Effects on Lutein Adsorption of Adding Polar Solvents to Silicic Acid or to Soy Oil/Hexane Miscellas

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Polar solvents limit adsorption of lutein from a soy oil miscella on silicic acid by competitive adsorption. Water and selected alcohols were added to miscella and adsorbent to observe if the mode of adding solvent affected lutein adsorption. No differences were produced by the method of addition. However, different mechanisms of limiting lutein adsorption were observed. Isopropanol limited lutein adsorption by competitive adsorption. Water was less competitive because it was less soluble in the miscella and was more thermodynamically stable as free water. Propanediol, a polyol, totally inhibited lutein adsorption by binding silica particles together. This study may have implications for silicic acid adsorption of oil components from hydrolyzed oils in which glycerides and free glycerol are present.

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

Oil is extracted from soy flakes with hexane, which is then evaporated to produce a crude oil. The crude oil contains a number of substances, including pigments, phospholipids and free fatty acids, which must be removed to produce a bland, light-colored product 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 pigments are almost exclusively the carotenoid lutein (2). Hassler and Hagberg (3) showed that adsorption of soy oil pigment onto bleaching clay follows a Freundlich isotherm, indicating monolayer adsorption, but the nature of the binding is not fully understood.

Adsorption studies have been performed with simple model systems of silicic acid and crude soy oil diluted with hexane, *i.e.*, miscella, to better understand the interaction of oil components with silicate adsorbents. The adsorption of phospholipid (4) and lutein (5) onto silicic acid, from soy oil/hexane miscellas, also follows a Freundlich isotherm. Adding 1% isopropanol (IPA) to the miscella promotes adsorption of phospholipid, which was proposed to occur by removal of triglycerides from the adsorption sites. Free fatty acid adsorption to amorphous cristobalite silica was also promoted by IPA (6). In contrast, IPA inhibited lutein adsorption to silicic acid (5). This was explained by competition between pigments and IPA for silanol binding sites. The polarity of miscella constituents was suggested to be the basis of the competition, with more polar constituents being strong competitors. Increasing the IPA concentration further decreased lutein binding. Addition of moisture to silica prior to adsorption had a similar effect. Proctor and Snyder (5) showed that triglycerides are the major species adsorbed. Chapman and Pfannkoch (7) later showed that triglyceride is an important competitive inhibitor of pigment adsorption in miscellas.

Comparison of alcohols, other than IPA, and water on the inhibition of lutein binding has been made with a 0.1M concentration of added polar solvents in the miscella (8). There was little difference between members of a homologous series of alcohols (C_1–C_{10}) in their ability to limit pigment adsorption. A comparison of the effect of a C_3 alcohol, aldehyde, ketone, acid and ester to inhibit lutein adsorption showed the following order: alcohol > acid > ketone > ester. The same result was obtained with C_4 molecules. This indicates that the ability to hydrogen bond, rather than polarity, may be the determining factor in adsorptive competitiveness. However, water inhibition of lutein binding was less effective than acetone (8). This was suggested to be due to water's low solubility in the hydrophobic environment. It was proposed that thermodynamically it would be more advantageous for water to hydrogen bond with other water molecules, to form free droplets, than to be adsorbed.

Silicic acid is hygroscopic. Therefore, moisture pre-bound to the adsorbent may better compete with lutein for adsorption sites than moisture in a miscella. The objective of this study was to observe lutein binding to silicic acid to which water, or selected alcohols, had been previously added. The isotherm would be compared with one obtained by adding the same amount of solvent to the miscella. Comparison of the two isotherms would give some indication of how preadsorbed solvent affects lutein binding, relative to solvent added to the miscella. Light microscopy was also used to observe differences in the appearance in the silica and to explain the adsorption data.

