THE ABSORPTION OF CHOLESTEROL, ERGOSTEROL, AND CALCIFEROL BY SUPERFILTROL FROM SINGLE AND BINARY SOLVENTS

By

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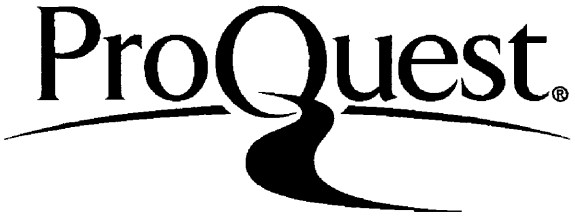

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THE ADSORPTION OF CHOLESTEROL, ERGOSTEROL, AND CALCIFEROL BY SUPERFILTROL FROM SINGLE AND BINARY SOLVENTS.

A study has been made of the adsorption of cholesterol, ergosterol, and calciferol by superfiltrol from single and mixed solvents. The single solvents were ethyl alcohol, diethyl ether, Skellysolve, hexane, and benzene. The binary solvents were made up from the single solvents mentioned above and are indicated in the tables. Diethyl ether and ethyl alcohol were chosen as representative polar solvents. Both showed a tendency to dissolve the adsorbent. In an effort to use a polar solvent which would not have this dissolving property, acetone was used alone and with varying concentrations of hexane. However, it was discovered that acetone had the same property; so its use was discontinued. Benzene, Skellysolve, and hexane, representative nonpolar solvents, were found to have no tendency to dissolve the adsorbent. Three other samples of superfiltrol varying in particle size and a sample of activated alumina were examined for solubility in the polar solvents. They were all found to be soluble in the polar solvents so were discarded as possible adsorbents.

Little work has been reported in the literature determining the amount of adsorption of each component of a ternary system over the entire concentration range of the binary solvent.

Studies have been reported of the adsorption of the components of binary systems over the entire concentration range. Bartell and Scheffler (1) reported that methyl, ethyl,

n-propyl, n-butyl, and isoamyl alcohols in benzene solution were preferentially adsorbed by silica up to Nalc = 1.00, 0.87, 0.87, 0.82, 0.72, respectively, while by carbon, the values were respectively 0.26, 0.20, 0.10, 0.10, and 0.10. They concluded that the degree of adsorption of one component (adsorbate) depends on the adhesion tension of that liquid as compared to the adhesion tension of solvent against solvent and also upon solubility of adsorbate in the solvent. It was found that the greater the solubility, the less was the tendency to be adsorbed. Bartell and Sloan (2) reported preferential adsorption by carbon of benzene from ethyl alcohol up to N_b = 0.8; of ethyl carbonate from ethyl alcohol up to N_{ec} = 0.55; of benzene from ethyl carbonate up to N_b = 0.995; and of alpha bromonapthalene from benzene up to N_{ab} = 0.46. Bartell, Scheffler, and Sloan (3) found preferential adsorption by silica from the systems ethyl carbonate-benzene, ethyl carbonate-dimethylaniline, and ethyl carbonate-methyl benzoate. Rao (4) reported that there was selective adsorption by silica of ethyl alcohol from benzene at all concentrations, of benzene from carbon tetrachloride at all concentrations, of ethyl alcohol from water rich solutions, of acetone from water rich solutions, of water from ethyl alcohol rich solutions, and of water from acetone rich solutions. Patrick and Jones (5) investigated the adsorption by silica of acetic acid from the solvents kerosene, carbon disulfide, gasoline, carbon tetrachloride, toluene, and nitrobenzene and found, as Bartell and Scheffler later did, that

the less soluble the solute was, the more it was adsorbed.

According to Kane and Jatkar (9), toluene was preferentially adsorbed at all concentrations from acetic acid by sugar charcoal, while acetic acid was preferentially adsorbed at all concentrations from toluene by silica gel. However, toluene was preferentially adsorbed at the lower toluene concentrations by animal charcoal while acetic acid was preferentially adsorbed at the lower acetic acid concentrations. The animal charcoal is a combination of polar ash and nonpolar carbon and the resultant S curve was attributed to that fact.

Ermolenka and Levina (10) investigated the adsorption of salicylic acid by charcoal from binary solvents of similar polarities as well as of polar-nonpolar mixtures. Adsorption from the binary mixture of similar polarities was independent of the relative concentrations of the solvent components. In the case of the polar-nonpolar binary solvents, the adsorption of the salicylic acid increased with the increase of the nonpolar concentrations.

Experimental

Material and Apparatus:- Eastman's P1135 hexane (practical grade) and the Skellysolve were purified to remove unsaturated hydrocarbons according to Ewing, Kingsley, Brown, and Emmet (6). Benzene (C.P.) was also purified according to the above authors. Diethyl ether (C.P.) was dried over sodium and redistilled. Absolute ethyl alcohol (C.P.) was used as obtained and its density corresponded to the density of 99.5% or higher in the handbook. The superfiltrol 643 and other adsorbents were used as purchased with the exception of one set of experiments where superfiltrol 643 had been treated with solvents. The ergosterol, cholesterol, and calciferol were of a high degree of purity. Acetone (C.P.) was dried over calcium chloride and redistilled. The stigmasterol was obtained from C.D. Ball of this department and was used without further treatment.

The densities were obtained using a special pycnometer similar to a type since described by Lipkin, Davison, Harvey, and Kurtz (7).

A pycnometer was made by blowing a bulb from 2 to 4 mls. in volume in a half mm. capillary tubing and then bending the tubing as shown in Fig. 10. Since it was necessary to determine the densities of mixed solvents, one or both of which were volatile, it was thought that the rate of evaporation would be appreciably reduced by use of capillary tubing. In using these pycnometers, there was no noticeable change in weight while on the balance, even when ether was

being weighed. A removable scale was used to read the height of the liquid in the capillary on the two sides. The scale was graph paper mounted on cardboard. A nick was etched into the capillary tube so that it acted as a concave lense. When a thick line on the scale was viewed through the "lense" in line with the eye, the thick line was focussed to a thin straight line. If it was not lined up with the eye, the thin line was distorted. Thus it was possible to clip the scale on exactly the same position each time. A removable scale was used because it was planned that the pycnometer was to be placed in a constant temperature water bath. However, a constant temperature air bath was used; but the removable scales were retained since they were satisfactory and probably more accurate than lines etched into the glass. The constant temperature air bath was used to eliminate the possibility of error introduced in wiping the pycnometer dry before each weighing. To assure thermal equilibrium, readings were taken on the capillary arms until there was no further change in levels. The pycnometer was weighed empty after each density determination in order to check its weight. In order, to obviate loss by evaporation when filling the pycnometer, a device pictured on Fig. 10 was used. Pressure on the rubber bulb forced the liquid into the pycnometer in a few seconds. The filling apparatus was so designed that the liquid to be transferred to the pycnometer occupied most of the volume, leaving very little free space for evaporation. One pycnometer was calibrated with water, and the others were cali-

brated with benzene whose density had been determined with the first pycnometer.

The components of each system used were weighed out in order to obtain the percentage by weight of each. For a given set of experiments, the same weight of superfiltrol and the same volume of solution were used for each adsorption. The solutions and the container holding the superfiltrol used for the adsorption were kept in a constant temperature bath at 25.00° C. The solutions were thus pipetted directly into the superfiltrol without change in temperaure. For the earlier experiments where longer periods of time were consumed in shaking the mixture, a shaker rigged from an automobile windshield wiper was used. It was possible to shake as many as 8 different solutions at one time while they were immersed in the bath. The shaking tubes used were of various volumes and were designed to provide no more space above the liquid than was necessary for efficient shaking. They were from 8 cms. to 15 cms. long and 1 cm. to 2 cms. in diameter. In the later experiments where shaking times of ½ hour were used, the shaking was carried out in 10 ml. volumetric flasks and by hand, keeping the solutions in the bath. After the shaking, the superfiltrol was allowed to settle. The liquid was then quickly decanted into centrifuge tubes, corked, and centrifuged. Aliquots were pipetted out for analysis and a portion of the remainder was distilled and used to determine the density. Owing to the fact that alcohol or ether rich binary solvents had

a much greater density than could be accounted for by adsorption of the lesser component, it was thought that the dissolved superfiltrol was responsible for the increase and could be removed by distillation.

In order to prevent loss by vaporization, an apparatus pictured in Fig. 11 was used to distill the liquids. The volume of each of the two parts of the apparatus was about 6 mls., which was slightly greater than the volume of liquid distilled. The part (A) into which the liquid was distilled was heated in the bunsen burner before connecting to the part of the apparatus containing the liquid to be distilled. On cooling, a partial vacuum was created so that the distillation could be carried out without blowing out the cork. The liquid in part (B) was heated in a water bath and distilled into part (A) which was surrounded by an ice water bath. The liquid was then transferred directly into the pycnometer. The liquids were distilled in the hope that whatever adsorbent was dissolved would be left behind. While some residue remained behind, the alcohol or ether rich binary solvent mixtures to a large extent retained their abnormally large densities. However, all of the adsorption of the ergosterol or calciferol of the ternary system took place in the alcohol or ether poor binary solvents where no apparent solution of the adsorbent took place.

Cholesterol was analyzed using a Cenco-Sheard spectrophotometer at 450 millimicrons. Advantage was taken of the color formed when a benzene solution of cholesterol was

treated with acetic anhydride and concentrated sulfuric acid. Two mls. of benzene solution, 2.75 mls. of acetic anhydride, and 0.1 ml. of sulfuric acid were mixed and placed in a cell whose volume was slightly less than the total of the above mixture. Each time before a reading was taken, the color was allowed to develop for 20 minutes. A reference liquid was made up of the same amounts of the above reagents but with the cholesterol absent from the benzene. Most of the concentrations analyzed from the adsorption experiments were within the range of the instrument. Where the concentrations were too great, double, triple, or quadruple the relative amounts of the reagents were used. In plotting the known concentrations against log I_o/I, a straight line was obtained as shown on Fig. 2.

