Diffuse Reflectance Fourier Transform Infrared Spectroscopy of Oleic Acid Adsorption on Silicic Acid

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Diffuse reflectance Fourier transform infrared spectroscopy was used to observe adsorption complexes of oleic acid and isopropanol (IPA) on silicic acid in hexane. The spectra provide definite evidence of the molecular nature of the surface interaction. In addition, the effect on oleic acid adsorption of modifying the solvent with IPA, which competes for adsorption sites and modifies the solvent polarity, was studied. Oleic acid adsorption was reduced in the presence of an equimolar IPA concentration in hexane, relative to that from hexane alone. This could be explained by a combination of competitive adsorption of IPA and IPA interacting with oleic acid in solution. IPA, in solution, and silica are probably competing for the lipid. This is additional evidence that suggests that lipid adsorption onto silicic acid is influenced by competitive adsorption. The adsorption of oleic acid and IPA, from a mixture of the two in hexane, was controlled by (i) the equilibrium between surface-bound species and molecules in solution and (ii) the polar interaction between oleic acid and IPA in solution. Thus, washing pre-bound oleic acid with hexane removed only a small amount of oleic acid, while washing with a solution of IPA in hexane removed most of the pre-bound oleic acid.

KEY WORDS: **Adsorption, Fourier transform infrared reflectance spectroscopy, isopropanol, oleic acid, silicic acid, triglyceride.**

Soy oil is extracted industrially from soy flakes with hexane, which is then evaporated to produce a crude triglyceride oil. The oil contains fatty acids, phospholipids and carotenoid pigments, which must be removed to obtain a bland, stable, light-colored product that is acceptable to consumers. Fatty acids and phospholipids are removed commercially by saponification and hydration, respectively, and then separated from the oil by centrifugation. The oil is subsequently bleached by adsorption of carotenoid pigments and the residues of previous refining steps onto alumino-silicate and silica adsorbents at 100°C under reduced pressure (1).

Adsorption studies with simple model systems of silicic acid (SA) and crude soy oil diluted with hexane (*i.e.*, miscella) have been useful in studying lipid/adsorbent interactions, which may be useful in better understanding the industrial adsorption process. 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) is according to 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 adsorption by SA (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, there is little difference between members of a homologous series of alcohols (C_1–C_{10}) in their ability to limit pigment adsorption (4). A comparison of the effects of C_3 compounds to inhibit pigment adsorption showed alcohol < acid < ketone < ester, indicating that the ability to hydrogen-bond rather than the polarity was the basis for competitive adsorption (4).

IPA treatment of SA, which has been exposed to crude soy oil miscellas, caused adsorption 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 species adsorbed, probably because they are the lipid present in largest concentrations. Chapman and Pfannkoch (5) later showed that triglyceride is an important competitive inhibitor of pigment adsorption in miscellas.

The reports outlined above studied adsorption by measuring miscella component concentration before and after adsorption, but there was no direct observation of the lipid/SA complex. In contrast, Sarier and Guler (6) and Guler and Tunc (7) used infrared spectroscopy to investigate beta-carotene (6) and chlorophyll (7) adsorption to acid-activated montmorillonite from benzene and hexane, respectively. Spectroscopy showed that beta-carotene was bound as a carbonium ion, while chlorophyll was chemisorbed as a result of interaction with Lewis and Brønsted acid sites on the surface of the clay.

The objective of this study was to use diffuse reflectance Fourier transform infrared spectroscopy (FT-IR) to examine the nature of a simple lipid binding to SA. The FT-IR spectrum of SA was obtained when oleic acid (OA) was adsorbed from hexane solution. The interaction of IPA with the lipid and silica in modifying the adsorption was explored. The desorption of lipid with IPA was also examined.

MATERIALS AND METHODS

Lipid, solvents and adsorbents. OA (Sigma Chemical Co., St. Louis, MO), hexane [high-performance liquid chromatography (HPLC) grade], IPA (HPLC-grade) and SA (Bio-Sil A., 100–200 mesh; Bio-Rad Laboratories, Richmond, CA) were used.

SA/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 SA 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}. These conditions were used throughout unless stated otherwise. This was done to obtain a baseline spectrum of the adsorbent, which was subtracted from subsequent spectra after lipid adsorption. The effect of exposing the adsorbent to hexane was examined by adding 0.5 g of silica to 100 mL of hexane in a sealed vessel and mixing for 15 min. the SA was recovered

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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 0.05M IPA in hexane.

OA adsorption. The FT-IR spectrum of oleic was obtained by direct transmission of the infrared beam through a film of pure OA on a salt window.

A volume of 100 mL of 0.05M OA in hexane was stirred with 0.5 g SA for 15 min before recovering and drying, as described earlier. FT-IR analysis was then performed. The procedure was repeated with 100 mL of 0.05M OA and 0.05M IPA in hexane.

OA desorption. Half-gram quantities of SA were recovered after OA binding from the hexane and hexane/IPA solvent systems. The FT-IR spectrum of the adsorbent was then examined after washing with 100 mL hexane for 15 min. The experiment was repeated, but washing was done with 100 mL 0.05M IPA in hexane. The experiment was then done after adsorbing and washing in 0.05M IPA.

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

RESULTS AND DISCUSSION

SA/solvent interaction. Figure 1 shows the FT-IR spectrum of SA. The literature indicates a broad band from 2800–3800 cm^{-1} as shown here for OH groups on silica gel (10). Removing all of the water and hydrogen-bonded OH groups requires evacuation at temperatures greater than 500°C. This treatment leaves a single sharp peak around 3748 cm^{-1} for nonhydrogen-bonded surface OH groups (10). This is typical behavior for silica and alumina surfaces (8). In Figure 1, the nonhydrogen-bonded surface OH groups are shown by the peak or shoulder at 3740 cm^{-1}. The broad band at 2800 to 3750 cm^{-1} is due to the OH stretch for surface OH groups and adsorbed water undergoing hydrogen bonding. The H-OH bending vibration is found at 1637 cm^{-1}. These are observed even in "dried" SA that is stored in a desiccator (data not shown) and are probably due to binding of atmospheric moisture when weighing and handling samples. For this reason, SA was not dried prior to use in the subsequent experiments. However, care was taken to replace the container lid promptly after use. Hydrocarbon peaks observed at 2860 and 2957 cm^{-1} are due to a hydrocarbon-containing material adsorbed on the SA as received. The peaks at 2354 and 667 cm^{-1} are due to atmospheric carbon dioxide and were found in almost all samples. The bands at 1989 and 1870 cm^{-1} are due to structural vibrations of SA. An intense structural siloxane peak (Si-O-Si) is found from 1250 to 1400 cm^{-1}, and structural "skeletal vibrations" at < 1300 cm^{-1}.

