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Identification of Certain Fatty Oils by Chromatographic Methods

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IDENTIFICATION OF CERTAIN FATTY OILS

BY CHROMATOGRAPHIC METHODS

This paper is submitted to the faculty of Ursinus College in partial fulfillment of requirements for departmental honors in chemistry.

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I. Introduction

Two problems facing the analytical chemist in the fatty oil industry are:

1. Identification of a fatty oil.
2. Separation of a fatty oil from a mineral oil.

This paper reports on the efforts to apply columnar chromatography to the solution of these two problems.

II. Purpose

1. To determine if the use of a column of adsorbent alumina will produce colored adsorption bands visible in natural or ultraviolet light which are characteristic for each substance concerned. The appearance of such bands is called a chromatogram.

2. To determine by chromatographic methods whether it is possible to separate fatty oils from mineral oils with subsequent recovery of both. Such a separation would make possible an examination of the fatty oil components without changing their structures. (Such an examination is not possible with the official American Oil Chemists Society procedure (1).

III. Procedure

The adsorbent, adsorptive alumina (80-200 mesh), was packed to a depth of 10 cm. in a glass column 30 cm. x 1.3 cm.

The packing was done in the following manner: After packing a ball of glass wool into the column, one poured in alumina until it filled the column to a height of approximately 2 cm. above the surface of the glass wool. The column was then tapped with the fingers until the adsorbent ceased to settle. This process of filling with alumina to a 2 cm. height and settling this adsorbent by tapping was repeated until the alumina height in the column stood at 10 cm. Upon the upper surface of the adsorbent column thus produced

was packed a ball of glass wool to keep the surface of the column intact during the subsequent pouring of solvent upon it.

This column was then washed with 15 ml. of petroleum ether (B. P. 30-60° C); whereupon, the weighed sample, consisting of a single fatty or mineral oil dissolved in 25 ml. of petroleum ether was poured into it. The weight of the sample varied between 0.450 and 0.550 gms.

The solvent issuing from the column after the addition of this liquid was collected in a weighed 50 ml. beaker. When flow thru the column had ceased, the column was further washed with two successive 35 ml. portions of petroleum ether followed by three successive 35 ml. volumes of diethyl ether. Finally 35 ml. of acetone was poured into the column. The procedure for the collection of the effluent caused by these six additions of solvent was the same as that used in collecting the flow caused by the sample solvent as described above.

After evaporation of the solvent from the beaker, the latter was weighed to the nearest milligram, and the per cent of the sample eluted into it was calculated.

It was hoped that an examination of the elution characteristics of individual oils would enable one to predict whether a particular fatty oil could be separated from a particular mineral oil by this procedure. A great difference in elution characteristics would indicate a ready separation; a similarity of such characteristics would indicate the impossibility of separation by this chromatographic method.

The oils examined by this method were:

1. A white mineral oil - U. S. P., 340 S. U. viscosity at 100° F.

2. A paraffinic mineral oil - "Topaz B" of Atlantic Refining Company, 100 S. U. viscosity at 100° F.

3. A naphthenic mineral oil - "561 Oil" of Gulf Oil Company, 100 S. U. viscosity at 100° F.

4. A glycerol trioleate - commercial grade.

5. Neatsfoot oil - 20 cold test.

6. Herring oil.

7. Salmon oil - British Columbian Crude.

8. Menhaden oil.

9. Coconut oil.

10. Soybean oil.

11. Linseed oil.

12. A blown (air-oxidized) soybean oil.

13. Castor oil - technical grade.

14. Sperm oil - 38 cold test.

15. A methyl oleate - technical grade.

In the case of blown soybean oil and castor oil, both of which are practically insoluble in petroleum ether, the procedure was altered somewhat. With these two oils the sample was dissolved in 35 ml. of diethyl ether and treated in the column with successive portions of 35 ml. of diethyl ether, and 35 ml. of acetone.

Following the termination of solvent flow from the column, the position and color of the chromatogram bands were noted. The chromatogram was observed in both visible and ultraviolet light.* The exhibition by an oil of a unique chromatogram would indicate that the oil could be identified by means of this chromatogram.

*The ultraviolet light source was a Hanovia Mercury Vapor Arc Lamp producing a wave length of 3660 A°.

IV. Explanation of Data Charts

A chart for each oil examined is given showing the observed elution data and ultraviolet chromatogram characteristics.

The vertical columns in a chart designated 1 to 7 refer to the chromatograms produced by the particular solvents added in the sequence shown:

Column #1 - Solvent in which sample is dissolved.

Column #2 - First petroleum ether addition.

Column #3 - Second petroleum ether addition.

Column #4 - First diethyl ether addition.

Column #5 - Second diethyl ether addition.

Column #6 - Third diethyl ether addition.

Column #7 - Addition of acetone.

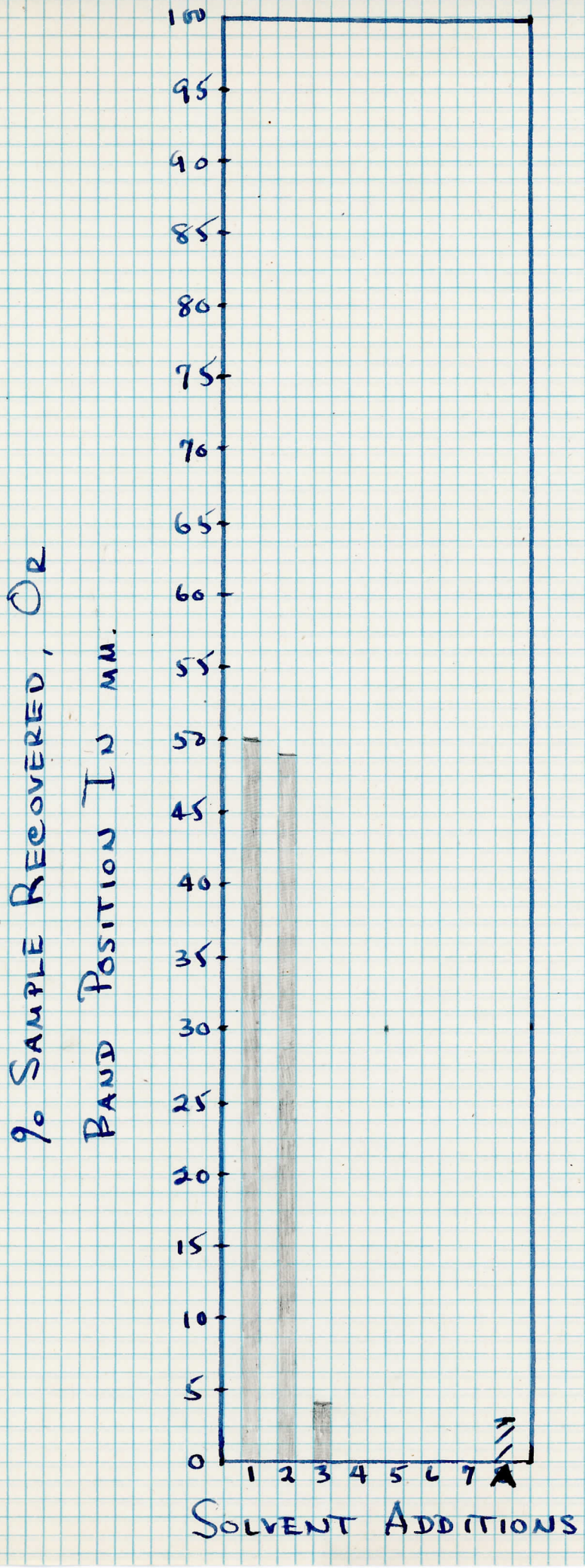
An upward extending gray bar in a column of the chart indicates that elution occured during that addition. The per cent of sample weight eluted is given by the height of the bar and can be read from the left-hand margin of the chart.