Ergosterol and calciferol were analyzed in some of the early experiments using the Cenco-Sheard spectrophotometer taking advantage of the color reaction of the chloroform-antimony trichloride reagent. (8) Measurements were made at 500 millimicrons. In the later experiments, the Beckman spectrophotometer was used at 261.5 millimicrons. A straight line curve was obtained when concentrations were plotted against the corresponding log I_o/I. (Fig. 3) One ml. samples of the original solutions were evaporated, taken up in alcohol, and diluted to a concentration within the range of the spectrophotometer.

Due to the fact that ether and alcohol seemed to dissolve out a portion of the superfiltrol, experiments were

carried out to determine the effect on the density change of binary solvents by pretreating the superfiltrol with alcohol and ether. Fifty gms. of superfiltrol were shaken with 100 mls. of absolute ethyl alcohol and shaken at intervals. After 20 hours, the mixture was shaken and the slurry poured into 5 chromatographic tubes fitted with absorbent cotton. These were allowed to settle out for a period of 12 hours. The alcohol was drawn out and an additional 10 mls. of alcohol were added to each tube and subsequently drawn out. This was repeated. The superfiltrol was then scraped out and placed in a flask with 50 mls. of absolute diethyl ether, shaken, and put back into the tubes. The ether was drawn out; 2-5 ml. portions of ether for each tube were passed through; and a third 5 mls. were allowed to stand in the tubes for 12 hours. Air which had been dried by passing through 2- 4 ft. columns of magnesium perchlorate, was pulled through the tubes in series for 48 hours. The superfiltrol was scraped out and placed in a vacuum desiccator, kept under vacuum for 3 hours, and then put back into the tubes, where dry air was drawn through for 24 hours. The superfiltrol was stored under vacuum in a desiccator. In spite of the above treatment, a slight odor of ether was noticeable each time the desiccator was opened. The results of use of treated superfiltrol are tabulated in Tables 17, 18, and 19.

Duplicate samples of 10 mls. of alcohol were shaken ½ hour with 1 g. of treated superfiltrol 643, centrifuged, and densities determined. The densities were 0.7875 and 0.7879

as compared to 0.7869 before shaking.

Due to the apparent tenacious adsorption of solvent and inability to avoid some solution of the adsorbent, the use of the superfiltrol treated with ether and alcohol was discontinued. No advantage was gained in merely having a decrease in the amount dissolved, and the experiments would have been complicated by having the ether or alcohol adsorbed on the adsorbent.

The solutions of ergosterol were at first shaken 3 hours or more, but there was evidence of slow decomposition as shown by the appearance of a yellow color in the solution. The log I_o/I values for the solutions after shaking were less than before, indicating that there was some adsorption. However, it is possible that the decomposition product or products formed compounds with the antimony trichloride reagent which had a smaller log I_o/I value than that formed with ergosterol. Therefore, the calculated concentrations (Table 2) after shaking may be merely apparent concentrations. There was no detectable yellowing of the solution in the first ½ hour of shaking, which was assumed to be an indication of no decomposition during that time. It was observed that when there was adsorption of calciferol or ergosterol, there was a definite color change in the superfiltrol, the intensity of which depended on the amount of adsorption. There was a series of color changes which were complete within ½ hour indicating that the adsorption was complete in that time. In ½ hour, there was no adsorption of ergosterol from alcohol or

ether solutions as compared to an apparent small adsorption with longer shaking as as pointed out above. There was 100% adsorption from hexane. Since there was no decomposition of ergosterol and since adsorption was complete in ½ hour of shaking, that time was used in the case of the systems ergosterol-hexane-diethyl ether and ergosterol-hexane-ethyl alcohol (Tables 10 and 11, Fig.5).

Discussion of results

It was thought that the relative adsorption of components of the binary solvents could be ascertained by means of changes in densities. An increase in the density would mean that the lighter component was adsorbed relatively more than the heavier, and the opposite would be true if the density decreased. This worked out very well for the benzene or hexane rich solvent; but a complication arose in the case of the alcohol or ether rich solvents. These solvents as well as the single alcohol or ether solvent showed an unexpectedly large increase in density. It was expected that there would be an increase which would taper off as the concentration approached that of pure alcohol or ether. However, there was no such tapering off and the increase in density was maintained right up to the concentration of pure alcohol or ether. It was thought then that something from the superfiltrol was dissolved out to give this increase. In order to check this, alcohol was passed through a chromatographic column and densities taken from time to time.(Tables 21-24 inclusive). The density increase was very large for the first

eluate, but fell off for succeeding eluates and approached that of the alcohol (C.P.) but never quite reached it. Hulett (12) has found that small particles of a substance have a greater solubility than larger particles of the same substance. This phenomena may have occurred in the solubility of the superfiltrol in the polar solvents. The first eluates may have taken out the finer particles, as evidenced by the increased density, and left the larger particles for succeeding eluates. These succeeding eluates had steadily decreasing densities which indicated decrease in solubility of the superfiltrol.

Another explanation of the steady decrease in solubility of the superfiltrol in each eluate may be that a minor soluble component of superfiltrol was gradually washed out.

It was found that on standing, white crystals, later turning slightly yellowish, were formed. These crystals were heated in a hot flame but did not char, indicating that they were probably inorganic in nature. The same treatment described above was employed using ether in place of alcohol and similar results were obtained. Air which had been dried over calcium chloride was drawn through the superfiltrol. The superfiltrol was transferred to a glass dish and placed in a desiccator which was then evacuated. It was kept there 12 hours. Under ordinary circumstances, the ether presumably should have been all evaporated off. However, there was a faint odor of ether from the superfiltrol. The superfiltrol was then placed back into the column and alcohol passed

through. It was expected that there would be very little change in density due to the fact that the ether supposedly had dissolved out the readily soluble portion. Approximately the same density for the first eluate was obtained as in the case where the superfiltrol had not been previously treated with ether (Table 25). This might be explained by postulating that some of the particles retained a layer of ether molecules which rendered them soluble in alcohol.

While the dissolving effect of alcohol and ether was annoying, the ergosterol and calciferol adsorbed only in the alcohol or ether poor binary solvents so that it was relatively unimportant. It was found that (Table 7) alcohol, ether, and hexane which had been shaken with the adsorbent when measured against the original solvent showed absorption in the ultraviolet. Although this absorption was minimized in most cases due to dilution of samples, the reference liquid in each case was made up from the solvent which had gone through the same process as the solvent which contained the ergosterol or calciferol. One ml. samples of the above mentioned solution were evaporated, taken up in alcohol, and diluted with alcohol in the same way as were the solutions containing calciferol and ergosterol.

When ergosterol or calciferol was adsorbed from solutions containing hexane or benzene, the superfiltrol became colored. The color was proportional to the amount of ergosterol or calciferol adsorbed. In the case of erosterol, the change was from a bright blue to a dark purple, whereas the

change for the calciferol was from yellow to tan, to brown, and finally to dark green. Cholesterol did not show a similar change in color. In order to ascertain whether cholesterol was unique among the sterols in this respect, a sample of stigmasterol was investigated. As in the case of cholesterol, no color was found to develop.

The dielectric constants of ethyl alcohol, diethyl ether, benzene and hexane are 25.7, 4.33, 2.28 and 1.87 at 20° C.(11). The order of adsorption of cholesterol from the four different solvents was identical to the order of the values of their dielectric constants,with the adsorption least in the case of the alcohol (Fig.1, Table 1). The same general rule was followed in the adsorption of the components of binary solvents. Fig. 6, Tables 12, 13 and 15 show that there was a preferential adsorption of the component which had the largest dielectric constant. Moreover, the relative adsorption of alcohol was greater than that of acetone which in turn was greater than that of ether. The dielectric constant of acetone is 20.7 which is less than that of alcohol and greater than that of ether. As illustrated in Fig. 7, and tabulated in Table 16, ether was preferentially adsorbed from benzene which has the lower constant. Adsorption of calciferol and ergosterol from the binary mixtures of hexane-alcohol and hexane-ether are shown in Figs. 4 and 5, Tables 8, 9, 10 and 11. The adsorption of both calciferol and ergosterol fell off more rapidly in the alcohol-hexane mixtures. Assuming hexane to be a neutral factor, alcohol

which has a higher dielectric constant than ether, eliminated ergosterol (or calciferol) as a competitor in the adsorption at a much lower concentration than did ether. The curves for ergosterol and calciferol seemed to coincide with each other in the corresponding binary solvents.

General Conclusions:- Cholesterol was found to be adsorbed more from the nonpolar solvents, Skellysolve and benzene, than from the polar solvents, ethyl alcohol and diethyl ether.

Calciferol was adsorbed entirely from hexane and Skellysolve, almost entirely from benzene, and not at all from ethyl alcohol and diethyl ether.

The adsorption of calciferol from hexane fell off rapidly as ethyl alcohol was added until at 2.3% or more by weight of alcohol there was no adsorption. The same was true for the diethyl ether-hexane mixture except that the decrease in adsorption was more gradual and the point of zero adsorption was about 64% ether. This difference in adsorption between the alcohol and ether seemed to be related to the relative adsorption of the solvents. Both alcohol and ether were preferentially adsorbed from hexane in solutions rich in hexane. Alcohol was relatively more so.

The adsorption of ergosterol from the alcohol-hexane and ether-hexane mixture was very similar to that of calciferol. The rate of decrease of adsorption from alcohol-hexane mixtures was about the same for calciferol and ergosterol but more rapid for calciferol in the ether-hexane mixtures.

Diethyl ether was preferentially adsorbed at all concentrations from benzene.

Skellysolve was preferentially adsorbed from benzene at the lower concentrations; but above 35% Skellysolve, benzene was adsorbed in the greater proportion.

Summary

1. The adsorption of cholesterol from diethyl ether, ethyl alcohol, benzene, and Skellysolve on superfiltrol was determined at 25° C over a range of concentrations from 0.16 to 10.02 mg. per ml.

2. The adsorption of ergosterol from diethyl ether, ethyl alcohol, and benzene on superfiltrol was determined at 25° C over a range of concentrations from 0.204 to 3.26 mg. per ml.

