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POLYETHYLENE POWDER AS A STATIONARY PHASE FOR PREPARATIVE-SCALE REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Polyethylene powder has been shown to function effectively as a stationary phase in reversed-phase high-performance liquid chromatography. The low cost and durability of this material make it particularly attractive for use in preparative-scale separations. The applicability of preparative reversed-phase techniques to difficult separations of natural products is demonstrated by the isolation and separation of bacteriochlorophylls from green sulfur bacteria.

INTRODUCTION

Five years after the introduction of reversed-phase chromatography by Howard and Martin[1], the use of polyethylene powder as both a solid support and a stationary phase was described by Green *et al.*[2]. These investigators achieved good separations of C_6–C_{20} fatty acids using aqueous acetone as the mobile phase. Polyethylene column chromatography was first utilized for the separation of plant extracts by Anderson and Calvin[3]. Richards and Rapoport[4] used polyethylene columns to separate polycarboxylated porphyrins. Such applications revealed the advantages of polyethylene powder as a packing material for reversed-phase chromatography, but its use has not been widely adopted.

More recently, reversed-phase packing materials have been developed especially for use in high-performance liquid chromatography (HPLC). Chemically bonded stationary phases[5] formed by attachment of long hydrocarbon chains to the surface of silica particles have proved extremely useful, and are now available commercially[6]. A survey of the chromatographic literature has revealed no application of powdered polyethylene as a packing material in HPLC. Polyethylene, however, possesses several properties that make it, in many respects, a more suitable packing material than the commercially available chemically bonded materials. The extreme inertness of polyethylene is highly desirable for the separation of chemically reactive species. This chemical inertness also makes a polyethylene column nearly indestructible. The low cost of polyethylene powder permits the application of reversed-phase HPLC techniques to preparative separations that in some instances would not be economi-

cally feasible with chemically bonded packing materials. This paper describes the performance of an inexpensive, large-scale, HPLC system that utilizes a column packed with polyethylene powder and a pneumatic pump. The resolution attainable from this system is demonstrated by the notoriously difficult separation of bacteriochlorophylls *c* (ref. 7). The separation of mixtures of long-chain alcohols with the same packing material illustrates the versatility of the system.

APPARATUS

Column

The column was constructed from a 9-ft. (275-cm) section of 3½-in. (9-cm), schedule 40, stainless-steel pipe fitted at each end with a 4-in. (10-cm) stainless-steel flange and end-plate (see Fig. 1). A cylindrical extension on each end-plate protrudes into the column and supports a stainless-steel frit (Mott Metallurgical Corp., Farmington, Conn., U.S.A.). Shallow grooves machined in the surface of the cylindrical extension ensure an even distribution of the mobile phase across the entire cross-section of the column. This column is routinely operated at 2000 p.s.i. (140 atm)*. A 1¼-in. (3-cm) column of similar design has been constructed for smaller scale separations.

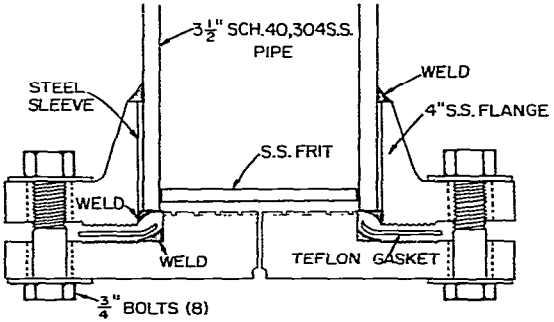

Fig. 1. Construction details of the HPLC column.

Pump

The eluting solvent is delivered to the column with an inexpensive Haskel MCP** pneumatic reciprocating pump. The displacement of this pump is sufficiently small in comparison with the column volume that no pulsation can be detected at the column outlet with a sensitive refractive index detector. The small displacement also permits the introduction of most samples at the pump inlet via a three-way stopcock, thereby eliminating the need for a high-pressure sample injection valve.

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* A column of this size would, of course, represent a considerable hazard if inadvertently pressurized with gas rather than liquid.

** MCP-188 (not presently available), MCP-110 and MCP-71 pumps manufactured by Haskel Engineering Corp., Burbank, Calif., U.S.A., have been found satisfactory for the application outlined in this paper. For most purposes the pumps of smaller displacement (MCP-110 or MCP-188) are more desirable. These pumps are available with necessary air controls for less than $ 300.

