COMPARATIVE STUDIES ON THE ADSORPTION BEHAVIOR OF CRUDE VITAMIN A, CAROTENE, AND CHOLESTEROL

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INTRODUCTION

Work in this laboratory on the adsorption behavior of vitamin A and related substances was initiated in 1929-30 when two of us (H. N. H., V. G. L.) attempted the concentration of this vitamin from the non-saponifiable portion of cod liver oil. Almost from the first we directed our efforts to adsorption methods of separating vitamin A in order to minimize oxidation or other undesired chemical reactions.

Little has been published on the adsorption of vitamin A. Kobayashi and Yamamoto (1) observed a color reaction with Japanese acid clays, Florida clays, and fuller's earth in contact with cod liver oil or solutions of vitamin A in volatile solvents. Lachat, Dutcher, and Honeywell (2) stated that vitamin A in cod liver oil may be adsorbed on highly activated silica gel so tenaciously that it cannot be rendered available when the silica gel is fed to rats. Toluene, they observed, removes the adsorbed vitamin A from the silica gel, while acetone extracts are inactive. Still more recently von Euler and Karrer (3) made reference to the use of fibrous alumina as an adsorbent in concentration studies on this vitamin.

It should be thoroughly understood that mere removal of vitamin A from a solvent is not necessarily due to adsorption, but may result from chemical destruction or from both. Effective adsorption implies the possibility of later liberating or recovering the vitamin from the porous solid used.

Willstätter has made most effective use of various forms of alumina in concentrating enzymes from water systems, and various
workers have used clays and similar materials in concentrating enzymes and even the water-soluble vitamins from water. However, it is a very different matter to adsorb and recover the fat-soluble vitamins; new difficulties, such as the problem of evaporating fats, present themselves.

Throughout our 1st year of work, vigorous efforts were made to improve the adsorption technique. In all of the work the Norris and Church (4) modification of the Carr-Price antimony trichloride reaction was used in estimating the amount of vitamin A present. It was felt that this chemical determination was sufficiently specific and accurate for the work since cod liver oil is known to contain the active chromogen, and since the determinations were in all cases a comparison of the intensity of color produced by different amounts of the same chromogen.

An important step in the study was the adsorption of vitamin A from petroleum ether and chloroform solutions of cod liver oil by silica gel and norit (Fig. 1). Petroleum ether proved to be the better solvent from which to adsorb, while norit showed greater removal of the vitamin. However, this combination proved to be unfortunate for recovery.

Solutions of the non-saponifiable fraction of cod liver oil offered more satisfactory material for these studies since so much of the extraneous material could be removed prior to adsorption. The cold saponification method of Marcus (5) was used to obtain this concentrate.

A new technique was here developed, early in 1930, based on the different adsorption results obtained with different solvents. The material was adsorbed from a solvent which permitted good adsorption and recovered, or liberated, by use of a better solvent from which there was poor adsorption. With this earlier work as the foundation, more extensive experiments have been carried out in the last 2 years.

**Materials**

*Cod Liver Oil*—In the most recent experiments, material which had been obtained by nearly complete removal of cholesterol from a Squibb concentrate of the non-saponifiable fraction, and by distillation of this residue under 2 to 3 mm. pressure, at 176–196°, was used in petroleum ether solution.
Carotene—Preliminary experiments exhausted the supply of carotene prepared in this laboratory by the methods of Holmes and Leicester (6). In the work represented by Fig. 3 the carotene used was a pure product of The British Drug Houses Ltd., presented to us by Dr. Ellice McDonald, of the Cancer Research Laboratories, University of Pennsylvania.

Cholesterol—The cholesterol was a pure product prepared by the Eastman Kodak Company.

Solvents The solvents used were dried over anhydrous calcium chloride and filtered just before use. In the most recent experiments, special care was taken to redistil the solvents in a stream of carbon dioxide or nitrogen, since they were suspected of containing dissolved air which might cause oxidation of some of the carotene or vitamin.

Adsorbents—The adsorbents were ultraporous solids, most of which were prepared in this laboratory. They were ground to pass a 200 mesh sieve and activated for 2 hours in a stream of carbon dioxide to remove air and moisture.

Alumina I was a gel made by adding a slight excess of NH₄OH to AlCl₃ solution. The gel was allowed to dry slowly—over a period of 2 or 3 months—then washed free from chloride ion, dried again, and activated.

Alumina III was a commercial product of the Aluminum Company of America, made by slow crystallization from dilute NaOH solution, followed by heating to drive off most of the water of crystallization. The commercial product was carefully washed to remove any remaining traces of NaOH, but the wash water gave no alkaline test with litmus.