The spectrum of SA incubated with hexane (data not shown) was similar to that of SA in Figure 1. The impurity hydrocarbon peak at 2860 cm^{-1} was reduced a little relative to SA, suggesting complete solvent evaporation and nonadsorption of hexane.

Figure 2 is the unprocessed spectrum of IPA adsorbed to SA, and it shows that IPA in hexane does remove the 2860 cm^{-1} peak, which suggests that this peak is due to polar hydrocarbon molecules adsorbed on the SA because it is removed by a polar solvent, but only slowly by a nonpolar solvent.

Figure 3 shows the spectrum of IPA bound by SA minus the SA spectrum (Fig. 1). The negative peak at 3740

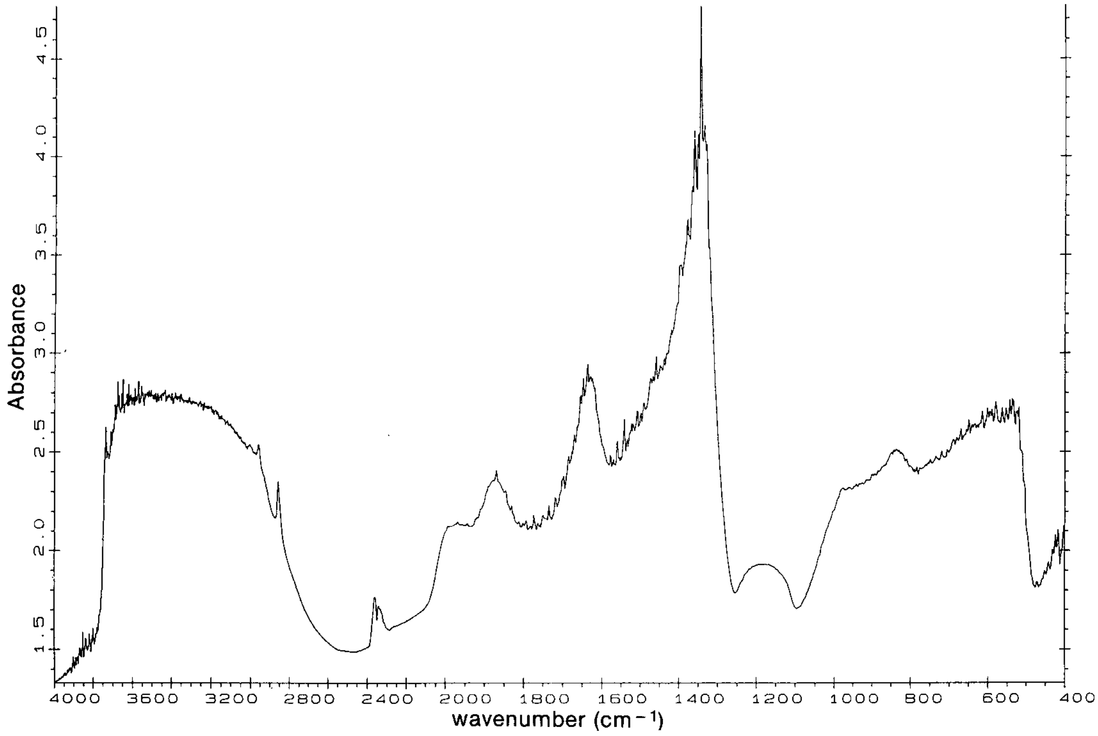


FIG. 1. The Fourier transform infrared spectroscopy spectrum of silicic acid.

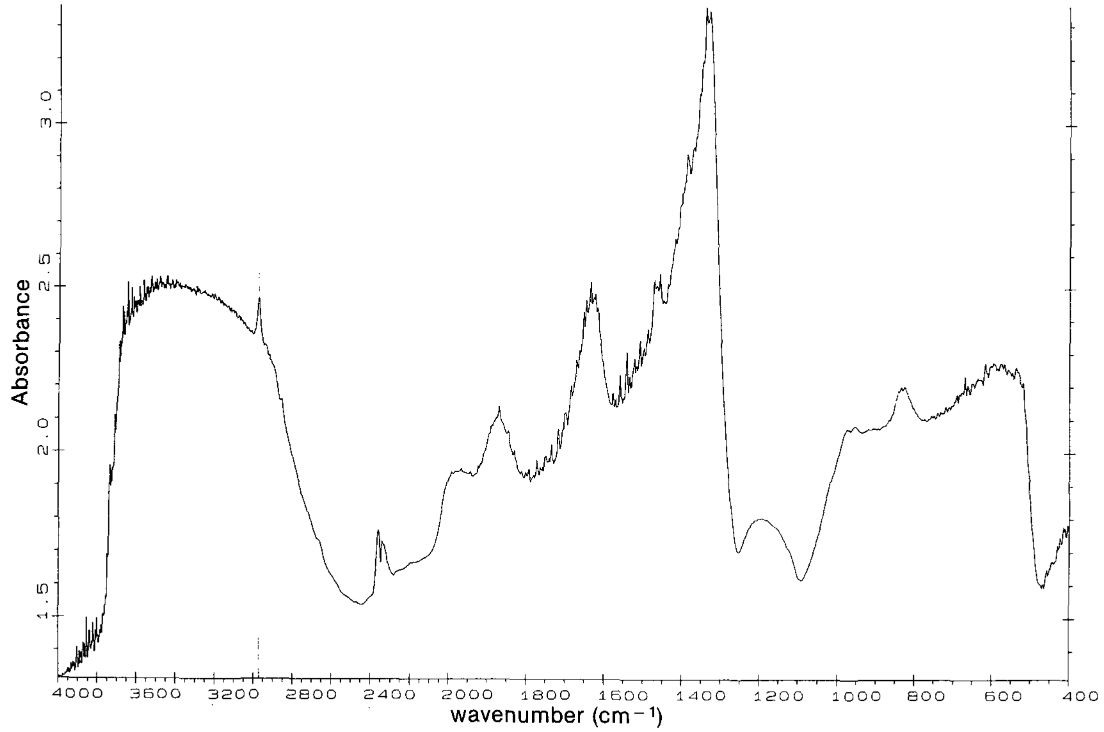


FIG. 2. Fourier transform infrared spectroscopy spectrum of isopropanol (IPA) adsorbed on silicic acid (SA).

