# Adsorption of Lutein From Soybean Oil on Silicic Acid I. Isotherms

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**Adsorption of lutein from crude soybean oil miscella on dispersed silicic acid resulted in three different Freundlich type isotherms, depending on the amount of adsorbent used. Normally, changing the amount of adsorbent gives a new point on the same isotherm. Further investigation of the lutein adsorption revealed that addition of 1% isopropanol to the hexane solvent and deactivation of the silica acid with water decreased lutein adsorption. Purification of the lutein resulted in increased adsorption, indicating that triglyceride was competing for adsorption sites. The triglyceride competition was confirmed by calculating an adsorption isotherm for triglyceride from crude soybean oil miscella. Use of silicic acid as a column rather than dispersed yielded multiple isotherms for lutein adsorption based on the amount of adsorbent used. Also, lutein adsorption on columns was concentrated at the entrance to the column. Experiments showed that lutein adsorption was not due to partitioning between bound triglyceride and hexane solvent.**

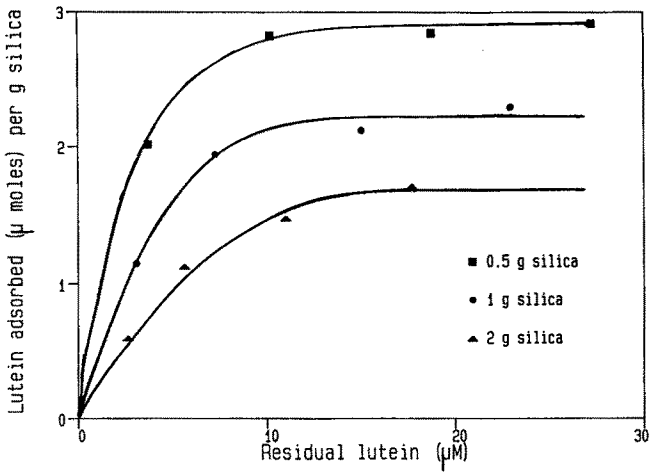


**FIG. 1. Lutein isotherms were determined by incubating 200 ml of 40%, 30%, 20% or 10% vol/vol crude soy oil/hexane miscella for 15 min at 22 C with variable amounts of silica. Lutein concentration was obtained by measuring absorbance at 445 nm.**

Freshly extracted soybean oil contains a number of components, such as pigments, phospholipids and 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 using bleaching clays (1). Soybean oil pigment is almost exclusively the carotenoid lutein (2). Hassler and Hagberg (3) showed that the adsorption of soybean oil pigment on bleaching earth occurs according to a Freundlich isotherm. The adsorption of soybean oil phospholipids and pigment on silicic acid from a soybean oil miscella also conforms to a Freundlich isotherm. (4). Conditions favoring adsorption of phospholipid are 1-2% isopropanol (IPA) in the miscella and 5% water-deactivated silicic acid (4).

The purpose of this investigation was to study the conditions favoring the adsorption of lutein on silicic acid. We wanted to know if a simultaneous adsorption of pigment and phospholipid was possible or, alternatively, the conditions required for consecutive adsorption of these oil components. In addition, the interaction between pigment and triglyceride during adsorption was explored. The long term goal is to determine the feasibility of establishing a soybean oil refining process based on adsorption that might reduce energy costs and improve soybean oil quality when compared with conventional high temperature practices.

## MATERIALS AND METHODS

*Oil solvents and adsorbent.* Unless stated otherwise, crude soybean oil obtained by commercial oil extraction and stored at 4 C was used throughout the investigation.

All solvents were of high performance liquid chromatography (HPLC) grade. Soybean oil miscellas were prepared by diluting crude soybean oil with hexane. In some experiments isopropanol (IPA) was added to increase the polarity of the miscella.

The adsorbent used was Bio-Sil A (100-200 mesh silicic acid, Bio Rad Laboratories, Richmond, California), and the term "silica" is used as a synonym for silicic acid.

