# Inhibitory Effect of 1,3-Diglycerides During Adsorption of β-Carotene onto Attapulgite and Sepiolite

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**ABSTRACT:** The adsorption isotherms of β-carotene on attapulgite and sepiolite were measured in the presence of 1,3-diglyceride in *n*-hexane to elucidate the relative inhibitory power of the diglycerides, hydroxyl group, carbon number, and double bond for adsorption bleaching. The adsorption mode of β-carotene was the Langmuir type. The inhibition of β-carotene adsorption may be caused by the polarity of diglycerides and the formation of diglyceride micelles. The relative inhibitory power of 1,3-diolein, 1,3-distearin, and 1,3-dipalmitin toward β-carotene adsorption was 1.49, 1.40, and 0.99, respectively. Therefore, the relative inhibitory power due to the hydroxyl group and increase in the carbon atom of the fatty acid in glyceride was 0.41–0.49.

Paper no. J9793 in *JAOCS 78*, 733–736 (July 2001).

**KEY WORDS:** Attapulgite, β-carotene adsorption, 1,3-diglyceride, relative inhibitory power, sepiolite, triglyceride.

Crude vegetable oil is processed by degumming, alkali-refining, bleaching, and deodorizing. During the bleaching process, adsorption of the triglyceride onto the adsorbent is responsible for "neutral oil loss." Proctor and Snyder (1) showed that triglycerides compete with lutein on silicic acid in crude soybean oil miscella. Chapman and Pfannkoch (2) later showed that triglycerides suppress the adsorption of porphyrin on acid-activated clay in a noninteracting solvent. Competitive adsorption from sesame oil indicated that the adsorption process of vegetable oils (i.e., bleaching) has to be considered a multicomponent adsorption system (3). Studies of competitive adsorption bleaching on attapulgite and sepiolite showed that β-carotene is more strongly adsorbed than triolein (4), that one molecule of triolein inhibits the sorption of 0.84 molecules of β-carotene (5), and that trilinolein inhibits 1.2 times more strongly than triolein (6,7). However, none of these studies attempted to quantitatively determine the inhibitory power of diglycerides for adsorption bleaching.

During bleaching, up to approximately 40% of the oil is retained in the adsorbent (8). In order to cope with adsorption during bleaching, we attempted to determine the competitive amount of glyceride, as well as the β-carotene, adsorbed from the vegetable oil. Our attempt was unsuccessful because the adsorbent surface is covered with multiple layers of oil. Therefore, the theoretical inhibition power of glyceride upon the β-carotene adsorption was evaluated in a dilute glyceride/β-carotene hexane solution in which their mutual interaction could be investigated. The information can be used in adsorption bleaching and miscella bleaching studies (3).

The purpose of this investigation was to study quantitatively the relative inhibitory power (due to the 1,3-diglyceride, hydroxyl group, carbon number, and double bond) that is basically common to the β-carotene adsorption onto the different types of attapulgite and sepiolite suitable for bleaching from the alkali-refined vegetable oils (9,10). The adsorption isotherm of β-carotene from the 1,3-diglyceride hexane solution was measured to determine the adsorption capacity of the adsorbent (11). The inhibitory power was statistically calculated using the maximal amounts of β-carotene adsorbed per unit surface area for eight kinds of adsorbents. The relative inhibitory power was discussed by using the significant difference in the maximal amounts adsorbed between no glyceride and 1,3-diglyceride or triglyceride.