The colored bands in the upper part of a column of the chart show the color and position of the ultraviolet chromatogram bands present following the treatment represented by the column. In order to fix the position of these bands the alumina column was graduated from 0 mm. at the bottom to 100 mms. at the top. This alumina column graduation is represented in the left-hand margin of the charts, so a band position can be read from this margin. An uncolored band marked "W" represents a white chromatogram band.

The striped bar in the vertical column designated "A" gives the per cent of sample unrecovered (as read from the left-hand margin) if it is red. If it is blue, it gives the per cent of sample recovered in excess of the sample weight. This excess recovery (Fig. #1 and #2) may be caused by insufficient drying of the eluted samples.

FIG 1

WHITE MINERAL OIL



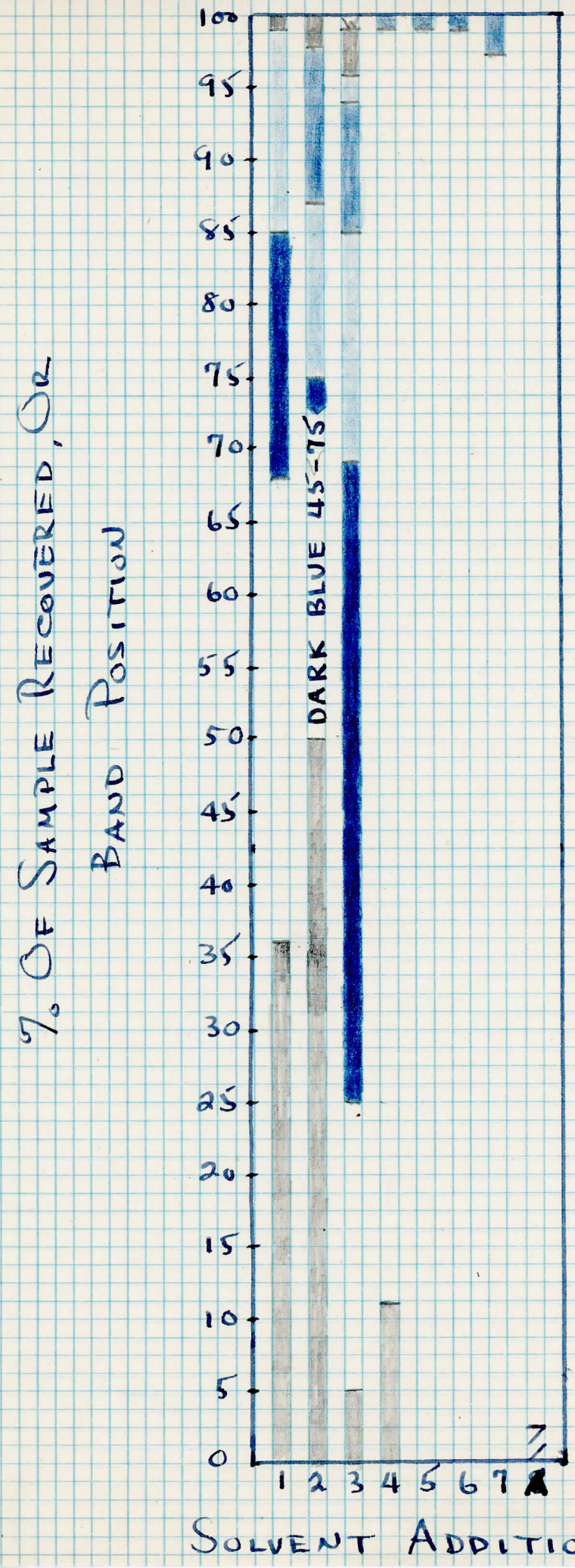


Fig 2

Paraffinic
Mineral Oil

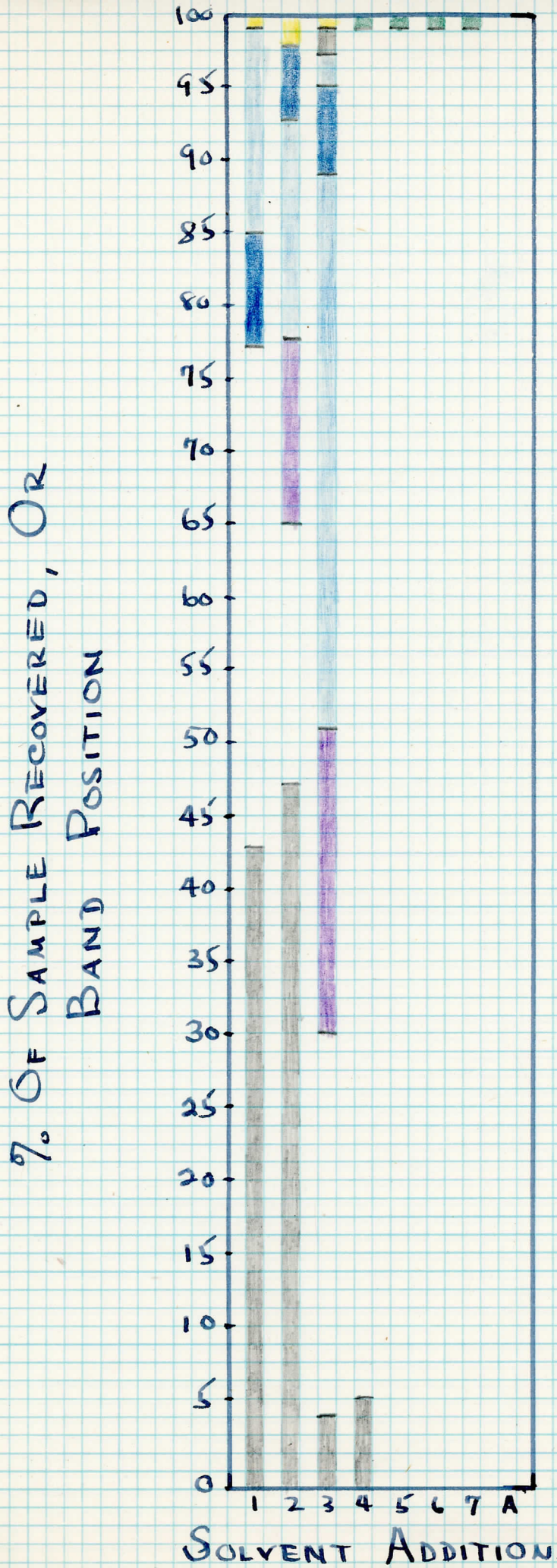


Fig 3 ?