*Lutein measurement.* Pigment concentration was measured as lutein by measuring optical absorbance at 445 nm, which is the wavelength of maximum absorbance of lutein in hexane (5). Measurements were made with a Varian model 634S double beam spectrophotometer. Lutein concentration was calculated using the absorbance value and appropriate $E^{1\%}_{7cm}$ (2).

*Lutein isotherms.* Lutein isotherms were determined by preparing 200-ml volumes of 10, 20, 30 or 40% (v/v) concentrations of soybean oil miscellas in hexane. The lutein content of each miscella was found before a known weight of silica was added to each miscella. The miscellas were agitated with a magnetic stirrer for 15 min in a closed vessel at 22 C with 2, 1 or 0.5 g of silica. The residual lutein concentration of the miscella was measured, and the amount of lutein adsorbed was calculated by difference. Isotherms were plotted as μmol of lutein adsorbed per g of silica vs residual μmolar concentration of lutein for each weight of silica used.

*Miscella polarity.* The adsorption experiment was repeated with 1% IPA in the miscella, and the results were compared with those obtained in the absence of IPA.

*Degree of silica deactivation.* Isotherms were determined using 1-g quantities of water-deactivated silica. Deactivation was achieved by adding a known volume of water to 100-g quantities of silica. The flask containing the silica was rotated several times to break up the water droplets, then stoppered and left to equilibrate overnight. Deactivated silicas with 15%, 10% and 5% water were prepared.

Isotherms using 1-g quantities of deactivated silicas

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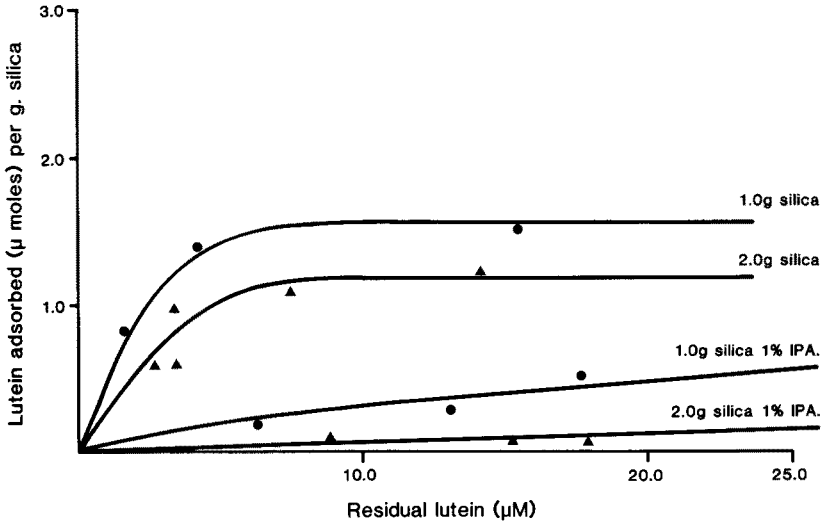


**FIG. 2. Lutein isotherms were determined by incubating 200 ml crude soy oil/hexane miscellas of variable oil concentrations for 15 min at 22 C with variable silica and IPA. Lutein concentration was obtained by measuring absorbance at 445 nm.**

were obtained, and the results were compared with the isotherm produced using active silica.

*Partial purification of lutein.* A lutein-rich extract was obtained for isotherm analysis. Six g of silica were stirred with a miscella of 200 ml soybean oil and 200 ml hexane for 20 min. The silica was allowed to settle, and the miscella was decanted from the adsorbent. The silica was washed with 20 ml of hexane and the hexane removed. Adsorbed species were desorbed with 100 ml of 10% ethanol in hexane, and the solvents were removed by rotary evaporation. The residue was placed in 4 ml hexane at 0 C for 36 hr, and a colorless precipitate was filtered out periodically. The sample was placed on a hexane wetted Sep-Pak silica cartridge (Waters Associates, Milford, Massachusetts) and subjected to a polarity gradient in hexane as follows: 2%, 3%, 5%, 7%, 10% acetone and 1.5%, 5% and 10% ethanol. The ethanol and 10% acetone fractions were pooled and rotary dried, and the residue was placed in 2 ml hexane until a red precipitate appeared; this happened after 12 hr.