3. The adsorption of calciferol from diethyl ether, ethyl alcohol, Skellysolve, and benzene on superfiltrol was determined at 25° C over a range of concentrations from 0.0124 to 0.1272 mg. per ml.

4. Using 1 mg. of calciferol per ml., its adsorption from the binary mixtures of diethyl ether-hexane and ethyl alcohol-hexane was determined over the entire concentration range.

5. Using 1 mg. of ergosterol per ml., its adsorption from the binary mixture of diethyl ether-hexane and ethyl alcohol-hexane was determined over the entire concentration range.

6. The preferential adsorption of the components of binary mixtures of ethyl alcohol-hexane, acetone-hexane, diethyl ether-hexane, Skellysolve-diethyl ether, Skellysolve-benzene, and diethyl ether-benzene on superfiltrol at 25° C over the entire concentration range was determined.

7. The preferential adsorption of the components of the binary mixtures of diethyl ether-benzene, diethyl ether-hexane, and ethyl alcohol-hexane on treated superfiltrol was

determined at 25° C over the entire concentration range.

8. The densities of ethyl alcohol and diethyl ether eluates from columns packed with three different superfiltrols and activated alumina were determined. Densities of hexane eluates from a column packed with superfiltrol 643 were determined.

9. The ultraviolet light absorption was determined for solutions of ethyl alcohol, which had been shaken with superfiltrol 643 and activated alumina. Determinations were also made where solutions of diethyl ether and hexane were shaken with superfiltrol 643, evaporated, and the residues taken up with ethyl alcohol.

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Changes in Concentrations of Cholesterol in the Single Solvents; Benzene, Diethyl Ether, Skellysolve, and Ethyl Alcohol. Five mls. solution shaken 3 hrs. with 0.250 g. Superfiltrol 643. Analysis Made Using Cenco-Sheard Spectrophotometer to Measure Optical Density of Colored Solution Obtained by Adding Concentrated Sulfuric Acid and Acetic Anhydride to a Benzene Solution of Cholesterol.

Table 1.

Solvent.	Conc, mg./ml. before adsorption.	Conc. mg./ml. after adsorption.	Mg. cholesterol adsorbed per g. S-643.
Benzene	0.16	0.13	0.6
	0.31	0.23	1.6
	1.25	0.90	7.0
	2.50	2.02	9.6
	5.00	4.24	15.2
	10.00	8.66	26.8
Diethyl Ether	1.25	1.19	1.2
	2.50	2.41	1.8
	5.00	4.87	2.6
	10.01	9.77	4.8
Skellysolve	2.01	0.45	31.2
	3.18	1.10	41.6
	4.01	1.47	50.8
	6.34	3.10	64.8
	7.97	4.35	72.4
Ethyl Alcohol	1.25	1.13	2.4
	2.51	2.13	7.6
	5.01	4.30	14.2
	10.02	9.02	20.0

Changes in Concentrations of Ergosterol 95752 in the Single Solvents: Benzene, Ethyl Alcohol, and Diethyl Ether after Adsorption upon 0.500 g. Superfiltrol 643 from 5 mls. Solution. Analyses Made Using Cenco-Sheard Spectrophotometer to Measure Optical Density of Colored Solutions Obtained by Adding Antimony Trichloride Reagent to a Chloroform Solution of Ergosterol.

Table 2.

Solvent.	Conc. mg./ml. before adsorption.	Apparent Conc. mg./ml. after adsorption.	Mg. Ergosterol adsorbed per g. S-643
Benzene (Shaken 8 Hrs.)	0.407	0.170	2.37
	0.815	0.261	5.54
	1.629	0.590	10.39
	3.26	1.64	16.2
Diethyl Ether (Shaken 8 Hrs.)	0.408	0.398	0.10
	0.816	0.757	0.59
	1.632	1.423	2.09
	3.26	2.98	2.8
Ethyl Alcohol (Shaken 3 Hrs.)	0.204	0.200	0.04
	0.408	0.329	0.79
	0.808	0.590	2.18
	1.603	1.206	3.97
Hexane (Shaken ½ Hr.)	1.028	0.065	9.63

Adsorption of Calciferol (Winthrop) from 5 mls. of Solution upon Shaking 2 hrs. with 0.500 g. Superfiltrol 643. Analyses Made Using Cenco-Sheard Spectrophotometer to Measure Optical Density of Colored Solution Obtained by Adding Antimony Trichloride Reagent to a Chloroform Solution of Calciferol.

Table 3.

Solvent.	Conc. mg./ml. before adsorption.	Conc. mg./ml. after adsorption.	Mg. Calciferol adsorbed per g. S-643.
Benzene	0.0124	0.0018	0.106
	0.0248	0.0046	0.202
	0.0496	0.0089	0.407
	0.0992	0.0181	0.808
Diethyl Ether	0.0124	0.0124	0.000
	0.0165	0.0167	-0.002
	0.0248	0.0244	0.004
	0.0330	0.0326	0.004
	0.0496	0.0491	0.005
	0.0660	0.0660	0.000
	0.0992	0.1000	-0.008
	0.132	0.136	-0.04
Skellysolve	0.0124	0.0000	0.124
	0.0248	0.0000	0.248
	0.0496	0.0000	0.496
	0.0992	0.0000	0.992
Ethyl Alcohol	0.0118	0.0116	0.0002
	0.0262	0.0268	-0.0006
	0.0571	0.0589	-0.0018
	0.1272	0.130	-0.0028

Calibration Data for the Determination of Cholesterol Using a Cenco-Sheard Spectrophotometer. Two mls. Benzene Solution of Cholesterol, 2.75 mls. Acetic Anhydride, and 0.1 ml. Concentrated Sulfuric Acid Mixed and Color Allowed to Develop for 20 Minutes.

Table 4.

Conc. mg./2 ml.	Log I_o/I	Conc./Log I_o/I
0.161	0.056	2.89
0.401	0.136	2.95
0.562	0.184	3.05
0.802	0.273	2.94
1.204	0.405	2.96

Calibration Data for the Determination of Calciferol Using a Cenco-Sheard Spectrophotometer. Calciferol Taken up by 1 ml. Chloroform and 5 mls. Antimony Trichloride in Chloroform Reagent Added.

Table 5.

Conc. mg./ml.		Log I_o/I		Conc./Log I_o/I
0.01275		0.279		
0.01275		0.281		
0.01275		0.281		
	(Av.)	0.280		0.0456
0.00992		0.210		
0.00992		0.213		
0.00992		0.220		
0.00992		0.207		
	(Av.)	0.213		0.0466
			(Av.)	0.0461

Calibration of the Beckman Spectrophotometer with Respect to Solutions of Ergosterol in Alcohol, with Alcohol in the Reference Cell.

Table 6.

Conc. mg./ml. ergosterol in alcohol.	Log I_o/I	Conc./Log I_o/I
0.01430	0.392	0.0365
0.01224	0.327	0.0366
0.01071	0.294	0.0364
0.00952	0.262	0.0363
0.00857	0.238	0.0360
	(Av.)	0.0364

Ten mls. Solvent Shaken 1 hr. with Adsorbent (1 g.) and the Log I_o/I of the Resultant against the Original Solvent Measured with the Beckman Spectrophotometer at 265 millimicrons.

Table 7.

Solvent	Adsorbent	Log I_o/I
Ethyl alcohol	Superfiltrol-643	0.286
Ethyl alcohol	Activated alumina	0.266
Diethyl ether	Superfiltrol-643	0.245
Hexane	Superfiltrol-643	0.090

Changes in Concentrations of Calciferol in the Binary Mixtures of Hexane and Diethyl Ether after Shaking 10 mls. of Solution with 1.000 g. Superfiltrol 643 for ½ hr. Analyses of Calciferol Made by Measuring Optical Density in the Ultraviolet of an Alcohol Solution, Using a Beckman Spectrophotometer.

Table 8.

% ether in hexane by weight.	Conc. mg./ml. before adsorption.	Conc. mg./ml. after adsorption.	Mg. Calciferol per g. S-643
0.00	1.028	0.000	10.28
2.06	0.998	0.092	9.06
4.90	0.998	0.371	6.27
15.31	0.998	0.720	2.78
37.83	0.998	0.926	0.72
63.15	0.998	0.998	0.00

Changes in Concentrations of Calciferol in the Binary Mixtures of Hexane and Ethyl Alcohol after Shaking 10 mls. of solution with 1.000 g. Superfiltrol 643 for ½ hr. Analyses of Calciferol Made by Measuring Optical Density in the Ultraviolet of an Alcohol Solution, Using a Beckman Spectrophotometer.

Table 9.

% alcohol in hexane by weight.	Conc. mg./ml. before adsorption.	Conc. mg./ml. after adsorption.	Mg. Calciferol per g. S-643
0.00	1.028	0.000	10.28
0.98	1.028	0.625	4.03
1.60	1.028	0.836	1.92
2.29	1.028	1.030	-0.02
4.71	1.028	1.010	0.10
14.94	1.028	1.044	-0.16
84.95	1.028	1.030	-0.02

Changes in Concentration of Ergosterol in the Binary Mixtures of Hexane and Diethyl Ether after Shaking 2 mls. of Solution with 0.200 g. S-643 for ½ hr. Analyses of Ergosterol Made by Measuring Optical Density in the Ultraviolet of an Alcohol Solution, Using a Beckman Spectrophotometer.

Table 10.

% ether in hexane by weight.	Conc. mg./ml. before adsorption.	Conc. mg./ml. after adsorption.	Mg. Ergosterol adsorbed per g. S-643.
0.00	1.028	0.065	9.63
9.73	1.028	0.184	8.44
20.13	1.028	0.489	5.39
30.12	1.028	0.595	4.33
44.00	1.028	0.953	0.75
66.18	1.028	1.017	0.11
73.91	1.028	1.019	0.09

Changes in Concentration of Ergosterol in the Binary Mixtures of Hexane and Ethyl Alcohol after Shaking 10 mls. of Solution with 1.000 g. S-643 for ½ hr. Analyses made as in Table 10.

Table 11.

