Diffuse-Reflectance Fourier-Transform Infrared Spectroscopy of Vegetable Oil Triglyceride Adsorption on Silicic Acid

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The adsorption of triglyceride by silicic acid from hexane miscellas was observed with diffuse-reflectance Fourier-transform infrared spectroscopy. Triglyceride was adsorbed by hydrogen bonding to silanol groups through the ester carbonyl group. Addition of isopropanol (IPA) to the tri-glyceride–hexane solution prior to adsorption resulted in unchanged triglyceride adsorption on silicic acid despite IPA's ability to adsorb on silicic acid and hydrogen-bond with triglyceride. Washing the triglyceride, adsorbed on silicic acid, with hexane containing IPA resulted in partial desorption of the triglyceride and a small amount of IPA being adsorbed. Triglyceride desorption into fresh hexane–IPA is due to the establishment of a new equilibrium of lipid and IPA between hexane and silicic acid. The relative strengths of all the possible pairwise interactions among triglyceride, IPA and silicic acid are revealed by the relative amounts of adsorption under various conditions.

KEY WORDS: Adsorption, Fourier-transform infrared spectroscopy, isopropanol, silicic acid, triglyceride.

Crude soy oil is industrially extracted from soy flakes with hexane. The hexane is evaporated to produce a crude triglyceride oil that contains fatty acids, phospholipids and carotenoids, which must be removed to produce a bland, stable oil. Bleaching is an important processing step after fatty acid and phospholipid removal. Carotenoids, residual fatty acids and phospholipids are adsorbed by bleaching clays at 100°C under reduced pressure during bleaching (1).

Adsorption studies with simple model systems of silicic acid and crude soy oil diluted with hexane, i.e., miscella, have been useful in studying lipid/adsorbent interactions. These studies have involved measuring the concentrations of oil components before and after adsorption and plotting the amount adsorbed per gram vs. the residual concentration after adsorption. Such studies have shown that the binding of phospholipid (2), carotenoids (3) and triglyceride (3) from a miscella follows a Freundlich isotherm.

Addition of 1% isopropanol (IPA) to the miscella promotes adsorption of phospholipid (2), which was proposed to occur by removal of triglyceride from the adsorption sites. IPA inhibited soy oil carotenoid pigment adsorption by silicic acid (3). This was explained by competition between pigments and IPA for silanol binding sites. The basis of the competition was suggested to be the polarity of the miscella constituents, with more-polar constituents being better competitors. However, a comparison of the effect of C_3 compounds to inhibit pigment adsorption showed that alcohol > acid > ketone > ester, indicating that ability to hydrogen-bond rather than polarity was the basis for competitive adsorption (4). There is little difference between the ability of members of a homologous series of alcohols (C_1–C_{10}) to limit pigment adsorption (4). IPA treatment of silicic acid, that had been exposed to crude soy oil miscellas, caused

desorption of carotenoids and restored much of the ability of the silica to adsorb pigment (5).

Proctor and Snyder (3) demonstrated that triglycerides are the major lipid adsorbed, probably because they are present in the largest concentrations. Chapman and Pfannkoch (6) later showed that triglyceride is an important competitive inhibitor of pigment adsorption in miscellas, despite the fact that added ester groups were the least effective functional group to competitively inhibit soy oil carotenoid adsorption (4). Fourier-transform infrared spectroscopy (FT-IR) has been useful in showing the mode of adsorption of oleic acid to silicic acid and how adsorption is affected by solvent interactions (7). Diffuse-reflectance FT-IR showed the formation of complexes of oleic acid and silicic acid by hydrogen bonding. IPA (0.05 M) reduced oleic acid adsorption from hexane, both because of competitive adsorption and the probable formation of IPA/fatty acid complexes in solution, which lowered the concentration of fatty acids in solution. However, fatty acids are a minor component of high-quality oil, which is almost totally triglyceride. Such studies may help in understanding triglyceride adsorption.