Dilutions of the extract were made for isotherm studies. Because of sample limitations, 50-ml volumes of lutein extract and 0.25 g of silica were used. The data obtained were compared with the isotherm from 200 ml of a soybean oil miscella with 1 g silica.

*Oil-free lutein.* An isotherm was determined using purified lutein (Carl Roth KG, D75 Karlesruhe 21, West Germany, distributed by Atomergic Chemetals Corp., Plainview, New York). Variable amounts of lutein were dissolved in 200-ml volumes of hexane, and the dissolved pigment was exposed to 1 g silica. This isotherm was compared with that of a soybean oil miscella exposed to 1 g silica.

*Triglyceride isotherm studies.* Triglyceride isotherms were determined by stirring 0.5, 1.0 or 2.0 g silica with 200-ml volumes of refined soybean oil miscellas for 15 min. Triglyceride concentrations were found by measuring the absorbance at 210 nm and were calculated as triolein with the aid of a standard curve.

*Triglyceride effect.* The effect of previously sorbed tri-

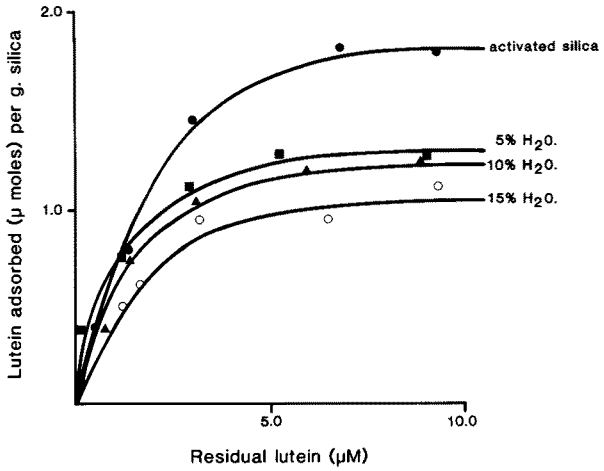


**FIG. 3. The effect of water-deactivated silica on lutein isotherms was determined by incubating 1 g silica, which had been water deactivated to varying degrees, with 200 ml of 40%, 30%, 20% or 10% vol/vol crude soy oil/hexane miscellas for 15 min at 22 C. Lutein concentration was obtained by measuring absorbance at 445 nm.**

glyceride on the lutein isotherm was evaluated. Five g silica were incubated with 400 ml of 40% refined oil miscella for 15 min at 22 C. The silica was recovered and washed with hexane. It was divided into five portions of equal weight (i.e., 1 g silica and adsorbed oil), which were used to prepare a lutein isotherm. The isotherm was obtained as previously described except in volume of 100 ml. This isotherm was compared with that of 1 g fresh silica.

*Column adsorption isotherms.* Variable quantities of silica were placed in a pyrex column of 16 mm i.d. and 3 mm wall thickness. The adsorbent bed was supported by Pharmacia AC adapters and column fittings (Pharmacia, Piscataway, New York). Two hundred ml of 10, 20, 30 or 40% v/v soybean oil miscellas in hexane were pumped over 0.5, 1.0 or 2.0 g of silica by a Masterflex Speed Con-

