Experiment	Extra-coil volume (mL)	Fit coefficient (R^2)
39, 3.73 mm, 59.1 mL, 600 rpm	7.77	0.9970
41, 3.73 mm, 59.1 mL, 800 rpm	8.21	0.9917
42, 3.73 mm, 59.1 mL, 1,000 rpm	8.70	0.9941
33, 3.73 mm, 59.1 mL, 1,200 rpm	8.39	0.9924
34, 3.73 mm, 59.1 mL, 1,200 rpm	8.47	0.9940
35, 3.73 mm, 59.1 mL, 1,200 rpm	8.44	0.9946
44, 3.73 mm, 59.1 mL, 1,200 rpm	9.00	0.9976
47, 5.33 mm, 120.5 mL, 600 rpm	6.90	0.9950
50, 5.33 mm, 120.5 mL, 700 rpm	7.50	0.9973
48, 5.33 mm, 120.5 mL, 800 rpm	8.16	0.9976
51, 5.33 mm, 120.5 mL, 900 rpm	8.47	0.9971
49, 5.33 mm, 120.5 mL, 1,000 rpm	8.80	0.9986
56, 7.73 mm, 259.5 mL, 600 rpm	7.59	0.9996
55, 7.73 mm, 259.5 mL, 700 rpm	7.83	0.9991
57, 7.73 mm, 259.5 mL, 800 rpm	8.67	0.9961
54, 7.73 mm, 259.5 mL, 800 rpm	8.30	0.9969
52, 7.73 mm, 259.5 mL, 900 rpm	8.38	0.9965
53, 7.73 mm, 259.5 mL, 1,000 rpm	8.27	0.9991

phase plotted against the square root of mobile phase flow. The equation of the fitted linear relationship is:

$$V_E = 8.8375\sqrt{F} + 7.287$$

and the coefficient of linear regression is 0.9992. Therefore, the extra-coil volume determined by the above equation is 7.287 mL, which is approximately 0.9 mL greater than measured in the traditional way. The 0.9 mL is due to the combination of extra-coil volume in the delivery tubes, the valve used to switch between stationary and mobile phases, and the extra-coil volume in a pressure transducer. The equation of the fitted linear relationship using an extra-coil volume of 7.287 mL is:

$$\text{Percentage retention of stationary phase} = -9.2153\sqrt{F} + 100$$

and the coefficient of linear regression is also 0.9992. The above equation shows that the retention will be 100% when there is no mobile phase flow.

The equation of the fitted linear relationship using an extra-coil volume of 6.4 mL is:

$$\text{Percentage retention of stationary phase} = -9.2153\sqrt{F} + 99.075$$

and the coefficient of linear regression is also 0.9992. The gradients of these fitted linear relationships are identical. However, the intercept on the *y*-axis is not 100% but is 99.075% for the extra-coil volume of 6.4 mL. This means that all calculated retentions would be approximately 1% lower than the actual retentions, leading to errors in predicting peak elution times.

These results confirm the derivation of Eq. (11) and the assumption that the stationary phase retention is 100% when the mobile phase flow rate is zero. This new method of determining the extra-coil volume for a J-type centrifuge was found to be more accurate than calculating the extra-coil volume from the length of the flying leads, since the intercept on the vertical axis passes through the 100% retention mark. The linear regressions of all fitted linear relationships depend upon experimental accuracy.

Normal Phase Mode in Three Stainless Steel Coils

The second column of Table 2 shows that the extra-coil volumes, determined by the method described in the theory section, are close to the 8.5 mL extra-coil volume determined by measuring the lengths of the flying leads and other associated tubing. These results also confirm the derivation of Eq. (11) and the method proposed in the theory section for determining extra-coil volume.

Closer examination of Table 2 shows that the extra-coil volume increases with rotational speed. This is due to stationary phase being retained in the flying leads at the lower rotational speeds. At the higher rotational speeds, the accelerations subjected to the flying leads are greater and cause all of the stationary phase to be displaced from the flying leads. This increases the extra-coil volume to that determined by measuring the lengths of the flying leads and associated tubing, i.e., 8.5 mL. In Table 2, there are three extra-coil volumes that are larger than the 8.5 mL volume; these suggest experimental errors or end effects in the coil (delivery tubes) or inaccuracy in determining the extra-coil volume by measuring the lengths of the flying leads and associated tubing.

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 from Table 2, shows that the

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B gradients are within $\pm 2.5\%$ of the mean of these four gradients.[6] It is also argued in Ref.,[6] that the B gradients of the other retention tests at lower speeds and in other coils are accurate within $\pm 2.5\%$. The extra-coil volume results for these 1200 rpm retention experiments are within -0.11 to $+0.5$ mL of the measured value of 8.5 mL, which are less than $\pm 1\%$ of the coil volume. The mean value of these extra-coil volumes is approximately 8.57 mL, which is within 0.1 mL of the measured extra-coil volume. This discrepancy of less that 0.1 mL is less than half of the 0.2 mL volumetric measuring accuracy of the graduations on the stationary phase collector. The fit coefficients (R^2) also vary between 0.9917 and 0.9996. Therefore, the experiment procedure used, see Ref.,[6] combined with the new method of determining the extra-coil volume, provides an accurate determination of the extra-coil volume.

CONCLUSION

The extra-coil volume results confirm the assumption that the intercept on the vertical axis, when the mobile phase flow rate is zero, is the extra-coil volume, when the displaced volume of stationary phase is plotted against the square root of the mobile phase flow rate.

These results also confirm the assumption that the stationary phase retention characteristic will pass through the 100% retention mark on the vertical axis when the mobile phase flow rate is zero, see Eq. (5), provided the extra-coil volume was determined as described in the theory section.

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REFERENCES

1. Conway, W.D. *Countercurrent Chromatography Apparatus, Theory and Applications*; VCH Publishers, Ltd.: UK, 1990; 197 pp.
2. Du, Q.; Wu, C.; Qian, G.; Wu, P.; Ito, Y. Relationship between the flow-rate of the mobile phase and retention of the stationary phase in counter-current chromatography. J. Chromatogr. A **1999**, *835*, 231–235.
3. Ettre, L.S. Nomenclature for chromatography (IUPAC recommendations 1993), paragraph 3.2.13. Pure & Appl. Chem. **1993**, *65* (4), 819–872.

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4. Sutherland, I.A.; Muytjens, J.; Prins, M.; Wood, P. A new hypothesis on the phase distribution in countercurrent chromatography, J. Liq. Chrom. & Rel. Technol. **2000**, *23* (15), 2259–2276.
5. Sutherland, I.A.; Wood, P. Countercurrent chromatography and its versatile application as an industrial purification and production process. J. Liq. Chromatogr. & Rel. Technol. **1998**, *21* (3), 279–298.
6. Wood, P.L.; Hawes, D.; Janaway, L.; Sutherland, I.A. Stationary phase retention in countercurrent chromatography: modelling the J-type centrifuge as a constant pressure drop pump. J. Liq. Chromatogr. & Rel. Technol. **2003**, *26* (9&10), 1373–1396.

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