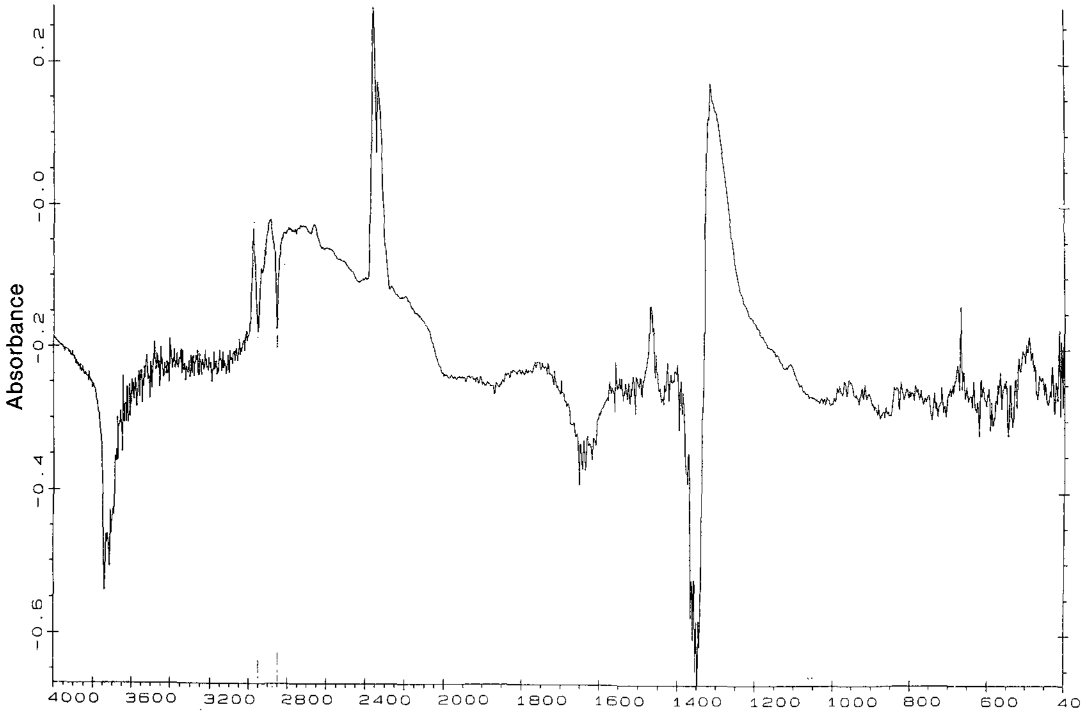


FIG. 3. The Fourier transform infrared spectroscopy spectrum of silicic acid-bound isopropanol with the silicic acid spectrum subtracted.

cm^{-1} indicates that IPA adsorbs by hydrogen-bonding to the nonhydrogen-bonded silanol group originally at 3740 cm^{-1}. Because the 3740 cm^{-1} band is absent in the adsorbed-IPA spectrum, but is present in the SA spectrum, subtraction results in the negative 3740 cm^{-1} band. The nonhydrogen-bonded silanol stretch at 3740 cm^{-1} was shifted down by hydrogen-bonding to IPA to form a broad band at 2500 to 2800 cm^{-1}, which shows as a broad positive band in the difference spectrum in Figure 3. The peak at 2978 cm^{-1} in Figures 2 and 3 is the asymmetric vibration of the methyl group of adsorbed IPA, while the C-H deformation vibration is seen at 1470 cm^{-1}. The data indicate hydrogen bonding of IPA to silanol groups in the presence of hexane. This was proposed in the presence of soy oil to inhibit carotenoid binding (3) and to promote that of phospholipid (2). In contrast, hexane does not bind to SA. The SA peak at about 1305 cm^{-1} occurs in Figure 3 and subsequent spectra because adsorption of OA or IPA causes a small shift in the large Si-O-Si band, so that the subtraction of a pure SA spectrum from the SA material leaves a difference peak at 1305 cm^{-1}.

The negative peaks at 2860 and 2955 cm^{-1} in the difference spectrum in Figure 3 are due to these bands being present in the spectrum of the original SA (Fig. 1), which is subtracted from the adsorbed IPA spectrum (Fig. 2). These bands are absent in Figure 2 because the polar IPA/hexane treatment removed the adsorbed material that caused these bands. The material is not removed from the SA because its removal requires a polar solvent, which would itself remain on the SA, as IPA does. Nevertheless, the negative peaks at 2860 cm^{-1} and 2955 cm^{-1} are not important because their source is understood, and they do not cause confusion.

OA adsorption. The spectrum of free OA is shown in Figure 4 and was obained for comparison with the adsorbed form. Important features of the spectrum include the C-H stretch for an R-C=C(H)R′ group at 3006 cm^{-1}, the asymmetric and symmetric C-H stretching vibration of a CH_2 group at 2926 and 2854 cm^{-1}, and the CH_2 deformation band at 1464 cm^{-1}. Other significant peaks are the carbonyl stretch at 1712 cm^{-1} and the peaks at 1285, 939 and 724 cm^{-1} due to skeletal vibration.

Figure 5 shows the FT-IR spectrum of OA bound by SA from hexane, while Figure 6 shows this spectrum with the SA spectrum (Fig. 1) subtracted. The negative peak at 3745 cm^{-1} indicates hydrogen bonding to SA as shown by the shift in the free silanol peak at 3740 cm^{-1} to form the broad band from 2500 to 2800 cm^{-1}. The involvement of the carboxyl oxygen of OA in hydrogen bonding is shown by the broadening of the carbonyl stretch at 1724 cm^{-1}, relative to the one in Figure 4 (1712 cm^{-1}). Marshall and Rochester (11) suggested that hydrogen bonding of an acid's carboxyl hydrogen to a silanol's oxygen and a simultaneous association of acid carbonyl oxygen with the silanol hydrogen is the most likely complex formed. The bands at 3008, 2921, 2858 and 1464 cm^{-1} for C-H stretching and deformation modes are only slightly shifted from those of pure OA, thus indicating adsorption of OA in a relatively unchanged form.