% alcohol in hexane by weight.	Conc. mg./ml. before adsorption.	Conc. mg./ml. after adsorption.	Mg. Ergosterol adsorbed per g. S-643.
0.00	1.028	0.065	9.63
0.99	1.025	0.247	7.78
1.92	1.025	0.750	2.75
3.72	1.026	0.993	0.33
20.82	1.026	1.016	0.10
41.88	1.026	1.026	0.00
78.19	1.026	1.026	0.00
95.84	1.028	1.023	0.05

Changes in Density of the Binary Mixtures of Hexane and Ethyl Alcohol upon Shaking 10 mls. with 1.000 g. S-643 for ½ hr.

Table 12.

% alcohol in hexane by weight.	Density before adsorption.	Density after adsorption.	Change in density.
0.99	0.6571	0.6566	-0.0005
1.92	0.6577	0.6566	-0.0011
3.72	0.6594	0.6572	-0.0022
5.42	0.6615	0.6584	-0.0031
9.41	0.6652	0.6607	-0.0045
17.78	0.6737	0.6677	-0.0060
20.82	0.6776	0.6722	-0.0054
22.97	0.6797	0.6751	-0.0046
28.31	0.6887	0.6902	0.0015
41.88	0.7044	0.7076	0.0032
60.88	0.7279	0.7322	0.0043
78.19	0.7526	0.7573	0.0047

Changes in Density of the Binary Mixtures of Hexane and Diethyl Ether upon Shaking 10 mls. with 1.000 g. Superfiltrol 643 for ½ hr. at 25.00° C.

Table 13.

% ether in hexane by weight.	Density before adsorption.	Density after adsorption.	Change in density.
0.00	0.6568		
2.07	0.6575	0.6574	-0.0001
15.31	0.6633	0.6629	-0.0004
37.83	0.6746	0.6745	-0.0001
63.15	0.6858	0.6865	0.0007
74.60	0.6922	0.6934	0.0012
88.20	0.7001	0.7022	0.0021
95.84	0.7050	0.7076	0.0026
97.61	0.7061	0.7088	0.0027
100.00	0.7076	0.7101	0.0025

Changes in Density of the Binary Mixtures of Skellysolve and Diethyl Ether upon Shaking 25 mls. with 1.000 g. Superfiltrol 643 for 2 hrs. at 25.00° C.

Table 14.

% ether in Skellysolve by weight.	Density before adsorption.	Density after adsorption.	Change in density.
0.00	0.6742		
20.67	0.6796	0.6792	-0.0004
41.06	0.6856	0.6856	-0.0001
61.22	0.6924	0.6928	0.0004
80.66	0.6996	0.7009	0.0013
100.00	0.7076	0.7101	0.0025

Changes in Density of the Binary Mixtures of Hexane and Acetone upon Shaking 10 mls. with 1.000 g. S-643 for ½ hr.

Table 15.

% acetone in hexane by weight.	Density before adsorption.	Density after adsorption.	Change in density.
7.05	0.6616	0.6601	-0.0015
13.34	0.6670	0.6646	-0.0024
19.60	0.6736	0.6713	-0.0023
40.40	0.6959	0.6957	-0.0002
60.63	0.7223	0.7243	0.0020
80.39	0.7518	0.7560	0.0042
87.87	0.7640	0.7689	0.0049
93.31	0.7731	0.7782	0.0051
100.00	0.7847	0.7899	0.0052

Changes in Density of the Binary Mixtures of Benzene and Diethyl Ether upon Shaking 10 mls. with 1.000 g. S-643 for 2 hrs. at 25.00° C.

Table 16.

% ether in benzene by weight.	Density before adsorption.	Density after adsorption.	Change in density.
0.00	0.8732	0.8732	0.0000
8.26	0.8582	0.8685	0.0003
16.78	0.8434	0.8440	0.0006
35.04	0.8116	0.8125	0.0009
54.82	0.7784	0.7807	0.0023
87.92	0.7264	0.7286	0.0022
93.84	0.7173	0.7198	0.0025
100.00	0.7077	0.7101	0.0024

Changes in Density of the Binary Mixtures of Benzene and Skellysolve upon Shaking 10 mls. with 1.000 g. S-643 for 2 hrs. at 25.00° C.

Table 17.

% Skellysolve in benzene by weight.	Density before adsorption.	Density after adsorption.	Change in density.
16.24	0.8313	0.8353	0.0035
34.07	0.7914	0.7915	0.0001
53.68	0.7520	0.7509	-0.0011
75.59	0.7132	0.7130	-0.0002
100.00	0.6778		

Changes in Density of the Binary Mixtures of Benzene and Diethyl Ether upon Shaking 10 mls. with 1.000 g. Treated S-643 for 3½ hrs. at 25.00° C.

Table 18.

% ether in benzene by weight	Density before adsorption.	Density after adsorption.	Change in density.
4.88	0.8644	0.8641	-0.0003
8.26	0.8586	0.8584	-0.0002
16.78	0.8431	0.8434	0.0003
35.04	0.8118	0.8127	0.0009
54.82	0.7787	0.7796	0.0009
76.44	0.7443	0.7453	0.0010
87.92	0.7263	0.7272	0.0009
93.84	0.7173	0.7183	0.0010
100.00	0.7077	0.7084	0.0007

Changes in Density of the Binary Mixtures of Hexane in Ethyl Alcohol upon Shaking 10 mls. with 1.000 g. Treated S-643 for 3½ hrs. at 25.00° C.

Table 19.

% alcohol in hexane by weight.	Density before adsorption.	Density after adsorption.	Change in density.
66.94	0.7359	0.7355	-0.0004
78.35	0.7526	0.7527	0.0001
89.38	0.7700	0.7702	0.0002
95.84	0.7799	0.7812	0.0006
100.00	0.7869	0.7877	0.0008

Changes in Density of the Binary Mixtures of Diethyl Ether in Hexane upon Shaking 10 mls. with 1.000 g. Treated S-643 for 3½ hrs. at 25.00° C.

Table 20.

% ether in hexane by weight.	Density before adsorption.	Density after adsorption.	Change in density.
10.25	0.6605	0.6603	-0.0003
20.13	0.6647	0.6644	-0.0004
44.00	0.6755	0.6755	0.0000
66.18	0.6876	0.6875	-0.0001
73.91	0.6920	0.6918	-0.0002
88.94	0.7009	0.7011	0.0003
97.95	0.7070	0.7073	0.0003
100.00	0.7076	0.7085	0.0009

Hexane Passed Through a Chromatographic Column Containing S-643 and Densities of Successive Eluates Determined at 25.00° C.

Table 21.

Eluate	Density
1	0.6581
2	0.6578
3	0.6581
4	0.6581
Unchromatographed	0.6581

Ethyl Alcohol Passed Through Chromatographic Columns Containing 3 Different Superfiltrols and an Activated Alumina and Densities of Successive Eluates Determined at 25.00° C.

Table 22.

Eluate	S-1	S-2	S-3	Alumina
1	0.8330	0.8436	0.8625	0.7905
2	0.7880	0.7876	0.7884	0.7879
3	0.7871	0.7872	0.7870	0.7873
Unchromatographed	0.7863	0.7863	0.7863	0.7863

Diethyl Ether Passed Through Chromatographic Columns Containing Dried Superfiltrols and Alumina from Table 22. Densities of Successive Eluates from Each Column Determined at 25.00° C.

Table 23.

Eluate	S-1	S-2	S-3	Alumina
1	0.7121	0.7113	0.7145	0.7100
2	0.7083	0.7083	0.7085	0.7089
Unchromatographed	0.7077	0.7077	0.7077	0.7077

Diethyl Ether Passed Through Chromatographic Columns Containing Superfiltrol 643. Densities of Successive Eluates Determined at 25.00° C.

Table 24.

Eluate	Density
1	0.7343
2	0.7114
3	0.7118
4	0.7119
5	0.7110
6	0.7113
7	0.7111
8	0.7114
9	0.7114
10	0.7110
Unchromatographed	0.7077

Superfiltrol from Treatment Described in Table 24 Dried out with Air Drawn Through a Calcium Chloride Tube, Allowed to Stand in an Evacuated Desiccator over Night. Ethyl Alcohol Passed Through Column Filled with the Dried Superfiltrol. Densities at 25.00° C for Successive Eluates Determined.

Table 25.

Eluate	Density
1	0.8384
2	0.7888
Unchromatographed	0.7863

Benzene Passed Through Superfiltrol 643 in a Chromatographic Column. Densities of Successive Eluates Determined at 25.00° C.

Table 26.

Eluate	Density
1	0.8732
2	0.8732
Unchromatographed	0.8732

Skellysolve Passed Through Superfiltrol 643 in a Chromatographic Column. Densities of Successive Eluates Determined at 25.00° C.

Table 27.

