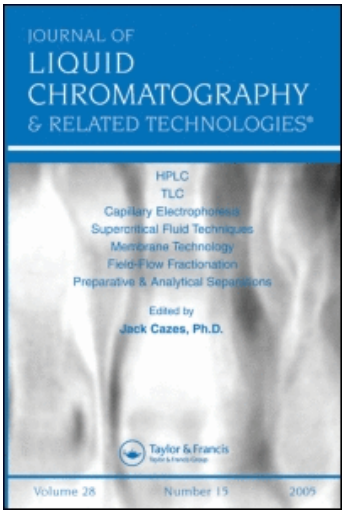

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MICROTECHNIQUE TO REMOVE PARTICLES FROM HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SAMPLES[1]

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ABSTRACT

A high speed (13,000 x G) microhematocrit centrifuge rapidly (1 min) removes particles from small (10-60 µL) volumes of sample solution prior to high performance liquid chromatographic analysis. The described technique, using glass capillary tubes, effectively cleans up aqueous biochemical solutions and/or concentrates of environmental samples. Advantages of the method are speed, small sample size, and moderate equipment cost.

INTRODUCTION

Small (5-10 µm), uniform packing materials and sophisticated packing techniques have improved the efficiency and selectivity of

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high performance liquid chromatographic (HPLC) columns (1). One problem associated with refined high performance columns is high back pressure caused by clogging of column frits or in-line protection filters. Particles must be removed from suspension before sample injection to prevent blockage of frit pores. Particles can be removed by filtration or centrifugation. The disadvantages of filtration are the large (generally >1 mL) sample volumes required and, for some compounds, potential contamination from the filtering apparatus. Common low-speed (1500-3000 x G) centrifuging requires long spin times (several min) and can be cumbersome for small samples. Centrifugal Microfilters™ can filter small volumes, but require plastic filter holders and low-speed centrifugation. Refrigerated centrifuges are required for biochemically labile compounds (e.g., nucleotides) (2). The above inconveniences in preparing particle-free chromatographic samples can be overcome by a high-speed centrifugation of small samples. We describe a 1-min capillary technique to clean up biochemical and environmental samples for HPLC analyses.

METHOD AND APPLICATIONS

Plain (without heparin) microhematocrit glass capillary tubes (length: 75 mm, inside diameter: 1.2 mm; Fisher Scientific) hold the samples during centrifugation (3). A controlled volume of sample (10-60 μL) is drawn into the capillary tube with suction provided by a 0.5-mL plastic syringe (e.g., insulin syringe) attached to the tube with an open-holed septum in a short (1.5 cm) length of rubber tubing (Fig. 1). After the sample is drawn into

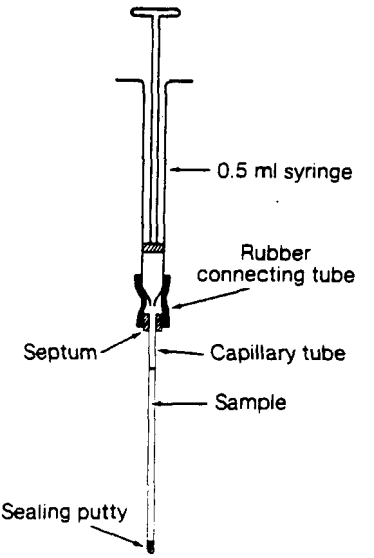

FIGURE 1.

Schematic diagram of syringe-septum device for drawing sample into capillary tube.

the tube, the lower tip is closed with sealing putty (Critoseal™) and the tube is removed from the septum device. The tube is centrifuged (International ICE microcapillary centrifuge, model MB™) for 1 min. The sample tube is removed and supernatant is drawn into a syringe for injection into the liquid chromatograph. If preferred, the supernatant can be eluted from the capillary tube by reconnecting the tube to the syringe-septum device (with the plunger drawn), cutting it with a file above the liquid-solid interface, and forcing the solution into a collection vessel.

Using Stokes formula for settling velocity (4), we calculated the expected fate of suspended particles of varying density and

diameter in water and acetonitrile during a 1-min centrifugation at 13,000 x G (Fig. 2). Water and acetonitrile were chosen because they span the range of viscosities for common HPLC solvents. Calculations were based on Stokes law formula (4).

$$V = \frac{2G\,(P - Ps)r^2}{9n} \qquad (1)$$

where V = velocity of settling (cm s^{-1}),

$P - Ps$ = density difference between particle and solvent (g cm^{-3}),

G = gravitational acceleration (981 cm sec^{-2}), and

r = radius of particle (cm),

n = viscosity of solvent (g cm^{-1} s^{-1}).