The objective of this investigation was to use diffuse-reflectance FT-IR to examine the nature of triglyceride binding by silicic acid. The FT-IR spectrum of silicic acid was obtained when triglyceride was adsorbed from hexane solution. The interaction of IPA with the triglyceride and silica in modifying the adsorption was explored. The desorption of triglyceride with IPA was also examined.

MATERIALS AND METHODS

Lipids, solvents and adsorbents. Commercially refined triglyceride soy oil, hexane [high-performance liquid chromatography (HPLC) grade], IPA (HPLC-grade) and silicic acid (Bio-Sil, A., 100-200 mesh; Bio-Rad Laboratories, Richmond, CA) were used.

Triglyceride soy oil spectra. The infrared spectrum of the triglyceride oil was obtained by direct transmission of the infrared beam through a film of pure triglyceride oil on a salt window.

Silicic acid/solvent interaction. Control experiments were conducted to examine the nature of the adsorbent and the adsorption of solvents in a lipid free system. The FT-IR spectrum of silicic acid was observed with a Nicolet Model 205 FT-IR instrument (Nicolet, Madison, WI) with a diffuse-reflectance unit 0030-002 (Barnes Analytical, Stamford, CT) having a resolution of 4 cm^{-1}. Spectra were obtained by co-adding 100 interferograms before transformation of the spectra. This was done to obtain a baseline spectrum of the adsorbent, which was subtracted from subsequent spectra following lipid adsorption. The effect of exposing the adsorbent to hexane was examined by adding 0.5 g of silica to 100 mL hexane in a sealed vessel and mixing for 15 min (3). The silicic acid was recovered and dried under air flow in a hood for 15 min prior to FT-IR analysis as described above. This was repeated with 100 mL of 0.05 M IPA in hexane. The infrared spectrum showed that no hexane remained on the

silicic acid after 15 min drying. We have published the spectra of the fresh silicic acid and of the IPA adsorbed on silicic acid previously (7).

Triglyceride adsorption. A 100-mL vol of 0.05 M triglyceride oil in hexane was stirred with 0.5 g silicic acid for 15 min before recovering and drying. FT-IR analysis was then performed. The procedure was repeated with 100 mL of 0.05 M triglyceride oil and 0.05 M IPA in hexane.

Triglyceride desorption. Silicic acid (0.5 g) was recovered after triglyceride binding from the hexane and washed with 100 mL hexane for 15 min. The FT-IR spectrum of the lipid–silicic acid complex was obtained. The experiment was repeated with (i) 100 mL 0.05 M IPA in hexane as a desorption solvent, (ii) with 100 mL 0.05 M IPA as the adsorption system and hexane for desorption and (iii) with 100 mL 0.05 M IPA for both adsorption and desorption.

The spectral data were interpreted by examining the literature values of infrared absorption (8,9).

RESULTS AND DISCUSSION

Triglyceride oil spectrum. Figure 1 shows the infrared spectrum of pure triglyceride oil. The peak at 3009 cm^{-1} is the C-H stretch alpha to the double bond of an esterified unsaturated fatty acid. The peaks at 2926 and 2854 cm^{-1} are the asymmetric and symmetric stretching vibrations of methylene (CH$_2$) groups. Carbonyl stretch is at 1745 cm^{-1}, and methylene wagging is shown by the peak at 1462 cm^{-1}. The peaks at 1378, 1239 and 723 cm^{-1} were unidentified. All the major peaks are narrow and well-defined.

Triglyceride adsorption from hexane. Figure 2 shows the FT-IR spectra of triglyceride bound from 0.05 M triglyceride in hexane by 0.5 g silicic acid. The silicic acid spectrum has been subtracted to better observe the bound lipid. The negative peak at 3737 cm^{-1} is a result of subtracting the free silanol peak of silicic acid (Si-OH), at

this wavenumber, from silicic acid with adsorbed triglyceride in which the band is absent due to triglyceride binding. The reduction in the peak relative to that of silicic acid suggests that triglyceride is hydrogen-bonded to the silanol group (7), which shifts the free SiOH band at 3737 cm^{-1} to a broad flat band around 2800 cm^{-1}. The broadening and shifting of the carbonyl stretch (1745 cm^{-1}), relative to the free form (Fig. 1), is evidence that the carbonyl group of the triglyceride is hydrogen-bonding to the silanol groups (9). Therefore, triglyceride competes for hydrogen-bonding sites on the adsorbent by means of the carbonyl of the ester. These findings provide a rationale for the report that triglycerides affect the adsorption of other crude oil components for reasons other than viscosity (5). As well as competing for adsorption sites, the hydrocarbon chains of esterified fatty acids may sterically hinder the adsorption of other lipids.