Eluate	Density
1	0.6733
2	0.6739
3	0.6740
4	0.6740
Unchromatographed	0.6741

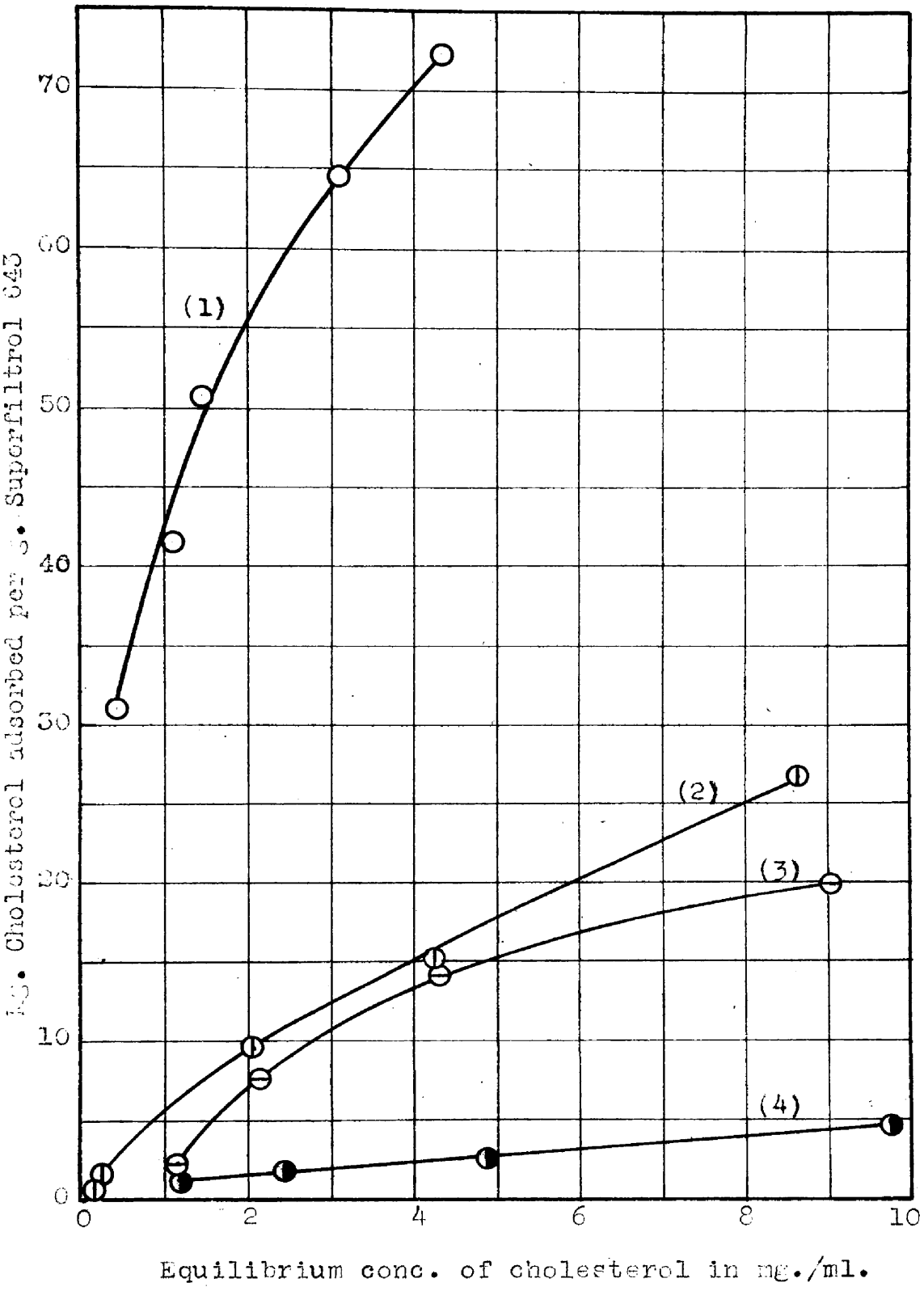

Fig. 1. Adsorption of cholesterol from (1) skellysolve, (2) benzene, (3) ethyl alcohol and (4) diethyl ether.

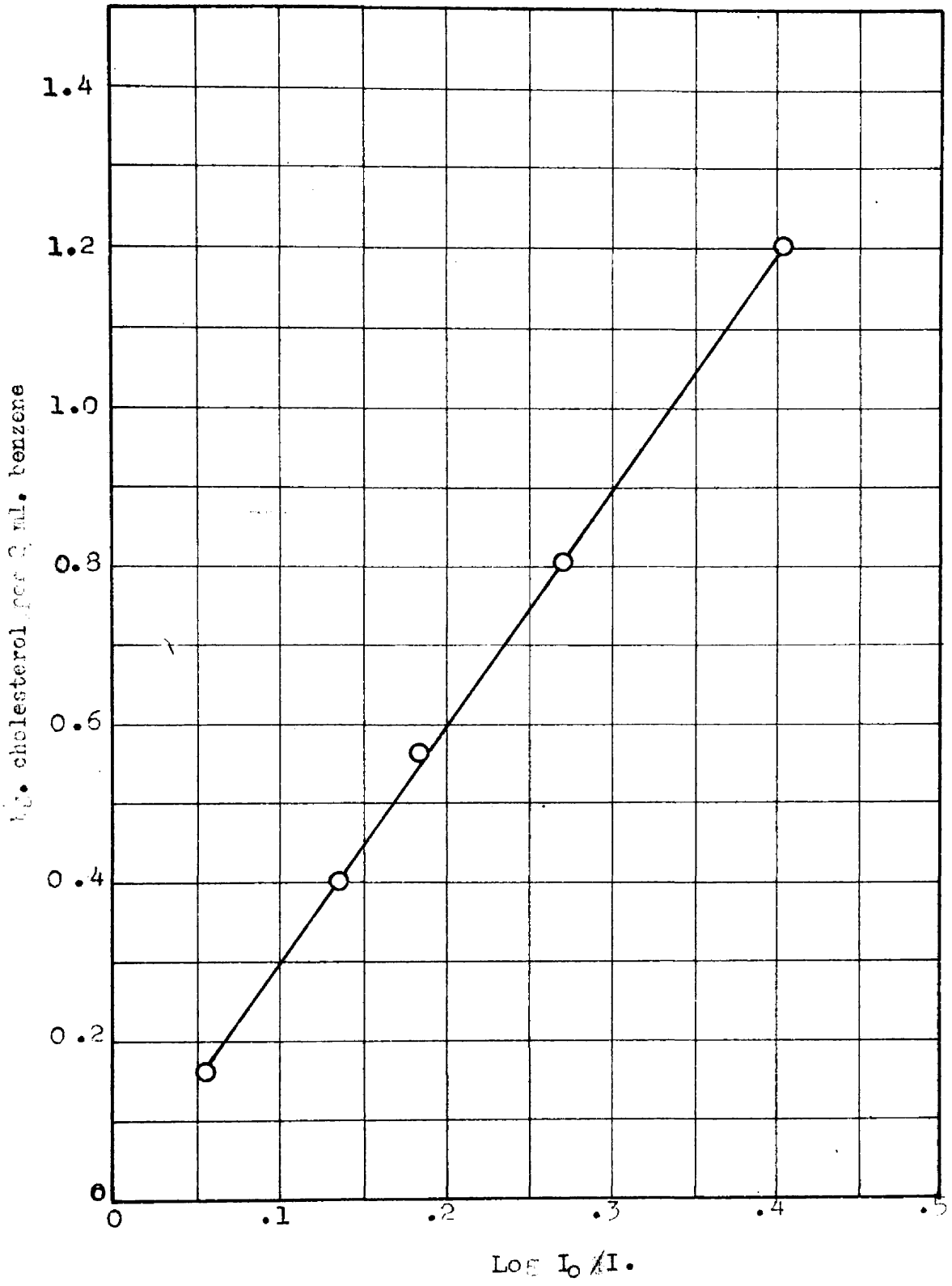

Fig. 2. Calibration curve for cholesterol, using a Cenco-Sheard Spectrophotometer.

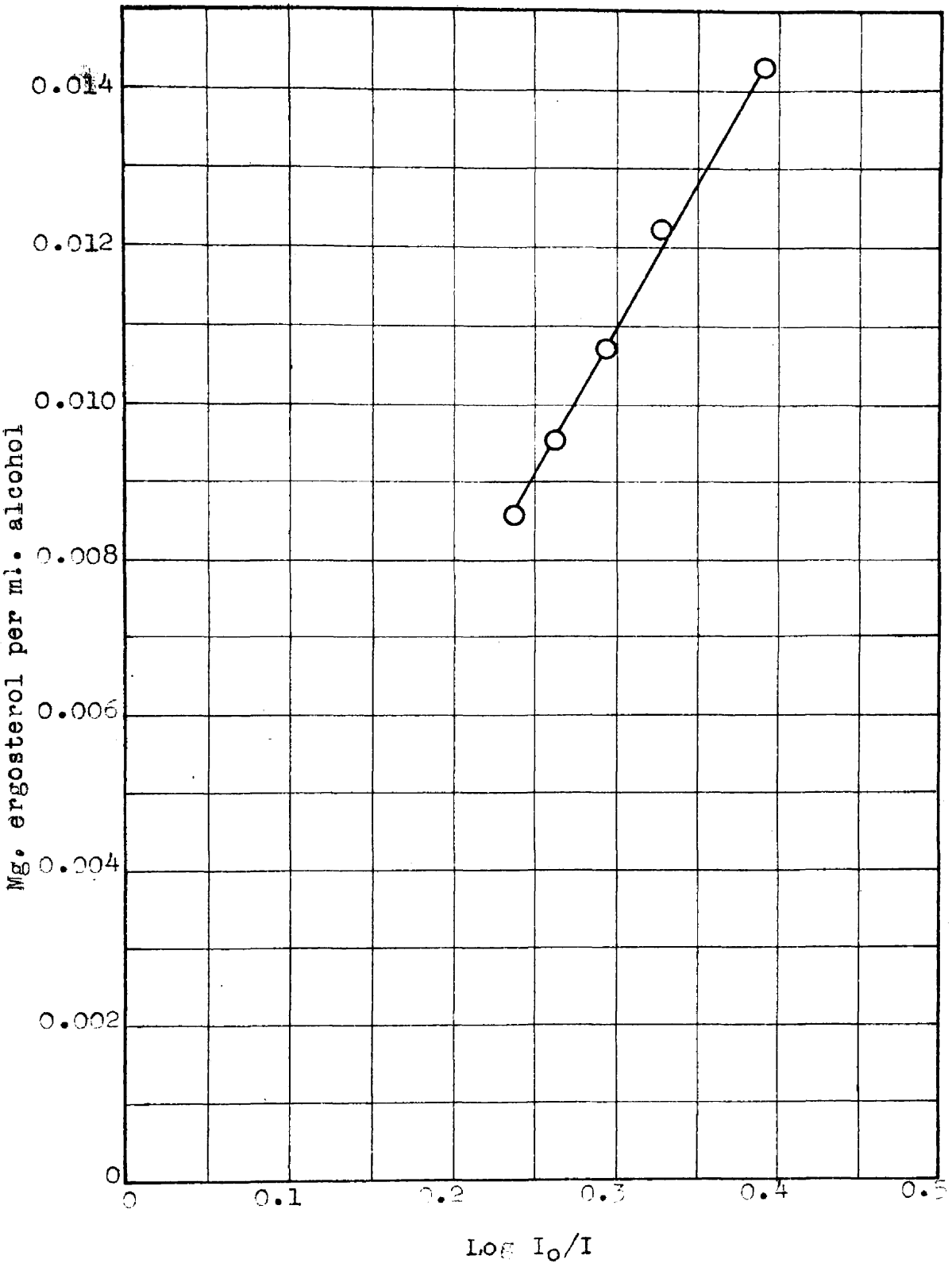

Fig. 3. Calibration curve for ergosterol in ethyl alcohol using the Beckman spectrophotometer.