Figure 7 illustrates the FT-IR spectrum of SA mixed with equimolar (0.05M) OA and IPA in hexane. The

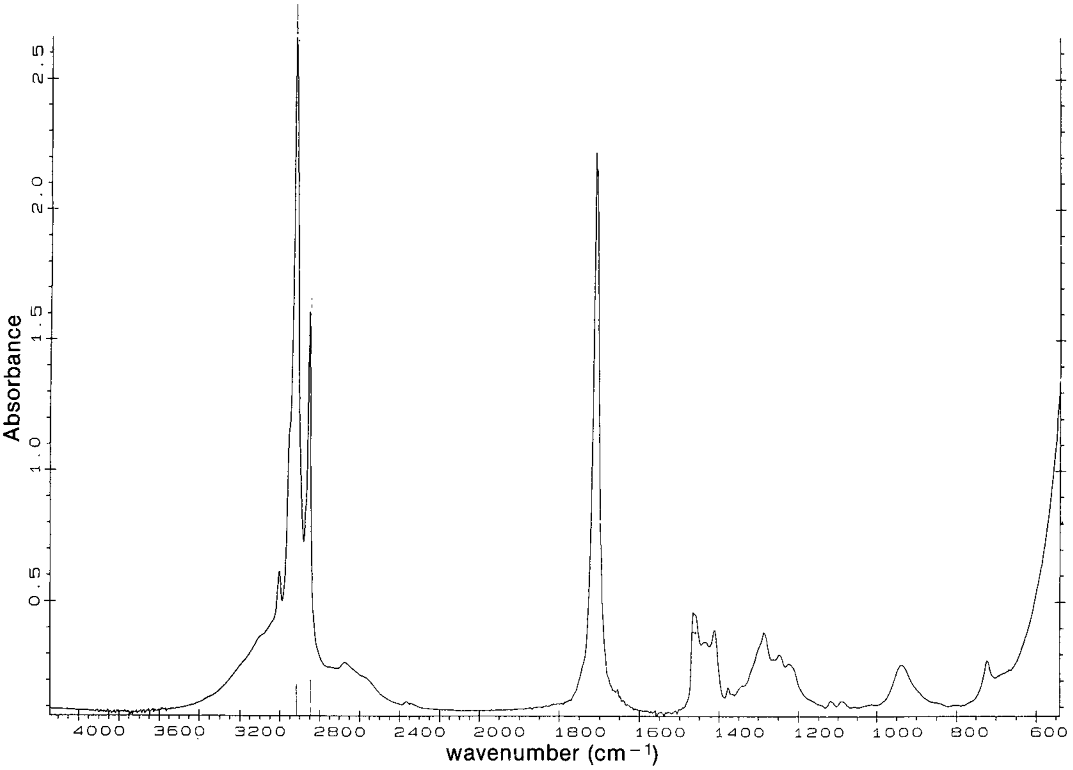


FIG. 4. **The transmission Fourier transform infrared spectroscopy spectrum of oleic acid on a salt window.**

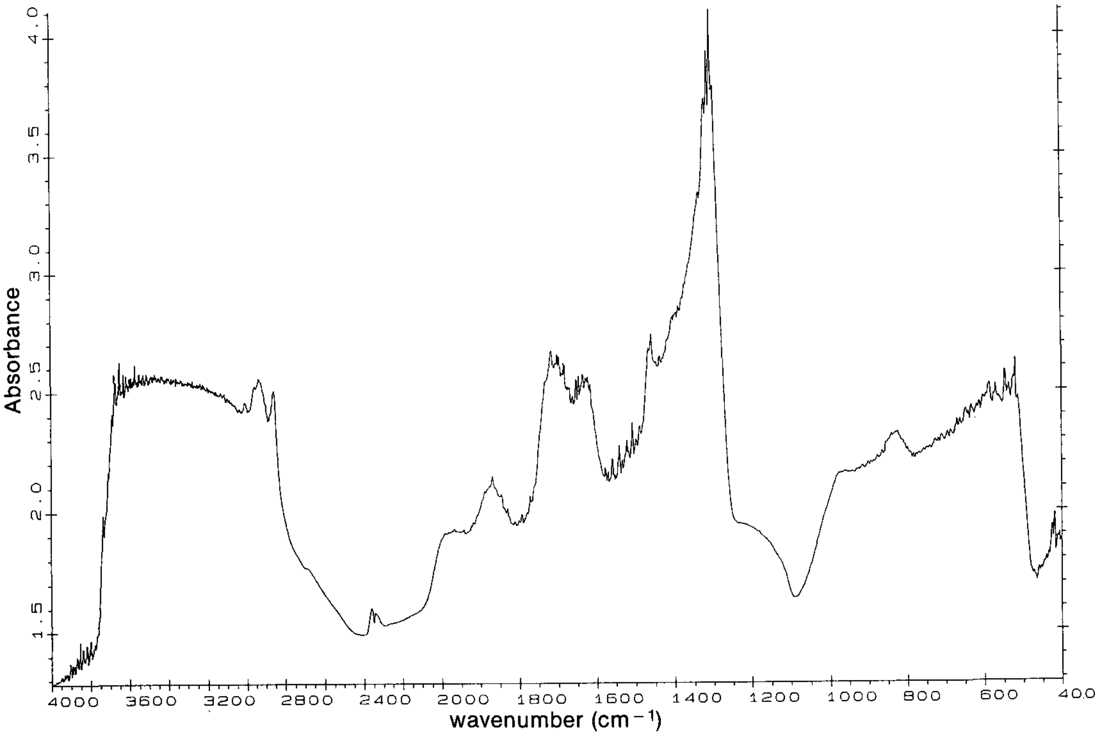


FIG. 5. The Fourier transform infrared spectroscopy spectrum of oleic acid bound to silicic acid obtained by incubating 0.05M oleic acid in 100 mL hexane with 0.5 g silicic acid for 15 min and subsequent air drying.