## EXPERIMENTAL PROCEDURES

*Materials.* β-Carotene (Type I, 95%; Sigma Chemical Co., St. Louis, MO), 1,3-dipalmitin, 1,3-distearin, 1,3-diolein (Sigma, 99%), and *n*-hexane (analytical reagent grade, Wako Pure Chemical Industries, Tokyo, Japan) were used. The attapulgites were: A-1 [low-temperature-dried, particle diameter (PD) < 75 μm], A-2 (high-temperature-dried, PD < 150 μm), A-3 (regular-volatile-matter sorptive, PD < 75 μm), A-4 (pharmaceutical, PD < 150 μm), A-5 (cosmetic, PD < 150 μm), and A-6 (low-volatile-matter sorptive, 250 < PD < 500 μm). Attapulgites were donated by Tsuchiya Kaolin Industries, Ltd. (Tokyo, Japan). The sepiolites were: S-1 (powder, American origin, PD < 75 μm), S-2 (powder, Turkish origin, PD < 75 μm), S-3 (spherule, Turkish origin, PD < 75 μm), S-4 (fiber, Chinese origin, size, 1000 × 1 μm), S-5 (fiber, Chinese origin, size, 5000 × 1 μm), S-6 and S-7 (powder, American origin, PD < 75 μm). Sepiolites were donated by Fuji Talc Industries, Ltd. (Tokyo, Japan). Standard activated clay (SAC) was supplied by Wako (PD < 75 μm). Bleaching media were dried at 110°C for 15 h before use.

*Procedure for adsorption isotherm.* The adsorption isotherm of β-carotene was measured by a previously described method (5). The *n*-hexane solutions of different con-

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centrations of β-carotene with 850 μM 1,3-diglyceride were prepared. The adsorbent (0.10 g) was added into a light-resistant hermetic glass container containing the solution (8.0 mL), and the slurry was incubated in a water bath shaker at 25°C for 200 min. The residual β-carotene in the supernatant was then determined by using a UV-1200 Shimadzu spectrophotometer at 453 nm (Shimadzu Corporation, Kyoto, Japan). The adsorption isotherm obtained could be fitted to the Langmuir equation. The maximum amount adsorbed ($V_m$) and the constant ($K_a$) were calculated from the slope and intercept, respectively, of the linearized isotherm. The adsorption free energy ($\Delta G$) was obtained by the following equation, $\Delta G = -RT \ln K_a$ ($R$, gas constant; $T$, absolute temperature).

*Statistical analysis.* Since the amounts adsorbed per gram of attapulgite and sepiolite were proportional to their surface areas (4), the maximal amounts adsorbed per surface area were calculated (9,10). They were used to statistically infer the relative inhibitory power of glycerides toward β-carotene adsorption. The significance level was performed by multiple comparison using Scheffe's method in the SuperANOVA program (Abacus Concepts, Inc., Berkeley, CA).

## RESULTS AND DISCUSSION

Figures 1 and 2 show the adsorption isotherms of β-carotene on attapulgite and sepiolite, respectively, from solution in the presence of no diglyceride, 1,3-dipalmitin, 1,3-distearin, and 1,3-diolein. The amount adsorbed decreased to half in the presence of 1,3-dipalmitin. The order of decrease was 1,3-dipalmitin > 1,3-distearin > 1,3-diolein. These decreases were caused by the inhibitory power of each diglyceride.

The adsorption data could be fitted to the Langmuir isotherm. Figure 3 shows typical examples of the linear Langmuir plots of A-1 and S-1 in Figures 1 and 2. These results indicate that the adsorption mode of β-carotene is not varied by the presence of 1,3-dipalmitin, 1,3-distearin, or 1,3-diolein. The maximal amounts adsorbed, being the Langmuir constant, are shown in Table 1. The fitness of the Langmuir equation suggests that β-carotene is adsorbed as a monolayer on the adsorbent.

Figure 4 shows the amounts of β-carotene adsorbed per surface area as statistically obtained using the data of A-1–A-3, A-5, S-1, S-2, S-6, and S-7 in Table 1. The amounts adsorbed in the presence of triolein and trilinolein were calculated using the data from previous reports (5,6). Since the maximal amounts adsorbed (μmol/m$^2$) represent the values obtained under the condition that two compositions exist, each difference in maximal amount adsorbed (μmol/m$^2$) between none and 1,3-dipalmitin, 1,3-distearin, 1,3-diolein, triolein, or trilinolein in Figure 4 is defined as the inhibitory power of the dissolved composition.