Naphthenic Mineral Oil

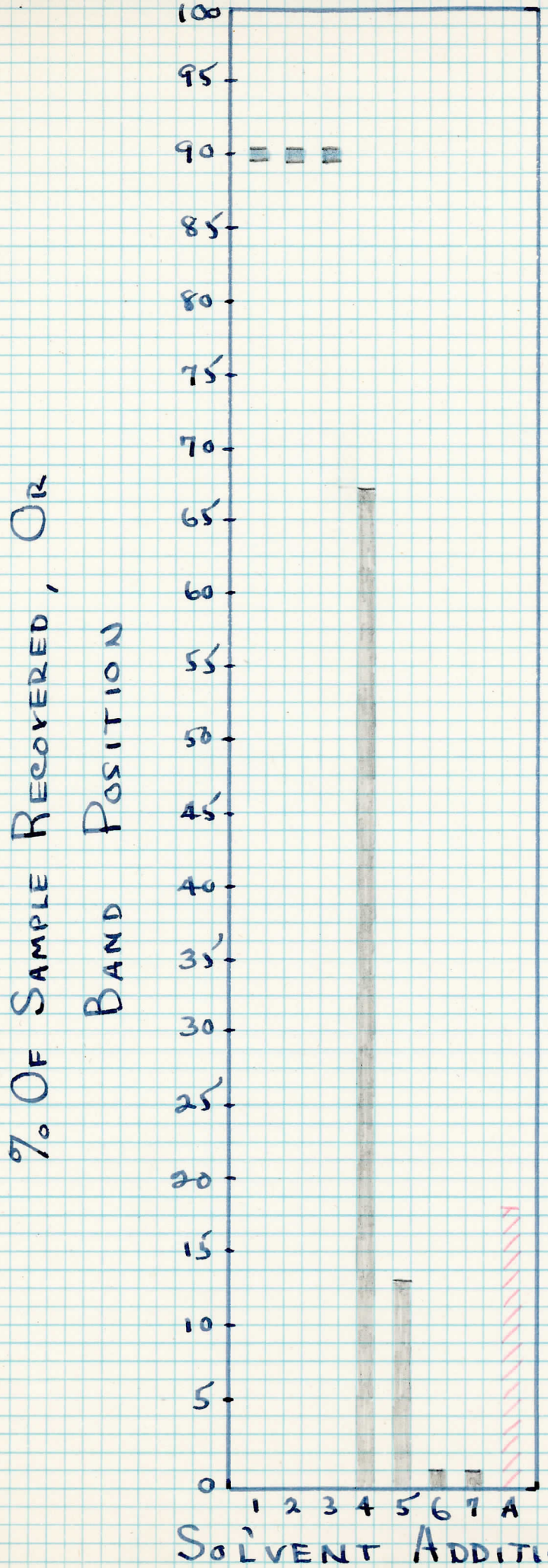


Fig 4 8

Glycerol Trioleate

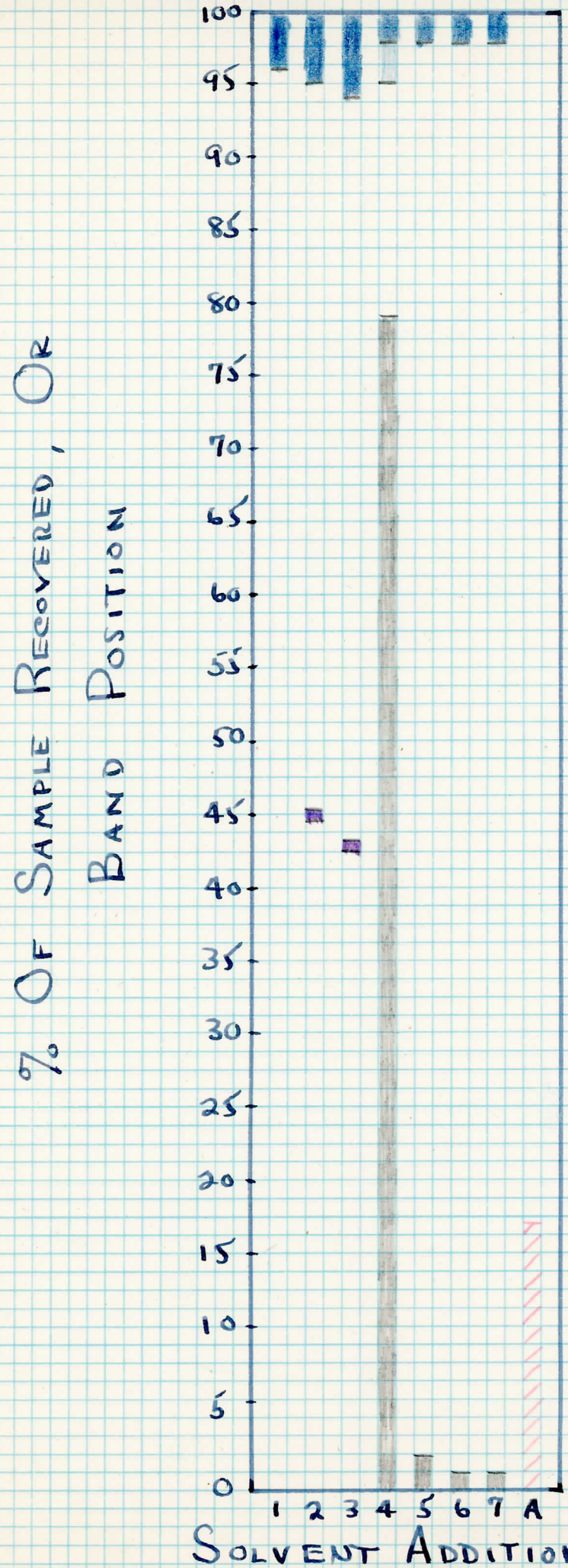


