The Effect of Added Solvents on Soy Oil Lutein Adsorption by Silicic Acid

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It has been reported that addition of isopropanol to a soy oil miscella inhibits the binding of soy lutein to added silicic acid by competitive adsorption. It was suggested that the competition was based on the polarity of the miscella constituents. This investigation studied the effects of a homologous series of lower alcohols to competitively inhibit lutein binding to silicic acid from a soy oil hexane miscella. Lutein binding inhibition by molecules of carbon chains with the same lengths, but with different functional groups, was also examined. Minor differences were found between members of a homologous series of alcohols. A similar result was found with short-chain fatty acids. The ability of various functional groups to displace lutein from silicic acid was dependent on the molecules' ability to form hydrogen bonds, rather than on polarity.

KEY WORDS: Adsorption, lutein, miscellas, silicic acid, solvents, soy oil.

Oil is extracted from soy flakes with hexane, which is then evaporated to produce the crude oil. Crude soy oil contains a number of substances, such as pigments, phospholipids and free fatty acids, which must be removed to produce a bland, light-colored oil that is acceptable to consumers. The commercial removal of pigments is achieved by an adsorption process at 100°C on bleaching clays under reduced pressures (1). Soy oil pigment is almost exclusively the carotenoid lutein (2). Hassler and Hagberg (3) showed that the adsorption of soy oil pigment on bleaching clay occurs according to a Freundlich isotherm.

Adsorption of phospholipids (4) and lutein (5) onto silicic acid from soy oil/hexane miscellas also conforms to a Freundlich isotherm. An advantage of this bleaching technique, relative to conventional methods, is that it is conducted at ambient temperatures, and the binding of these oil components is modified by the addition of a polar solvent to the miscella. One percent isopropanol in the miscella promotes adsorption of phospholipid, which presumably occurred by removal of triglyceride from adsorption sites. This then facilitates phospholipid binding (4). Free fatty acid adsorption to amorphous cristobalite silica was also promoted by isopropanol in a similar system (5). In contrast, isopropanol inhibited adsorption of lutein to silicic acid (5). This inhibition was explained in terms of competition between miscella constituents for silanol sites. Polarity was suggested to be a basis for competition because of the isopropanol effect. However, reducing triglyceride concentration promoted lutein adsorption, suggesting that concentration and/or molelcular weight of competing species may also be factors that determine adsorption.

The objective of this investigation is to study the effect of alcohol molecular weight on lutein binding from a soy oil miscella and the adsorption isotherm. The effect on the lutein isotherm of C_3 and C_4 compounds with different functional groups was also examined.

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MATERIALS AND METHODS

Oil and solvents. Commercially extracted alkali-refined soy oil was stored at 4°C and used throughout the investigations. Soy oil miscellas were prepared by diluting soy oil with hexane.

Water and the following alcohols were added to modify miscella polarity (0.1 M concentration) prior to lutein adsorption: methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, isobutanol, 2-octanol, 1-nonanol and 1-decanol. The following aldehydes, ketones, acids and esters were also used: propanal, acetone, 2-butanone, acetic acid, propionic acid, *n*-butyric acid, isobutyric acid, octanoic acid, methyl acetate and ethyl acetate.

Adsorbents. The adsorbent used was silicic acid (Bio-Sil A., 100–200 mesh, Bio-Rad Laboratories, Richmond, CA). Silicic acid was heated in a drying oven to remove moisture and was stored in a desiccator until used. The term "silica" is used as a synonym for silicic acid.

Lutein measurement. Pigment concentration in the miscellas was measured as lutein by reading optical absorbance at 445 nm, according to the method of Proctor and Snyder (5).

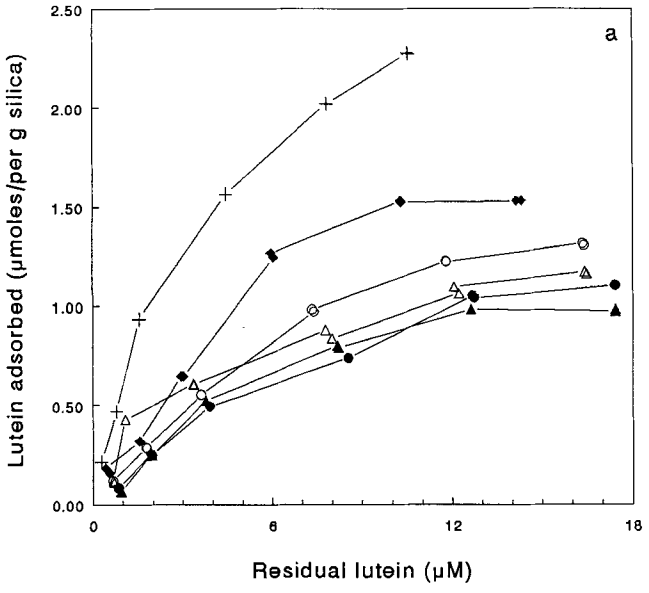
Lutein isotherms. Lutein isotherms were determined by preparing 100-mL volumes of 2.5, 5, 10, 20, 30 and 40% (vol/vol) concentrations of soy oil miscellas in hexane. The lutein content of each miscella was measured before 0.5 g of silica was added. The miscellas were agitated with a magnetic stirrer in a closed vessel at 22°C for 15 min. The concentration of residual lutein remaining unadsorbed was then found, and the amount of lutein adsorbed was calculated by difference. Isotherms were plotted as the amount of lutein adsorbed, per gram of silica, *vs.* the residual concentration of lutein. Duplicate determinations were made. This was the control experiment.