The subtraction process produces the negative and positive peaks around 1300 cm^{-1} because triglyceride adsorption causes a shift in the large Si-O-Si stretch in this region (7).

Triglyceride/IPA adsorption from hexane. Figure 3 shows the FT-IR spectrum of silicic acid exposed to a hexane solution of 0.05 M triglyceride and equimolar IPA with the silicic acid spectrum subtracted. The negative peak at 3732 cm^{-1} shows that the adsorbent silanol group is involved in hydrogen-bonding. In the presence of IPA, the carbonyl peak at 1745 cm^{-1} and the hydrocarbon peaks at 2840 cm^{-1} and 1462 cm^{-1} are about 15% less intense than those shown in Figure 2, thereby indicating a small reduction in the amount of adsorbed triglyceride. This small reduction is believed to be real because previous work (7) indicated a reproducibility to within 5 to 10%. The exact extent of this decrease is unimportant because the notable discovery here is that, with equimolar amounts of triglyceride and IPA present, the triglyceride composes most of the adsorbed material. The presence of small amounts of adsorbed IPA was
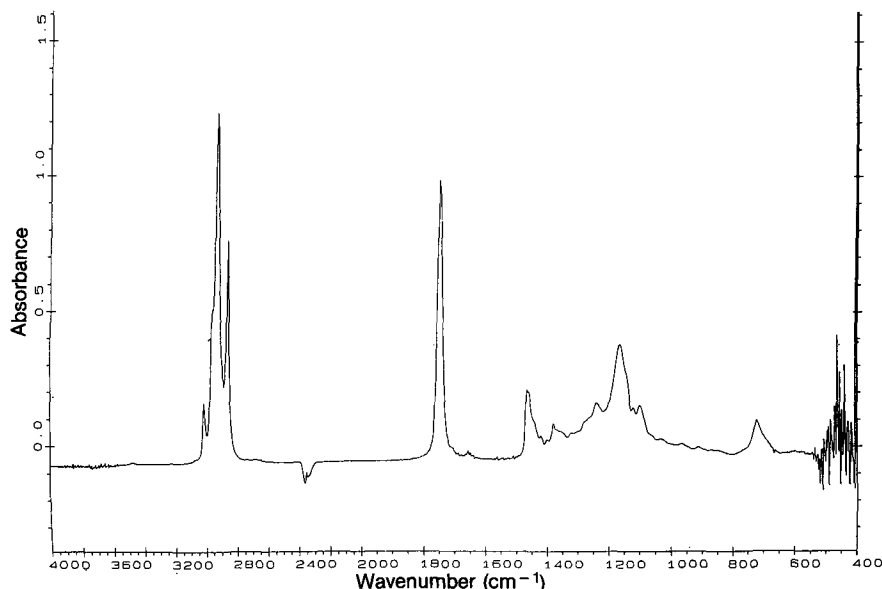