Significant differences in the maximal amount adsorbed were observed between no glyceride and 1,3-dipalmitin, 1,3-distearin, 1,3-diolein, triolein, or trilinolein. The inhibition power was in the order 1,3-diolein and 1,3-distearin > trilinolein, triolein, and 1,3-dipalmitin. Adsorption of β-carotene was inhibited by the competitive adsorption of triolein or trilinolein on the adsorbent's surface (5,6). However, from the results in Figure 4, it is not clear whether the inhibition due to the diglyceride is affected by the interaction of β-carotene with it in the hexane phase, the competitive adsorption of them on the adsorbent surface, or both. Table 2 shows the apparent adsorption free energy of β-carotene. The $\Delta G$ value indicates that β-carotene is physically adsorbed (12) on the adsorbent surface. The adsorption free energy in decreasing order was none, 1,3-dipalmitin, and 1,3-distearin, as well as in the reverse order of their inhibitory power. The results suggest that the binding force for the adsorption of β-carotene was decreased by the presence of diglyceride; the greater the inhibitory power was, the weaker the binding force. This can best be explained by assuming that β-carotene partially or fully covers the adsorbed diglyceride, being the same as that

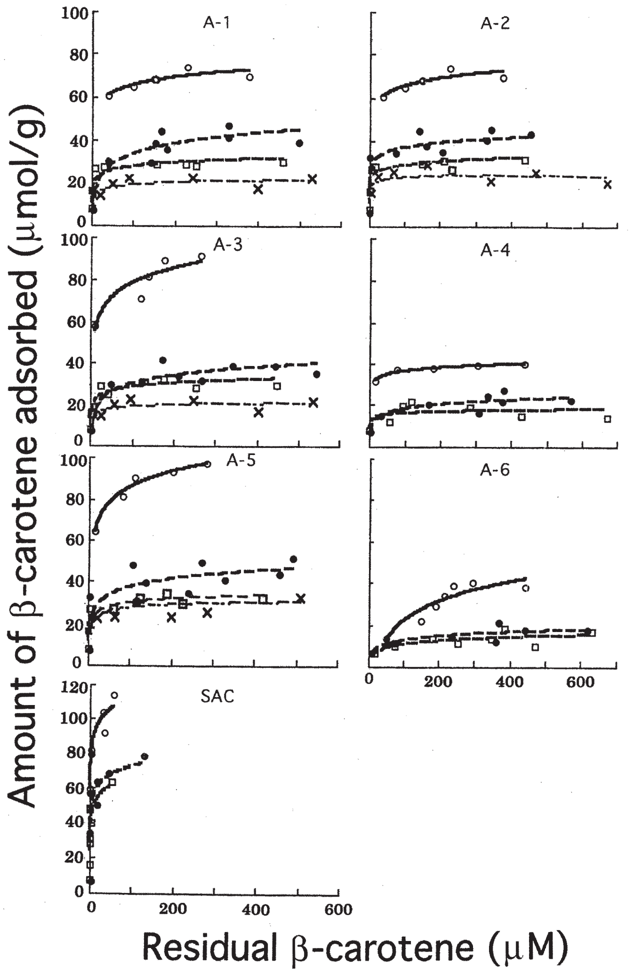

**FIG. 1.** Effect of diglyceride on adsorption isotherm of β-carotene onto attapulgite and standard activated clay (SAC). ○, no diglyceride; ●, 1,3-dipalmitin; □,1,3-distearin; ×, 1,3-diolein; A-1, low-temperature dried attapulgite (A), particle diameter (PD) < 75 μm; A-2, high-temperature dried, PD < 150 μm; A-3, regular-volatile-matter sorptive; PD < 75 μm; A-4, pharmaceutical, < 150 μm; A-5, cosmetic, PD < 150 μm; A-6, low-volatile-matter sorptive, 250 < PD < 500 μm. Attapulgites donated by Tsuchiya Kaolin Industries, Ltd. (Tokyo, Japan).