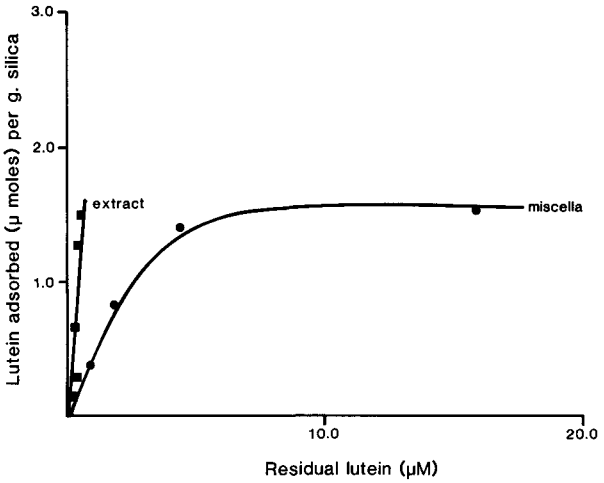


**FIG. 4. The isotherm of a lutein extract of crude soy oil miscella in hexane was determined relative to that of a crude soy oil/hexane miscella. Miscella concentrations were 40%, 30%, 20% or 10% vol/vol. 1.0 g silica/200 ml miscella or lutein extract was incubated for 15 min at 22 C. Lutein concentration was obtained by measuring absorbance at 445 nm.**

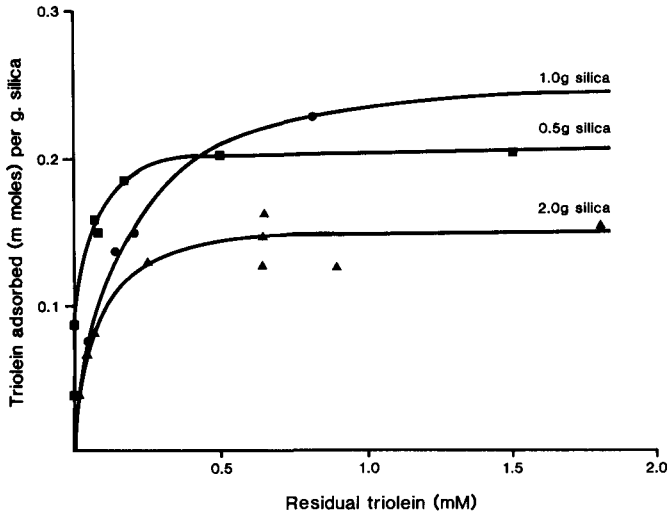


**FIG. 5. Triglyceride isotherms were determined with different amounts of silica by incubating 200 ml oil/hexane miscellas of different triglyceride concentrations for 15 min at 22 C. Triglyceride concentration was obtained by measuring absorbance at 210 nm and was calculated as triolein.**

troller Pump (Cole Parmer Instrument Co., Chicago, Illinois) at a speed of 11 ml/min. The amount of lutein adsorbed was measured and isotherms calculated.

*Lutein and oil distribution on a column.* Two hundred ml of 20% soybean oil/hexane miscella was passed over a 7-g column of silica. The cylinder of silica was removed from the Pyrex column intact, and the solvent present was allowed to evaporate overnight. Four 2-g sections of the silica were taken, proceeding from the exit of the column. The fifth section was in excess of 2 g. Oil and pigment were eluted from each 2-g section of silica with 25 ml IPA. Solvent was evaporated in a drying oven at 100 C, and the oil was weighed. The oil fractions were each dissolved in 25 ml hexane for lutein measurement.

## RESULTS AND DISCUSSION

Figure 1 shows three different Freundlich type isotherms for the three different amounts of silicic acid used to adsorb lutein in a dispersed system. A similar phenomenon was observed for phospholipid adsorption in silicic acid (4). The multiple isotherms for lutein and phospholipid adsorption are unique and to our knowledge have not been reported or discussed previously.

The phospholipid adsorption on silicic acid (4) is irreversible, but lutein adsorption was reversible as indicated by release of lutein from silicic acid when incubated with lutein-free miscella (data not shown). The usual interpretation of reversible adsorption is that the residual or free solute is in equilibrium with adsorbed solute. With the results shown in Figure 1, a single residual lutein concentration would have to be in equilibrium with three different concentrations of adsorbed lutein. Because adsorption from complex mixtures is an important industrial procedure and an important procedure in analysis, subsequent experiments were designed to understand this complex adsorption behavior better.