FIG. 1. The Fourier-transform infrared spectrum of commercially refined soy oil on a salt window in the absence of diffuse reflectance.
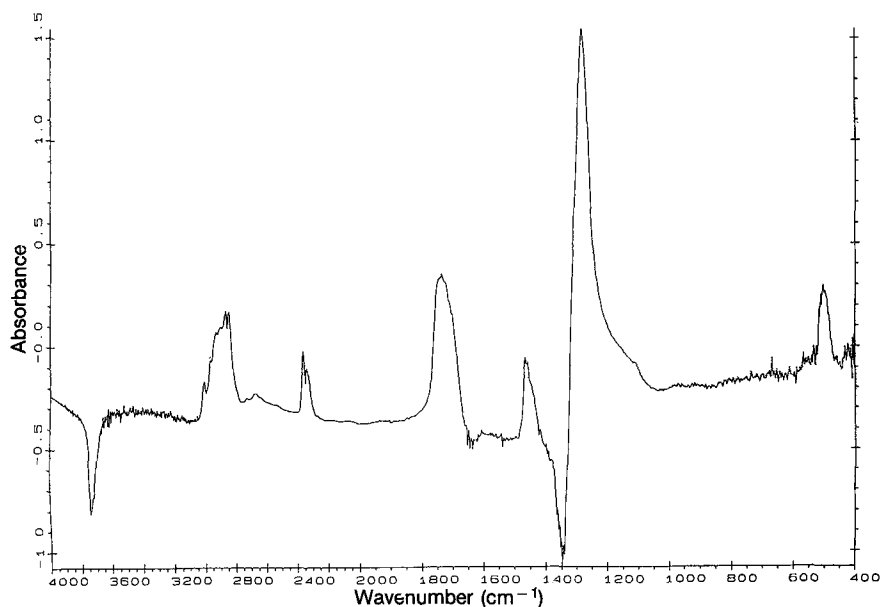
FIG. 2. The Fourier-transform infrared spectrum of triglyceride bound to silicic acid obtained by incubating 0.05 M triglyceride in 100 mL hexane with 0.5 g silicic acid for 15 min, and then air-drying the adsorbent. The spectrum of silicic acid is subtracted.
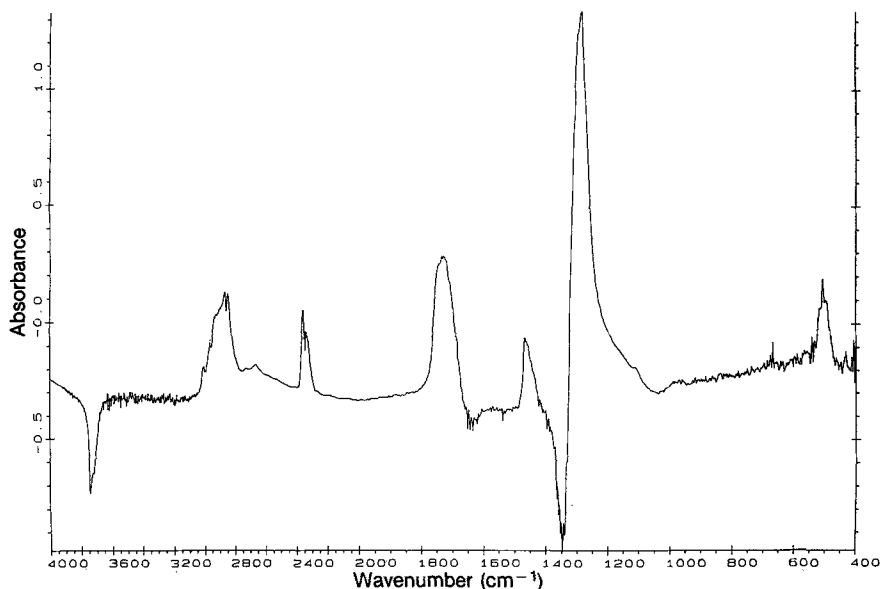

FIG. 3. The Fourier-transform infrared spectrum of triglyceride bound to silicic acid obtained by incubating 0.05 M triglyceride and 0.05 M isopropanol in 100 mL hexane with 0.5 g silicic acid for 15 min, and then air-drying the adsorbent. The spectrum of silicic acid is subtracted.

confirmed by the presence of a small band at 2975 cm^{-1} [found in IPA (7) but not in triglyceride] in a spectrum produced by subtracting Figure 2 from Figure 3.

Triglyceride desorption by hexane. Figure 4 is the FT-IR spectrum of bound triglyceride washed with 100 mL hexane. The C-H stretch, alpha to the double bonds at 3009 cm^{-1}, the CH_2 symmetrical stretch at 2854 cm^{-1} and the carbonyl stretch at 1745 cm^{-1} are all reduced relative to that of Figure 2. This shows a little decrease

in adsorbed triglyceride, which occurs when a new equilibrium is set up between the solvent and adsorbent on exposure to fresh solvent.

