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Notes on Methodology

An improved technique for dialysis of lipids

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» In 1957, Van Beers, De Iongh, and Boldingh (1) described a method for the separation of crude lipid mixtures into a phospholipid fraction and a neutral fraction by dialysis through a rubber membrane. The procedure is based on effecting aggregation of the polar compounds in a nonpolar medium, thus producing di-, oligo-, and higher polymers depending upon the type and number of ionogenic groups in the given molecule. The resulting increase in the molecular size restricts the movement of these aggregates through the pores of the membrane. The neutral lipids, since they are monomeric, may be easily removed from the system by dialysis. Olmsted (2) has reported a similar method of separation in the case of certain other high molecular weight lipid compounds.

The procedure of Van Beers et al. suffers from a number of disadvantages. Relatively large quantities of solvent are required even for small amounts of the given substance and the separation is also somewhat tedious. It therefore seemed desirable to use a continuous liquid-liquid extractor for this process. The apparatus shown in the figure was devised for this purpose. A closed system is used, in which the solvent is continuously recovered and maximum flow of the dialysis liquid is maintained. In this way, lipid mixtures from 100 mg to 7 g can be separated in less than 10 hr, using 250 ml of solvent. The apparatus requires little attention and may be left to run overnight. The dialyzer consists of the dialysis vessel (A), the funnel component (B), a device (C) for the suspension of the membrane, the condenser (D), and the flask (E) with a volume of 250–500 ml. The solvent for dialysis is placed in E, distilled via the tube A_1 into the funnel B_1, and flows to the bottom of A via B_2.

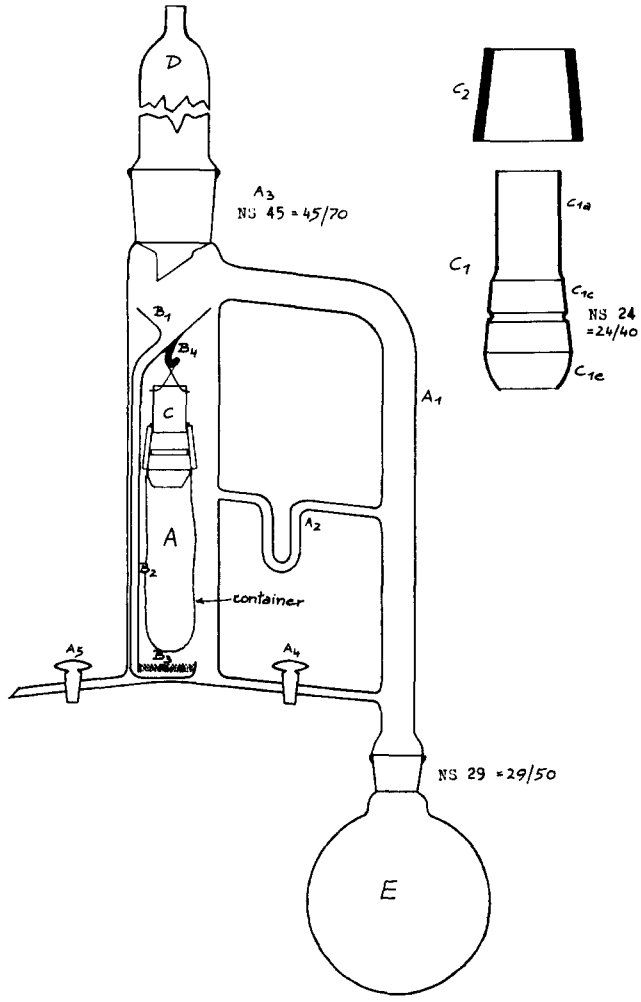

FIG. 1. Diagram of the dialyzer, consisting of the dialysis vessel (A), the funnel component (B), a device (C) for the suspension of the membrane, the condenser (D), and the flask (E) with a volume of 250–500 ml.

It then passes through the sintered glass disk B_3 into the dialysis vessel. After closing the stopcocks A_4 and A_5, A fills with solvent up to the level of the connecting capillary A_2. The excess solvent runs back into the round bottom flask E. The U-shaped bend in A_2 prevents the entrance of vapor from A_1. In order to empty the dialysis vessel A, stopcock A_4 or A_5 is opened.

The containers for dialysis, which are made from rubber or cellulose, possess a thickened rim. When following the procedure suggested by Van Beers et al., we had greatest success with the commercial type of rubber fingerstalls used in rectal examinations. Because of differences in the suitability of products of the fingerstalls, each brand has to be tested. Suitable fingerstalls may be used three times. Cellulose membranes were obtained from Fa. Kalle & Co, Wiesbaden,

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TABLE 1. EXPERIMENTS DEMONSTRATING COMPLETE SEPARATION OF MODEL MIXTURES OF TRIGLYCERIDES, PHOSPHOLIPIDS, AND FATTY ACIDS

	Initial Quantity	Recovery*	
		Dialysate	Residue
	g	%	%
Mixture I			
Triglycerides†	3.00	99.2	...
Phospholipids‡	0.09	...	97.8
Mixture II			
Triglycerides†	3.00	99.5	...
Phospholipids‡	0.15	...	98.0
Mixture III			
Triglycerides†	7.00	99.3	...
Phospholipids‡	0.70	...	99.5
Mixture IV			
Triglycerides†	0.100	99.0	...
Phospholipids‡	0.005	...	102.5
Mixture V			
Triglycerides†	3.00	99.5	...
Stearic acid	0.15		
Phospholipids‡	0.15	...	97.5

* Checked gravimetrically and examined as to its purity by thin-layer chromatography.

† Pure corn oil (free of phosphatides).

‡ Soybean phosphatides (mixture of lecithin, phosphatidylethanolamine, phosphatidylinositol, and polyglycerophosphatides).

Germany. The rim of the dialysis container is drawn over the cone-shaped end C_{1e} of C_1 and then up to the top of the socket joint C_{1c}. By pushing the ground-glass sleeve C_2 over the joint C_{1c} a solvent-tight seal between the membrane and C results. The membrane can be prevented from working loose by binding the central groove of C_{1c} with thread or by subsequently tying the membrane to C_{1c}. The lipids to be dialysed may be added via the opening in C either in solution or in solid form. The dialysis equipment is then attached to hook B_4 by means of a wire sling. The components B and C are placed in the dialysis vessel A via joint A_3, and then the dialysis can proceed. Redistilled, aldehyde-free petrol ether (b.p. 40°–60°) is applied.

After completion of dialysis, the internal components are removed again, the solvent is emptied into the flask E from A via the tap A_4, and then the dialysate is freed from solvent by distillation. The distilled solvent in A is removed via tap A_5.

The apparatus can also, of course, be used as a normal liquid-liquid extractor after removal of component C.

Examples are given in the table of the separation obtained by overnight dialysis of various lipid mixtures (respective compounds and quantities indicated). The purity of the substances and the completeness of the separation achieved were followed by thin-layer chromatography, which we have found to be more reliable than simply a determination of the phosphorus content. While the separation of the phosphatide-triglyceride mixtures proceeds relatively rapidly, the efflux of mono- and diglycerides is much slower. Dialysis for at least 4 days is necessary in order to remove the last chromatographically detectable trace of monoglycerides from the dialysis container, while the diglycerides are effectively removed in 1 to 2 days. The different rates of dialysis for these compounds can be used to good effect for their separation for preparative purposes, as studied by one of us (H. B.).

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