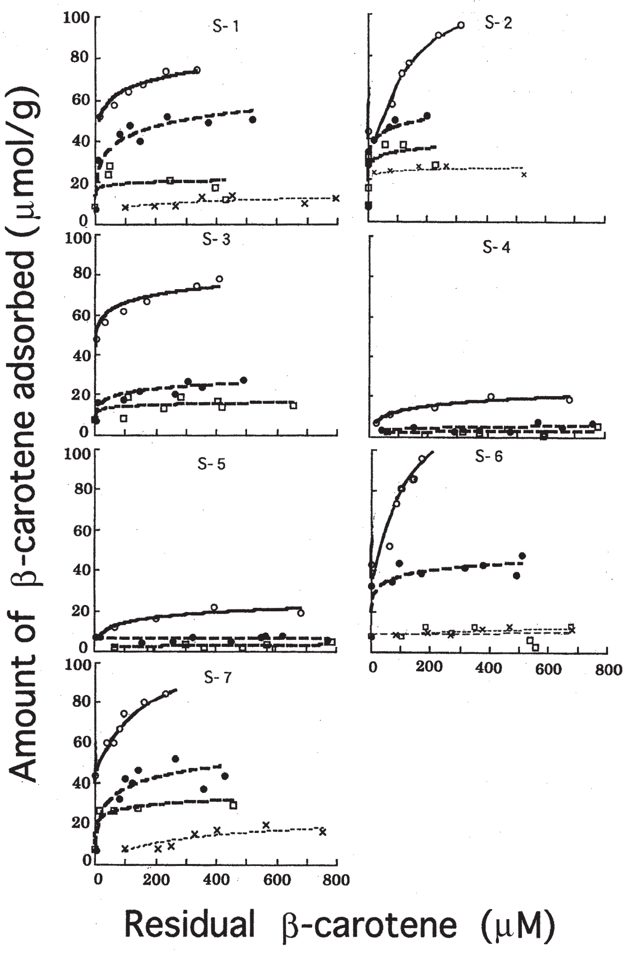

**FIG. 2.** Effect of diglyceride on adsorption isotherm of β-carotene onto sepiolite. ○, No diglyceride; ●, 1,3-dipalmitin; □, 1,3-distearin; ×, 1,3-diolein; S-1, sepiolite (s)-powder, American origin, PD < 75 μm; S-2, powder, Turkish origin, PD < 75 μm; S-3, spherule, Turkish origin, PD < 75 μm; S-4, fiber, Chinese origin, size 1000 × 1 μm; S-5, fiber, Chinese origin, size 5000 × 1 μm; S-6 and S-7, powders, American origin, PD < 75 μm. Sepiolites donated by Fuji Talc Industries, Ltd. (Tokyo, Japan). For other abbreviation see Figure 1.

described for the competitive adsorption of β-carotene and triolein (13).

Significant differences were observed between triolein and 1,3-diolein or 1,3-distearin, as shown in Figure 4. In order to compare the inhibitory power of diglycerides or functional groups, the relative inhibitory power was expressed relative to triolein. Triolein was selected as a standard because it is one of the main triglycerides in vegetable oils and it is the optimal one in comparing with the diglycerides in structural difference. The relative inhibitory power was obtained by dividing the differences between none and 1,3-diglycerides by the difference (0.228 μmol/cm$^2$) between none and triolein in Figure 4. The relative inhibitory power of 1,3-diolein and 1,3-distearin with the same chainlength fatty acids was 1.49 and 1.40 times, respectively, compared to triolein. One of their structural differences is the hydroxyl group at the 2-position carbon of the glyceride. These diglycerides are supposed to be smaller than triolein in molecular size, due to their tuning fork-type structure. If both β-carotene and diglyceride or triolein are adsorbed on the adsorbent surface according to their molecular sizes, the amount adsorbed will be larger in the presence of 1,3-diglyceride rather than triolein, that is, the inhibitory power of diglyceride will be smaller than that of triolein. The data showed exactly the opposite effect. Therefore, the results suggest that the polarity of the diglyceride significantly inhibits the β-carotene adsorption. This seems to indicate that the diglyceride interacts with the β-carotene as a polar basic compound (14) in the hexane phase and on the ad-