Triglyceride desorption by IPA/hexane. The FT-IR spectrum, obtained by incubating 0.5 g silicic acid with 0.05 M triglyceride in hexane and subsequent washing of the adsorbent with 0.05 M IPA in hexane, is shown in Figure 5. The spectrum of silicic acid has been subtracted. The intensity of the IPA peak at 2975 cm^{-1} is similar to that
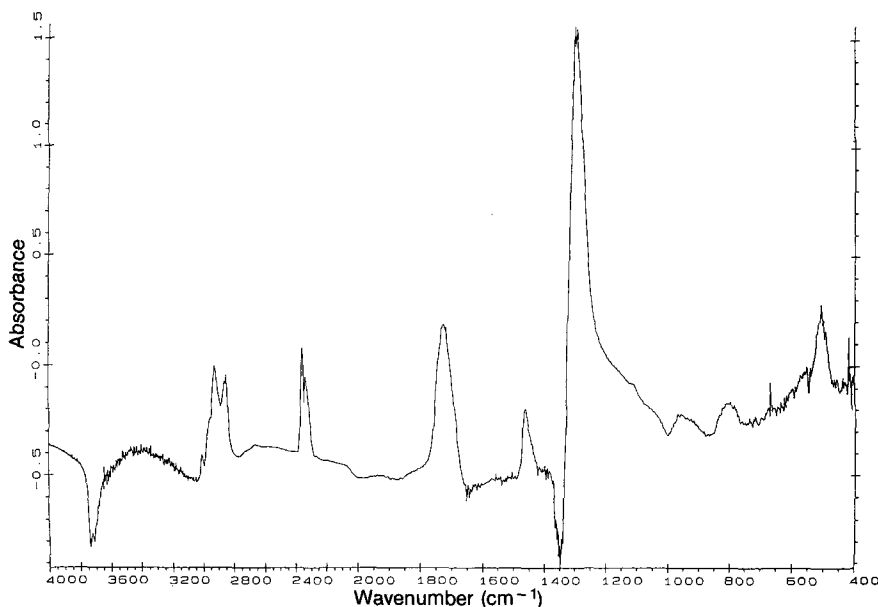
FIG. 4. The Fourier-transform infrared spectrum of triglyceride bound to silicic acid obtained by incubating 0.05 M triglyceride in 100 mL hexane with 0.5 g silicic acid for 15 min, and then air-drying the adsorbent. The adsorbent was then washed in 100 mL hexane for 15 min and air-dried. The spectrum of silicic acid is subtracted.
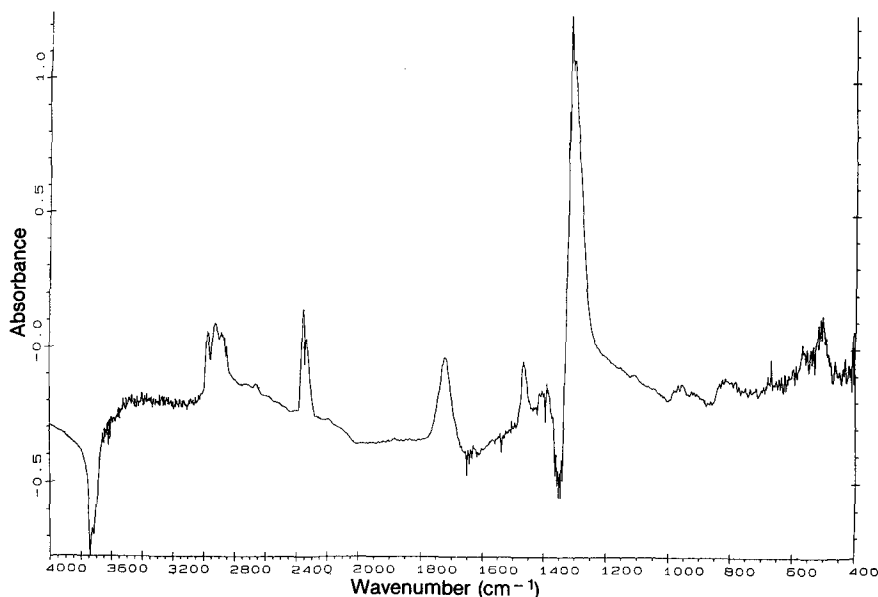