FIG 5

NEATSFOOT OIL

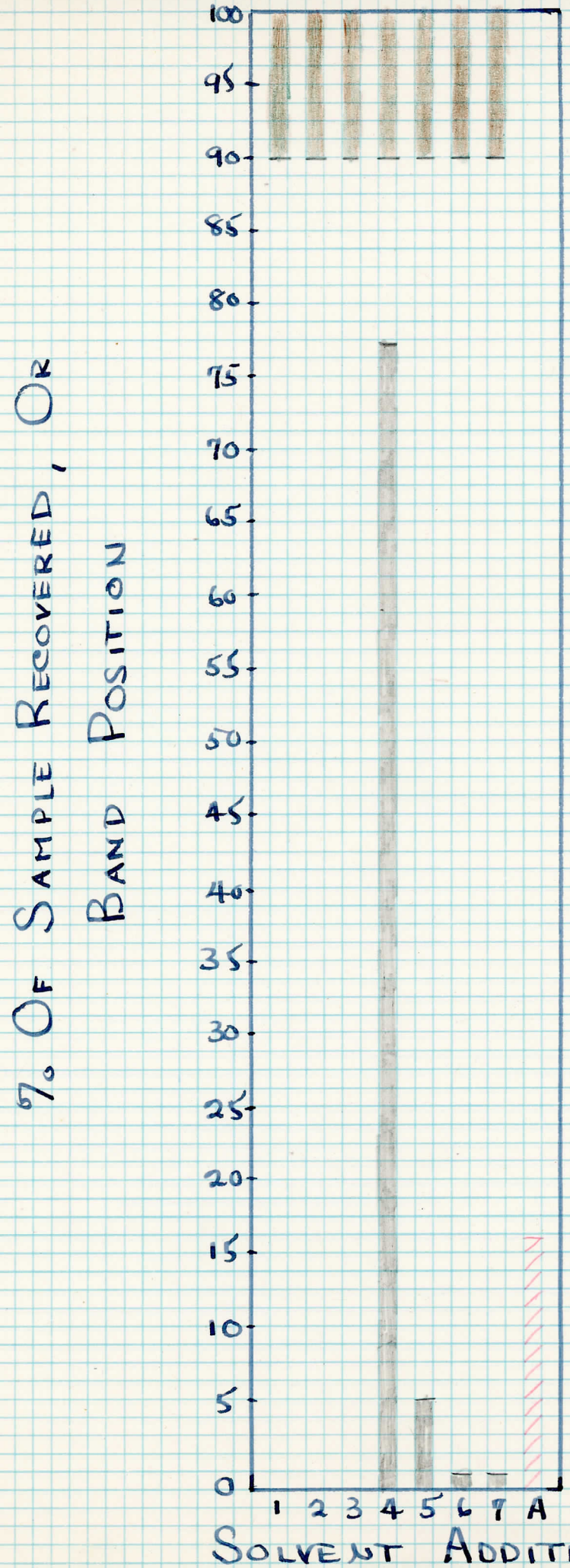
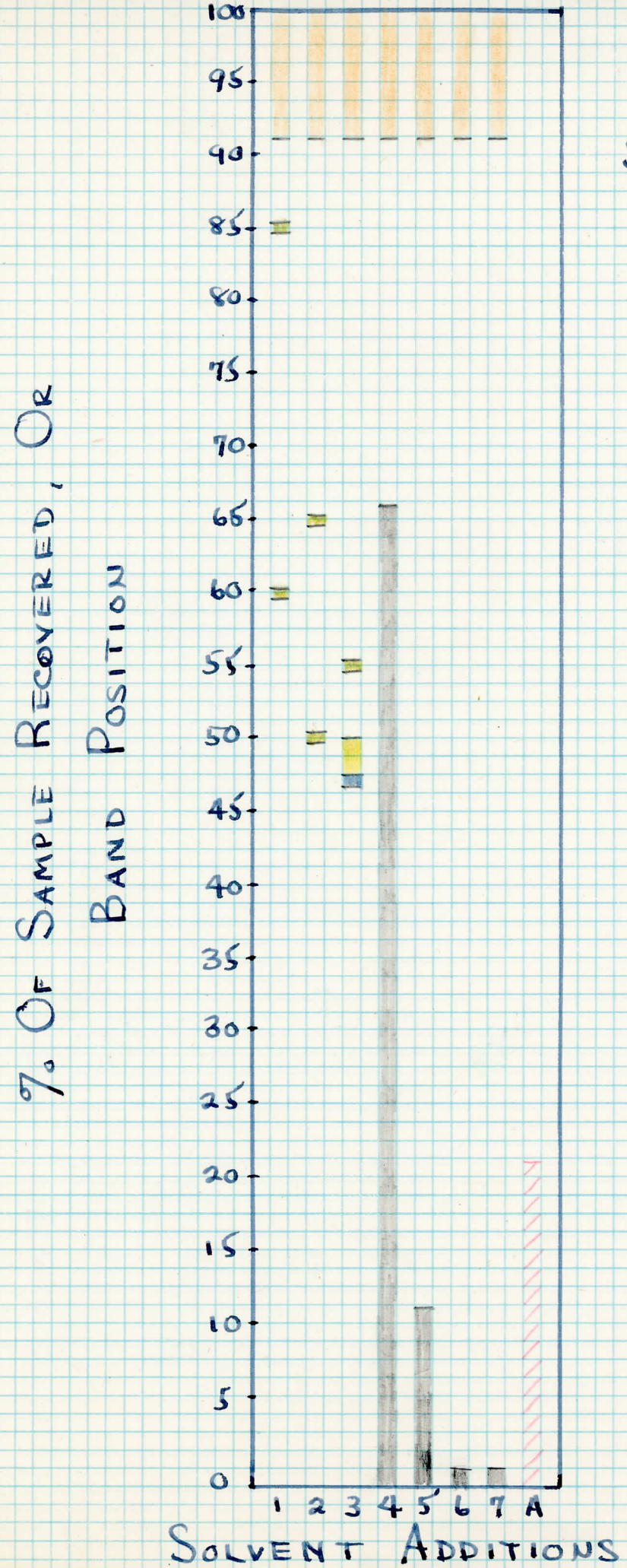