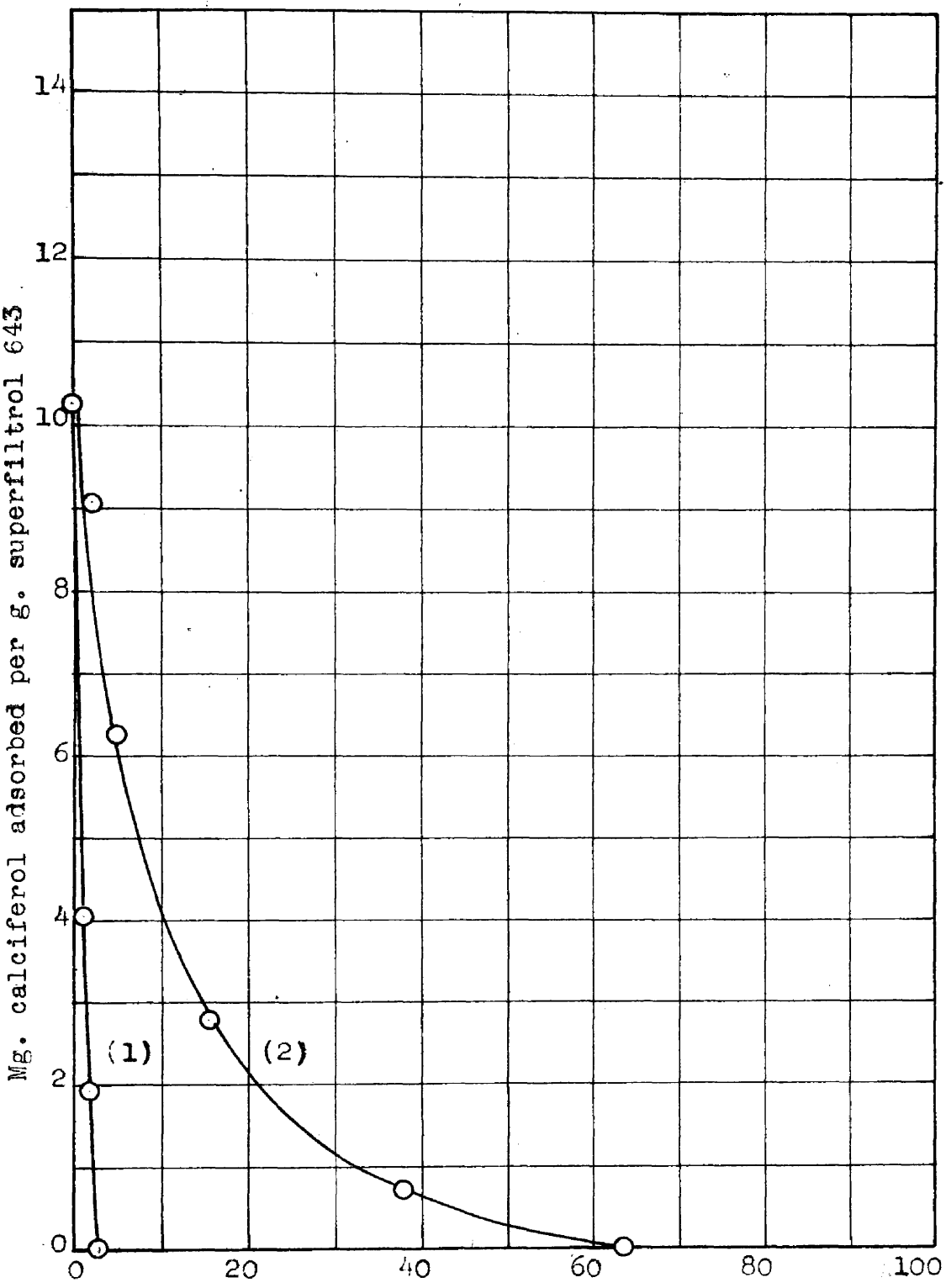

Fig. 4. Adsorption of calciferol from (1) ethyl alcohol-hexane and (2) diethyl ether-hexane mixtures.

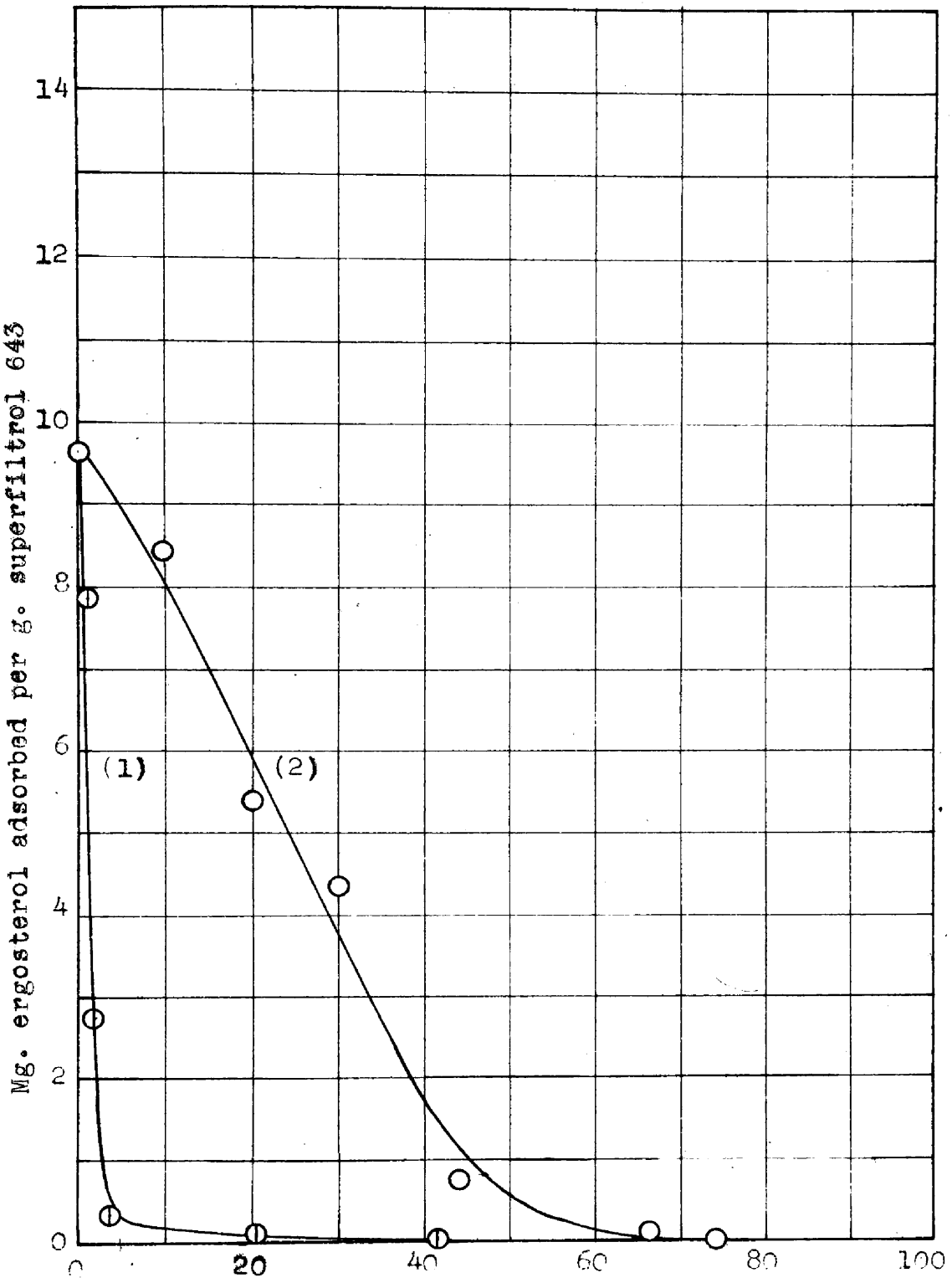

Fig. 5. Adsorption of ergosterol from (1) ethyl alcohol-hexane and (2) diethyl ether-hexane mixtures.

