presence of net repulsive charges on the particles. This would also account for the change in equilibrium with voltage. A more sophisticated theory of dielectric distribution which would take this effect into account awaits future development.

REFERENCES

1. Lowden, J. J. (Henry G. Thomas), U.S. 465,822 (1891).
2. Dow, D. B., U.S. Bur. Mines Bull. No. 250 (1926).
3. Stevens, E. W. (Petroleum Hydrogen Co. of America), U.S. 1,533,711 (1925).
4. Eddy, W. G., and H. C. Eddy, Ind. Eng. Chem. *13*: 1016–1017 (1921).
5. Pearce, C. A. R., Brit. J. Appl. Phys. *5*, 136–143 (1954).
6. Bates, J. R. (Sun Oil Co.), U.S. 2,665,246 (1954).
7. Föppl, A., and M. Abraham, "Einfuhrung in die Maxwellsche Theorie der Elktrizitat," 4th ed., Vol. 1, B. G. Teubner, Leipzig, 1912, p. 146.
8. Müller, V. F. H., Wiss. Veroffentl. Siemens-Werken *17*, 20–37 (1938).
9. Pohl, H. A., J. Appl. Phys. *22*, 869–871 (1951).
10. *Ibid.*, *29*, 1182–1188 (1958).
11. Pohl, H. A., and J. P. Schwar, *Ibid.*, *30*, 69–73 (1959).
12. *Ibid.*, J. Electrochem. Soc. *107*, 383–385 (1960).
13. Pohl, H. A., *Ibid.*, 386–389.
14. Pohl, H. A., and C. E. Plymale, *Ibid.*, 390–396.
15. Loesche, A., and H. Hultschig, Kolloid Z. *141*, 177–187 (1955).
16. Bailey, A. E., "Industrial Oil and Fat Products," 2nd Ed., Interscience Publishers, New York, 1951.
17. Black, B. C., and E. G. Hammond, Correspondence, JAOCS, in press.
18. Kolthoff, I. M., R. J. Elving and E. E. Sandell, "Treatise on Analytical Chemistry," Vol. 4, John Wiley and Sons, New York, 1963, p. 2641.
19. Black, B. C., and E. G. Hammond, JAOCS, in press.
20. Furedi, A. A., and I. Ohad, Biochem. Biophys. Acta *79*, 1 (1964).

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Separation by Dielectric Distribution: Application to the Isolation and Purification of Soybean Phosphatides and Bacterial Spores[1]

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Abstract

The redistribution of particle suspensions which occurs in a nonuniform electric field was used to separate soybean phosphatide micells from crude soybean miscella. Two arrangements of the nonuniform field were used. This method provides a convenient laboratory method for isolating and concentrating polar lipids. At present the method is too slow for commercial application but this may become feasible with improvements in apparatus design.

A simple apparatus was described that gave efficient separation of bacterial spores from vegetative cells.

Introduction

DIELECTRIC DISTRIBUTION may be defined as the distribution which mobile materials with different dielectric constants will assume in a nonuniform electric field (1). The more polar material moves toward regions of high field intensity, and less polar material moves toward regions of low field intensity. The application of dielectric distribution to analytical and preparative separations has received little attention in the past. Dielectrophoresis, the motion of particles in a nonuniform electric field, has been studied by several workers (2-8). They concluded the effect would not be appreciable for particles of molecular size, but for particules of 1 to 2μ or larger, the effect would be quite large.

There are a number of methods which may be used to separate small molecules (chromatography, distillation, partition, etc.). As the particle size increases, these methods become impractical for various reasons. Density separation (centrifugation) and screening (filtration) are essentially the only methods available to work with large particles. Dielectric distribution should be useful in the same range of particle size where density separations and filtration are employed and should supplement these methods.

This paper reports the use of dielectric distributions for the separation of soybean phosphatides from soybean miscella and for the separation of bacterial spores and vegatative cells.