FIG. 5. The Fourier-transform infrared spectrum of triglyceride bound to silicic acid obtained by incubating 0.05 M triglyceride in 100 mL hexane with 0.5 g silicic acid for 15 min, and then air-drying the adsorbent. The adsorbent was then washed in 0.05 M isopropanol in 100 mL hexane for 15 min and air-dried. The spectrum of silicic acid is subtracted.

of the triglyceride peak at 2927 cm^{-1}. This shows that IPA has displaced part of the triglyceride from the adsorbent. Additional evidence is the small carbonyl group stretch at 1722 cm^{-1}, showing less lipid binding. The smaller CH$_3$ and CH$_2$ wagging peak at 1464 cm^{-1} indicates less hydrocarbon from IPA and triglyceride on the surface.

Triglyceride and IPA adsorption with subsequent hexane wash. The spectrum in Figure 6 was obtained by incubating 0.5 g silicic acid with 0.05 M triglyceride/0.05 equimolar IPA in hexane, and then washing the recovered adsorbent in hexane. The carbonyl peak is less intense and shifted to a lower frequency relative to those in Figures 1 and 2. This suggests that physically adsorbed lipid,
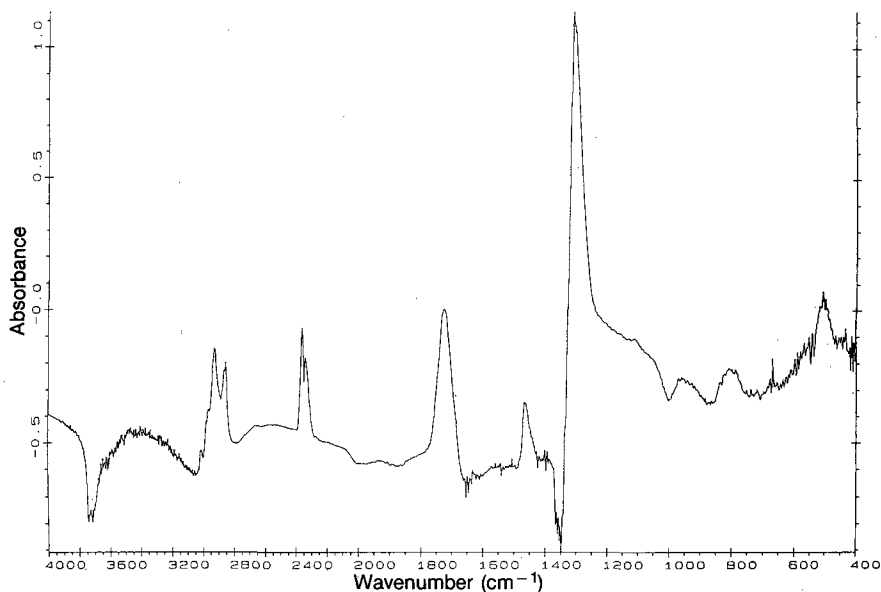
FIG. 6. The Fourier-transform infrared spectrum of triglyceride bound to silicic acid obtained by incubating 0.05 M triglyceride and 0.05 M isopropanol in 100 mL hexane with 0.5 g silicic acid for 15 min, and then air-drying the adsorbent. The adsorbent was then washed in 100 mL hexane for 15 min and air- dried. The spectrum of silicic acid is subtracted.

which would have an unshifted carbonyl peak position and is held by Van der Waals forces, has desorbed into the hexane wash.