Accessories

For the pigment separations, an absorbance detector was constructed from a flow cell with a path length of 1 cm, a red light-emitting diode and a phototransistor with a response maximum in the infrared region. A Micromeritics Model 770 refractometer was used as a detector in the alcohol separations. The refractometer was insulated and the inlet tubing was provided with a massive heat sink to minimize the effects of fluctuations in room temperature and concomitant changes in the refractive index of the eluate. A large fraction collector accomodating 270 bottles of 120-ml capacity has been constructed for use in conjunction with the $3\frac{1}{2}$-in. diameter column. Aqueous acetone composition gradients were produced with a two-compartment solvent reservoir[9]. Thorough stirring of both compartments and careful adjustment of the specific gravity of the solvent was found to be essential for good resolution and reproducibility.

COLUMN PREPARATION

Microthene FN 500 polyethylene (U.S. Industrial Chemical Co., New York, N.Y., U.S.A.) consists of spherical particles ranging from 3 to 45 μm in diameter with a typical weight-average particle size of 9 μm. This material was thoroughly washed with pentane, dried and slurried with aqueous acetone. The column was packed at a pressure higher than the normal operating pressure. For columns packed at the operating pressure it was found that after extended use the packing material compressed, leaving a void at the top of the column. The use of a short column extension greatly facilitates the column packing operation.

Properties of polyethylene

Polyethylene is very resistant to and insoluble in most organic solvents, mild acids and bases at room temperature, while most chemically bonded reversed-phase packing materials[6] are stable only in the pH range 3–10. Not only is the polyethylene packing resistant to damage, it is also very unlikely to cause any alteration of the sample under investigation. Silica gel or alumina columns, on the other hand, often degrade reactive materials[9].

An important advantage of polyethylene over chemically bonded phases is its low cost. In reversed-phase separations of relatively non-polar compounds of large molecular weight, the capacity of the chromatography column is often limited by the solubility of the sample in a solvent polar enough to effect the desired separation. In situations of this type, where large column volumes are required, the low cost of polyethylene in comparison with that of chemically bonded reversed-phase packing materials makes preparative-scale separations economically feasible. The polyethylene required to pack the large column described above can be purchased for about US $ 50 whereas, the cost of an equivalent amount of chemically bonded material would be of the order of US $ 50,000.

SEPARATIONS OF BACTERIOCHLOROPHYLLS AND THEIR ESTERIFYING ALCOHOLS

Holt and co-workers[10,11] first characterized the homologous series of light-harvesting pigments isolated from green sulfur bacteria. These pigments were found

to differ in the nature of the alkyl substituents on the chlorin ring. Considerable controversy, however, has arisen concerning some of the details of Holt and co-workers' structural assignments[7]. Much of the difficulty associated with the identification and structural characterization of these pigments can be attributed to the fact that a suitable procedure was not available for the isolation of the unaltered chlorophylls. The chromatographic apparatus described in this paper was originally constructed so as to permit the preparative-scale separation of these materials.

The chromatogram in Fig. 2 shows the first successful separation of bacteriochlorophylls *c*. A detailed description of the structural characterizations of these pigments will appear elsewhere[12], but the major components in this chromatogram are identified in Table I. This separation has revealed that the bacteriochlorophylls *c* differ not only in the peripheral substituents on the chlorin ring, but also in the esterifying alcohol. The conditions chosen for this experiment were not optimized for the separation of any particular pair of components, but rather to give the best

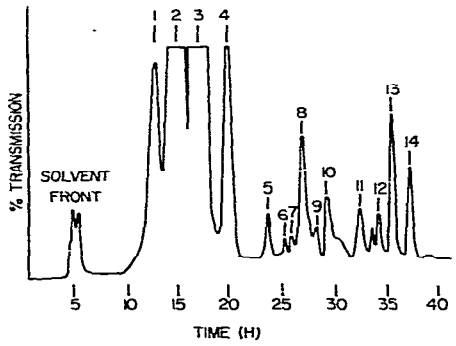
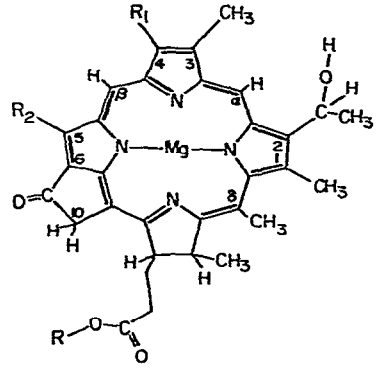