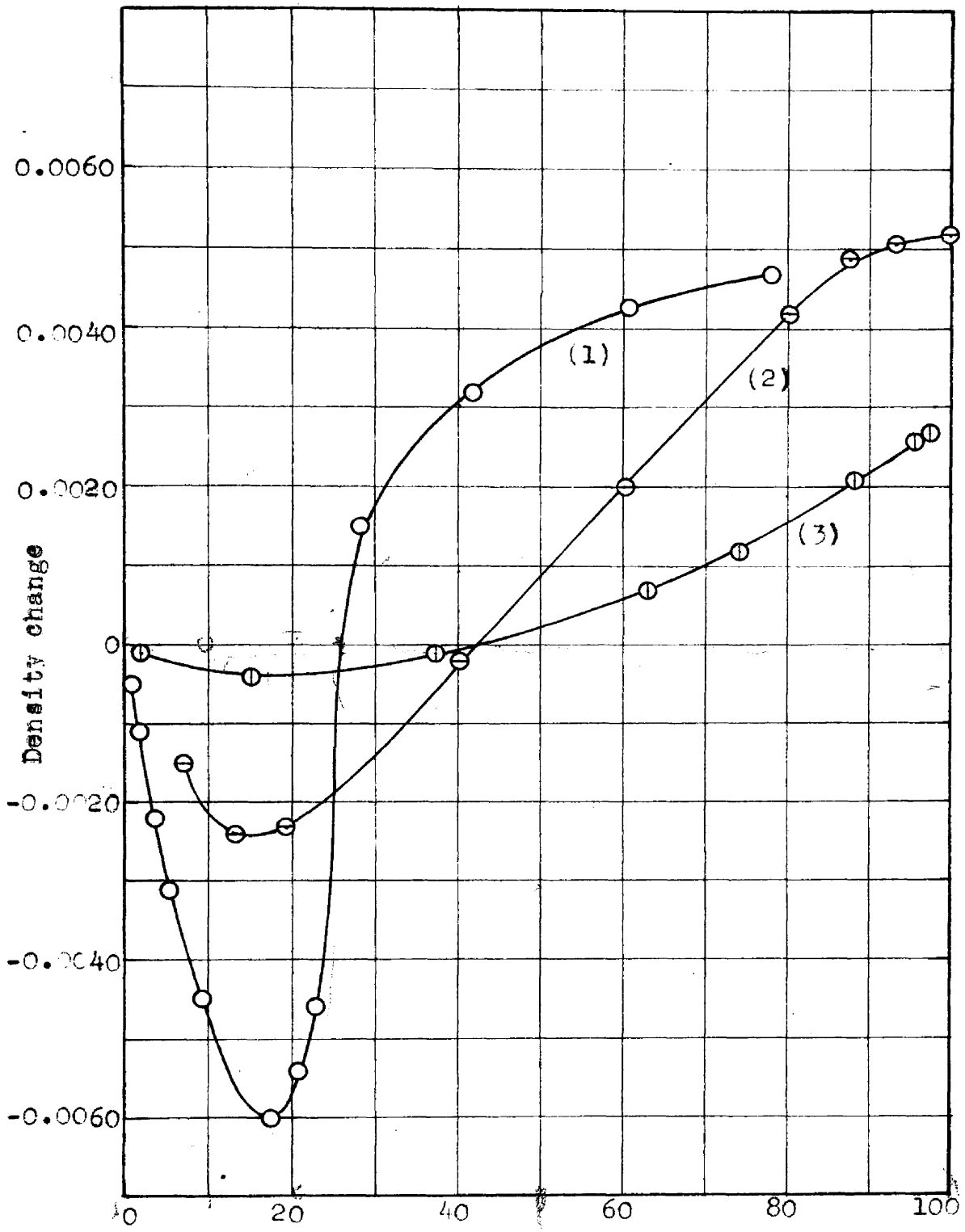

Fig. 6. Change in densities of (1) ethyl alcohol-hexane, (2) acetone-hexane and (3) diethyl ether-hexane mixtures after shaking with superfiltrol 643.

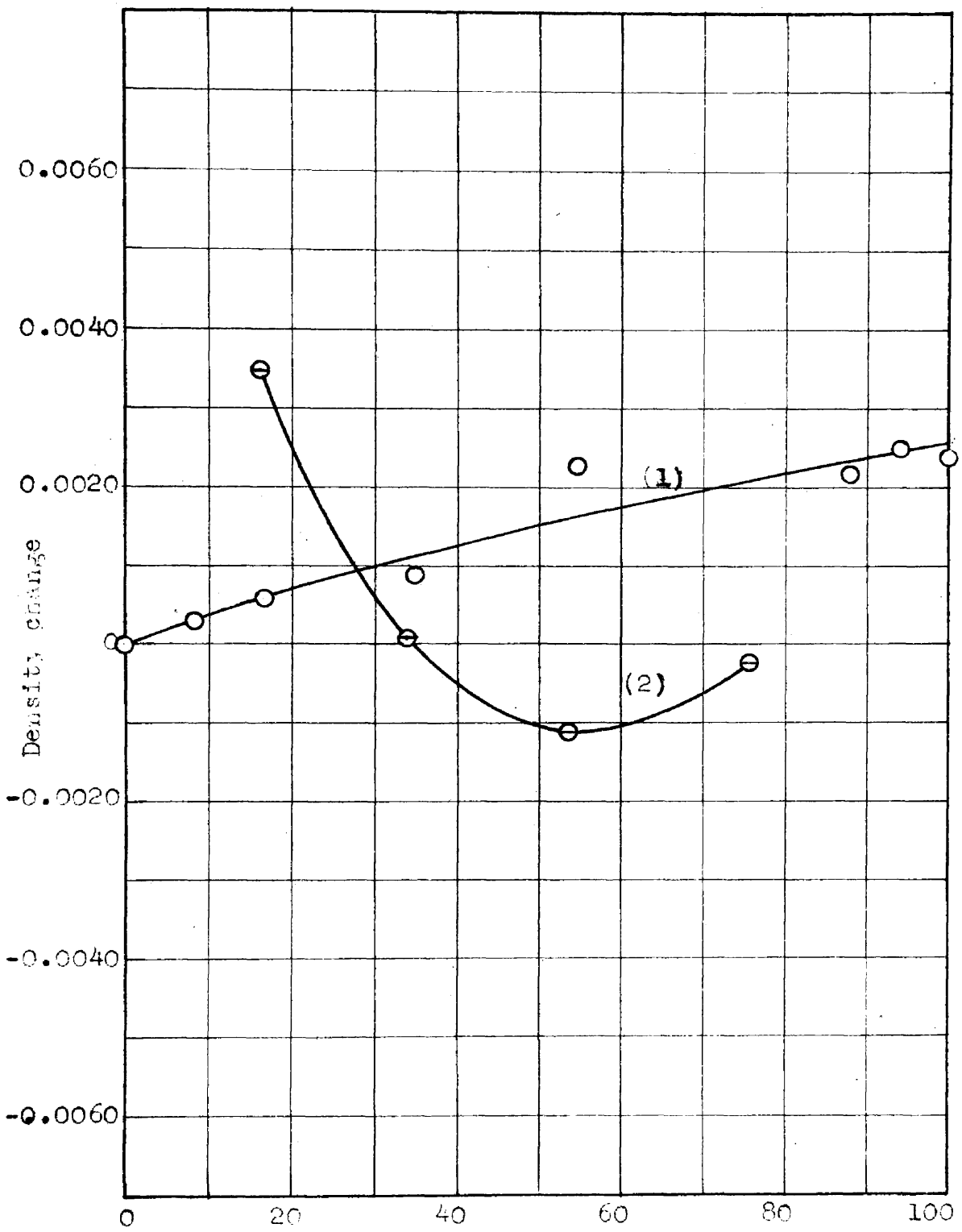

Fig. 7. Density changes in (1) diethyl ether-benzene and (2) skellysolve-benzene mixtures after shaking with superfiltrol 643.

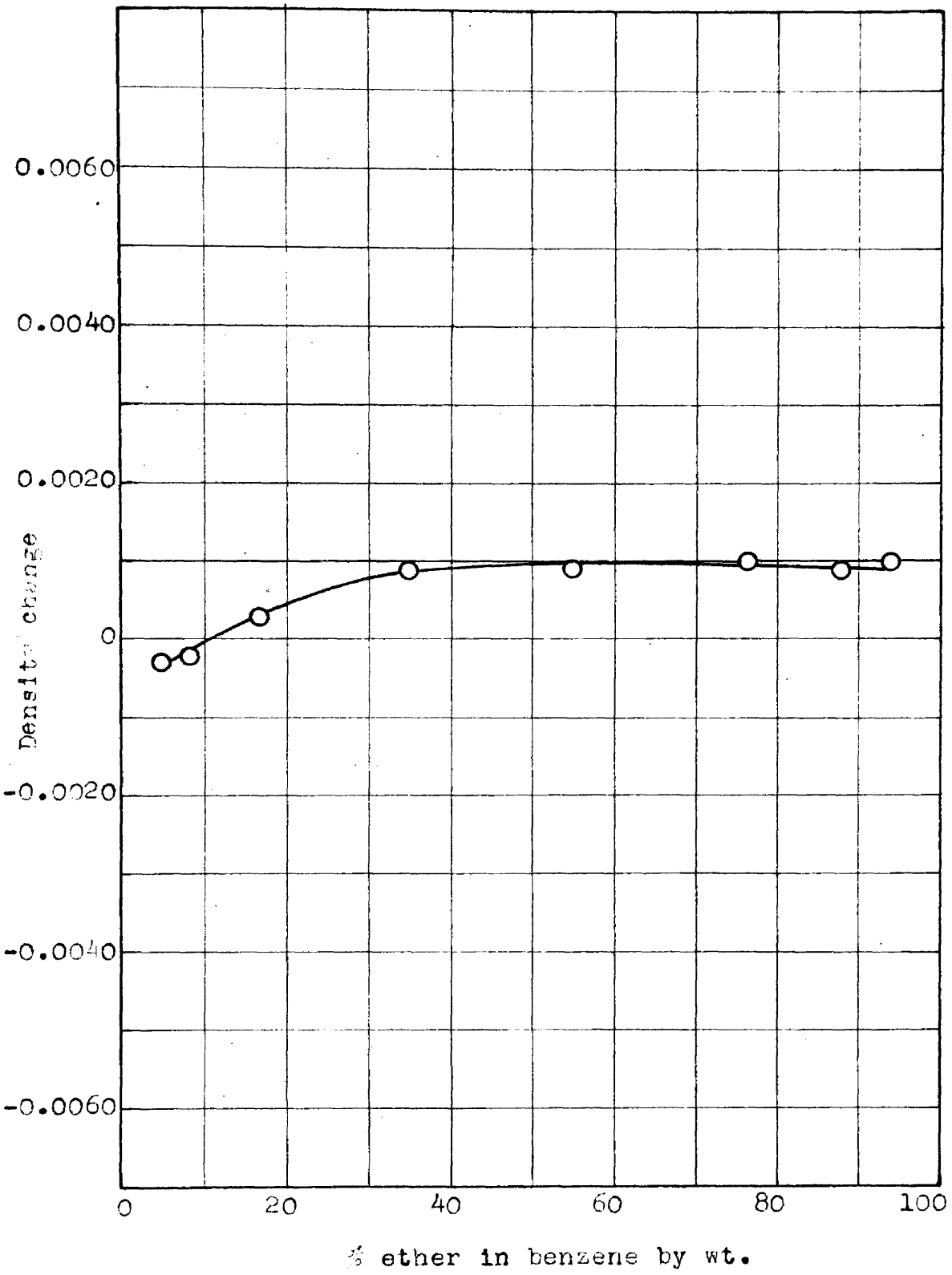

Fig. 8. Density changes in diethyl ether-benzene mixtures after shaking with treated superfiltrol 643.