FIG 6

HERRING OIL

Fig 7

Salmon Oil



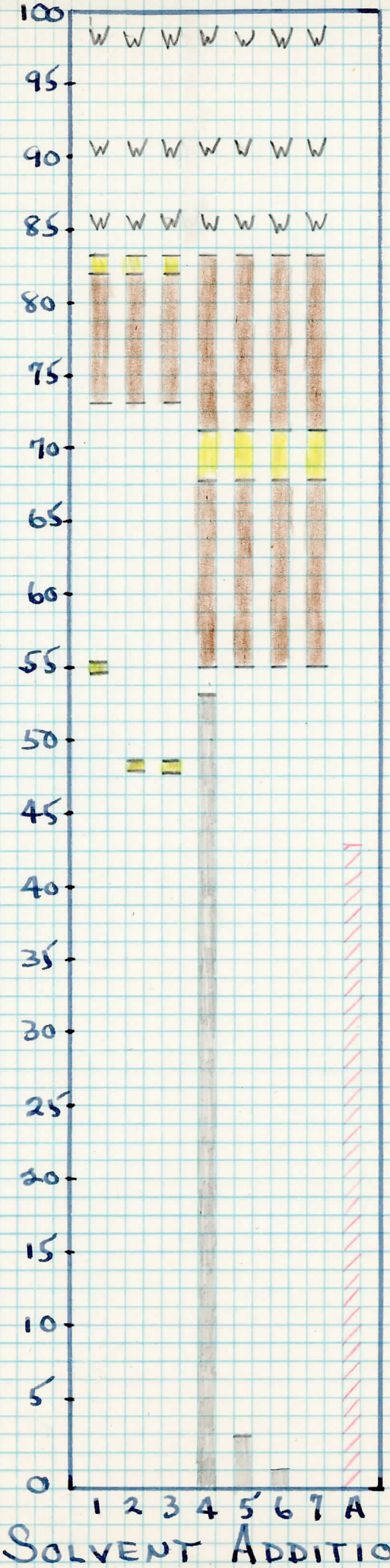
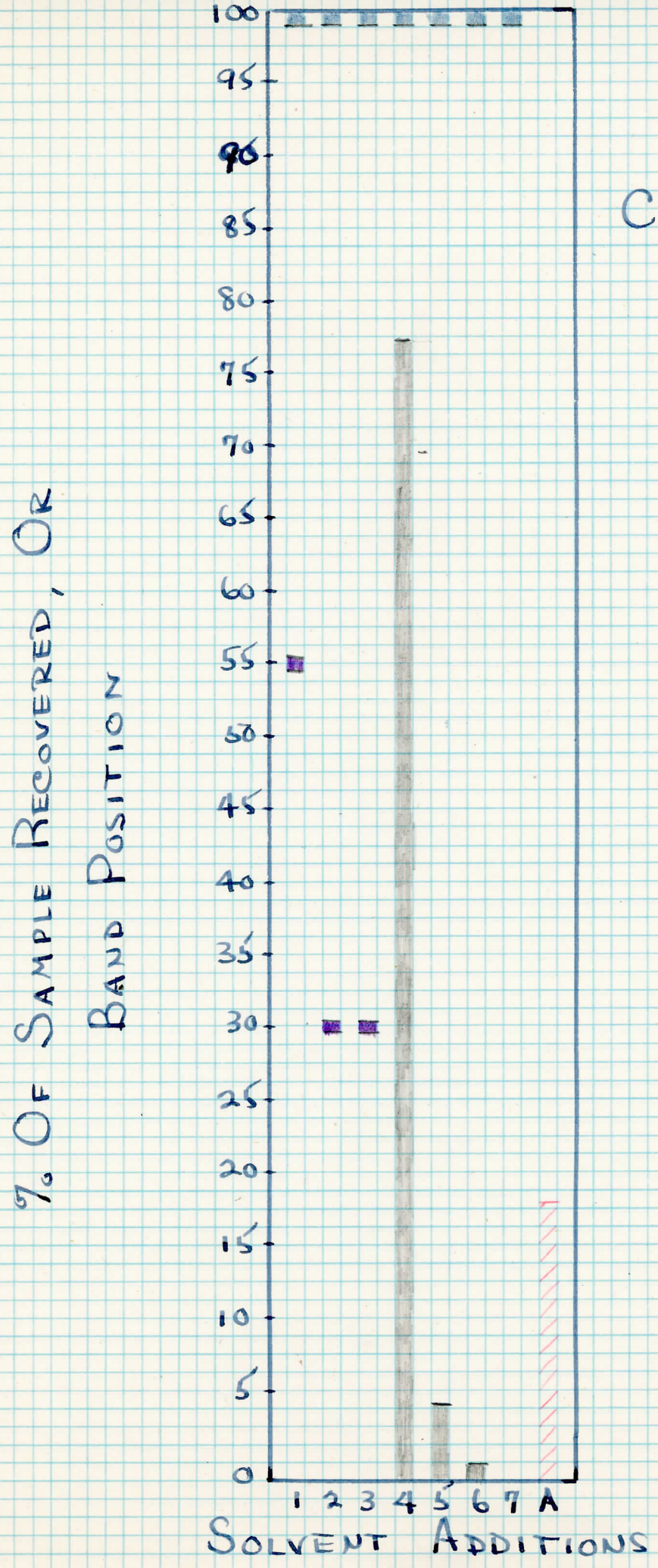