Experimental

Cylindrical Condenser Apparatus

The cylindrical condenser that was used to separate the phosphatides from neutral fat was similar to that used previously (1). It consisted of a Pyrex glass cylinder 0.8 cm I.D. and 78 cm long. The barrier separating the inner and outer portions of the con-

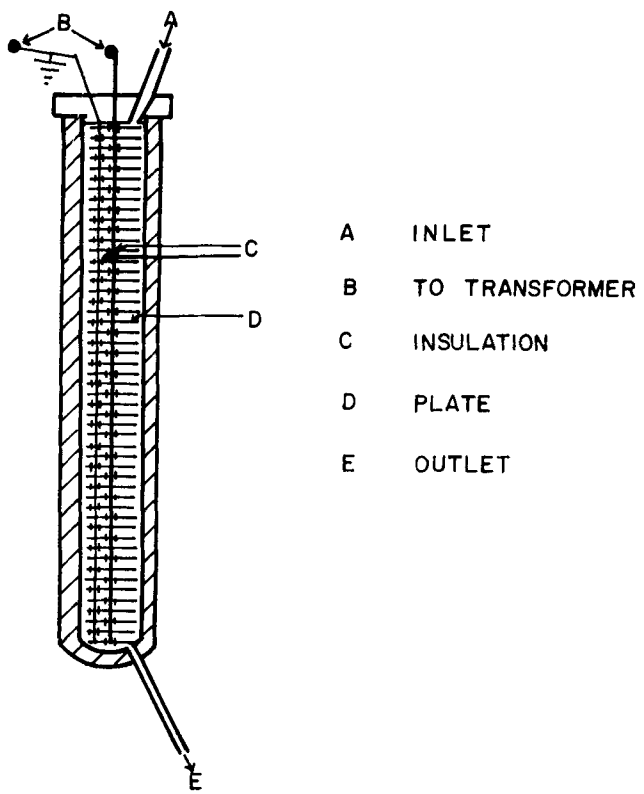

Fig. 1. The plate stack apparatus.

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denser at the bottom was 0.4 cm in diameter. The central electrode was an iron wire insulated with teflon tubing 0.41 mm thick so that the total diameter was 0.31 cm. Metal foil wrapped around the outside of the cylinder served as the peripheral electrode. A potential difference was applied between the outside and inside electrodes with a high voltage transformer as described previously (1).

The soybean miscella (prepared as previously described [1]) was introduced continuously into the apparatus through an inlet at the top. The material with the higher dielectric constant (the phosphatide micells) was attracted to the place of highest field strength around the center electrode. The material with the lower dielectric constant(solvent and neutral fat) moved toward the periphery of the cylinder where the field is weakest. Central and peripheral fractions were collected through outlets at the bottom. The flow rate was controlled by small delivery tubes drawn to the desired diameter. The flow rate was regulated to give 21 min residence time in the apparatus which was long enough for equilibrium to be attained. Fractions were collected and analyzed for phosphorous (9).

The thin layer chromatography of the phosphatides was accomplished by the method of Rouser et al. (10) using the solvent system of chloroform/methanol 2/1 plus 20 ml of water/liter.

Parallel Plate Stack Apparatus

A schematic diagram of the parallel plate stack apparatus is shown in Figure 1. The principle of separation using the plate stack condenser is similar to that of the cylindrical condenser. The polar material tends to move to the region of high field between the plates. This condenser was prepared by stacking plates that were 12 mm in diameter 1 mm apart. There were 60 plates in the stack, and it was put into a Plexiglass cylinder that was 15 mm I.D. The odd-numbered plates were connected to one terminal of the transformer and the even-numbered plates to the other. The wires connecting the plates were insulated. There was an opening in the top of the condenser to allow the feed solution to enter. The polar material was collected between the plates by the fringe field, while the nonpolar material passed beside the plates and was collected via the outlet at the bottom. The apparatus was used to separate phosphatides from soybean micella. Successive fractions were collected and analyzed for phosphorous (9). The apparatus volume was 9 ml.