Alumina V was made by igniting the salt Al(NO₃)₃·9H₂O for several hours at 200–300° until no more red-brown NO₂ was given off. The resulting pure white solid passed a 200 mesh sieve without further grinding, and gave no test for nitrate ion with ferrous sulfate and sulfuric acid.

Alumina VI was made from aluminum ethoxide (7) which was broken up and exposed on a glass plate in a warm, moist room to secure hydrolysis. It was rolled and crushed with a glass rod once a week, and after 5 weeks, was removed. It was heated 3 hours in dry CO₂ at 210° until no further condensation of vapor occurred in the exit tube of the activating flask. It was then ground and activated 2.5 hours in CO₂.
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Alumina VII was alumina (7) deposited on pumice. The pumice was broken to 8 to 14 mesh pieces and mixed with an equal weight of aluminum ethoxide. The whole was then heated until the ethoxide melted (some of the latter was here lost as dense white fumes). After cooling, the melt was broken up and exposed to the air of a moist, warm room. After 2 weeks, it was removed and heated to 200° for 3 hours in dry CO₂. It still appeared grayish in color and was therefore steamed 3.5 hours to accelerate hydrolysis, dried, and reheated at 200–230° for 2 hours.

Alumina VIII was a porous alumina made from aluminum butoxide (7) treated similarly to Alumina VI except that after exposure to the warm, moist air, it was steamed for 7 hours before being activated.

Patrick's silica gel of the commercial type (8), which had not been entirely freed from iron, was used.

Vitreous silica gel was made by the method of Holmes and Anderson (9). It was found very important to free acid-treated gels of every trace of acid. The gels were washed with water until apparently acid-free, then with 0.02 N NaOH, and again with water.

Norit carbon was purified by the hydrofluoric-hydrochloric acid treatment of Miller (10) to render it practically ash-free. It was activated in a silica crucible at 800–900° in an electric furnace as directed by Miller.

EXPERIMENTAL

Methods

Vitamin A—1 gm. of the activated adsorbent and 10 cc. of a solution of the vitamin were placed in bottles or tubes under an atmosphere of carbon dioxide. The bottles were shaken 10 minutes and allowed to stand overnight at room temperature to insure satisfactory settling. Blanks for each concentration were treated in all ways as in the regular runs except that porous solids were not present. In the numerous instances where duplicates were run they checked closely.

In order to recover the adsorbed vitamin the supernatant liquid was decanted and 10 cc. of chloroform added to the adsorbate while carbon dioxide was passed in. The tubes were then stoppered and, after shaking 10 minutes, allowed to stand overnight. In
the cases of adsorption from chloroform and ethylene dichloride a correction was made for the volume of liquid held mechanically by the adsorbent when the supernatant layer was poured off. This was not so satisfactory in the case of the petroleum ether solutions, however, owing to the extreme volatility of this solvent.

Carotene—Because of the high adsorption of carotene by some porous solids it was necessary in most cases to use 20 cc. of carotene solution with 1 gm. of adsorbent. To obtain some of the higher points on the curves 0.5 and 0.25 gm. samples of adsorbent were used with 20 cc. of solution, and the adsorption observed was multiplied by 2 or 4 respectively to obtain the specific adsorption.

The tubes were filled with carbon dioxide, the adsorbent rapidly weighed in, and carbon dioxide passed into the tubes again while the 20 cc. of solution were added. After closing the tubes with tin-foil-wrapped corks and shaking 10 minutes, the solutions were allowed to stand overnight to settle, although equilibrium was reached in much shorter time. The recovery was effected in the same way as in the case of vitamin A; that is, the supernatant liquid was poured off the adsorbent, and 20 cc. of chloroform were added, an atmosphere of carbon dioxide being maintained. The tubes were shaken 10 minutes and allowed to stand overnight before the concentration of the recovery solution was determined.

The amount of carotene present was determined colorimetrically with a dipping colorimeter. For the petroleum ether solutions the method of Willstättter and Stoll (11) with a standard 0.2 per cent solution of potassium dichromate was employed. For the solutions in other solvents it was found that the color of the carotene solution was shifted toward the red in chloroform and carbon disulfide especially, and would not match the dichromate standard, so it was necessary to make up known concentrations of carotene in the desired liquid. Ten readings were made and averaged in determining the concentration of each solution.

Cholesterol—With cholesterol adsorption, 20 cc. of solution and 2 gm. of activated adsorbent were used. The solutions in the various solvents contained 1, 2, 5, and 10 gm. per 100 cc. of total volume. The solutions were shaken 1 hour and then filtered through dry filter paper. The blanks were treated in the same way, except for omission of adsorbent. 10 cc. of each solution were evaporated on a water bath to constant weight, and the concentration of the
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Equilibrium solutions and of the blanks determined. The procedure followed in recovery was essentially the same as with the vitamin and carotene. The equilibrium solution was decanted and 20 cc. of chloroform added. The solutions were shaken 1 hour, filtered, and 10 cc. of the filtrate evaporated to constant weight to determine the equilibrium concentration.

