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THE APPLICATION OF VACUUM LIQUID CHROMATOGRAPHY TO THE SEPARATION OF TERPENE MIXTURES

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Vacuum liquid chromatography [vlc, a name coined by Targett *et al.* (1)] was developed because of the impatience of Australian chemists with classical column chromatography.[1] We have applied the technique extensively and virtually exclusively to the separation of complex terpene and lipid mixtures derived from soft corals (2-5). Pelletier *et al.* (6) have recently provided an excellent and detailed report on the application of the technique to the separation of alkaloids using aluminium oxide adsorbents. The technique is essentially a preparative layer chromatographic (plc) separation run as a column, the flow of which is activated by vacuum. It differs from flash chromatography (7), in that vlc involves step gradient elution, and the column is allowed to run dry after each fraction is collected. The similarity with plc then becomes obvious, because plc separation may be enhanced by repetitive running of plc plates dried between runs. Because Pelletier *et al.* (6) have so clearly described the details of the vlc experiment, it is not necessary to restate the same details that were given in our earliest papers (2-5). However, it is felt that some additional notes on the application of this technique to silica gel chromatography may be of assistance to potential users.

[1]In the late 1960s and early 1970s, this technique appears to have developed independently in several centers. One of us (JCC) used it extensively during his Ph.D. studies (University of Sydney, 1969), and Dr. R.J. Wells appears to have used similar techniques at James Cook University (until 1971) and subsequently at RRIMP. Its origins are not entirely clear, although its exploitation is obvious (2-5, 8, 9).

THE TECHNIQUE

APPARATUS.—The apparatus described by Professor Pelletier (6) is simple and practical, in contrast to that developed earlier by others (1). We find two additional set-ups of considerable utility for different applications. Figure 1a shows a small scale apparatus suitable for the chromatography of extracts or reaction mixtures of less than 1 g. It is also suitable for undergraduate experiments. In this case only 10-15 ml fractions are collected at each polarity step, and a complete chromatography can be readily contained within 0.5 M^2 of bench space. Figure 1b shows our variation of the apparatus used for larger scale separations. In this case, the base is cut out of a 500-ml filter flask and the cut edge ground smooth and flat. It will then seal onto a flat glass plate (discarded plc plate), and the collection flasks can be 25-, 50-, or 100-ml erlenmeyer flasks. The column is a standard sintered glass filter funnel (100, 150, or 250 ml volume).

ADSORBENT.—Silica gel (Merck 60H or 60G) is packed to a hard cake under applied vacuum as described by Pelletier *et al.* (6). The directions as given are extremely important and very precise in this matter. In our experience, this layer should not exceed 5 cm in height. Thus, for small scale separations (<100 mg), a column of 0.5-1.0 cm internal diameter and 4 cm high should be used. For 0.5-1.0 g, a column approximately 2.5×4 cm is appropriate, while for 1-10 g separation, a 5 cm diameter$\times5$ cm high column would be adequate. Larger amounts are best separated on a 250 ml sintered glass filter funnel

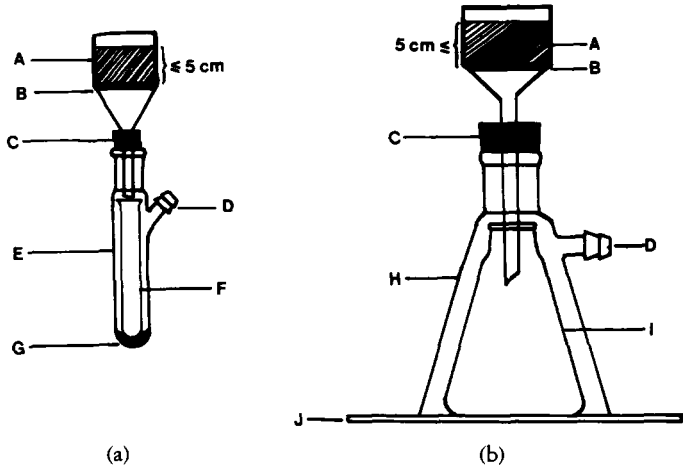

FIGURE 1. Laboratory vlc apparatus

A—Adsorbent-E. Merck, tlc grade silica gel 60G or 60H
B—Sintered glass Buchner filter funnel; porosity 2 fritted disc.
C—Rubber gasket (or ground glass joints).
D—To vacuum aspirator or pump.
E—Sidearm test tube.
F—Test tube, 20-25 ml.
G—Cotton "wool."
H—Filter flask (500 ml) with base removed.
I—Erlenmeyer flask.
J—Flat glass plate.