Triglyceride and IPA adsorption with subsequent IPA/hexane wash. The spectrum in Figure 7 was obtained by exposing 0.5 g silicic acid to equimolar (0.05 M) trigly-

ceride and IPA and subsequently washing the adsorbent with a similar solvent system. The spectrum shows IPA adsorption is greater than that of triglyceride. This is shown by the peak at 2975 cm⁻¹, which indicates considerable IPA adsorption on silicic acid, with smaller triglyceride peaks at 2925 and 2856 cm⁻¹ for triglyceride
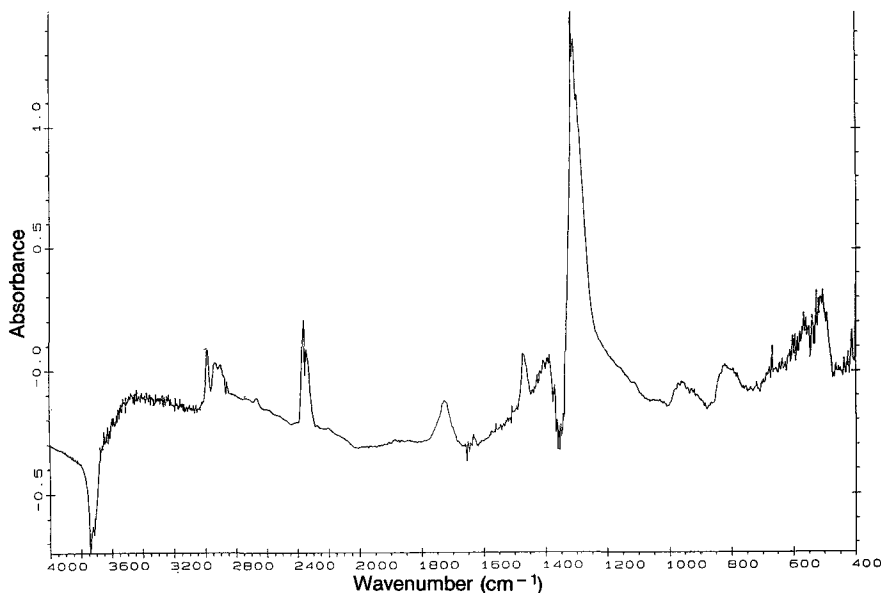

FIG. 7. The Fourier-transform infrared spectrum of triglyceride bound to silicic acid obtained by incubating 0.05 M triglyceride and 0.05 M isopropanol (IPA) in 100 mL hexane with 0.5 g silicic acid for 15 min, and then air-drying the adsorbent. The adsorbent was then washed in 0.05 M IPA in 100 mL hexane for 15 min and air-dried. The spectrum of silicic acid is subtracted.

adsorption. The carbonyl peak (1722 cm⁻¹) is also small, indicating by comparison to Figure 3 that an IPA-in-hexane wash is effective in removing lipid from silicic acid.

In summary, the data show that triglycerides hydrogen-bond to silicic acid in hexane solution. When equimolar IPA was added to the triglyceride, the triglyceride is more strongly hydrogen-bonded to the silicic acid than IPA. Hexane was able to remove some of the physically adsorbed lipid, probably by establishing a new lipid equilibria between adsorbent and fresh solvent. IPA treatment of bound triglyceride also displaces lipid into the solvent, but IPA then occupies the available adsorption sites. Thus, IPA and triglyceride in the miscella directly compete with each other for adsorption sites, but in equimolar hexane solution the triglyceride is the most abundant adsorbed species. However, when adsorbed lipid on silicic acid is washed with IPA in hexane, the lipid is displaced from the surface and replaced with IPA. Three factors contribute to the desorption, but their order of relative importance is not known at this time. First, an equilibrium between adsorbed lipid and the solution phase will result in some lipid desorbing into a fresh liquid hexane phase that does not contain lipid. Second, the IPA will compete with the lipid for hydrogen-bonding sites on the silicic acid surface. Third, the IPA will compete by hydrogen-bonding with the lipid to effectively reduce the free lipid concentration in the hexane solution.

Further work is being done to investigate lipids in solution and adsorption to better understand lipid/adsorbent relationships.

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