

The preparative isolation of lecithin

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Summary Lecithin can be prepared on a relatively large scale, free of colored impurities, by a simple two-column procedure. Commercial crude egg lecithin is partially purified by a single-step passage through an alumina column. It is then purified by a two-step passage through a prepacked, commercial silica gel column. The lecithin is prepared in solvent-free form for weighing by lyophilization from cyclohexane. Toxic solvents (chloroform and methanol) are avoided by the use of ethanol, isopropanol, hexane, and water. The elutions are easily monitored by a flow cell in an ordinary spectrophotometer set at 215 nm. Study of the column parameters has made it possible to use heavy loads with a relatively small column and minimal solvent.

Supplementary key words lyophilization from cyclohexane · optical column monitoring

The first useful isolation method for lecithin called for the use of columns of alumina (1), a material which still seems to be the preferred adsorbent. Alumina separations, unfortunately, tend to yield yellow lecithin preparations and unknown materials from the alumina itself. Wells and Hanahan (2) stated that fines are eluted when large lecithin samples are applied to the alumina packing, and that this cannot be prevented by a preliminary washing of the column. The fines are probably caused by the softness of alumina particles, which are readily abraded in handling, and lecithin in high concentrations may act as a peptizing (suspending) agent. Some investigators have used basic alumina and there is the possibility that some lecithin is degraded during long contact in a slow-flowing column. It is therefore important to operate the column rapidly.

Most investigators have used chloroform and methanol in their procedures, but contemporary safety standards call for the avoidance of chloroform, which is believed to be a carcinogen (3). Methanol, as is well known to the lipid researcher, has its own toxic qualities. Geurts van Kessel et al. (4) recently showed that a high-performance analytical column of silica gel could produce a narrow lecithin peak when eluted with hexane-isopropanol-water, the elution being monitored at 206 nm. However their lecithin co-eluted with sphingomyelin.

Marsh and Holzbach (5) introduced the idea of starting with crude commercial egg lecithin instead of eggs. While highly purified commercial egg lecithin is quite expensive, the lower grade material (about 40% pure) is quite inexpensive. Their procedure involves two alumina columns, both of which are run rather slowly and which yield a material that contains some yellow impurity. By collecting fractions, one can obtain half the yield in colorless form and rework the other half.¹

Marsh and Holzbach (5) applied their total crude lecithin directly to the first alumina column, but we found that our lecithin did not dissolve completely in the small volume of ethanol recommended. The column clogged and could not be operated at a convenient speed. We turned to a larger volume of ethanol and removed the remaining insoluble material by centrifugation. Further improvement in the column flow rate was obtained by removing some of the fine particles of alumina.

To obtain a high level of final purification, we turned to a commercially packed preparative column of silica gel. This could be operated at a high speed, yet could be heavily loaded because most of the impurities were sharply separated. We were thus able to obtain colorless lecithin in good yield. By monitoring the elution with a simple flow cell in a spectrophotometer, we were able to avoid the handling involved in collecting column fractions.

Materials and Equipment

Crude egg lecithin (Cat. #102146) and Woelm basic alumina, activity I, were obtained from ICN Pharmaceuticals, Cleveland, OH. A prepacked silica gel column (Lobar, size B, 2.5 × 31 cm) was obtained from EM Laboratories, Elmsford, NY. The Lobar column was operated with a chromatographic pump (Model RPSY, Fluid Metering, Inc., Oyster Bay, NY) and pulsations in flow were reduced with an FMI pulse dampener. The operating pressure was about 60 psi. Hexane and isopropanol were ACS reagents that were redistilled in this laboratory with a simple still. Cyclohexane was redistilled commercially (Burdick & Jackson Laboratories, Muskegon, MI).

A guard column was connected between the pulse dampener and the Lobar column to trap highly polar impurities. This was a short column of adjustable height, filled with 2 g of silica gel 60,230/400 mesh, EM Laboratories. Just above the guard column was a simple tee valve for adding the dissolved sample with a syringe.

The column effluent was passed through a flow cell

Abbreviation: TLC, thin-layer chromatography.

¹ Holzbach, R. T., and M. Marsh, personal communication.

with quartz windows, with path length of 2.5 mm, mounted in a spectrophotometer set at 215 nm (Beckman Model 24 with recorder set at 2 AFS).

Thin-layer chromatography was carried out on silica gel plates with chloroform–methanol–water 60:35:8 (we have not yet discarded this use of chloroform and methanol). The spots were examined with a phospholipid spray (6) followed by a charring spray (7).

