The Effect of Relative Concentrations on the Efficiency of Separation of Polar and Nonpolar Lipids by Alumina Column Chromatography[1]

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Columns were packed with aluminas from various sources and of low and high adsorptive power. Polar and nonpolar lipids were eluted from them with strong and weak solvents, and the fractions were tested for phosphorus, nitrogen, sterols, and triglycerides. The conditions used by the 1956 Technical Committee of the N.S.P.A. for the chromatography of linseed oil gave the most complete separation of polar from nonpolar lipids when the former were present in low concentration. When the lipid phosphorus load approached 0.4 mg./gm. alumina however, none of the alumina systems tried was satisfactory although silicic acid columns resolved the mixtures completely.

Gamma alumina had greater resolving power than *alpha* alumina monohydrate. Brockmann Grade I alumina with a strong solvent system gave better separation than weaker aluminas with weaker solvent systems.

Both silicic acid and alumina have established places in the column chromatography of lipids. Silicic acid of great resolving power can be prepared and excellent separations made with it (2,3). However finely ground silicic acid makes a slow-running column, which tends to release silicic acid into the eluate. This disadvantage plus the need for a rapid means of separating total lipid into polar and nonpolar fractions prompted this present investigation of the limitations of alumina columns.

Although alumina is an active catalyst of chemical reactions and is reputed to hydrolyze phospholipids, success has been achieved in the use of alumina columns to determine refinery loss in crude and refined vegetable oils (4,7). The size and composition of the polar charge however cannot always be estimated in advance with sufficient accuracy so that accidental overloading of a column can be avoided. The effect of relative concentrations of polar and nonpolar lipids on the resolving power of alumina columns was therefore investigated. *Alpha* and *gamma* aluminas of high and medium activity were tested with strongly polar and less polar solvents, using synthetic mixtures of triglycerides, fatty acids, and phospholipids.

Materials and Methods

Chromatography-grade alumina was used in all cases. X-ray diffraction patterns showed that North American alumina, as supplied by Fisher Scientific Company, Merck and Company, and the Aluminum Corporation of America, was of the *alpha* modification. German alumina, as supplied by Woelm Eschwege, proved to be a *gamma* alumina. All samples were activated at 400°C. for 2 hrs. While cooling, they were kept in a desiccator over a fresh surface of phosphorus pentoxide.

TABLE I

Characteristics of the Lipids Used in the Test Mixtures (percentage by weight)

Material	Neutral lipid	Polar lipid	Free fatty acid	Phospholipid
Lecithin 1	50	50	25	25
Lecithin 2	8	92	14	78
Safflower oil	99	1	0.4	0.46
Linseed oil	97	3	1.3	1.3
Oleic acid	nil	100	100	nil

The activity of all samples of alumina was checked by the method of Brockmann and Schodder (1). Activation at 400°C. for 2 hr. produced a Grade I alumina. When weaker adsorbents were required, the desired amount of water was added to the powder, either directly or by equilibration with salt solutions of known vapor pressure. To subdivide Grade I aluminas, the usual Brockmann column was packed and loaded with the prescribed *p*-methoxyazobenzene-azobenzene mixture. The eluent was added by burette at a rate that matched the rate of elution. The volume of eluent required to separate the bands was noted, and the distance of separation was recorded as a function of the volume of eluent.

Reagent-grade solvents were dried before use. Hexane and diethyl ether were distilled off fresh sodium ribbon. Methanol was treated with magnesium methoxide, then distilled. Chloroform was stored over anhydrous calcium sulphate before distillation.

The alumina was wet-packed into a glass column 17 mm. i.d., which was fitted with a glass-wool plug and a stopcock. The upper end of the column was fitted with a standard taper joint and a solvent reservoir with pressure equalizer. In this column 20 g. of alumina had a column volume of 24 cm^3. Each column volume was collected as a fraction. The solvent was removed; the residue was weighed and analyzed for phosphorus and nitrogen by standard methods. A portion of each fraction was used in a Lieberman-Burchard test.

Two samples of commercial vegetable lecithin were chromatographed on silicic acid columns according to Hirsch and Ahrens (3). One sample contained 50% polar lipid, the other 92%. These "lecithins" were used directly or diluted with alkali-refined and bleached safflower oil. In some experiments oleic acid was added to increase the free fatty acid content. The characteristics of these materials are shown in Table I.

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[1] Presented at the fall meeting, American Oil Chemists' Society, Los Angeles, Calif., September 28–30, 1959.