MATERIALS AND METHODS

Oil, solvents and adsorbent. Commercially extracted alkali-refined soybean oil was stored at 4°C and used throughout the investigation. Soy oil miscellas were prepared by diluting alkali-refined soy oil with hexane (5). Water, methanol, IPA and 1,3 propanediol (PDL) were used to either modify miscella polarity or to deactivate the silica before adsorption. Methanol was used because it is the alcohol that is most similar to water in size and structure. PDL resembles water in that both ends of the molecule are polar, and on hydrogen bonding to silica there would be an exposed hydroxyl group. Isopropanol was used for comparison with PDL because it is also a C_3 alcohol and a general indicator of maximum competitive inhibition of lutein adsorption by a mono-ol (8).

Bio-Sil A (100–200 mesh; Bio-Rad Laboratories, Richmond, CA) was the silicic acid used throughout. The moisture content of the adsorbent was determined by heating 5 g silica at 130°C for 1 h and subsequently measuring the weight loss. The water content was 4.2%.

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

Lutein isotherms—silica deactivation. Polar solvent was added to 10 g silica to give a concentration of 10 millimoles per gram and was left to equilibrate overnight. Isotherms were prepared with an adsorbent mass equivalent to 0.5 g silicic acid plus the weight of 5 millimoles

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of solvent. A control isotherm was obtained with unmodified silicic acid.

Isotherms were determined by preparing 100-mL vol of alkali-refined soy oil miscellas (6). The lutein content was measured before the adsorbent was added. The miscellas were agitated with a magnetic stirrer in a closed vessel for 15 min at 22°C to achieve equilibrium. The concentration of residual lutein remaining unadsorbed was measured, and the amount of lutein adsorbed was calculated by difference. Isotherms were plotted as the amount of lutein adsorbed per gram of silica *vs.* residual concentration of lutein.

Lutein isotherms—modified miscella polarity. Isotherms were prepared as described above but by modifying the miscella rather than the adsorbent. This was done by adapting the method of Minyu and Proctor (8). Isotherms were prepared with fully active silica, and 5 millimoles of solvent was added to the miscella (100 mL) before adsorption. This produced a 0.05 M concentration as compared to the 0.1 M concentration produced by Minyu and Proctor (8). A lower concentration was used to allow equimolar comparisons between modified miscella systems and modified silica without "overloading" the adsorbent.

Data were plotted to compare isotherms of altered adsorbent and miscellas for each solvent system relative to a control of unmodified silica in unchanged soy oil/hexane miscellas.

Light microscope studies. The silica in miscella was placed on a concave slide with a cover slip and observed with conventional and phase-contrast microscopy (Nikon Biological Microscope *Opti-phot*; Nikon Inc., Melville, NJ). Photomicrographs were prepared to illustrate differences in adsorbent appearance, which may provide insight into the adsorption process.

RESULTS AND DISCUSSION

The isotherms describing lutein adsorption by water-deactivated silica and adsorption by active silica from water-modified miscella are illustrated in Figure 1. In each case 5 millimoles of water was added. Less lutein was bound with moisture present than in the control experiment. This has been reported (5,8). However, in this study, water inhibition of lutein binding was independent of whether water was added to the miscella or directly to the adsorbent. Because adsorbent moisture is inversely proportional to lutein adsorption (5), the equilibrium distribution of water between silica and miscella would appear to be the same.

In both instances, when silica was added to a miscella in the presence of added moisture, a light gel formed that broke up on mixing. No gel was seen in the control. Microscopic observations, prior to mixing, showed that silica particles seem to adhere to each other in the presence of moisture (Fig. 2), but in the control experiment, the silica particles were discrete throughout (Fig. 3). Attachment between particles is seen in Figure 2B, which may be due to "water bridges" by bound moisture. The gel was destroyed after mixing, and the silica became separate particles (Fig. 4). Following adsorption, particles were either totally yellow or totally white. The light particles in Figure 4 are colorless, and the dark particles are yellow. Preliminary work showed the number of colorless particles was proportional to added moisture. In contrast,