Miscella polarity. Isotherms were prepared as described above but with 0.1 M concentration of additional solvent present in the miscella.

RESULTS AND DISCUSSION

Figure 1a shows the effect of adding water and low molecular weight alcohols (C_1–C_3), to the miscella on pigment binding. Lutein adsorption followed a Freundlich isotherm, and added solvent reduced lutein adsorption relative to the control in each case. There is little difference in the isotherms obtained with ethanol, *n*-propanol and isopropanol, which were more effective than water in inhibiting lutein binding. Methanol was slightly less effective in reducing lutein adsorption. The differences in the results obtained with different solvent systems were best seen at high residual lutein levels.

Water was the least effective solvent, but it did significantly reduce lutein adsorption relative to the control. Water can hydrogen bond to the silica or to other water molecules. In this lipid system it is probably more thermo-



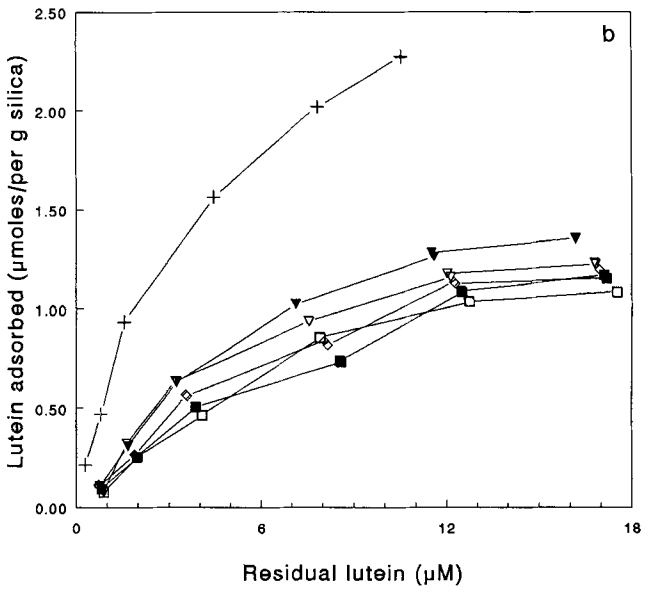


FIG. 1. Lutein isotherms were obtained by incubating 0.5 g of silicic acid with 100 mL of 2.5, 5, 10, 20, 30 and 40% (vol/vol) alkali-refined soy oil/hexane miscella for 15 min at 22°C in the presence of 0.1 M concentration of (a) water (◆), methanol (○), ethanol (●), *n*-propanol (△) or isopropanol (▲). A control (+) was prepared without added solvent; (b) *n*-butanol (□), isobutanol (■), 2-octanol (▽), 1-nonanol (◇) and 1-decanol (□). A control (+) was prepared without added solvent.