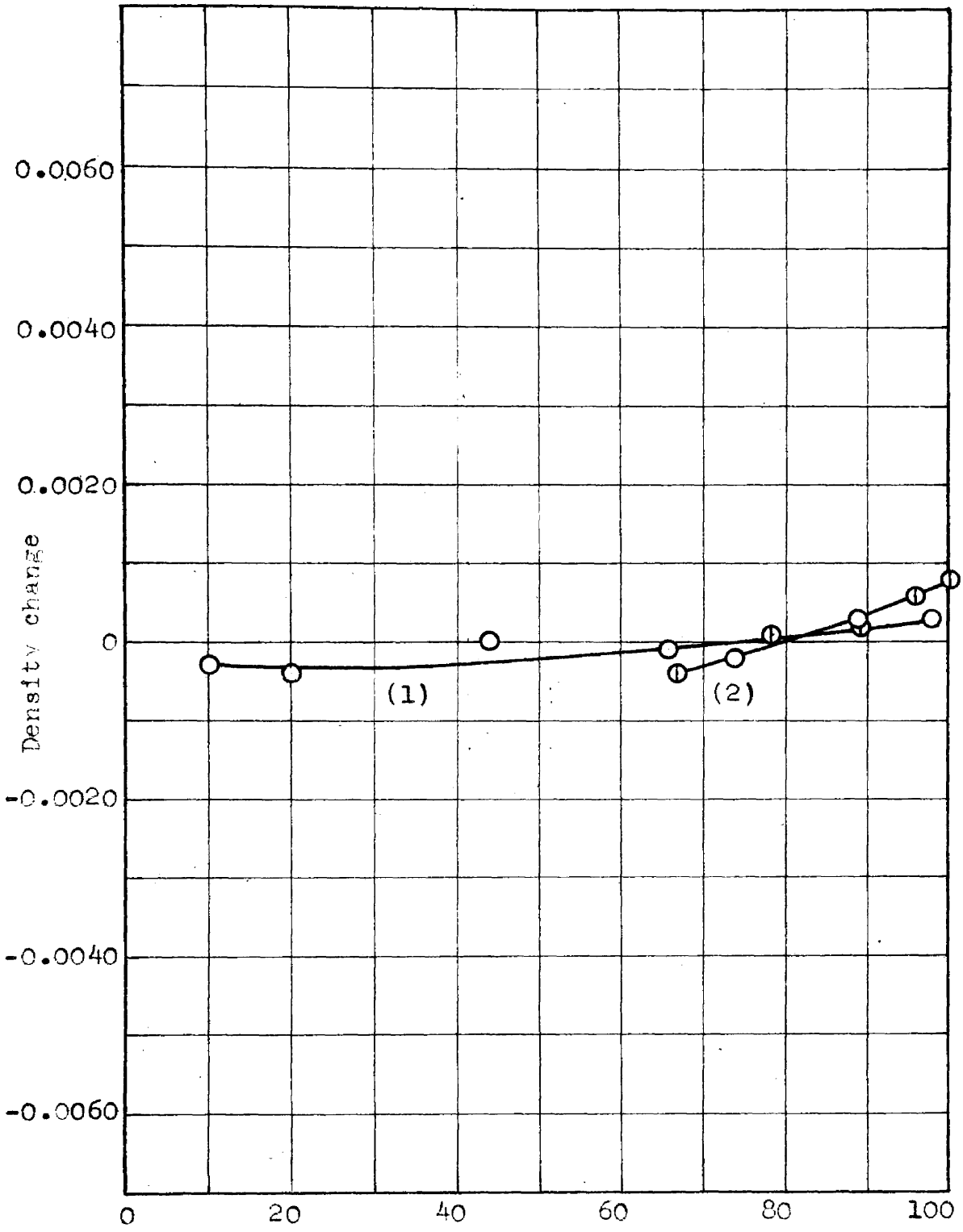

Fig. 9. Density changes in (1) diethyl ether-hexane and (2) ethyl alcohol-hexane mixtures after shaking with superfiltrol 643.(treated)

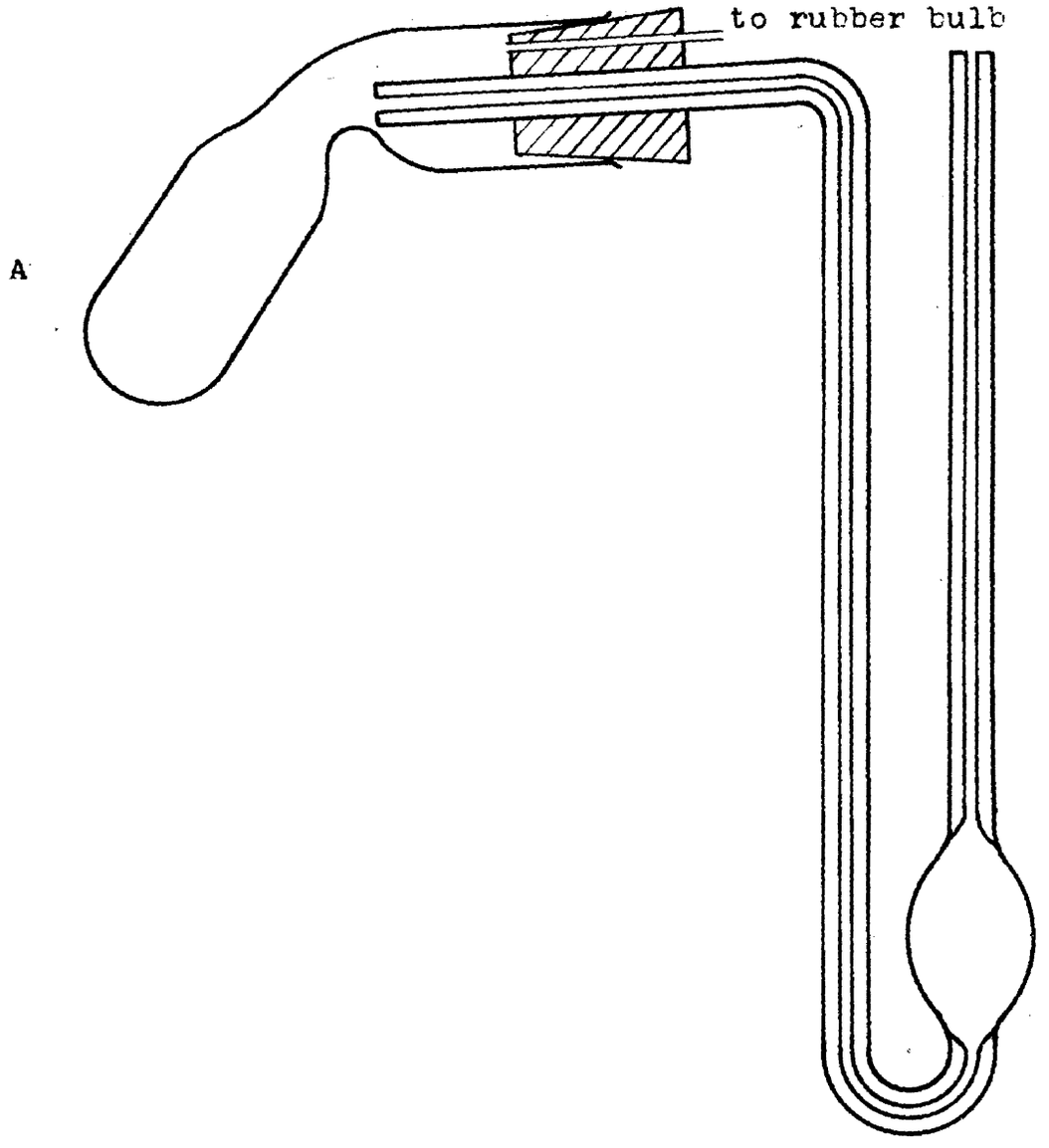

Fig. 10. Pycnometer and apparatus for filling pycnometer.

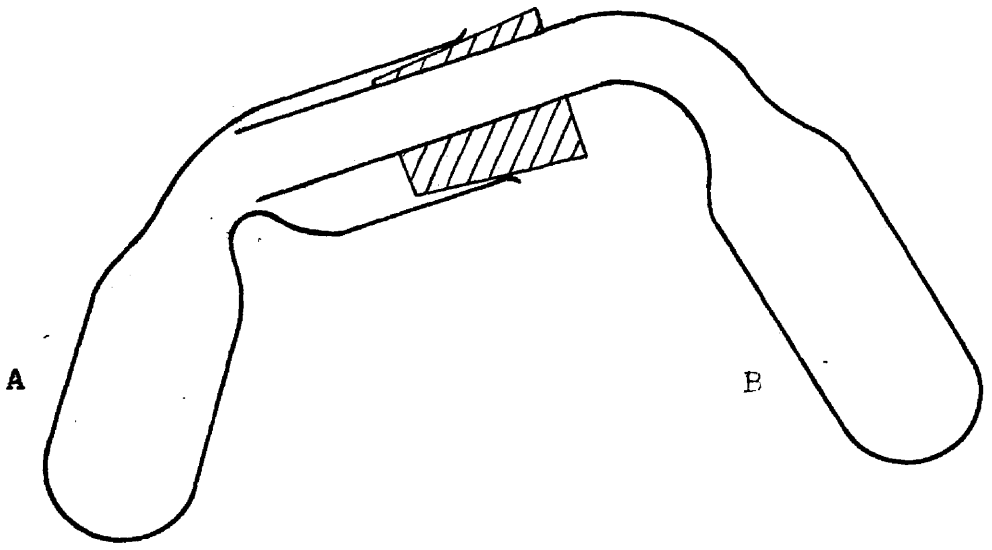

Fig. 11. Apparatus used to distill mixtures which had been shaken with superfiltrol 643.