Contribution No. 44 from the Genetics and Plant Breeding Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

TABLE II

Lipid Loads on Alumina Columns

Method	Linteris and Handschumaker		Rhodes and Lea		Sims and Mes	
			B.J. 65	B.J. 60		
Material	Good crude	Poor crude	Egg P-L	Egg P-L	Veg. P-L	Veg. P-L
Wt. adsorbent, g	20	36	40	160	20	20
Tube diam., mm	18	18	15	15	17	17
Wt. charge, g	2.5	0.50	2.0	3.91	0.5	0.5
Total load, g./g	0.125	0.0139	0.05	0.0244	0.025	0.025
Expected loss, %	2.5	95	98	98	46	95
Polar charge, g	0.062	0.475	1.96	3.84	0.23	0.475
Polar load, (g./g.) x 10^4	31.3	132	490	239	115	237
Phos. lipid load, (g./g.) x 10^4	25	2.78	490	239	97.5	97.5
Lipid-P. load, (g./g.) x 10^4	1.0	0.11	19.6	9.56	3.9	3.9

Experimental and Results

Initial Tests. A survey was made of the loads of polar lipid commonly applied to alumina columns. Representative data (Table II) indicated that where the method was being used to determine refinery loss, the phospholipid load was restricted to a small fraction of the total polar load. It should be pointed out however that in the work of Rhodes and Lea referred to (5) neutral lipid and lecithin were collected as one fraction to be separated later by silicic acid chromatography. The two lipid mixtures selected from our own work represented heavy column loading.

To test the effects of load on column behavior, the total load on a 20-g. column of alumina monohydrate Grade I was kept constant at 25 mg. of total lipid per g. of adsorbent while the polar lipid load was increased from 2 to 12 mg./g. The results (Table III) show that as the polar lipid load was increased, the quality of the resolution decreased and the yield of neutral oil recovered from the column was reduced. Similar results were obtained with *gamma* alumina.

Investigations Using Weaker Aluminas. Because the polar lipid load in the above experiments had been modest, weaker aluminas were tried as a means of increasing the yield and, with less strong solvents, of improving the resolution. Both *alpha* alumina monohydrate and *gamma* alumina were reduced to Grade II status and tested with 25-mg. loads of polar lipid. Solutions of diethyl ether in hexane were used to elute the lipid from these columns of weaker alumina. Ten column-volumes of 6% ether in hexane were followed by 10 at 10% ether in hexane and 10 at 50% ether in hexane. Finally 5 column-volumes of pure diethyl ether were used.

Tests with Grade I alumina showed that this solvent system moved only 18 to 20% of the neutral lipid. With Grade II aluminas the yield of neutral lipid rose to 60 to 80% recovery but never reached 100% recovery. Weaker aluminas than Grade II were not tried because, even at loads of 2.5×10^{-4} g. of lipid phosphorus per g. of adsorbent, the eluted neutral lipid was badly contaminated with both phosphorus and nitrogen. When the load was reduced to 5×10^{-5} g. of lipid phosphorus per g. of alumina Grade II, phosphorus-free neutral lipid was obtained but in only 81% yield.

TABLE III

Effect of Polar Lipid Concentration on Resolution
(Alcoa alumina, 2.5% methanol in ether,
10 column volumes)

Experiment No.	Load (mg./g.)			% recovery of neutral oil	Phos. lipid in neutral oil
	Neutral	Polar	Total		
					%
36	22.8	2.26	25.1	90.6	0
37	19.0	6.51	25.5	84.1	0
32	12.9	12.9	25.8	82.6	3

Reinvestigation of Grade I Aluminas. Attention was therefore refocussed on Grade I aluminas. The activation process was investigated, and weight loss with time of heating at 400°C. was measured by using Merck, Alcoa, and Woelm alumina. As expected, the *alpha* aluminas lost weight continuously during the 24-hr. heating period.

Heating *alpha* alumina monohydrate for 2 hrs. at 400°C. resulted in an average weight loss of 4 to 5% and a Grade I alumina. As attainment of a true equilibrium weight at 400°C. within finite time seemed unlikely, the above conditions were adopted for the *alpha* aluminas. Heating at 500°C. did not improve the activity, possibly because of the stimulation of crystal growth (6).

The *gamma* alumina was found to increase in weight after being heated for 2 hrs. and 4 hrs. at 400°C. Only after 8 hrs. at 400°C. did the sample return to its original weight. After 24 hrs. at 400°C. the Woelm alumina showed a 0.2% weight loss. It was therefore decided to use the Grade I *gamma* alumina directly from its sealed metal container.

Various solvent systems were tried with the different Grade I aluminas: chloroform followed by 50% methanol in chloroform (C:M); diethyl ether followed by C:M and pure methanol; and 2.5% methanol in diethyl ether. At a constant load of polar lipid of 25 mg./g. of adsorbent, inconsistent results were obtained. It was therefore decided to try to subdivide the broad class of Grade I aluminas with regard to adsorbent activity and to consider the effects on resolution of the various components of the polar lipid charge.

Subdivision of the aluminas was tried first. Using the procedure described in Materials and Methods, four samples of Grade I aluminas were examined, with the following results expressed as the mean of two determinations.