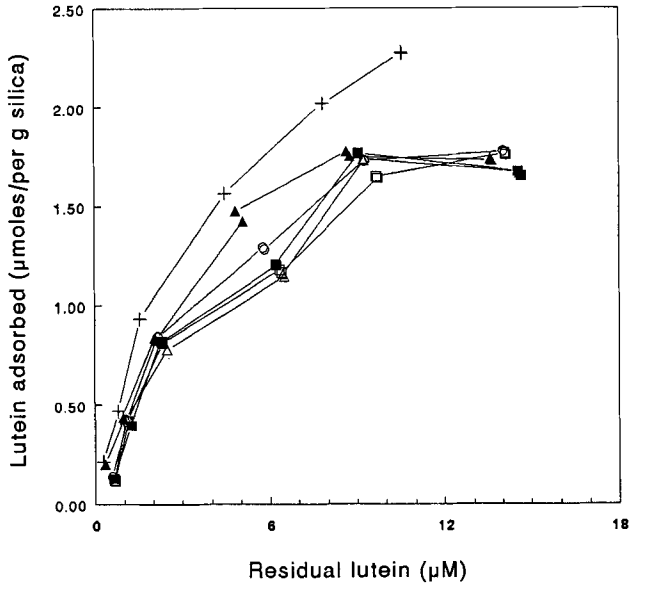


FIG. 2. Lutein isotherms were obtained by incubating 0.5 g of silicic acid with 100 mL of 2.5, 5, 10, 20, 30 and 40% (vol/vol) alkali-refined soy oil/hexane miscella for 15 min at 22°C in the presence of 0.1 M concentration of acetic acid (△); propionic acid (▲), *n*-butyric acid (□), isobutyric acid (■) and octanoic acid (○). A control (+) was prepared without added solvent.

dynamically stable for water molecules to associate together. The effect of added water in this system may be due to the strength of water/water hydrogen bonding and water's lipophobic nature. To enable water molecules to bind to silica, water hydrogen bonding has to be disrupted, and individual molecules migrate to the adsorption surface. This is probably not as energetically favorable as disruption of alcohol hydrogen bonding. Furthermore, an alkane structure would increase solubility in a lipid system and permit binding to an adsorbent. Therefore, competitive adsorption would be expected to be improved by addition of an alkyl group. Methanol is slightly less effective than ethanol at reducing lutein binding, but the effect of ethanol is similar to that of propanol isomers. Therefore, the methyl groups bound to the hydroxyl carbon of isopropanol do not sterically hinder adsorption relative to the primary alcohol.

Butanol isomers, octanol and nonanol also produced similar isotherms to those obtained with propanol (Fig. 1b). These data indicate that with lower alcohols there are several small differences in adsorption on the basis of isomerism or molecular weight. Molecular shape and size are reported to be important factors preventing hydrogen bonding to silica due to steric hinderance (6). In studies of long-chain species, Hau and Newar (7) reported that the number of moles adsorbed to silica decreases as chainlength increases. The results of studying the competitive adsorption of lutein suggest that with alcohols (C_0-C_{10}) (Fig. 1a and 1b) there is no large change in the isotherm, as alcohol length is increased but small differences are seen.

These isotherm studies give indirect evidence that lower alcohols bind largely independently of chainlength. This study is complicated by the presence of triglyceride, which is the major species bound overall (5).

Figure 2 shows the effect on lutein adsorption of adding members of a homologous series of fatty acids to the soy oil miscellas. Although lutein adsorption is reduced relative to the control, there is little difference between isotherms. Therefore, the ability of free fatty acids to compete with lutein for binding sites is independent of chainlength. The data conform to Wu and Mead's (8) findings that fatty acid adsorption to silica is independent of chainlength. This shows the importance of extracting free fatty acids from soy oil before pigment adsorption.

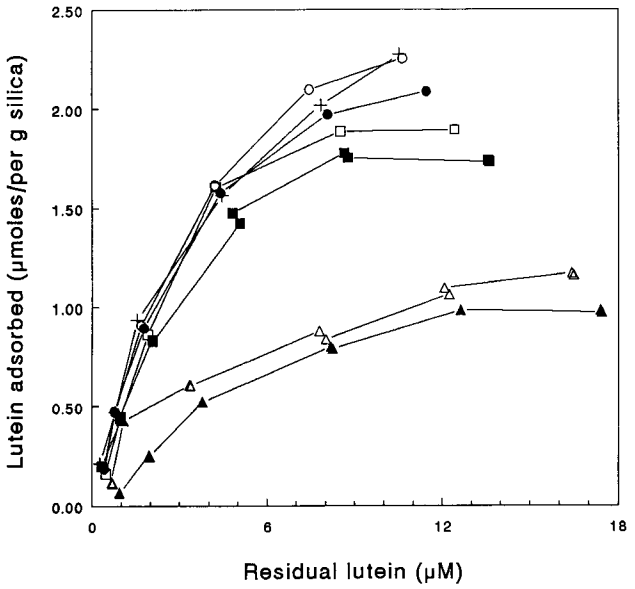


FIG. 3. Lutein isotherms were obtained by incubating 0.5 g of silicic acid with 100 mL of 2.5, 5, 10, 20, 30 and 40% (vol/vol) alkali-refined soy oil/hexane miscella for 15 min at 22°C in the presence of 0.1 M concentration of *n*-propanol (△), isopropanol (▲), propionic acid (■), acetone (□), propanal (●) and methyl acetate (○). A control (+) was prepared without added solvent.

