Factors Influencing Phospholipid Adsorption on Silica From Soybean Oil Miscella

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Phospholipids can be removed from crude soybean oil miscellas by adsorption on silica. The adsorption is not complete even though the silica has not been saturated. Micelle formation and calcium salts of phospholipids were ruled out as causes for the incomplete adsorption pattern, and competition for adsorption sites by triglyceride was judged to be the likely cause.

Crude soybean oil can be degummed (phospholipids removed) by treating the oil (1,2) or hexane miscella (3) with silica. Studies on the miscella degumming showed that adsorption of phospholipids on silica is not complete and follows a Freundlich isotherm. Thus, as concentration of phospholipids in the miscella increases, the amount adsorbed per gram of silica increases (3). This pattern of adsorption is normally the result of a reversible equilibrium between the adsorbed and nonadsorbed compound. With phospholipid adsorption, the process is irreversible (3). The expected pattern for irreversible adsorption would be complete adsorption of the phospholipid until the silica becomes saturated.

Sen Gupta (4) investigated phospholipid adsorption on silica in chromatography experiments and concluded that lack of adsorption is due to micelle formation. When phospholipid preparations are separated by column chromatography on silica, nonpolar solvents such as petroleum ether elute the phospholipids first, and more polar solvents such as petroleum ether-diethyl ether mixtures are required to elute triglycerides. These separations were done with a completely activated silica. When crude soybean oil is separated by the same column chromatography procedure, about 2/3 of the phospholipid is eluted from the column and about 1/3 is adsorbed (4).

The studies reported in this paper are directed at resolving the inconsistency of an irreversible adsorption of phospholipid from crude soybean oil on silica, but with only part of the total phospholipid being adsorbed, and the partial adsorption of phospholipid not saturating the silica adsorbent. We refer to this as the incomplete adsorption pattern for phospholipids.

MATERIALS AND METHODS

Crude soybean oils and lecithin (70% phospholipid plus 30% soybean oil) were obtained from Riceland Foods (Stuttgart, Arkansas) and Farmland Industries Cooperative (Van Buren, Arkansas). Oils and lecithin were used in miscellas of varying concentration (w/v oil in solvent) with 2% isopropyl alcohol (IPA) in hexane as solvent. Silica (100-200 mesh from BioRad Laboratories, Richmond, California) was used with 5% moisture added. The batch adsorption technique (1 g silica in 100 ml miscella) has been described previously (3). Phosphorus was analyzed by phosphomolybdate colorimetric reaction after wet ashing (5).

Column adsorption was done with a fixed bed of silica in a 2.5-cm glass column with no headspace. The miscella was pumped from the bottom of the silica bed through Tygon tubing. The miscella fractions were sampled both before and after adsorption and analyzed for total weight (triglycerides) and phosphorus. Desorption was achieved by pumping appropriate solvents over the column and collecting the eluant.

RESULTS AND DISCUSSION

The pattern (Freundlich isotherm) for phospholipid adsorption on silica was achieved after 15 min exposure of the miscella to silica (3). Perhaps 15 min was insufficient time for complete adsorption to occur, and so we explored the time needed for phospholipid adsorption. Figure 1 shows that adsorption was essentially complete after 15 min in comparison to 24 hr for three different concentrations of crude soybean oil miscellas. In these experiments one g of silica was used, and the increased adsorption of phospholipid for the highest concentration of soybean oil shows that the silica was not saturated at the two lower concentrations. Yet phospholipid was not completely adsorbed at the lower concentrations. The data also show that adsorption was not instantaneous but increased with time over the 15 min period. Because phospholipid remainded in solution after 24 hr, we concluded that time of adsorption was not a factor in the incomplete adsorption pattern for phospholipids.

Calcium salts of phospholipids are difficult to hydrate (6), and such salts may not be adsorbed by silica. We tested this idea by adding calcium chloride and calcium hydroxide (in separate experiments) to crude soybean oil

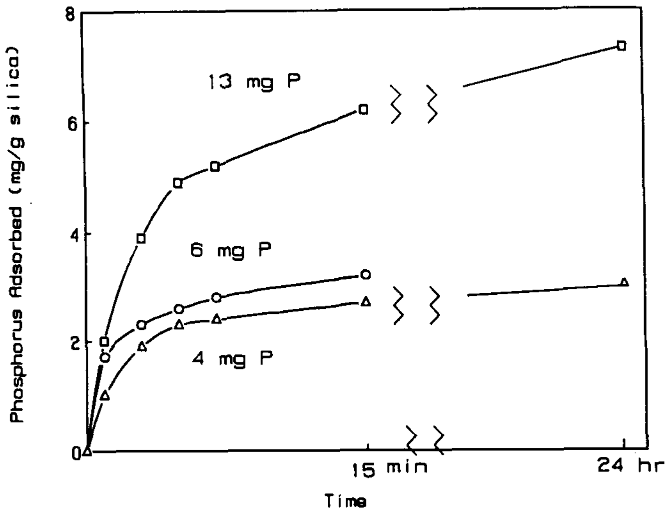

FIG. 1. Change in phosphorus adsorbed with time. One g of silica mixed with 100 ml miscella. 4 mg P = 4.6% soybean oil in hexane/IPA; 6 mg P = 6.9% soybean oil in hexane/IPA; 13 mg P = 15% soybean oil in hexane/IPA.