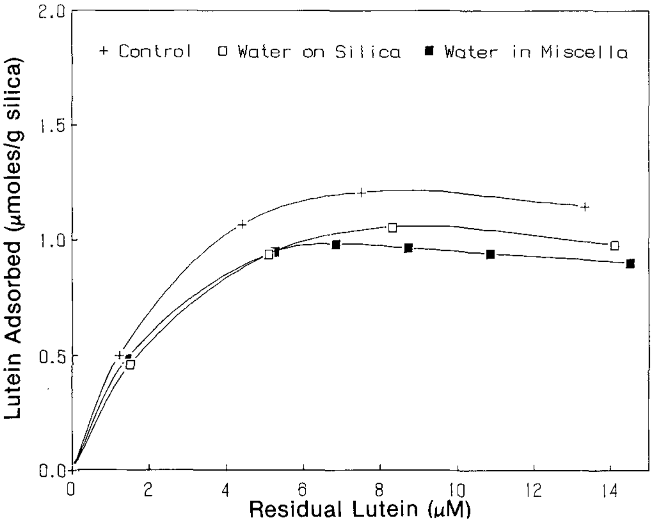

FIG. 1. Lutein isotherms were determined by incubating 100 mL alkali-refined soy oil miscellas with 0.5 g silica and stirred for 15 min in the presence of 5 mmoles water. Water was added either to the silica or the miscella before adsorption. Lutein concentrations were determined by absorbance at 445 nm. The control experiment was performed in the absence of added moisture.

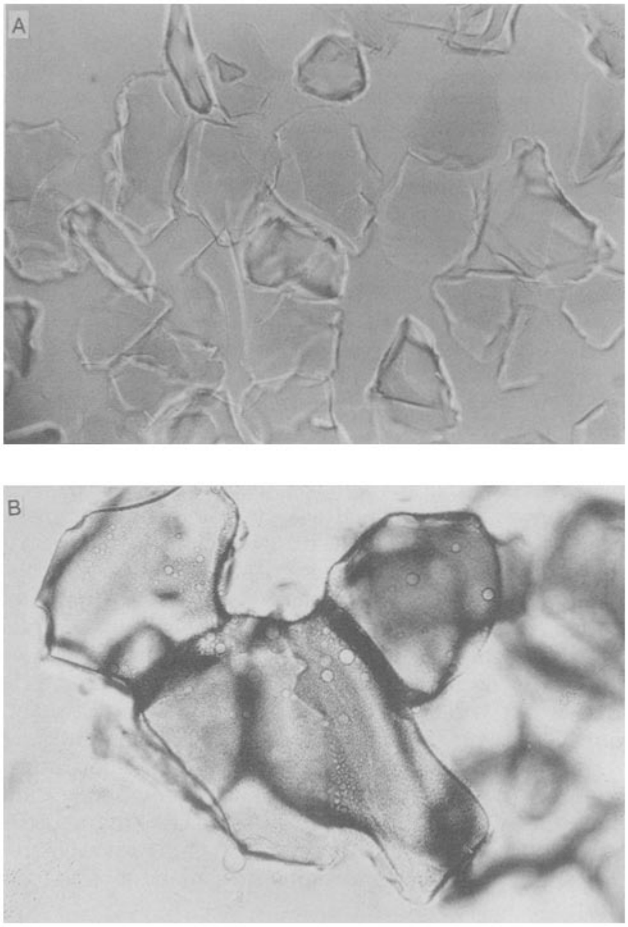

FIG. 2. A. Light photomicrograph of silica particles in the presence of moisture after adding to a soy oil miscella before mixing (×66). B. Phase-contrast photomicrograph of silica particles in the presence of moisture after adding to a soy oil miscella before mixing (×132).