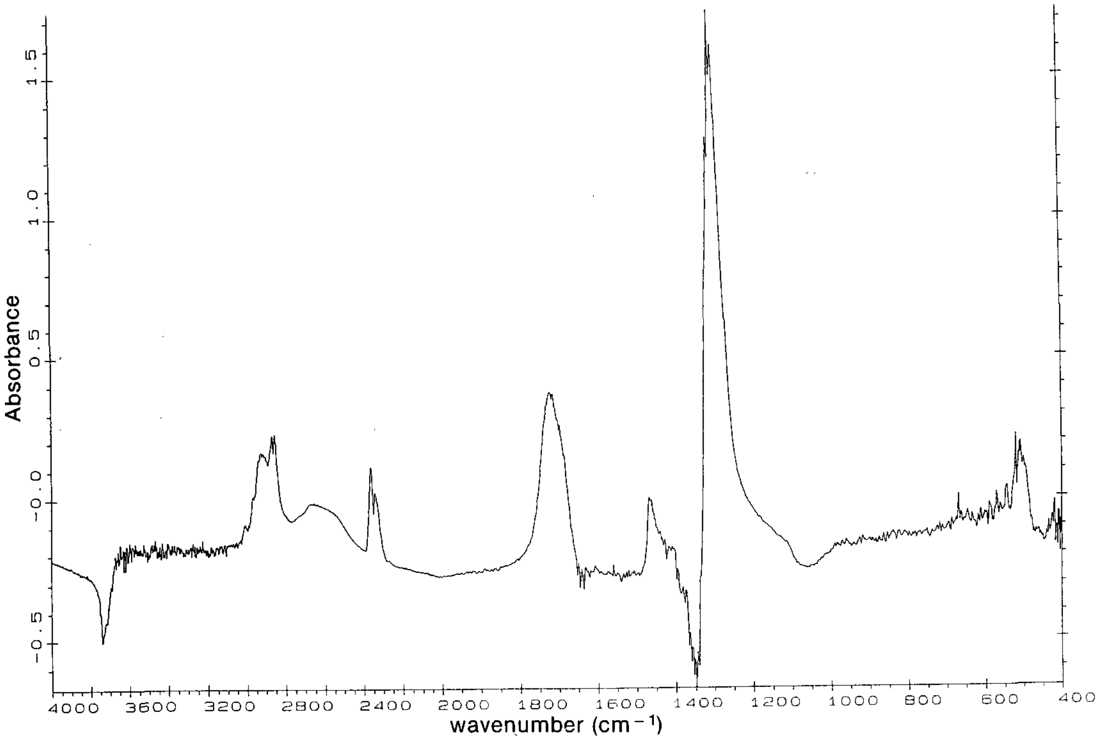


FIG. 6. Fourier transform infrared spectroscopy (FT-IR) spectrum of oleic acid bound to silicic acid (Fig. 5) with the FT-IR spectrum of silicic acid (Fig. 1) subtracted.

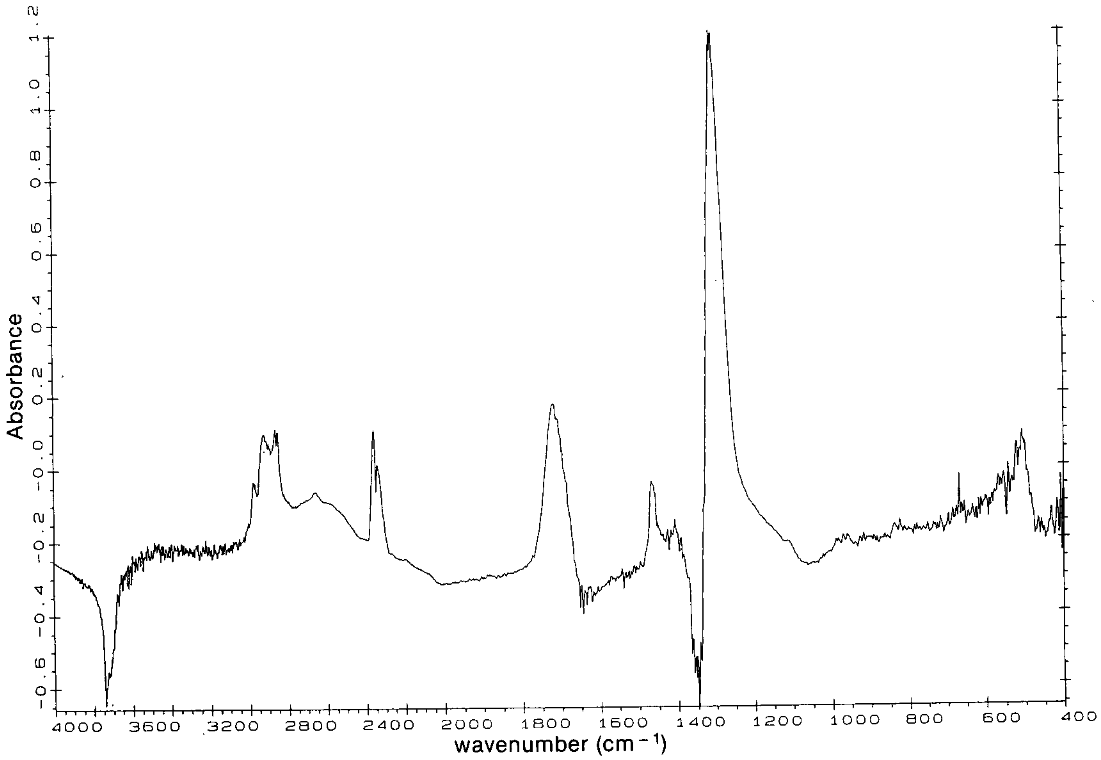


FIG. 7. The FT-IR spectrum of oleic acid and isopropanol bound to silicic acid. This spectrum was obtained by incubating 0.05M oleic acid and 0.05M isopropanol in 100 mL hexane with 0.5 g silicic acid for 15 min and subsequent air drying of the adsorbent. See Figure 6 for abbreviation.

negative peak at 3745 cm^{-1} and broad peak at 2500 to 2800 cm^{-1} again indicate a hydrogen-bonding interaction of OA and/or IPA with SA. The dominant bands in the C-H stretching region at 2926 and 2860 cm^{-1} indicate that OA is the principal adsorbed species. This is confirmed by the large carbonyl band at 1721 cm^{-1}, which again is broadened to indicate hydrogen-bonding of the OA to the surface hydroxyl groups. The presence of IPA on the surface is shown by the small band at 2975 cm^{-1}, which matches the C-H methyl stretch of IPA. The C-H stretching vibrations and carbonyl vibration for adsorbed OA are less intense in Figure 7 than in Figure 6. This suggests that less OA is adsorbed in the presence of IPA than in its absence, as is reasonable and expected. While these diffuse reflectance spectra are not expected to be exactly quantitative, they are highly consistent and reproducible, as shown in Table 1. This gives some support to the proposal that IPA displaces other adsorbates in lipid systems (2–4).

These systems may be discussed in terms of equilibria in which there is (i) competitive adsorption on the surface and (ii) competitive interactions in solution. The conclusion that species readily move between the surface and the solution phases is shown by the desorption experiments that follow. A further factor in this equilibrium system is the likelihood that the IPA hydrogen-bonds, or at least has polar interactions, with the carbonyl group of OA. This occurs when both OA and IPA are present in the hexane solution; some of the OA is prevented from adsorbing on the surface by interaction in hexane with IPA.

OA desorption. Figure 8 shows the effect of adsorbing OA from hexane and subsequently washing the SA with hexane. The FT-IR spectrum of SA (Fig. 1) has been

TABLE 1

Reproducibility of Carbonyl Band Intensities at 1712 cm Obtained with Fourier Transform Infrared Spectroscopy

Figure	Adsorbate	Absorbance units	Date of analysis (in 1993)
6	Oleic acid	0.63	June 22
8	Oleic acid (hexane-washed)	0.60	July 8
7	Oleic acid plus IPA[a]	0.50	June 28
10	Oleic acid plus IPA (hexane-washed)	0.51	July 15

[a] IPA = isopropanol.