FIG 8₁₃

MENHADEN OIL

Fig 9

Coconut Oil



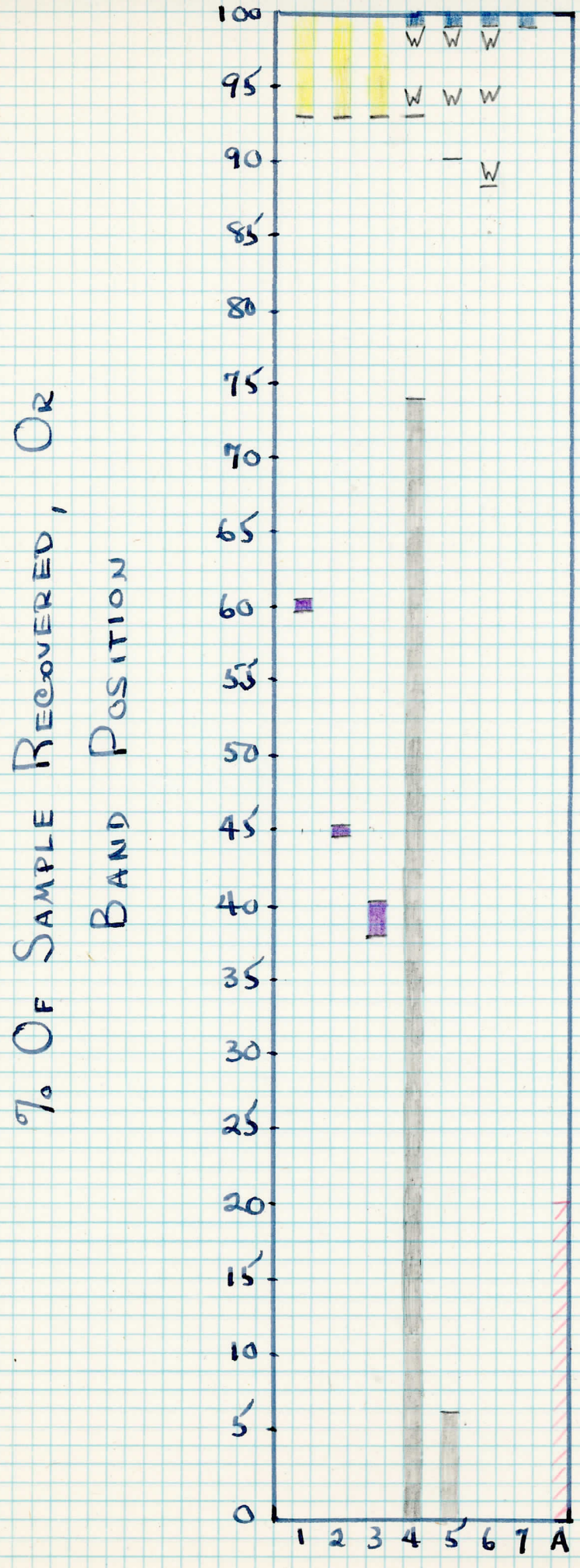


FIG 10₁₄

SOYBEAN OIL

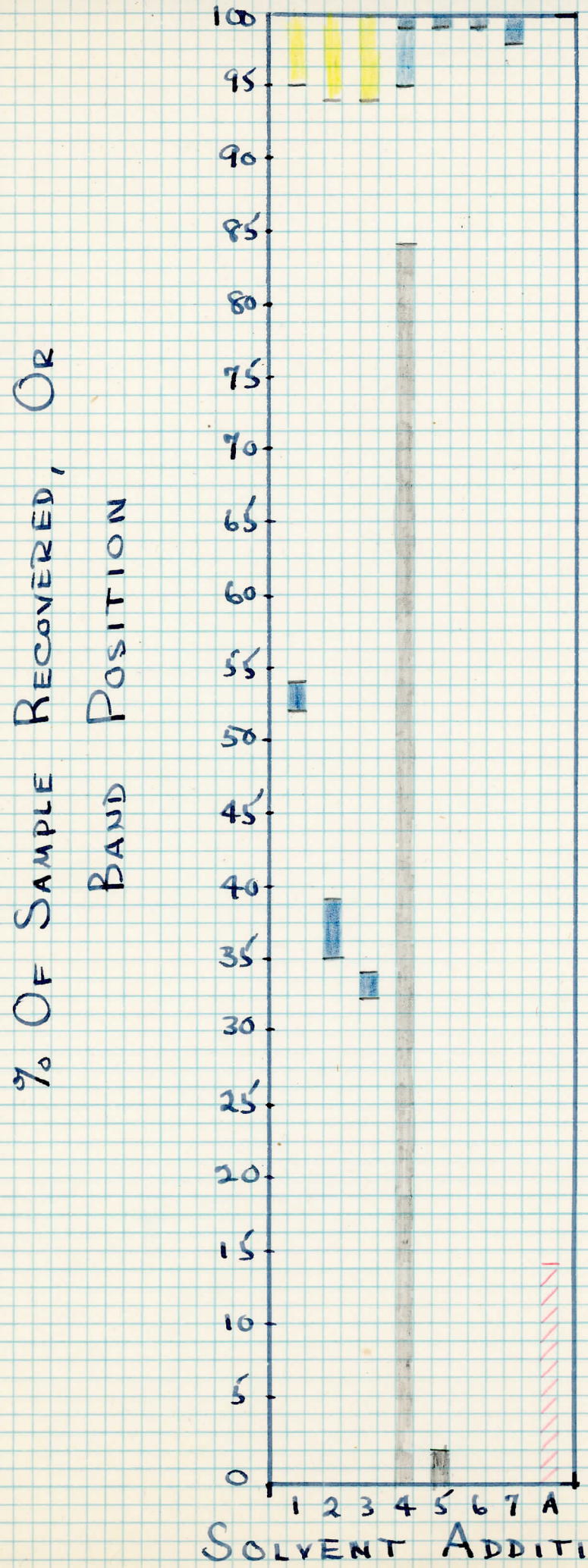


FIG 11[15]

LINSEED OIL

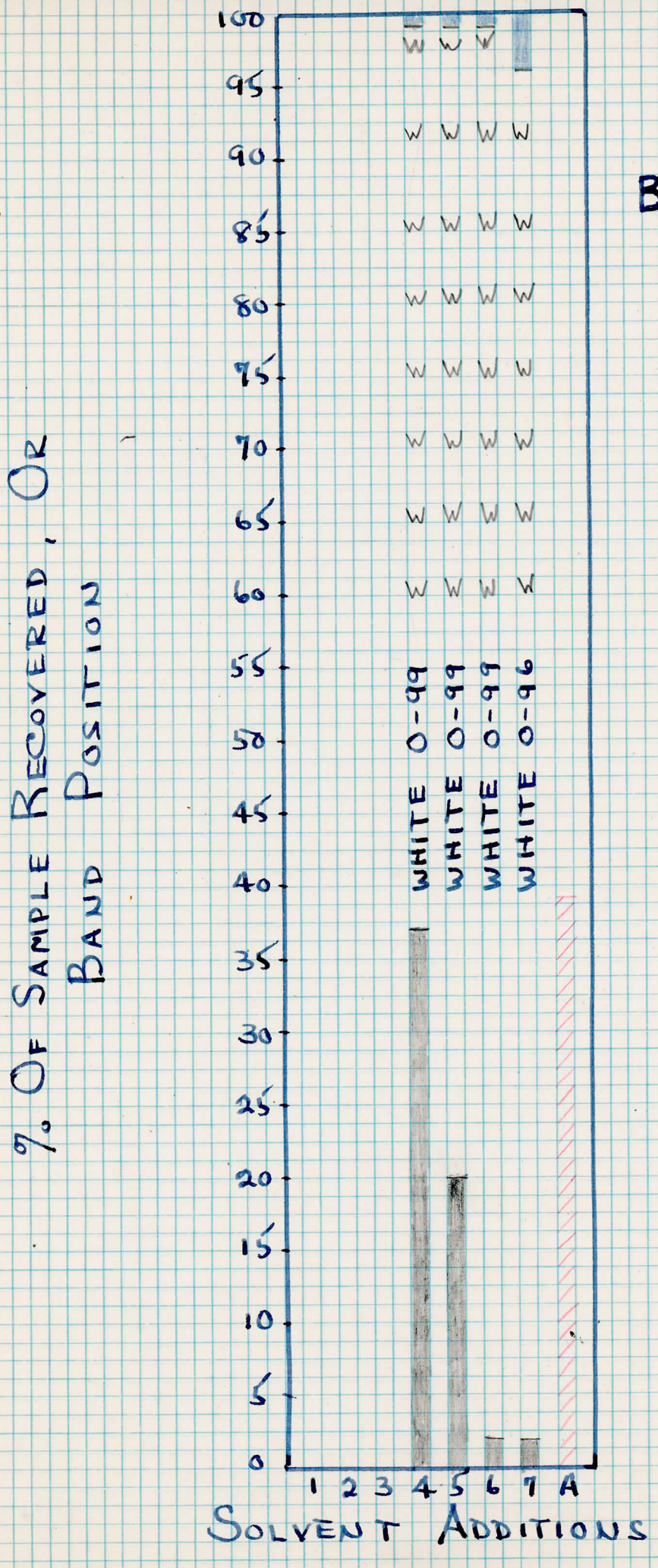


Fig 12[16]