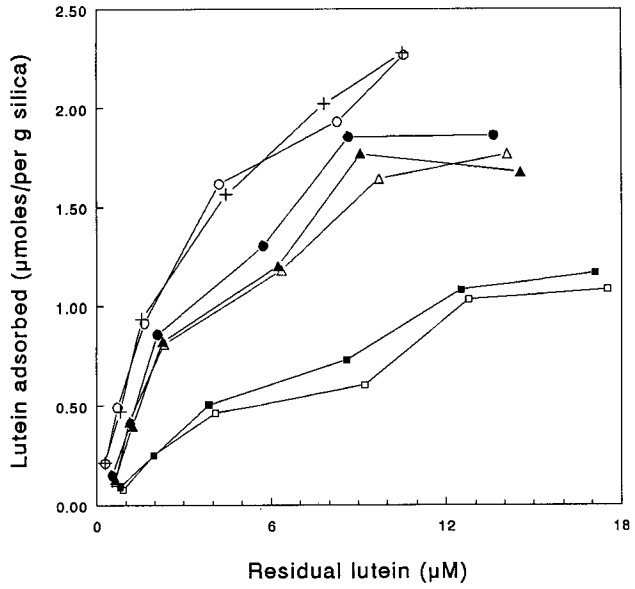


FIG. 4. Lutein isotherms were obtained by incubating 0.5 g of silicic acid with 100 mL of 2.5, 5, 10, 20, 30 and 40% (vol/vol) alkali-refined soy oil/hexane miscella for 15 min at 22°C in the presence of 0.1 M concentration of *n*-butanol, (□), isobutanol (■), *n*-butyric acid (△), isobutyric acid (▲), 2-butanone (●) and ethyl acetate (○). A control (+) was prepared without added solvent.

The effect of an added C_3 alcohol, aldehyde, ketone, acid and ester on lutein binding is illustrated in Figure 3. Freundlich isotherms were observed in the presence of each solvent, but there were differences in the solvent's ability to inhibit lutein binding. The alcohols were the most effective in inhibiting lutein binding, which is probably because they are best able to form hydrogen bonds with silanol groups. Hau and Newar (7) reported that the greater the tendency for hydrogen bonding, the stronger the adsorption. This study supports that premise, with the alcohols being most effective. A silanol hydroxyl is capable of forming two hydrogen bonds with a single alcohol hydroxyl group, or can hydrogen bond to two different alcohols (9).

Propionic acid was the most effective of the nonalcohol solvents, despite the fact that it has a lower polarity than the aldehydes and ketones used. This is most likely due to its ability to hydrogen bond. Each molecule is capable of forming two hydrogen bonds to a silanol group (9). Organic acids are not bound to silica to the same degree as alcohols, but the acid binding strength is greater (7).

At low miscella residual concentrations (<9 μM), the lutein isotherm, obtained with the remaining solvents, did not differ from the control or from each other. However, differences were evident in the 40% miscella. Acetone reduced lutein binding slightly more than propanal, probably because of the greater polarity in a similar system (10).

The isotherm obtained with methyl acetate was similar to that of the control. This indicates that this ester was not any more effective than triglyceride esters in competing with lutein for adsorption sites. This could be because the amount of added ester is negligible compared to the amount of triglyceride esters in the system. In any case, the position of the carbonyl group on an ester may make hydrogen bonding to a surface sterically difficult. Nevertheless, in this system triglyceride is the major oil component, and therefore, concentration is the overriding consideration (5).

The nonalcohol solvents were practically ineffective at reducing lutein binding at most miscella concentrations. Therefore, ability to hydrogen bond is more important than polarity in determining a molecule's ability to compete with lutein for adsorption sites.

The effectiveness of the solvents as competitors for lutein adsorption was alcohol > acid > ketone > aldehyde > ester. This is supported by Hau and Newar (7), who found that when comparing different classes of compounds of the same chainlength, the amount adsorbed was alcohol > acid > ester.

Figure 4 shows the effect of selected C_4 compounds on lutein adsorption by silica. The pattern of adsorption was similar to that obtained with C_3 compounds, *i.e.*, alcohol > acid > ketone > ester. In contrast to the isotherms obtained with C_3 compounds, at all residual lutein levels, ketones and acids produced isotherms that were dissimilar from the control but similar to each other.

In summary, alcohols can compete more effectively with lutein for adsorption sites on silica than other solvents because of their ability to form hydrogen bonds, rather than their polarity. Water is not as effective as alcohols because of the energy needed to overcome hydrogen bonding between water molecules in a hydrophobic environment. There is little difference between isotherms obtained within a homologous series of small molecular weight alcohols or fatty acid. Functional groups, other than alcohols, inhibit lutein binding to a lesser extent, even if polarity is greater than the corresponding alcohol.

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