Parallel Plate Condenser

Figure 2 shows a diagram of a column between two large parallel plates. This apparatus was constructed by using two copper plates, 1 mm thick and 9.5 cm x 9.5 cm. The two plates were held 7 mm apart by clamping 7 mm rubber spacers between them. The clamps were also insulated with rubber. The plates were insulated by covering them with sheets of Parafilm. A 6 mm Pyrex glass tube was fixed between the two plates. One of the plates was grounded, while the other was connected to the high voltage terminal of the AC transformer. The column was partially filled with benzene-carbon tetrachloride (2.5:1), and a mixture of polar and nonpolar particles were put in the top of the column. This mixture moved slowly toward the bottom of the column by gravity. The voltage was adjusted so the more polar particles were retained as they passed through the field while the less polar particles moved through. This apparatus was used to separate a crude mixture of the spores and vegetative

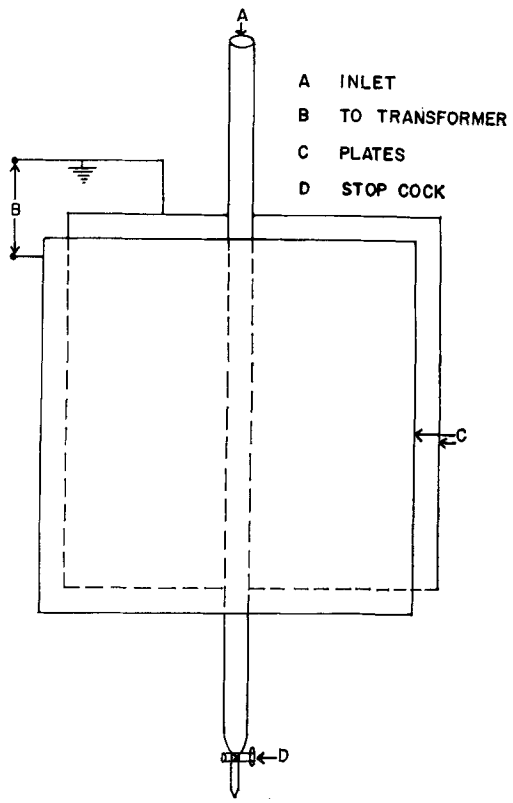

Fig. 2. The parallel plate condenser apparatus.

cells of *Bacillus megaterium*. This mixture was prepared according to the method of Walker et al. (11) and lyophilized. The relative number of spores and vegetative cells were determined by observation under a phase microscope.

Results and Discussion

Continuous Flow Cylindrical Condenser Apparatus

This apparatus was used to prepare pure phosphatide in high yield, starting with dilute, crude, soybean miscella. The flow rate was 1.5 ml/min. The voltage was 5,000 v, and the concentration of soybean oil in Skellysolve B was 1.08%. The crude soybean oil contained 0.17% phosphatide, assuming a molecular weight of 788 for the phosphatide (the value for a typical lecithin molecule). The drain ratio of the peripheral vent to the central vent was approximately 3:1. The barrier divides the condensers' volume in a 3:1 ratio, and it is most efficient to maintain the

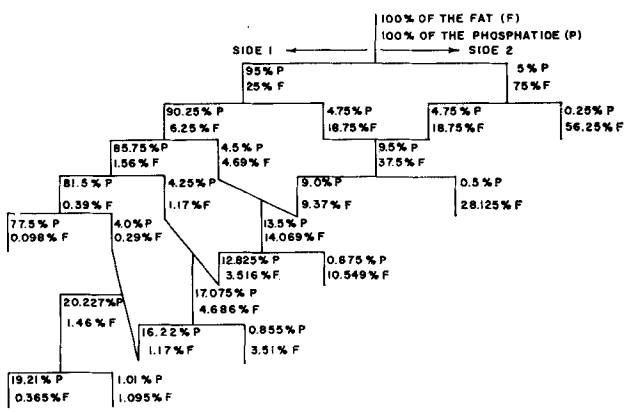

Fig. 3. The separation of soybean phosphatides from soybean oil in the cylindrical condenser. Side 1 is the volume around the central electrode and side 2 that near the periphery. P and F are the expected percentage of the original phosphatide and fat in the fraction.