Blown Soybean Oil

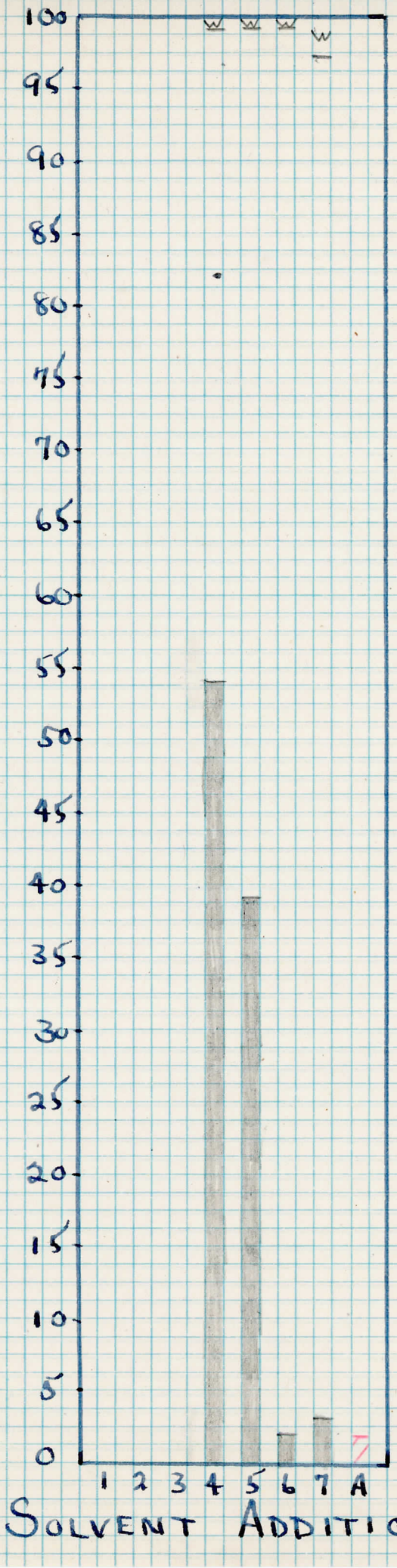


Fig 13

Castor Oil

FIG 14

SPERM OIL

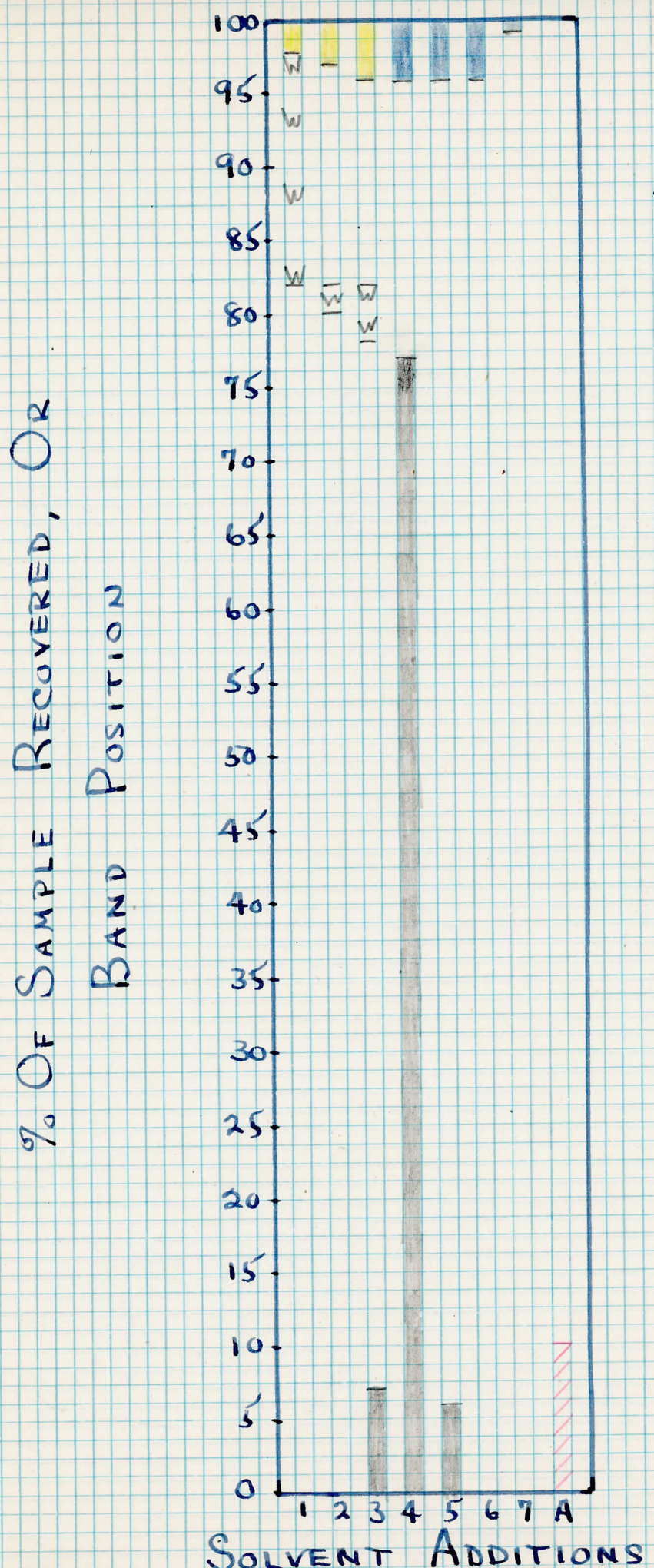


Fig 15

Methyl Oleate

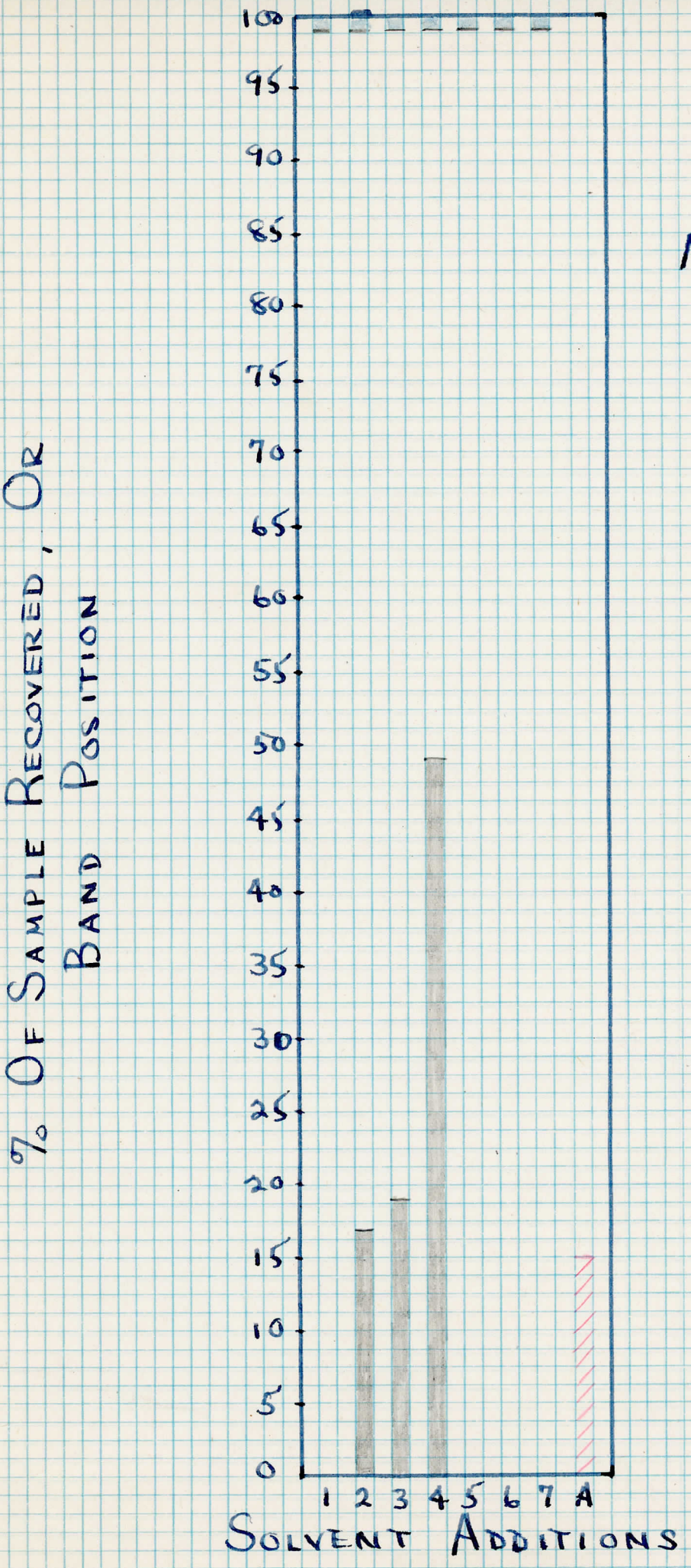


Fig 16

Neatsfoot Oil (51%)