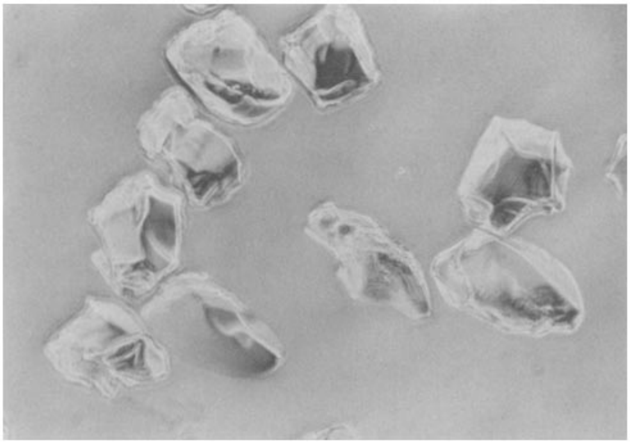

FIG. 3. Phase-contrast photomicrograph of silica particles, representative of the control experiment before and after mixing with a soy oil miscella for 15 min (×66).

the control silica fragments were various intensities of yellow. Water adsorption may occur by some cooperative binding mechanism in which bound water facilitates further binding at nearby positions. Iler (9) described moisture adsorption to dry silicic acid as clusters of water molecules rather than as a monolayer. If this occurs in the miscella, local concentrations of moisture may be created, and some particles may become more deactivated than others.

Figure 2B shows water droplets in water-treated miscellas before adsorption, which are not observed in the control at the same magnification (data not shown). This would support the suggestion of Minyu and Proctor (8) that moisture in a hydrophobic system is more stable as liquid water than as bound moisture and, thus, more slowly adsorbed. It is unlikely that these are air pockets because they did not appear in any other preparations.

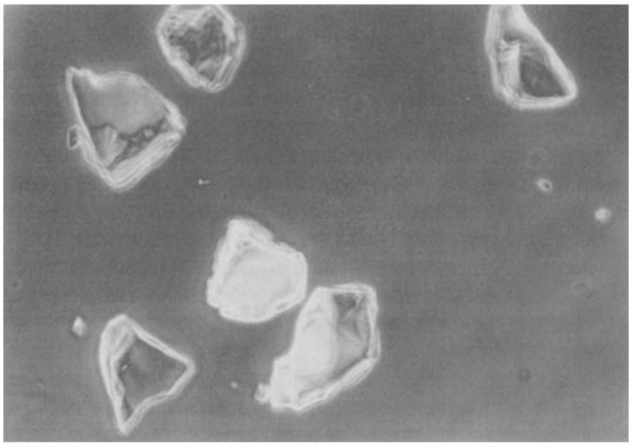

FIG. 4. Phase-contrast photomicrograph of silica particles in the presence of moisture after mixing with a soy oil miscella for 15 min (×66).

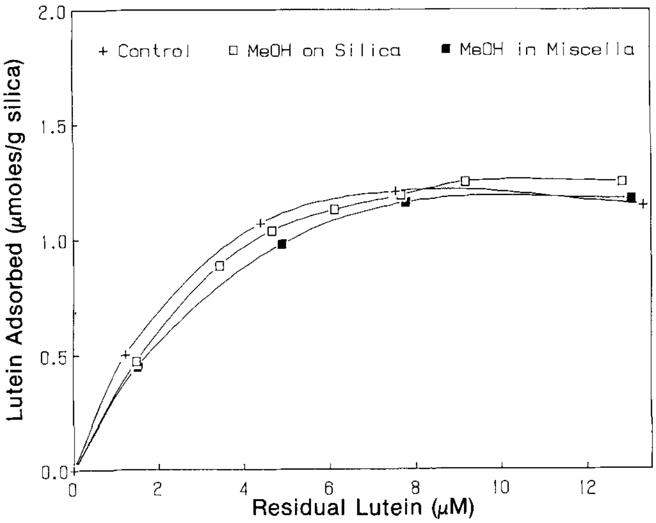

FIG. 5. Lutein isotherms were determined by incubating 100 mL alkali-refined soy oil miscellas with 0.5 g silica and stirred for 15 min in the presence of 5 mmoles methanol. Methanol was added either to the silica or the miscella before adsorption. Lutein concentrations were determined by absorbance at 445 nm. The control experiment was performed in the absence of added methanol.