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miscellas. The added calcium compounds were removed by filtration, and the miscellas were treated with silica and phospholipid adsorption was measured. The results in Table 1 show that treatment with calcium chloride did decrease the amount of adsorbed phospholipid, whereas calcium hydroxide did not. While this was a positive result that could possibly explain the incomplete adsorption pattern for phospholipids, upon analysis of crude soybean oil for calcium, we found insufficient calcium to account for the incomplete adsorption.

Phospholipids are known to form micelles in certain solvents (4), and Sen Gupta has used this information to explain lack of adsorption of phospholipids during chromatography on silica columns. Micelle formation could be an explanation for incomplete adsorption. Sen Gupta reported no micelle formation in chloroform solutions, and we found (3) that adsorption isotherms were identical in hexane and in chloroform. Thus, micelle formation is unlikely as a cause for incomplete adsorption, but we have no direct evidence to support or reject the micelle hypothesis.

Proctor and Snyder (7,8) showed that triglycerides in crude soybean oil compete with lutein for adsorptive sites on silica when a hexane miscella of crude soybean oil is exposed to silica. To investigate the possible competition between phospholipids and triglycerides we determined the adsorptive isotherm for soybean lecithin in which the phospholipid to triglyceride ratio would increase from about 0.03 to about 0.9. Figure 2 shows the results along with the usual adsorptive isotherm for crude soybean oil. By increasing the ratio of phospholipid to triglyceride, the isotherm pattern showed increased adsorption of phospholipid at low concentrations of lecithin. One interpretation of these results was that as the phospholipid to triglyceride ratio increased, there was less triglyceride to compete with phospholipids for sites on the silica. Thus, more phospholipid was adsorbed. As lecithin concentration increased in the miscella, more triglyceride was present and the adsorption pattern became essentially the same as for crude soybean oil.

We attempted to enhance the adsorption of phospholipid by passing miscellas over a 10-g column of silica. Table 2 shows results in which miscellas varied in phospholipid to triglyceride ratio from 0.9 for lecithin to 0.03 for crude soybean oil. By using 10 g of adsorbent rather than one g, much more phospholipid was adsorbed,

TABLE 1

Changes in Phospholipid Phosphorus Adsorption on Silica as a Result of Calcium Exposure

Treatment	Original P mg/100 ml	Residual P mg/100 ml	Adsorbed P mg/g
No added Ca	15.23	7.40	7.83
	17.98	12.23	5.75
1 g $CaCl_2$[a]	15.63	14.48	1.15
	19.10	17.22	1.88
	17.98	16.60	1.38
1 g $Ca(OH)_2$[a]	19.1	12.93	6.18
	17.98	13.43	4.55

[a] Ca removed by filtration before silica adsorption.

TABLE 2

Effect of Changing the Phospholipid/Triglyceride Ratio on Phosphorus Adsorption on a 10-g Silica Column[a]

Phospholipid/ triglyceride	Residual P mg/100 ml miscella	Adsorbed P mg/10 g silica	% adsorbed
0.90	0.775	50.9	98.5
0.59	0.75	54.9	98.6
0.40	1.05	49.6	97.9
0.13	1.40	46.5	97.1
0.03	1.77	7.1	80.1

[a] 100 ml of miscella pumped over each column.

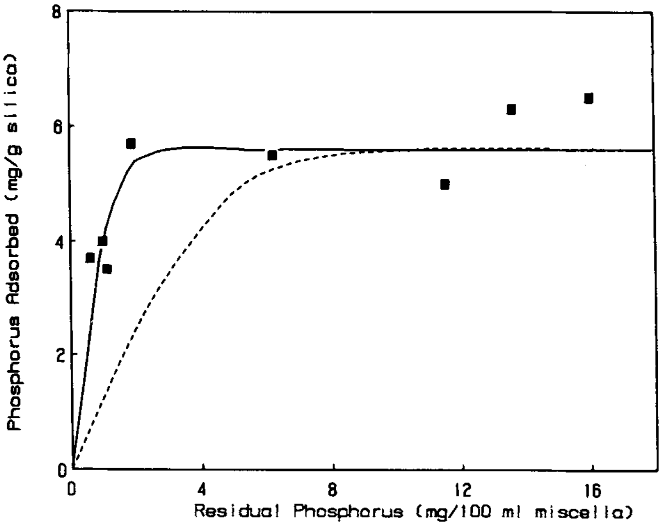

FIG. 2. Adsorption isotherms for phosphorus on silica from soybean oil miscellas. ——, soybean lecithin; – – – –, crude soybean oil.