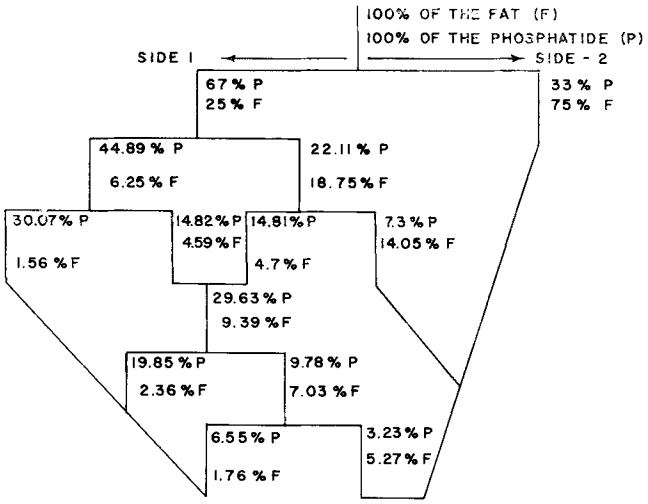

Fig. 4. The production of soybean oil with a 40% reduction in the original amount of phosphatide. Side 1 is the volume around the central electrode and side 2 that near the periphery. P and F are the expected percentage of the original phosphatide and fat in each fraction.

drainage of the two outlets in this same ratio.

Preliminary results with this apparatus indicated that approximately 95% of the total phosphatide would be in the central fraction.[1] With a 3:1 drain ratio, about 25% of the total fat should be collected in the central fraction and about 75% in the peripheral fraction. A scheme for purifying the phosphatide was worked out using these values, and it is shown in Figure 3. Side 1 and side 2 in the diagram correspond to the central and peripheral fractions, respectively. The side 1 fractions were recycled four times. At this point the central fraction was calculated to contain 77.5% of the original phosphatide and 0.98% of the original fat. This fraction should be 57.4% phosphatide. It analyzed 64%. Another fraction rich in phosphatide was obtained be recycling various fractions as indicated in the diagram. It was calculated to contain 19.2% of the total phosphatide and 0.365% of the total fat and thus be 8.24% phosphatide. It analyzed 7.8% phosphatide. Most of the deviation in the found and predicted purity is caused by the actual drain ratio being a little greater than 3:1. The two fractions richest in phosphatide were calculated to contain 96.7% of the total phosphatide and were found to contain 97%. A higher purity should be obtained by recycling further.

Thin-layer chromatography was used to detect any fractionation of types of phosphatides in the continuous flow experiments. The results indicated that both central and peripheral fractions contained the same types of phosphatides.

Next an experiment was carried out to demonstrate that the level of phosphatide could be reduced to about 40% of the original level, using a concentration of miscella similar to that in commercial refining processes. The distribution is less favorable at this high concentration, and preliminary results showed that with a 5.4% fat solution about 67% of the total phosphatide would accumulate in the central fraction (side 1) which contained about 25% of the total fat. Figure 4 shows a flow diagram for recycling the concentrated miscella and indicates the theoretical percentages of original fat and phosphatide in each fraction. As indicated, all of the final central fractions were combined as were all of the peripheral fractions. The percentage recovery of oil with reduced concentration of phosphatide was calculated to be 94.32%. Actually 93.3% was recovered. The level of phosphatide in this fraction was calculated to be 43.5% of the original level. Actually it was 39%. Any degree of separation of phosphatide from oil desired presumably could be achieved by further recycling.

[1] Some batches of soybean miscella give much less efficient separations in the apparatus. They may be activated by extracting the miscella with 1% aqueous sodium carbonate solution and 95% ethanol in a ratio of 100:50:25.

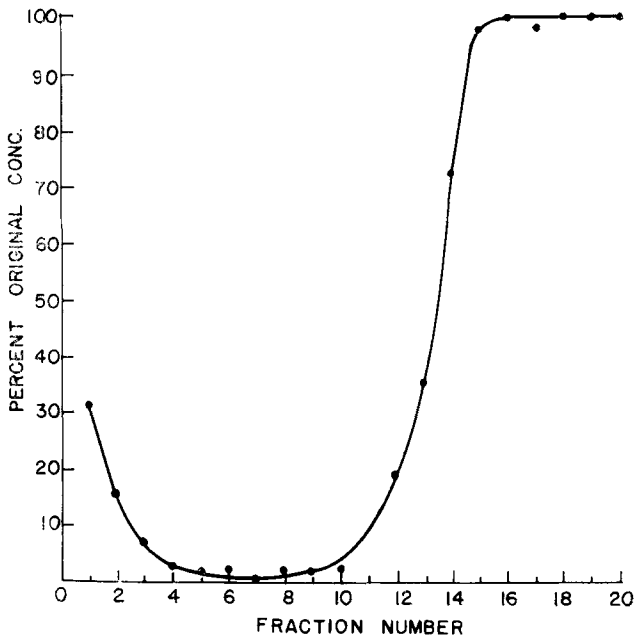

Fig. 5. Removal of phosphatide from miscella by the plate stack apparatus. Each fraction is 25 ml.