Fig. 2. Separation of 150 mg of bacteriochlorophylls *c* obtained from *Chlorobium limicola thiosulfatophilum* (Tassajara). The 3½-in. column was eluted with a 65–75% aqueous acetone gradient. The flow-rate at 2000 p.s.i. was approximately 14 ml/min.

overall resolution. Nevertheless, excellent resolution was obtained for components that in many instances differ by the addition of a single methylene group or by the saturation of one double bond. Overloading the column with 750 mg of mixed pigments resulted in a loss of resolution for the major fractions, but provided a pigment mixture enriched in the minor constituents. Hydrolysis of this enriched fraction produced a mixture of the six alcohols that were found to esterify bacteriochlorophyll *c*. The separation of these alcohols (Figs. 3 and 4) on a polyethylene column demonstrates the versatility of this method. In this separation an elution gradient was not used. The retention order of the alcohols parallels that of the corresponding chlorophylls. Sensitivity of the retention order to the degree of saturation is demonstrated by the relative retention times of geranylgeraniol, tetrahydrogeranylgeraniol and phytol (hexahydrogeranylgeraniol).

TABLE I

STRUCTURAL ASSIGNMENT OF THE BACTERIOCHLOROPHYLLS ISOLATED FROM *CHLOROBIUM LIMICOLA* (SEE FIG. 2)



No.	R_1	R_2	*ROH*
1	Et	Me	Farnesol
2	Et	Et	Farnesol
3	*n*-Pr	Et	Farnesol
4	*i*-Bu	Et	Farnesol
5	Et	Et	4-Undecyl-2-furanmethanol
6	Et	Et	Geranylgeraniol
7	*n*-Pr	Et	4-Undecyl-2-furanmethanol
8	Et	Et	*cis*-9-Hexadecen-1-ol
9	*n*-Pr	Et	Geranylgeraniol
10	*n*-Pr	Et	*cis*-9-Hexadecen-1-ol
11	Et	Et	10,14-Tetrahydrogeranylgeraniol
12	*n*-Pr	Et	10,14-Tetrahydrogeranylgeraniol
13	Et	Et	Phytol
14	*n*-Pr	Et	Phytol

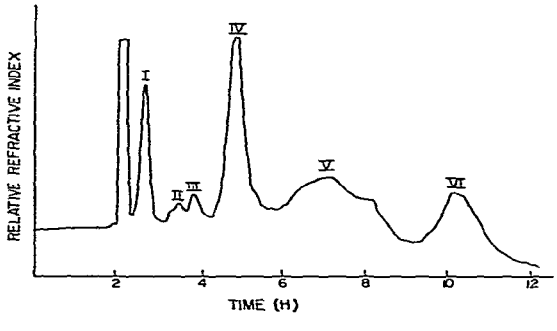


Fig. 3. Separation of 24 mg of mixed alcohols obtained from base hydrolysis of the minor components of bacteriochlorophylls *c*. The 1¼-in. column was eluted with 68% aqueous acetone. The flow-rate at 900 p.s.i. was 1.4 ml/min.

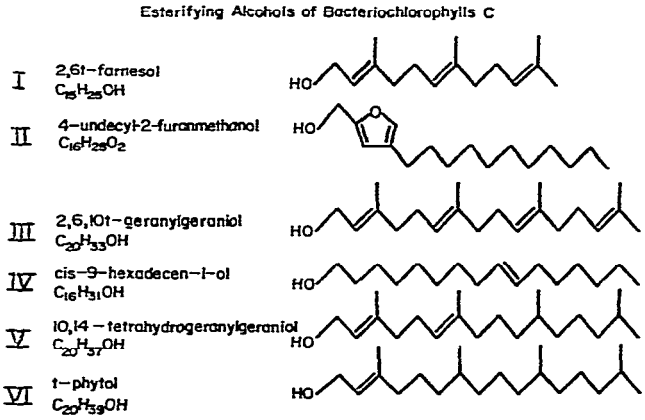


Fig. 4. Structural assignment of the esterifying alcohols of bacteriochlorophyll *c*.

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