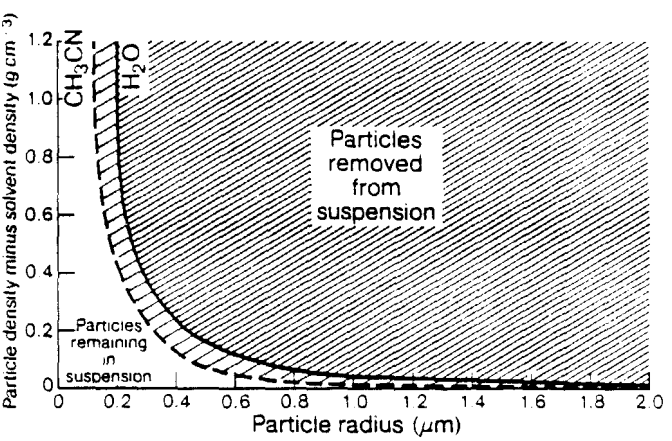

FIGURE 2.

Calculated distribution of particles in suspension in water and acetonitrile after centrifugation in capillary tubes for 1 min at 13,000 x G.

D, the length of the capillary tube (7.5 cm), is the maximum settling distance. Time of centrifuging, t, is 60 s and centrifuge coefficient, C, is 13,000. Then

$$D = V\,t = \frac{2\,G\,C(P - Ps)r^2t}{9n} \qquad (2)$$

$$\text{or} \quad P - Ps = \frac{9Dn}{2\,G\,C\,t\,r^2} = \frac{(9)(7.5)n}{(2)(981)(13{,}000)(60)r^2}$$

$$= \frac{4.4 \times 10^{-8}n}{r^2}\ \text{g cm}^{-3}. \qquad (3)$$

For calculations, particles were assumed to be spherical and wall effects of the tubes were considered to be negligible. Viscosities of acetonitrile ($n = 0.0035$ g cm^{-1} s^{-1}) and water ($n = 0.0089$ g cm^{-1} s^{-1}) were based on a temperature of 25°C (5). The calculated results indicated that particles with densities greater than the density of the solvent (by ca. 1%) and diameters greater than 2 μm would be removed from suspension in 1 min by the hematocrit centrifuge (Fig. 2). Particles left in suspension pass through analytical frits and filters, which normally have pore diameters of at least 2 μm.

The technique can be used in conjunction with microhomogenization techniques to rapidly remove particulate materials from aqueous homogenates. For example, we have examined interacellular free amino acids in zooplankters (unpublished data, Columbia National Fishery Research Laboratory). A daphnid (1-4 mg wet wt) was homogenized in

50 µL of acidified (1 drop 6 normal hydrochloric acid per mL solution) ethanol:water (0.6:1.0 V:V) with a microtissue grinder. The homogenate was drawn into a microhematocrit tube, which was then plugged and centrifuged (1 min). A portion of the supernatant was injected into an amino acid analyzer for analysis. The time required to homogenize, centrifuge, and inject the sample was about 2.5 min. We have also used the technique to remove traces of particulate material from mussel mantle tissue extracts after protein precipitation and to remove calcium carbonate crystals from concentrated lake water samples prior to HPLC molecular size fractionation of dissolved natural organic materials.

DISCUSSION

Although hematocrit centrifuges have been used in clinical laboratories for many years (6), their capability to provide rapid and efficient cleanup of HPLC samples has not to our knowledge been exploited. The high-speed (11,500 rpm; 13,000 x G) and horizontal sample position provide efficient and rapid (1 min) centrifugation, which can be done immediately prior to sample injection. In contrast to high-speed microcentrifuges which require plastic tubes, hematocrit units use capillary glass tubes that can be cleaned by precombustion. Sample handling is simplified because surface tension and tube configuration prevent sample spillage and mixing of particles with supernatant liquid after centrifugation. Sample contact with transfer glassware is minimal because samples are drawn directly into the capillary tubes for centrifugation. The

sample size is compatible with injection volumes commonly used for HPLC and is particularly useful when samples are limited to small volumes. If biochemical components are unstable over the short period required for centrifuging, the head can easily be removed and placed in cold storage prior to use. The cost of capillary hematocrit centrifuges is moderate (ca. $500) compared with HPLC columns and the space requirement (ca. 0.1 m^2 of bench space) is minimal. Although the unit operates on normal line voltage (115 V), we have found that its high power requirement can cause interference if the unit is plugged into the same electrical supply line as the HPLC detector.

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