Parallel Plate Stack Apparatus

Soybean miscella was used to demonstrate dielectric distribution with the plate stack apparatus. The polar phosphatide micells were attracted in and held between the plates while the fat solution passed along the edges and was collected through the outlet. A number of different concentrations of miscella were tried, and the lower concentrations gave better results. A concentration of 0.54% soybean oil in Skellysolve B was used to make the study reported in Figure 5. Fractions of 25 ml were collected at a flow rate of 0.3 ml/min. The voltage was 1,000 v. The level of phosphatide in the unfractionated miscella was designated as 100%. The quantity of phosphatide in the fractions decreased rapidly to practically nothing at fraction 4 and remained there until about fraction 11. The reason for the presence of phosphatide in fractions 2 and 3 is not clear. These fractions had as much residence time in the apparatus as any. The quantity of phosphatide in the fractions then increased again and finally reached the original concentration, indicating the condenser was filled to capacity with phosphatide. The factors determining the capacity of the plates are not clear. Certainly the plates were not physically full of phosphatides. Perhaps phosphatide chains formed at the edges of the plates and hindered the entrance of more particles, or perhaps the phosphatide particles accumulate similar charges and repel each other as suggested by Pohl and Schwar (7).

Parallel Plate Condenser

A bacterial spore and vegetative cell mixture (approximately 50:50) was separated with the parallel plate condenser to further demonstrate the usefulness

of dielectric distribution. A voltage of 3,000 v was applied. The spore cells were preferentially witheld in the column between the two plates while the vegetative cells and debris passed through the electric field. The material which was retained was refractionated under the same conditions. The particles retained between the plates the second time were 90% spores.

The particles fell freely in the solvent until they reached the upper edge of the condenser. Then there was a tendency for the particles to be attracted to the ungrounded side of the condenser. There was a strong tendency for the particles to form chains across the tube in the direction of the field. Chain formation is to be expected (1), but we have no explanation for the tendency of the particles to move to the ungrounded side of the apparatus. There was no evidence that the particles accumulated net charges and repelled each other as Pohl and Schwar (7) postulated.

REFERENCES

1. Black, B. C., and E. G. Hammond, JAOCS, (in press.)
2. Muller, V. F. H., Veroeffentl. Siemens-Werken *17*, 20–37 (1938).
3. Loesche, A. and H. Hultschig, Kolloid-Zeitschrift *141*, 177–187 (1955).
4. Pohl, H. A., J. Appl. Phy. *22*, 869–871 (1951).
5. Pohl, H. A., *Ibid.* *29*, 1182–1188 (1958).
6. Pohl, H. A., J. Electrochem. Soc. *107*, 386–390 (1960).
7. Pohl, H. A., and J. P. Schwar, J. Appl. Phy. *20*, 69–73 (1959).
8. Pohl, H. A., and J. P. Schwar, J. Electrochem. Soc. *107*, 383–385 (1960).
9. Black, B. C., and E. G. Hammond, JAOCS, in press.
10. Rouser, G., G. Kritschvsky, D. Heller and E. Lieber, JAOCS *40*, 425–454 (1963).
11. Walker, H. W., J. R. Matches and J. C. Ayres, J. Bacteriol. *82*, 960–966 (1961).

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Dihydroxy Fatty Acids in *Cardamine impatiens* Seed Oil[1]

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Abstract

The oil of *Cardamine impatiens* L. (Cruciferae) seed includes glycerides of a series of saturated long-chain vicinal dihydroxy fatty acids, which make up 25% by weight of the mixed fatty acids. The mixture of diols, after transesterification of the oil with methanol, can be crystallized from an ether solution of the mixed methyl esters and has the following composition: methyl 13,14-dihydroxydocosanoate, 66%; methyl 15,16-dihydroxytetracosanoate, 24%; methyl 9,10-dihydroxyoctadecanoate and methyl 11,12-dihydroxyeicosanoate, about 5% each. Chemical proof is presented showing that essentially all the diols have the *erythro* configuration.