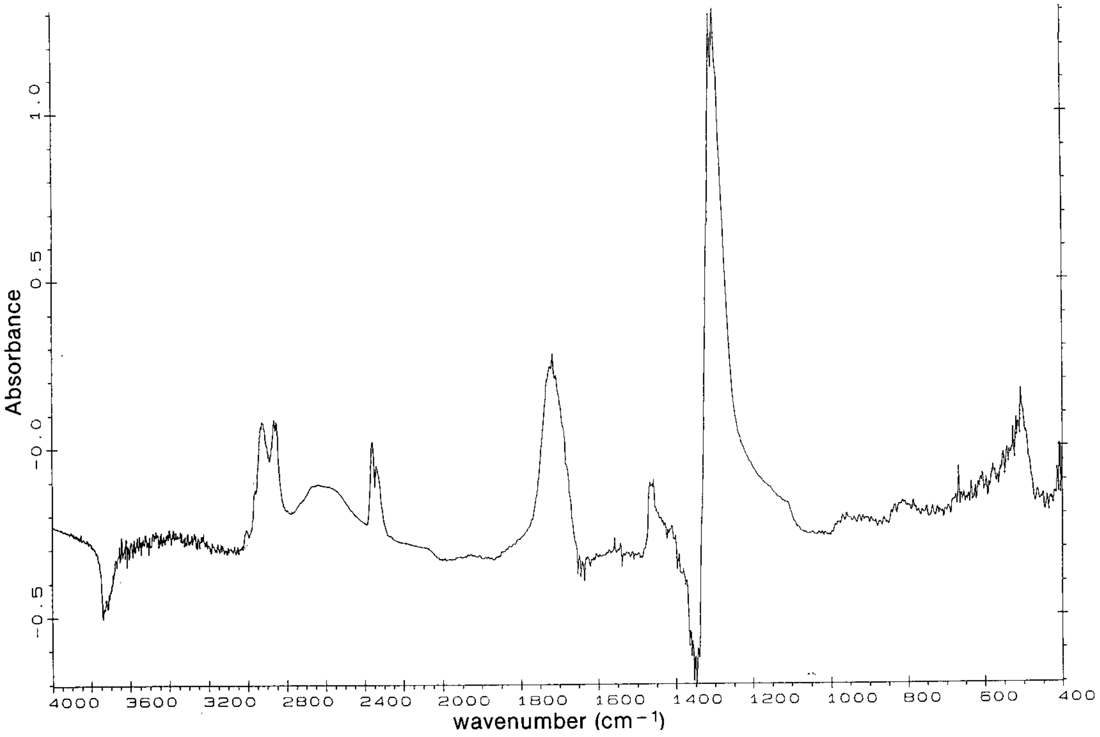


FIG. 8. The FT-IR spectrum of silicic acid binding oleic acid. This spectrum was obtained after incubating 0.05M oleic acid in 100 mL hexane with 0.5 g silicic acid for 15 min, washing with 100 mL hexane, and then air drying the adsorbent. See Figure 6 for abbreviation.

subtracted from that of the hexane-washed adsorbent binding OA. The spectrum is similar to that obtained after adsorption before washing (Fig. 6), except that the carbonyl stretch and C-H vibrations are reduced a little, although quantitative accuracy is not claimed to this level. This would indicate a small loss of OA from the adsorbent. Because hexane cannot displace OA, the most probable reason for this observation is that an equilibrium between solution and surface OA with fresh pure hexane solvent results in some OA going into the solution phase.

The FT-IR spectrum of SA, which has adsorbed OA from hexane and has been subsequently washed with 0.05M IPA in hexane, is given in Figure 9. The spectrum of SA has been subtracted. The carbonyl peak found on adsorption in hexane (1719 cm^{-1}) (Fig. 6) is much smaller, which indicates much reduced OA adsorption. However, the C-H stretch at 2980 cm^{-1} for methyl groups indicates adsorption of IPA. The negative peaks at 2955 and 2860 cm^{-1} are an artifact of the subtraction, as explained above. The C-H deformation stretch at 1465 cm^{-1} is also reduced relative to Figure 6 and has a band shaped like the adsorbed IPA in Figure 3. The OA desorption caused by IPA increases the proportion of bound IPA relative to bound OA. This is consistent with IPA competing for surface sites and interacting with OA in solution, which makes OA carbonyl groups less available for adsorption.

Figure 10 shows the adsorption of OA with IPA in hexane followed by washing with hexane. The OA binding is less than in Figure 8, where no IPA was used, as shown by the smaller C-H stretches at 2955 and 2860 cm^{-1} and smaller carbonyl stretch at 1712 cm^{-1}. There is little adsorbed IPA, as shown by the small intensity at 2975 cm^{-1} and the shape of the 1465 cm^{-1} band. As in Figure 8, washing with pure hexane has only a small effect on the adsorbed OA.

Figure 11 shows the adsorption of OA with IPA in hexane, followed by washing with 0.05M IPA in hexane. As shown in Figure 7, adsorption from an equimolar mixture of OA and IPA yields a spectrum that shows mostly OA on the SA surface. Washing with IPA in hexane removes most of the OA from the surface and deposits IPA, as shown by the IPA methyl band at 2975 cm^{-1} and the lack of an appreciable carbonyl band at 1712 cm^{-1}. This confirms the effect of washing with IPA in hexane shown in Figure 9.

The diffuse reflectance spectra are highly reproducible. When two silica samples from the same source were analyzed at a three-month interval, both spectra were like the one in Figure 1. Subtraction of the two spectra produced a reasonably flat baseline with a noise level of approximately 0.05 absorbance units in the OH stretching region from 3000 to 4000 cm^{-1} and a high noise level in the unusable Si-O stretching region from 1250 to 1400 cm^{-1} (data not shown). There was a low noise level in all other regions down to 600 cm^{-1}. The signal-to-noise ratio in the region of the peaks used for interpretation is good. The spectrum of adsorbed OA with SA (Fig. 5) shows that, even in the presence of SA (Fig. 1), the C-H bands (2800–3000 cm^{-1}) and the carbonyl band (1712 cm^{-1}) are obvious. However, their precise shape is better revealed by subtracting the SA spectrum (Fig. 7), which is used