packed to a height of about 5 cm. As the size of the extract increases, the diameter of the column is increased with very little increase in the height of the silica bed. If the column stops flowing under applied vacuum after application of the extract, which may be due to crystallization of more polar components on top of the column or blockage of the column by polymer, it may be necessary to stir the first 0.5 cm of the column with a spatula, under which condition, the column will recommence to flow.

ELUTION SCHEME.—Wherever possible, the mixture to be separated is added to the silica column in light petroleum, and increasing amounts of a more polar solvent (CH_2Cl_2, Et_2O, EtOAc, or Me_2CO) added to each successive solvent fraction. Early fractions increase slowly in polarity (1%, 2%, 3%) with the increments increasing more quickly (5%, 10%, 20%, 50%) until 100% of the more polar component is reached. A more polar solvent (e.g. MeOH) is then added slowly at first and then in increasing proportions until elution is complete. Usually 20-25 fractions will remove all components. We find this routine solvent elution scheme suffices to reduce complex mixtures into fractions suitable for ^{1}H-nmr assessment. Interesting fractions are then rechromatographed on a smaller column using smaller fractions and more gradual solvent polarity steps, especially near the solvent mixture which eluted the fraction from the first column.

If the crude mixture cannot be dissolved (or suspended) in light petroleum, it should be dissolved in a volatile solvent (e.g. CH_2Cl_2) and mixed with an equivalent weight of silica gel. The solvent is *carefully* removed under vacuum after which the dried powder is packed onto the top of the column. Elution then commences with light petroleum. Whenever possible, visual cues (e.g. pigments, etc.) may be used to gauge when to change the collection flask or

when to run several fractions of the same polarity.

The vlc method works extremely well and is very quick. Our publications evidence its success (8,9); these were all achieved before we entered the hplc era. The apparatus is simple and universally available, and the separations are efficient in terms of time, amount of adsorbent, and volume of solvent (6). It provides an ideal pretreatment of small samples prior to hplc separations, and at the other end of the scale, enables the fractionation of up to 30 g of extract in several hours.

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ACKNOWLEDGMENTS

1. N.M. Targett, J.P. Kilocyne, and B. Green, *J. Org. Chem.*, **44**, 4962 (1979).
2. J.C. Coll, G.B. Hawes, N. Liyanage, W. Oberhänsli, and R.J. Wells, *Aust. J. Chem.*, **30**, 1305 (1977).
3. J.C. Coll, S.J. Mitchell, and G.J. Stokie, *Aust. J. Chem.*, **30**, 1859 (1977).
4. J.C. Coll, N. Liyanage, G.J. Stokie, I. van Altena, J.N.E. Nemorin, S. Sternhell, and R. Kazlauskas, *Aust. J. Chem.*, **31**, 157 (1978).
5. B.F. Bowden, J.C. Coll, N. Liyanage, S.J. Mitchell, G.J. Stokie, and I. van Altena, *Aust. J. Chem.*, **31**, 163 (1978).
6. S.W. Pelletier, H.P. Chokshi, and H.K. Desai, *J. Nat. Prod.*, **49**, 892 (1986).
7. W.C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
8. J.C. Coll, "Proc. Fourth Asian Symp. Medicinal Plants and Spices, UNESCO Spec. Publ. Bangkok, 1981, pp. 197-204 (and references therein).
9. J.C. Coll, B.F. Bowden, D.M. Tapiolas, R.H. Willis, P. Djura, M. Streamer, and L. Trott, *Tetrahedron*, **41**, 1085 (1985) and references therein.

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