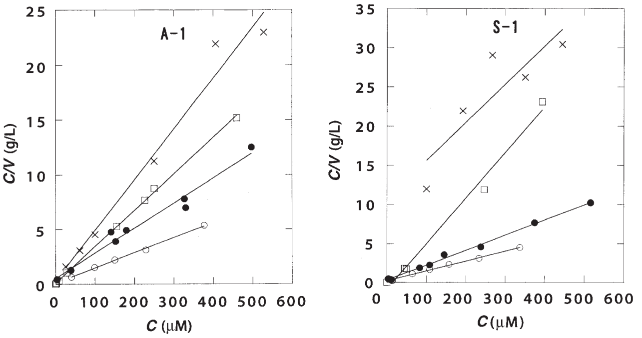

**FIG. 3.** Langmuir plots of β-carotene adsorption isotherms on adsorbents A-1 and S-1. ○, no diglyceride; ●, 1,3-dipalmitin; □, 1,3-distearin; ×,1,3-diolein. For abbreviations see Figures 1 and 2.

**TABLE 1**
**Maximal Amounts of β-Carotene Adsorbed from Diglyceride/Hexane Solution at 25°C**

| | Maximal amount of β-carotene adsorbed (μmol/g) | | | |
|---|---|---|---|---|
| Adsorbent[a] | None | 1,3-Dipalmitin | 1,3-Distearin | 1,3-Diolein |
| A-1 | 72.5 | 43.2 | 30.0 | 25.7 |
| A-2 | 91.5 | 44.9 | 31.2 | 24.0 |
| A-3 | 95.8 | 37.6 | 29.5 | 21.5 |
| A-4 | 40.5 | 24.2 | 14.0 | — |
| A-5 | 100.4 | 48.5 | 32.4 | 34.4 |
| A-6 | 55.8 | 26.4 | 18.9 | — |
| S-1 | 77.5 | 51.8 | 17.2 | 13.4 |
| S-2 | 101.2 | 61.4 | 28.5 | 23.5 |
| S-3 | 79.3 | 27.9 | 14.8 | — |
| S-4 | 21.3 | 7.3 | 1.8 | — |
| S-5 | 20.6 | 7.2 | 4.2 | — |
| S-6 | 114.0 | 43.6 | 14.0 | 12.9 |
| S-7 | 88.4 | 43.1 | 27.2 | 25.5 |
| SAC | 110.7 | 83.5 | 65.1 | — |

[a] A-1, low-temprature dried attapulgite (A), particle diameter (PD) < 75 μm; A-2, high-temperature dried, PD < 150 μm; A-3, regular-volatile-matter sorptive, PD < 75 μm; A-4, pharmaceutical, PD < 150 μm; A-5, cosmetic, PD < 150 μm; A-6, low-volatile-matter sorptive, 250 < PD < 500 μm; S-1, sepiolite (S)-powder, American origin, PD < 75 μm; S-2, powder, Turkish origin, PD < 75 μm; S-3, spherule, Turkish origin, PD < 75 μm; S-4, fiber, Chinese origin, size 1000 × 1 μm; S-5, fiber, Chinese origin, size 5000 × 1 μm; S-6 and S-7, powders, American origin, PD < 75 μm. Attapulgites donated by Tsuchiya Kaolin Industries, Ltd. (Tokyo, Japan); sepiolites donated by Fuji Talc Industries, Ltd. (Tokyo, Japan). The amounts adsorbed on A-4, A-6, and S-3–S-5 were not tabulated because they were comparatively small and widely varied.