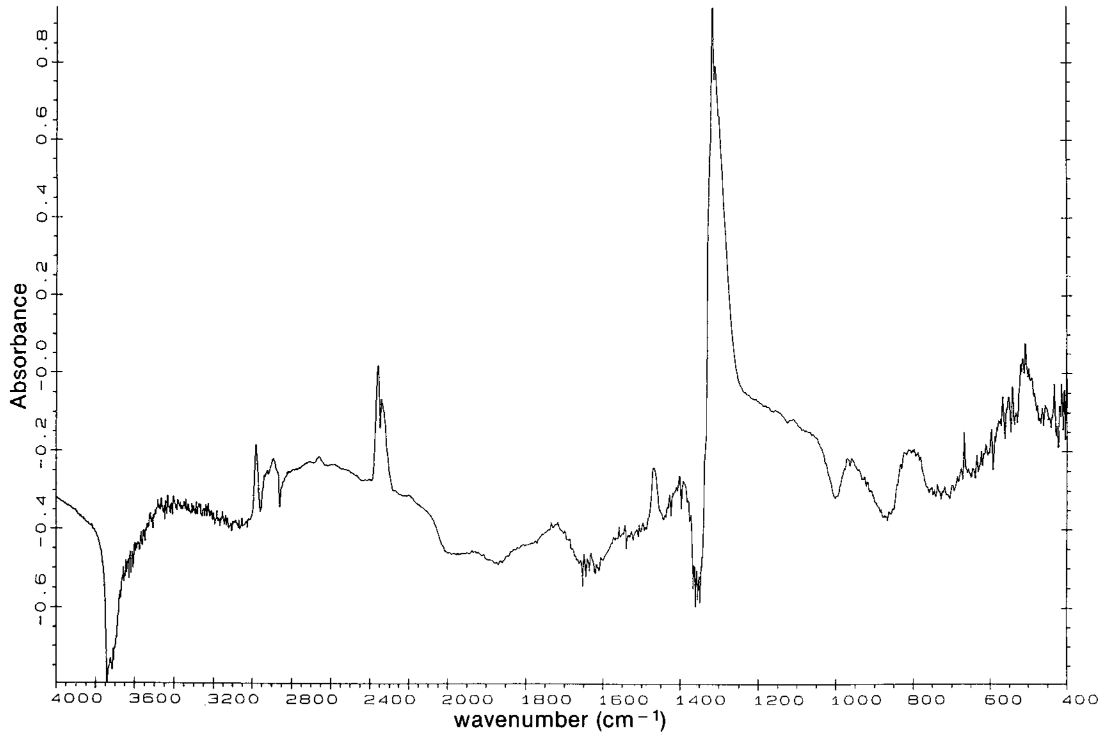


FIG. 9. The FT-IR spectrum of silicic acid binding oleic acid. This spectrum was obtained after incubating 0.05M oleic acid in 100 mL hexane with 0.5 g silicic acid for 15 min, washing with 100 mL 0.05M isopropanol in hexane, and then air drying the adsorbent. See Figure 6 for abbreviation.

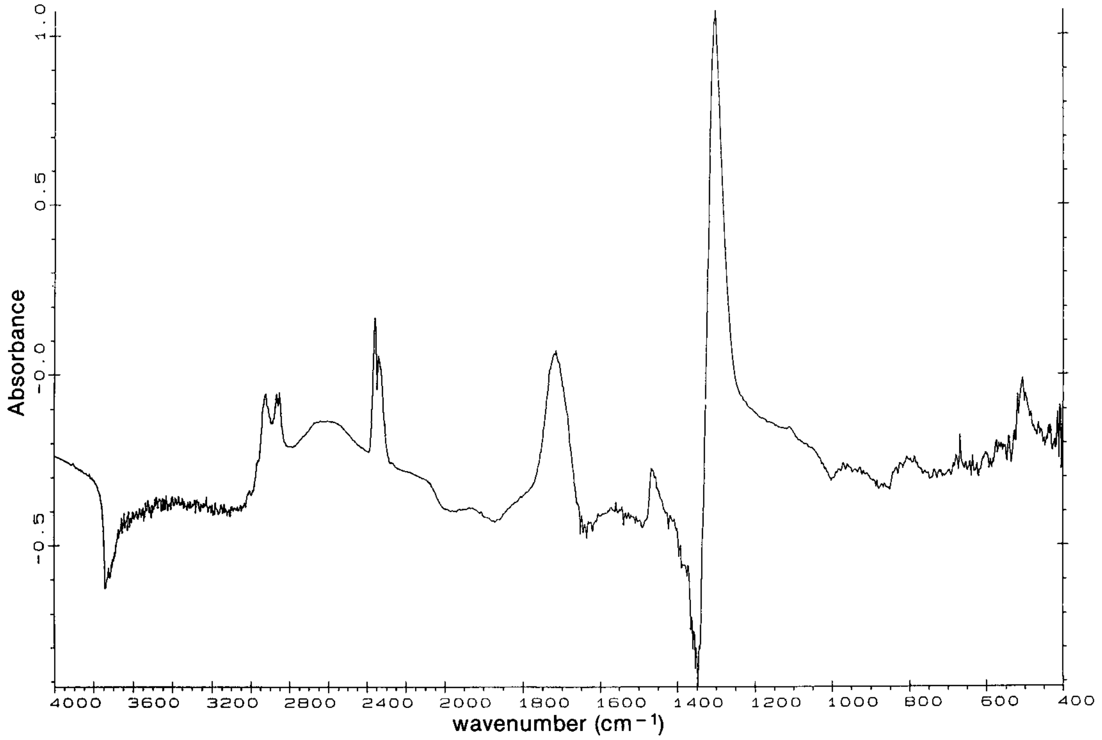


FIG. 10. The FT-IR spectrum of silicic acid binding oleic acid. This spectrum was obtained after incubating 0.05M oleic acid in 100 mL 0.05M isopropanol in hexane with 0.5 g silicic acid for 15 min, washing with 100 mL hexane, and then air drying the adsorbent. See Figure 6 for abbreviation.