Introduction

NUMEROUS PLANT SOURCES have been shown to contain various monohydroxy acids in substantial quantities. Among these are seeds oils from the genus *Lesquerella,* family Cruciferae, which contain large amounts of either 12-hydroxy-*cis*-9,*cis*-15-octadecadienoic acid (12) or 14-hydroxy-*cis*-11-eicosenic acid (13).

The presence of 25% of saturated long-chain vicinal dihydroxy acids in the seed oil of *Cardamine impatiens,* a crucifer, is reported here. This seed oil is the first known that contains a large amount of vicinal dihydroxy acids. Castor oil (4,14), *Strophanthus* seed oil (5) and *Cephalocroton* seed oil (3) have been reported to contain vicinal dihydroxy acids in amounts of 4% or less. Some other examples of fatty acids with vicinal hydroxyl groups have been found in extracts of plant parts other than seeds (6,7), in wheat stem rust uredospores (15) and in *Lycopodium clavatum* L. spores (10).

The unusual infrared (IR) band at 8.1 μ in the spectrum of *Cardamine* oil has not been assigned to any structural feature. We feel that it is due to a deviation from the usual pattern of glyceride structure found in seed oils. This possibility is being investigated, and the results will be reported later.

Experimental

Gas-Liquid Chromatographic Conditions

Analyses by gas-liquid chromatography (GLC) were done on either a nonpolar Apiezon L column or a polar LAC-2-R 446 column, or both. The operating parameters of the polar column were the same as described previously (8). The nonpolar column (275 × 0.6 cm) was packed with 10% Apiezon L on 60–80 mesh Celite and was operated under the following conditions: Injection port, 287C; column bath, 257C; detector bath, 268C; detector current, 200 ma; fraction collector, 277C; helium flow rate, 102 ml/min at an inlet pressure of 40 psi. Identification of components was based on their equivalent chain lengths (9) as compared to equivalent chain lengths of similar, known materials. All percentages reported are area percent.

Oil Preparation and Analysis

Oil was obtained from ground seeds (36.0 g) of *Cardamine impatiens* L. by Soxhlet extraction with petroleum ether (bp 30–60C). The solvent was removed *in vacuo* at ca. 40C, yielding 11.9 g or 33.2% of oil. The ultraviolet (UV) spectrum showed a maximum at 230 mμ, $E^{1\%}_{1cm} = 14$ (in absolute ethanol) and weaker maxima at 270 and 276 mμ. IR analysis of the oil as a film on sodium chloride plates showed hydroxyl absorption at 2.88 μ and a rather strong unidentified band at 8.1 μ. Analysis of *Cardamine* oil by thin-layer chromatography (TLC) on silica gel G plates, with petroleum ether-ethyl ether (70–30) as the developing solvent, showed a large unknown spot with an R_f intermediate between those of the spots obtained for triglycerides containing one and two hydroxyl groups per molecule.

Preparation of Methyl Esters and Crystallization of Diols

A 1.958 g sample of *Cardamine* oil was refluxed 3 hr under nitrogen, with 75 ml of 1% hydrochloric acid in methanol; the solution was diluted with water; and the esters were recovered by ether extraction; yield, 1.876 g. The mixed methyl esters were dissolved in 10 ml of ethyl ether and crystallized overnight at −18C. The crystals (0.459 g) were filtered off, washed with cold ether and dried. A second crystallization yielded only 0.005 g of material. The liquor remaining from the crystallization was concentrated *in vacuo* and GLC gave the following composition: $C_{16:0}$, 4.4%; $C_{18:0}$, 0.4%; $C_{18:1}$, 14.6%; $C_{18:2}$, 26.6%; $C_{18:3}$, 4.1%; $C_{20:0}$, 0.8%; $C_{20:1}$, 7.0%; $C_{20:2}$,

[1] Presented at AOCS meeting, Chicago, Ill., October 1964.
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