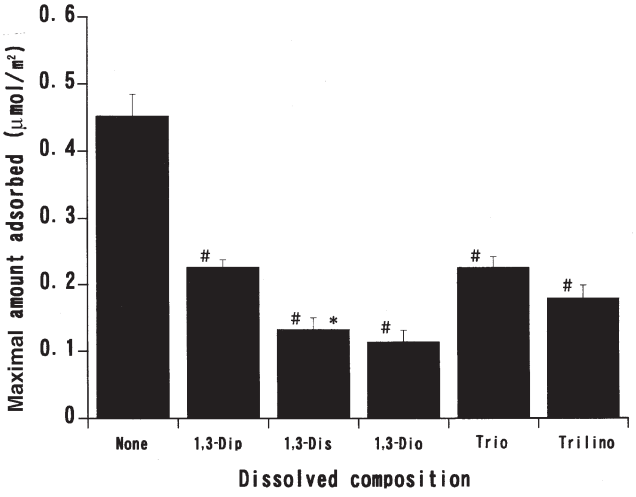

**FIG. 4.** Maximal amount of β-carotene adsorbed per surface area in the presence of different glycerides (values for triolein and trilinolein calculated from data in References 5 and 6). #, $P < 0.01$: none *vs.* 1,3-dipalmitin (Dip), 1,3-distearin (Dis), 1,3-diolein, (Dio), triolein (Trio), and trilinolein (Trilino); 1,3-diolein vs. triolein; 1,3-distearin vs. triolein. *, $P < 0.05$ : 1,3-distearin vs. 1,3-dipalmitin.

sorbent surface. The results also suggest that diglyceride miscelles are formed in hexane and that a portion of β-carotene may be taken into the micelle structures (15,16). Formation of the diglyceride micelles in solution may limit the β-carotene adsorption. The relative inhibitory power of the hydroxyl group was 0.41–0.49 times as strong as that of triolein.

Significant differences were observed between 1,3-dipalmitin and 1,3-diolein or 1,3-distearin. These results indicate that the increase in the carbon number of the fatty acids with even carbon atoms increases the inhibitory power, being 0.41–0.49 times as strong as that of triolein. The fact that the inhibitory power of 1,3-dipalmitin is almost the same as that of triolein can be explained by assuming that the inhibitory power due to the hydroxyl group is compensated by the decrease in the carbon numbers.

**TABLE 2**
**Apparent Adsorption Free Energy of β-Carotene from Diglyceride/Hexane Solution**

| | Adsorption free energy (kcal/mol) | | |
|---|---|---|---|
| Adsorbent[a] | None | 1,3-Dipalmitin | 1,3-Distearin |
| A-1 | −7.01 | −6.44 | −5.45 |
| A-2 | −6.68 | −6.50 | −5.86 |
| A-3 | −6.40 | −6.62 | −6.40 |
| A-4 | −6.92 | −6.52 | −6.27 |
| A-5 | −6.68 | −6.16 | −6.10 |
| A-6 | −5.45 | −5.39 | −5.45 |
| S-1 | −6.51 | −6.50 | −6.10 |
| S-2 | −6.10 | −6.93 | −5.86 |
| S-3 | −6.51 | −6.26 | −6.27 |
| S-4 | −5.86 | −5.32 | −5.86 |
| S-5 | −6.10 | −5.89 | −5.86 |
| S-6 | −6.10 | −6.74 | −5.86 |
| S-7 | −6.51 | −7.06 | −6.60 |

[a] For abbreviations see Table 1.

Since no significant differences were observed between the triolein and trilinolein or between the 1,3-distearin and 1,3-diolein, one may conclude that the double bond of the glyceride had no significant inhibitory power.

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[Received October 24, 2000; accepted April 13, 2001]