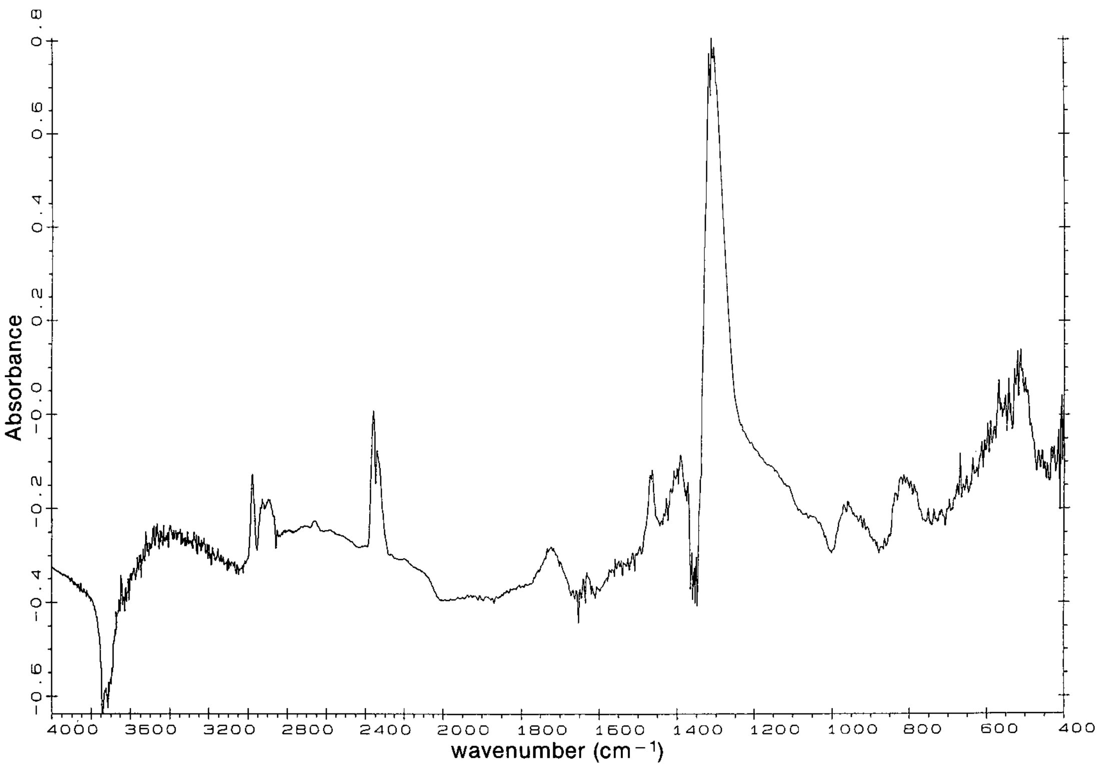
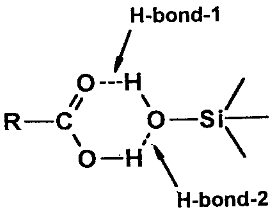


FIG. 11. The FT-IR spectrum of silicic acid binding oleic acid. This spectrum was obtained after incubating 0.05M oleic acid in 100 mL 0.05M isopropanol in hexane with 0.5 g silicic acid for 15 min, washing with 100 mL 0.05M isopropanol in hexane, and then air drying the adsorbent. See Figure 6 for abbreviation.

in the discussion. In subsequent studies, only the subtraction data are presented. OA adsorption was repeated three months after the original experiment and produced essentially the same spectrum, illustrating the reproducibility of the technique. Table 1, describing the intensity of the carbonyl stretch at 1712 cm^{-1}, also demonstrates the consistency of the data. Because washing with hexane has relatively little effect on the amount of OA adsorbed, the runs made with and without washing are essentially duplicate determinations. Table 1 shows that absorbances for the two OA adsorption runs are close together, as are the runs with OA and IPA. However, the runs with OA and the runs with added IPA are significantly different. Thus, the FT-IR diffuse reflectance technique, with subtraction, gives highly reproducible data.

It has been previously suggested that binding of an organic acid to a silica surface occurs through the double hydrogen bond structure shown in Scheme 1 (11). The spectra in this paper show clearly the formation of H-bond 1 because of the broadening of the carbonyl band of OA and the shift in the O-H band of SA. However, there is no direct spectral evidence either for or against the formation of H-bond 2. The complexing of IPA to OA in hexane solution may occur through a complex like that in Scheme 2.

The experimental data may be understood in terms of equilibria between OA, IPA and SA as shown in Equations 1–3, where subscripts indicate a surface-bound species.

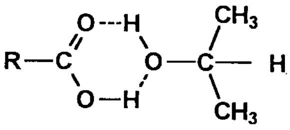


SCHEME 1

$$\mathrm{OA} + \mathrm{IPA} \rightleftharpoons (\mathrm{OA^{*}IPA}) \qquad [1]$$

$$\mathrm{OA} + \mathrm{SA} \rightleftharpoons \mathrm{OA_s} \qquad [2]$$

$$\mathrm{IPA} + \mathrm{SA} \rightleftharpoons \mathrm{IPA_s} \qquad [3]$$



SCHEME 2

The species OA*IPA may represent a hydrogen-bonded species, as shown in Scheme 2, or it may represent polar interactions between OA and IPA.

To summarize, FT-IR studies showed that OA and IPA both hydrogen-bond to SA from a hexane solution. The OA interaction is through the carbonyl group. Even though IPA does competitively desorb OA, the data indicate that OA is bound to a greater extent than IPA when they are present in equimolar amounts in solution. The extent of OA adsorption on SA is also affected by a solution interaction between OA and IPA.

ACKNOWLEDGMENT

Authors wish to acknowledge the Arkansas Science and Technology Authority for funding the work.

REFERENCES

1. Brekke, O.L., in *Handbook of Soybean Oil Processing and Utilization*, edited by D.R. Erickson, E.H. Pryde, O.L. Brekke, T.L. Mounts and R.A. Falb, American Oil Chemists' Society, Champaign, 1980, pp. 71–88.
2. Brown, H.G., and H.E. Snyder, *J. Am. Oil Chem. Soc. 62*:753. (1985).
3. Proctor, A., and H.E. Snyder, *Ibid. 64*:1163. (1987).
4. Minyu, J., and A. Proctor, *Ibid. 70*:575. (1993).
5. Chapman, D.M., and E.A., Pfannkoch, *Ibid. 69*:1009 (1992).
6. Sarier, N., and C. Guler, *Ibid. 66*:917 (1989).
7. Guler, C., and Tunc, F., *Ibid. 69*:948 (1992).
8. Hair, M.L., *Infrared Spectroscopy in Surface Chemistry*, Marcel Dekker Inc., New York, pp. 79–137.
9. Nakanishi, K., in *Infrared Absorption Spectroscopy*, Holden-Day, Inc. San Francisco and Nankodo Company Limited, Tokyo, 1062, pp. 20–45.
10. McDonald, R.S., *J. Phys. Chem. 62*:1168 (1958).
11. Marshall, K., and C.H. Rochester, *J. Chem. Soc. Faraday Trans. I. 71*:1754 (1975).

[Received August 24, 1993; accepted December 7, 1993]