	Alcoa A	Alcoa B	Merck	Woelm
Rate of band movement (mm./ml.)	0.80	0.43	0.29	0.17

Alcoa B was selected to represent the *alpha* aluminas on the basis of reproducibility of results and the Woelm material because of its high adsorptive power.

These two aluminas were then tested with mixtures in which the neutral lipid, free fatty acid, and phospholipid contents were varied systematically. The columns proved to be sensitive to both lipid phosphorus load and load of free fatty acid. A lipid phosphorus load of 4×10^{-4} g./g. could be accompanied safely by an oleic acid load of 16×10^{-4} g./g. However, when the lipid phosphorus load was as low as 1×10^{-4} g./g., a free fatty acid load of 32×10^{-4} g./g. could be tolerated.

Discussion

Although this investigation of the suitability of Grade II aluminas for separating mixtures of polar and less polar lipids at high column loading could not be considered complete, it would seem that high recovery of neutral oil was always accompanied by high contamination with phosphorus and nitrogen. Even at low polar lipid loads, 5×10^{-5} g. lipid phosphorus per g. alumina, the yield of neutral, phosphorus-free lipid was only 80 % of theory. This suggests that the mutual solubility of phospholipids and glyceride oils is stronger than the separating power of a Grade II alumina.

If the slope of the plot of mm. of band displacement as a function of volume of eluent describes adsorptive power, then apparent inconsistencies in results obtained with Grade I aluminas can possibly be explained on the basis of this more refined assessment of Grade I properties. The experiments with lipid mixtures however suggest that subdivision of polar lipids is also necessary for a true assessment of column performance. Separation of uncontaminated glycerides proved to be increasingly less satisfactory as the free fatty acid content of the polar material increased. The controlling factor however, in recovering neutral from polar lipids, would seem to be the load of lipid phosphorus on the column.

Reference to the work of Linteris and Handschumaker, whose general recommendations on column loading seem to have been followed by subsequent investigators, shows that although the total load of low quality oil applied to the column is just 11% of the load of good crude oil, the polar lipid load is 42% greater. Of this however the phospholipid load comprises only 2.1%. In the light of the results of this investigation the conditions suggested by Linteris and Handschumaker would seem both safe and conservative.

Acknowledgments

The authors wish to thank S.A. Forman for providing and interpreting the x-ray diffraction patterns of the alumina used in this investigation. A gift of alkali-refined and bleached safflower oil by the Pacific Vegetable Oil Corporation, Los Angeles, Calif., is gratefully acknowledged as are gifts of linseed oil from Toronto Elevators Ltd., Toronto, Ontario.

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[Received October 18, 1960]

Polymerizable Derivatives of Long-Chain Fatty Alcohols. III. Copolymers of Some Alkyl Acrylates and Acrylonitrile[1]

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Coplymers of acrylonitrile containing 5 to 28 mole % (11 to 70 weight %) of three alkyl acrylates (butyl, octyl, and octadecyl) were prepared in t-butanol solution. The copolymers were capable of being molded at about 300–400°F. under a pressure of about 2,000 p.s.i. The magnitude of both the tensile strength and the characteristic low temperature of the copolymers varied inversely with the weight percentage of the acrylate present without regard to its alkyl type. The copolymers were oriented with an accompanying three-fold increase in the tensile strength. Good resistance to several selected reagents was shown by all the copolymers.

IT HAS BEEN shown previously that monomers whose homopolymers crystallize provide copolymers with long-chain vinyl monomers (5,10,11,13) which are flexible through a fairly wide temperature range. To some degree these products have approximately the properties of plasticized polymer compositions and have been referred to as "internally plasticized." In general, the deficiencies of the internally plasticized copolymers (particularly of the vinyl chloride copolymers) have been a shorter useful thermal range, greater cold flow, and lower resilience than externally-plasticized polymers. It was reasoned (13) that in the copolymers the distribution of the "plasticizing" comonomer was necessarily random whereas, in the externally-plasticized composition, the plasticizer solvated principally the amorphous regions. The greater retention of crystallinity in the compositions, it was believed, would account for their better properties. A consequence of this reasoning was that modification (through copolymerization) of highly crystalline polymers (such as polyvinylidene chloride) should give products with sufficient retention of crystallinity to show improved properties. It was found that copolymers of vinylidene chloride and octadecyl acrylate (5) did indeed have high resilience. Furthermore it was shown that the long-chain alkyl acrylates were more efficient, both on a molar and on a weight basis, than the short-chain alkyl monomers in lowering the characteristic low temperatures of the copolymers. It was concluded therefore that both a monomer, the homopolymer of which exhibits backbone crystallinity, and a long-chain "plasticizing" monomer were desirable for internal plasticization. Crystallinity, it was believed, was important because the crystallites provided temporary cross-links which would dissolve under stress.

It became of interest to determine whether homopolymers possessing temporary cross-links arising

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[1] This paper was presented, in part, at the fall meeting, American Oil Chemists' Society, New York, October 1960.

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