Procedure

A 10 g sample of crude lecithin was shaken for an hour in 40 ml of absolute ethanol. Meantime the alumina column was prepared by gently mixing 100 g of adsorbent with 400 ml of absolute ethanol. When the mixture cooled it was poured into a 2.2 × 92 cm column without letting any fluid run out the bottom. The cloudy supernatant liquid was promptly suctioned off to remove much of the fines. Fresh ethanol (about 100 ml) was passed through the column to rinse out additional fines.

The lecithin mixture was centrifuged 20 min at low speed and the clear supernatant solution was allowed to pass through the column, followed by 250 ml of absolute ethanol. A slight pressure of helium produced a flow rate of about 6 ml/min. All of the effluent was collected, evaporated under vacuum to a small volume with additions of cyclohexane to displace the ethanol, and then lyophilized. The yellow preparation weighed about 5.7 g and TLC examination showed (besides the major spot for lecithin) the presence of three fast-moving components, a small amount of sphingomyelin, and a trace of a slower phospholipid, possibly lysolecithin. The alumina column removed a group of lipids that migrated between the fast-moving and lecithin spots, as well as a brown material.

The Lobar and guard columns were equilibrated with some hexane–isopropanol–water 60:80:12 v/v/v and 2.5 g of the lecithin from the alumina column dissolved in 2.5 ml of the same solvent was slowly injected into the column system. An additional 100 ml of the same solvent was pumped in, followed by 1400 ml of hexane–isopropanol–water 60:80:15. The yellow impurity eluted promptly, followed by a small peak for some other material, then by the lecithin, which eluted as a rather flat, long peak. The lecithin eluted between approximately 300 and 1300 ml and was collected in a single flask for subsequent lyophilization as above. The column was reequilibrated by pumping in 100–200 ml of the 60:80:12 mixture and then used for another purification cycle. The lecithin was stored in hexane–isopropanol 80:20 under nitrogen at –20°C.

Evaporation of the hexane–isopropanol–water mixture was fast at first, but slow when the major part of the hexane was removed; a water bath set at 48°C (higher than usual for chloroform–methanol) has to be used.

The yield of lecithin from the second column was 1.75–1.87 g (70–75%); since the yield from the first step was 57%, the overall yield from the commercial lecithin was 40–43%. Examination by TLC showed that there was no removal of sphingomyelin, which persisted as a very minor component comprised of short chain and long chain sphingomyelins. It was also possible to detect a very small amount of phospholipid that appeared to be lysolecithin.

Virtually all the sphingomyelin and all of the lysolecithin could be removed by using a lighter load: 0.5 g of the partially purified lecithin. By sacrificing the very end of the lecithin peak, we removed all detectable sphingomyelin. No difference in sphingomyelin content was noticeable when the load was between 1 and 2.5 g.

After the last chromatographic run of the day, the water-containing solvent in the Lobar column was replaced by pumping 150 ml of hexane–isopropanol 60:80 to help reduce irreversible deactivation of the silica gel. The guard column was repacked with fresh silica gel after every three or four runs.

Discussion

The brochure from EM Laboratories describing the Lobar column size B suggests 1.5 g of lipid as the maximum that can be applied, but such an estimate must consider the weight of the early-eluting lipid (about 0.75 g in this case). While the heavy load we applied did not allow removal of sphingomyelin, it is fortunate that the starting material was already very low in sphingomyelin content. With the above procedure, one can prepare about 5.4 g of high quality lecithin in 2 days.

Of course it is not necessary to monitor the elution with a spectrophotometer if fractions are collected. It would not be necessary to collect small fractions except in the region of 200 to 400 ml. An interesting aspect of the lecithin peak shape is that it was rather flat with loads above 0.5 g and the height of the peak was not affected by the load size, only the length of the peak. If a cuvette with longer path length is to be used, it will be necessary to use a longer wavelength to keep the recorder on scale.

We used to lyophilize lipids from benzene but now use the equally effective (and more rapidly evaporating) cyclohexane, which is presumably much less toxic.

While the analytical separation of lecithin by high-performance liquid chromatography, monitored at

206 nm (4), requires highly purified hexane and isopropanol, the preparative procedure described in this paper can be run with ordinary reagent grade solvents after a simple distillation. Jungalwala, Evans, and McCluer (8) have achieved a good separation of lecithin and sphingomyelin by low-wavelength, high-performance silica gel chromatography using acetonitrile-methanol-water and the method could probably be adapted to preparative scale work, but acetonitrile is rather toxic and extra precautions would be needed. ■■

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