The effect of added methanol on lutein adsorption is shown in Figure 5. The mode of methanol addition does not affect lutein adsorption, as was also the case with water (Fig. 1). However, the methanol isotherms are identical with that of the control. The effect of 0.05 M methanol, as used in this study, is different from the effect of 0.1 M methanol in the miscella in which there was inhibition of lutein binding relative to the control (8). However, methanol was the least effective alcohol examined by Minyu and Proctor (8). Nevertheless, the equilibrium distribution of methanol affecting lutein binding is the same for both addition to the silica and to the miscella.

Visual and light microscope appearances of silica in the methanol systems were identical to the control (data not shown).

The inhibitory effect of IPA on lutein adsorption was also independent of whether it was added to the silica or miscella (Fig. 6), and the microscopic appearance of the

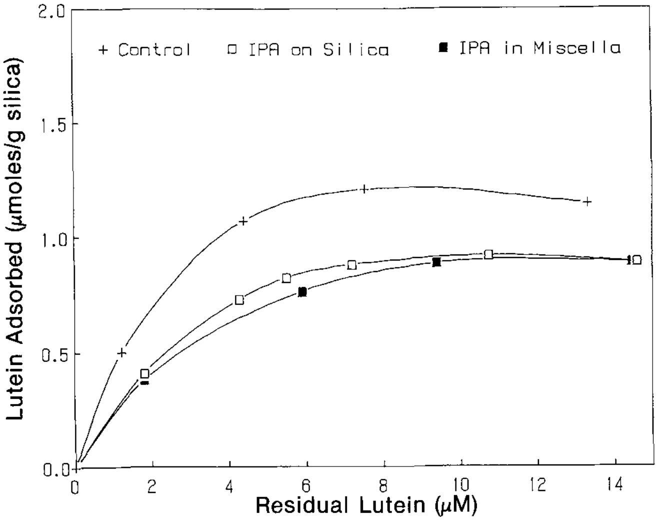

FIG. 6. Lutein isotherms were determined by incubating 100 mL alkali-refined soy oil miscellas with 0.5 g silica and stirred for 15 min in the presence of 5 mmoles isopropanol (IPA). IPA was added either to the silica or the miscella before adsorption. Lutein concentrations were determined by absorbance at 445 nm. The control experiment was performed in the absence of added IPA.

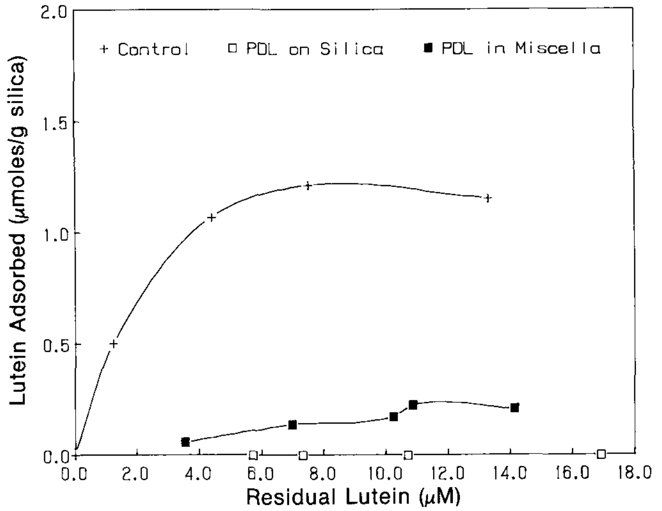

FIG. 7. Lutein isotherms were determined by incubating 100 mL alkali-refined soy oil miscellas with 0.5 g silica and stirred for 15 min in the presence of 5 mmoles propanediol (PDL). PDL was added either to the silica or the miscella before adsorption. Lutein concentrations were determined by absorbance at 445 nm. The control experiment was performed in the absence of added PDL.