Fig. 1. Adsorption of vitamin A with non-fatty residue from chloroform. This graph indicates that in the case of the less chemically active silica, true adsorption (increasing with fall in temperature from +24° to -24°) was the predominant influence with much less chemical reaction taking place, while with the more active norit carbon, chemical reaction (increasing with rise in temperature from -24° to +24°) of some sort was decidedly the predominant influence, with true adsorption as a very minor phenomenon. The results represented in Fig. 1 were obtained by earlier work in which air was not rigorously excluded from the apparatus.
Results

Vitamin A—Only one gel, Patrick’s silica, excels Alumina III in ability to adsorb from petroleum ether. When these two porous

Fig. 2. Adsorption of crude vitamin A. It is evident that aluminas prepared in different ways vary widely in adsorbing power. Adsorption of crude vitamin A from petroleum ether by several porous solids is high but with carbon chemical reaction accounts for much of the removal from solvent. Alumina III is broadly effective.
solids are compared in their ability to liberate, however, Alumina III is far superior. Alumina V, which was made by igniting the nitrate, gave excellent adsorption and good recovery, but Aluminas VI and VIII, which were made by hydrolysis of aluminum ethoxide and aluminum butoxide respectively, each gave poor recovery. 

![Graph](Image)

**Fig. 3.** Adsorption of carotene from petroleum ether

that from the ethoxide being higher in per cent than that from the butoxide. An analysis of these curves indicates that the use of Alumina III with petroleum ether as solvent for adsorption and chloroform for recovery should be the most effective combination (Fig. 2).

A recent article by Marcus (12) noted that contact with fine
powders destroyed the vitamin. The recoveries from several adsorbents make it evident that adsorption per se does not destroy it entirely, and in the case of Alumina III not to any marked extent.

![Graph showing adsorption of cholesterol](http://www.jbc.org/)

**Fig. 4. Adsorption of cholesterol**

*Carotene*—Some preliminary experiments were run with rather dilute solutions to determine which solvents were most, and which least effective for adsorption and recovery. It was found that adsorption from petroleum ether was excellent for nearly all adsorbents, thus paralleling the behavior of the vitamin, but from benzene, ethylene dichloride, and chloroform it was very poor.
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except in the case of norit, which showed some adsorption from benzene and even more from chloroform. More quantitative studies were made, therefore, with petroleum ether as solvent to adsorb from, and chloroform as liberating solvent, the same combination found most effective in the vitamin work.

In Fig. 3 it is shown that Alumina VIII is superior to Alumina III. The adsorption secured with Alumina V is very poor, in contrast with the high adsorption of vitamin A from petroleum ether by this adsorbent.

As in the case of vitamin A, the excellent adsorption shown by some gels was not followed by excellent recovery. The recovery secured from Alumina III was superior to that from Alumina VIII, as well as the recovery from any other gel studied.

**Cholesterol**

Cholesterol claimed our attention in these studies because of its presence in many sources of vitamin A, and because of the suggested chemical relationship between cholesterol and the vitamin.

The curves in Fig. 4 summarize the adsorption experiments made with cholesterol. The importance of the polarities of the components of the systems becomes apparent at once. Norit is non-polar, silica gel is polar and acidic, and alumina, polar and amphoteric. Silica gel adsorbs the slightly polar cholesterol quantitatively from petroleum ether, very highly from benzene, and in decreasing amounts down to ether, from which adsorption is low. The adsorption from benzene by chalky silica gel is much higher than by Patrick’s gel; the adsorption by norit is decidedly lower, and shows a partial reversal of the order with silica gel. The polar ether and very non-polar benzene show the expected exchange of position clearly. The failure of petroleum ether solution to show a decided lowering of adsorption may be related to its slight solubility for cholesterol. The adsorption by alumina is lower, but follows the same order as that for silica. These results agree very well with the reversal of Traube’s rule of adsorption as developed by Holmes and McKelvey (13).

Cholesterol in petroleum ether solution was found to be much like vitamin A in its adsorption behavior. For this reason the separation by adsorption of the vitamin from cholesterol and cholesterol-like matter in the non-saponifiable fractions of oils is a problem of great difficulty.
SUMMARY

Several adsorption curves for vitamin A, carotene, and cholesterol are presented.

Attention is directed to a new type of porous alumina of low cost and great value for such adsorption work.

Adsorption from one solvent and recovery by another have been developed as an important research technique in vitamin work.

Contrary to some statements in the literature, a high percentage of vitamin A has been recovered from porous solids upon which it had been adsorbed.

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