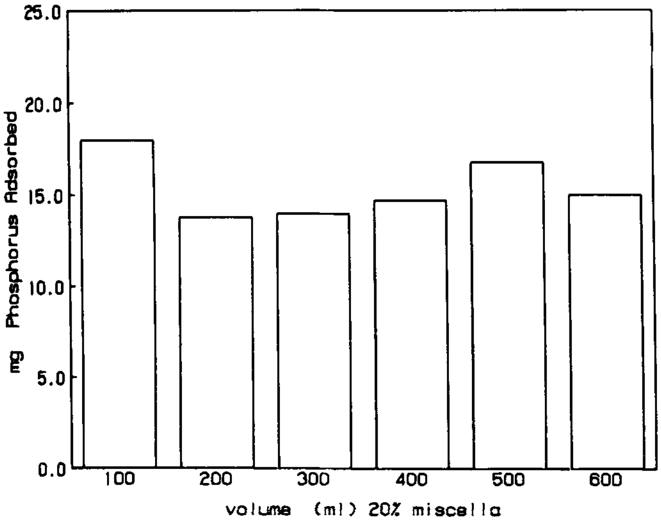

FIG. 3. Phosphorus amounts adsorbed on a 10-g silica column, showing results after 6 successive passes of 100 ml 20% soybean oil miscellas.

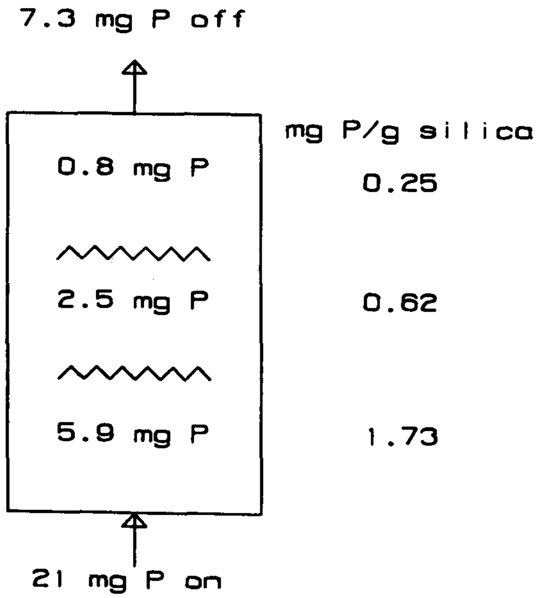

FIG. 4. Adsorption and recovery of phosphorus from a 10-g column of silica showing distribution of adsorbed phosphorus on the column. Adsorption was from 100 ml of 20% soybean oil miscella.

and except for the crude soybean oil miscella more than 97% of the original phospholipid was adsorbed.

When successive 100-ml aliquots of crude soybean oil miscellas were passed over a 10-g column of silica, the results of Figure 3 were obtained. Phospholipid continued to be adsorbed in each successive 100-ml portion of miscella in approximately the same amounts as the first 100-ml portion.

When we extruded the column packing after one 100-ml portion of miscella had passed over it, divided it into three equal sections, and eluted the adsorbed phospholipid, the results of Figure 4 were obtained. The phospholipids tended to be adsorbed at the entry to the column, and the amount adsorbed decreased in the distal portions of the column packing. Even though the silica was not saturated with phospholipid, phospholipid came through the column without being adsorbed. The total phospholipid coming off the column does not equal the amount put on because of incomplete desorption from the silica.

The best explanation we have for these results is that phospholipid and triglyceride were competing for available adsorption sites on the silica. Because there was much more triglyceride present than phospholipid, some of the phospholipid was excluded from adsorption. There did not appear to be anything different between the adsorbed and the nonadsorbed phospholipids based on thin layer chromatography (TLC) (data not shown).

To be useful as a refining process, the adsorbed phospholipid would need to be eluted from the silica and the silica reused. We found that appreciable phospholipid could be desorbed with hexane:IPA:water (6:8:1.5). After desorption, the silica could be reused directly for another adsorption cycle. We have not determined the number of cycles of adsorption-desorption that were possible with this two-solvent system.

The only process in current refining of crude soybean oil that makes use of adsorption is the bleaching step. The enhanced bleaching that occurs during filtration of the oil to remove bleaching earth is referred to as "press bleaching." "Press bleaching" is caused by a relative increase in concentration of the adsorbent compared to oil (9). This is the same effect that we noted in this study: namely, an increased adsorption due to increased concentration of the adsorbent compared to oil.

The study of selective adsorption of unwanted components of crude soybean oil could lead to increased efficiency of refining methods and to better understanding of bleaching procedures.

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