Previous results with phospholipid adsorption (4) showed that increasing the polarity of the solvent system by adding isopropanol (IPA) increases phospholipid adsorption. Also, deactivating silicic acid with water in-

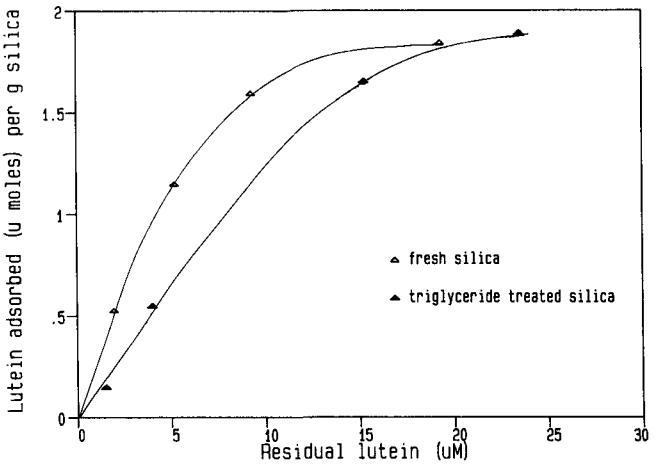


**FIG. 6. The lutein isotherm was determined by incubating 100 ml of 40%, 30%, 20% or 10% vol/vol crude soy oil/hexane miscella for 15 min at 22 C with 1-g quantities of silica/triglyceride complex obtained by incubating 5.0 g silica with 400 ml of a 40% refined oil miscella. Lutein concentration was obtained by measuring absorbance at 445 nm.**

creases phospholipid adsorption (4). Figure 2 shows that 1% IPA addition to the miscella decreased lutein adsorption. Figure 3 shows that deactivating silicic acid with water decreased lutein adsorption. So, although both phospholipid and lutein showed multiple isotherms based on the amount of silicic acid used, the adsorption of the two components of crude soybean oil responded differently to IPA and silicic acid deactivation.

Lutein was isolated from crude soybean oil and partially purified. When this partially purified lutein was exposed to silicic acid adsorption, the results shown in Figure 4 were obtained. By increasing the purity of the lutein (decreasing triglyceride in the miscella), much greater adsorption on silicic acid was demonstrated. A subsequent adsorption experiment with highly purified lutein under the same conditions as used for Figure 4

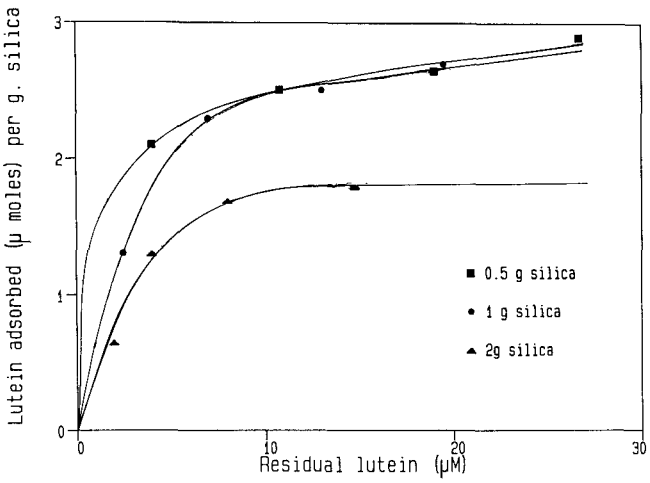


**FIG. 7. Lutein isotherms were determined by passing 200 ml of 40%, 30%, 20% or 10% vol/vol crude soy oil/hexane miscella over silica columns of variable height at a speed of approximately 11 ml/min. Lutein concentration was obtained by measuring absorbance at 445 nm.**