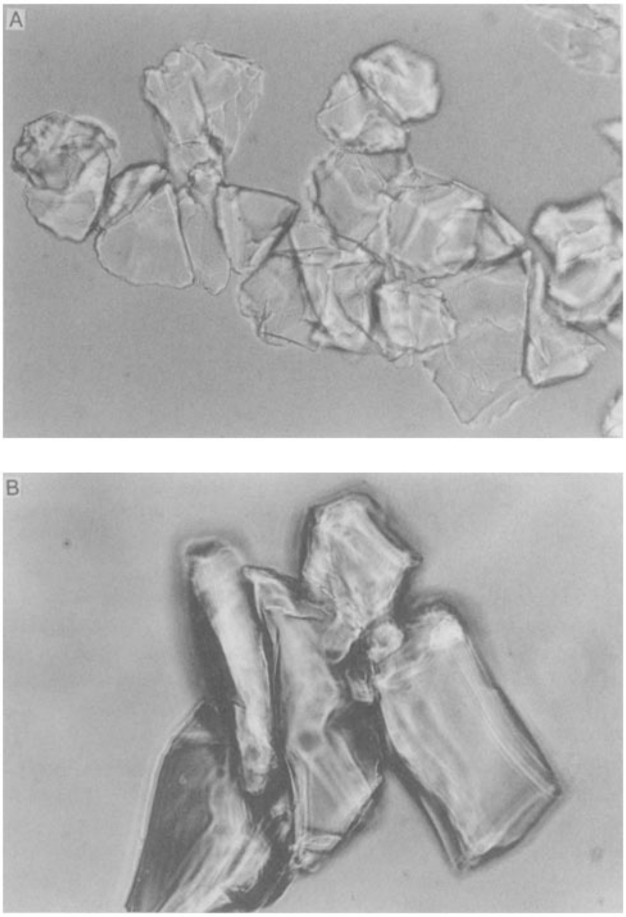

FIG. 8. A. Phase-contrast photomicrograph of silica particles in the presence of propanediol (PDL) after mixing in a soy oil miscella for 15 min (×66). B. Phase-contrast photomicrograph of silica particles in the presence of PDL after adding to a 20% soy oil miscella for 15 min (×132).

adsorbent was similar to that of the control (data not shown). IPA probably competes with lutein by binding to the adsorbent by means of the OH group, with the alkyl group exposed in the miscella. This is the most thermodynamically stable configuration and forms a "reverse-phase" surface. Iler (9) described the adsorption of tertiary butyl alcohol to silica in this manner. This seems to occur if the solvent is added to either the miscella or adsorbent (Fig. 6), and the IPA equilibrium distribution and subsequent lutein binding are also the same.

Addition of PDL caused negligible lutein adsorption or eliminated it altogether (Fig. 7). PDL caused an immediate formation of a stable white gel in the miscella, which was still intact after mixing for fifteen minutes. The microscopic appearance of the silica after incubation was similar to that of the water-treated system prior to mixing (Fig. 8). Particles aggregated and were unable to bind pigment. This is probably due to the polyol structure of PDL. Perhaps one end of the molecule hydrogen bonded readily to the silica, and the exposed terminal OH group then crosslinked to another silica particle. A network would be set up to reduce the surface area and effectively exclude lutein molecules.

To summarize, the inhibitory effect of added solvents on lutein binding is independent of whether solvent is added to the miscella or to the silica. This indicates that a similar lutein equilibria is set up between adsorbent and miscella irrespective of how the solvent is added. Inhibition of lutein adsorption can occur by a molecule occupying an adsorption site, as in the case of IPA, or by molecules with two hydrogen-bonding moieties effectively reducing the adsorbent surface area by binding adsorbent particles together. No pigment was adsorbed at the miscella/gel interface, indicating irreversible PDL binding at that surface. Possibly, IPA and other alcohols bind irreversibly, which would explain the small differences among isotherms obtained with a homologous series of alcohols (8). Evidence indicates that water binds reversibly, which may be why it is a poor competitor with lutein for adsorption sites.

These results may have implications for the adsorption of materials with significant levels of free fatty acids, monoglycerides and glycerol from lipid hydrolysis, *i.e.*, for recycling hydrolyzed frying oils. The glycerol may reduce the effectiveness of silicic acid adsorbents by crosslinking adsorbent particles.

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