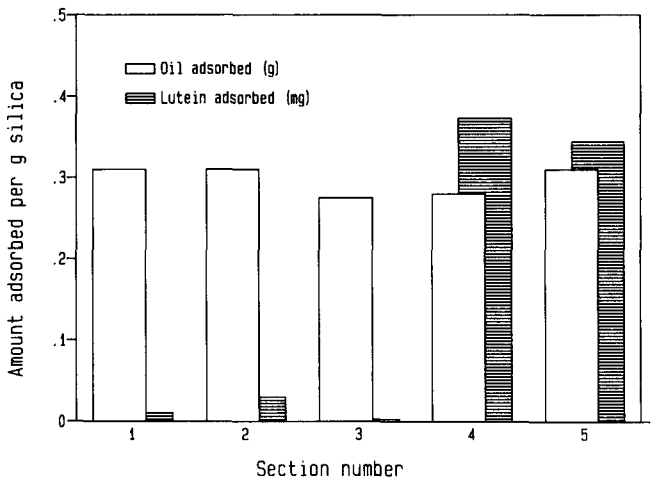


**FIG. 8. Oil and lutein content of a 7.0-g silica column over which 200 ml of a crude soy oil/hexane miscella had been passed was measured. Oil content of each section of the silica column plug was found by eluting fractions with IPA and weighing the dry residue. Lutein content was found by absorbance at 445 nm of each residue in 25 ml hexane. Numbers 1-4 refer to 2.0-g weights of silica sectioned from the exit of the column. Number 5 was a 2.43-g section nearest the entrance to the column.**

demonstrated that silica completely adsorbs highly purified lutein (no residual) up to 1μmol/g of silicic acid (data not shown).

The change in the lutein adsorption isotherm upon purification of lutein indicated that triglyceride might play a role in the adsorption of lutein on silicic acid. Therefore, we analyzed for total and free triglyceride in crude soybean oil miscellas after adsorption by silicic acid and found that triglyceride was bound to silicic acid under the same conditions as for lutein adsorption and that triglyceride adsorption was much greater than lutein adsorption (Fig. 5). Approximately 200 μmol triglyceride was adsorbed per gram of silicic acid (1 g in 200 ml) compared to 2 μmol lutein.

The results shown in Figures 4 and 5 indicated there was competition between lutein and triglyceride for adsorption sites on silicic acid. We experimented with exposing silicic acid to triglyceride miscella containing no lutein, washing all nonadsorbed triglyceride free, and exposing that silicic acid to a crude soybean oil miscella. The results in Figure 6 showed that prior exposure to triglyceride decreased the adsorption of lutein at low residual lutein but had little effect at high residual lutein concentrations.

For bleaching crude soybean oil miscella, it might be convenient to flow the miscella over a fixed bed of adsorbent rather than disperse the adsorbent in miscella. Therefore, experiments were done in which 0.5, 1 or 2 g of silicic acid were prepared in the form of small columns and crude soybean oil miscella was pumped over the columns. The results are shown in Figure 7. With packed silicic acid, the lutein adsorptions for 0.5 and 1 g silicic acid were essentially the same, but for 2 g silicic acid, the lutein adsorption/g silicic acid was definitely lower.

With the silicic acid in the form of a column, we observed that a band of lutein was adsorbed initially at the entrance to the column. That band broadened with continued pumping of miscella, but unadsorbed pigment was flowing off the column at the same time that areas of the adsorbent bed were visibly colorless.

A possible explanation for the localization of lutein at the entrance to the column could be that most adsorption of triglyceride occurred at the entrance, and lutein was partitioning between the solvent and bound triglyceride. To test this possibility a 7-g column of silicic acid was prepared, and miscella was pumped over it. Then the column material was removed as a plug, divided into five sections and analyzed for lutein and triglyceride. The triglyceride was evenly distributed across the column, but lutein was concentrated at the entrance (Fig. 8). Thus, partitioning of lutein between bound triglyceride and solvent was not a likely possibility for the concentration of lutein at the column entrance.

The results of adsorption experiments with silicic acid and crude soybean oil miscellas showed that in the presence of triglyceride, the lutein adsorption was different than with purified lutein. The competition between lutein and triglyceride adsorption may also explain why multiple isotherms for lutein were obtained with different amounts of adsorbent.

The concentration of adsorbed lutein at the entrance of a column of adsorbent has no ready explanation, but the concentration could be used to maximize lutein removal from crude soybean oil. The results suggest that a shallow bed of adsorbent with a large area would adsorb more lutein than the same amount of adsorbent in the form of a narrow column.

We plan to experiment further with this system by measuring kinetics of lutein and triglyceride adsorption to learn more about the anomalies observed.

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