Mineral Oil (49%)

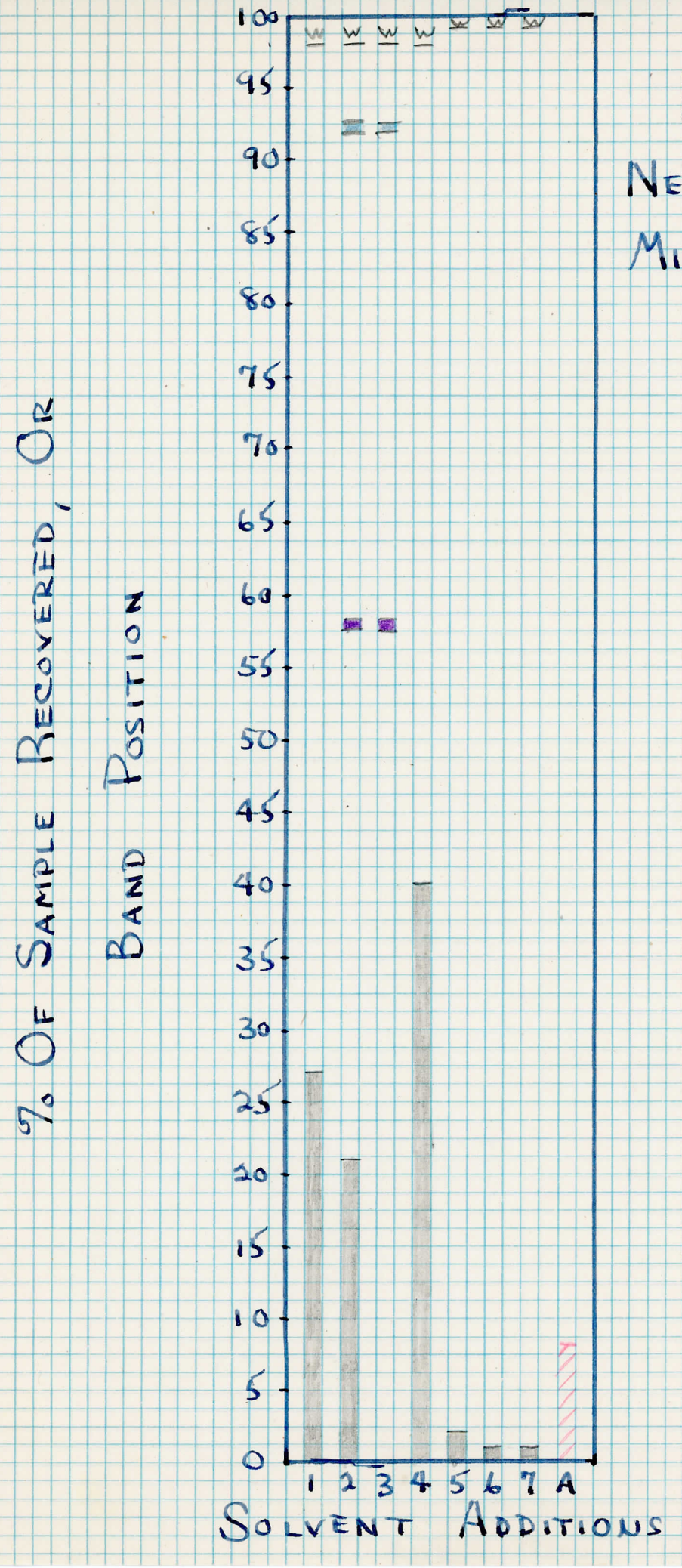
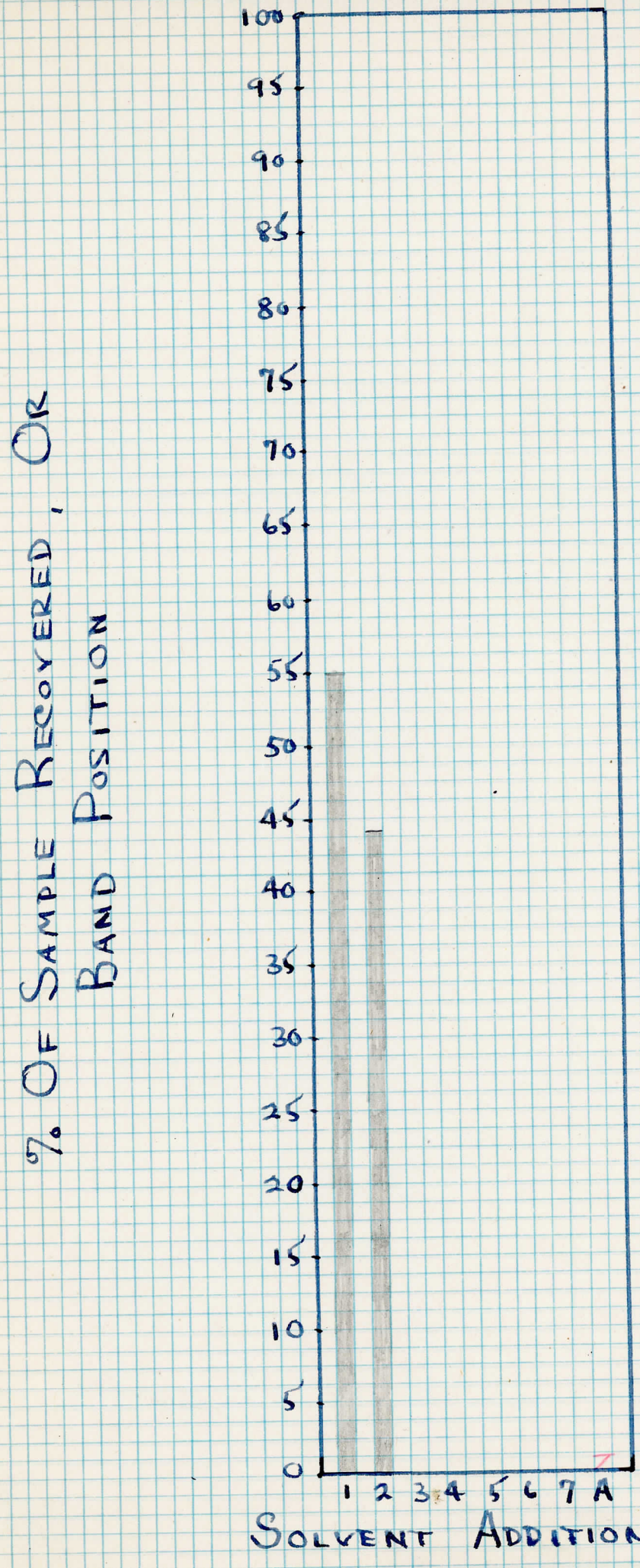


Fig 17

Mineral Oil From Preceding Mixture (Fig 16)



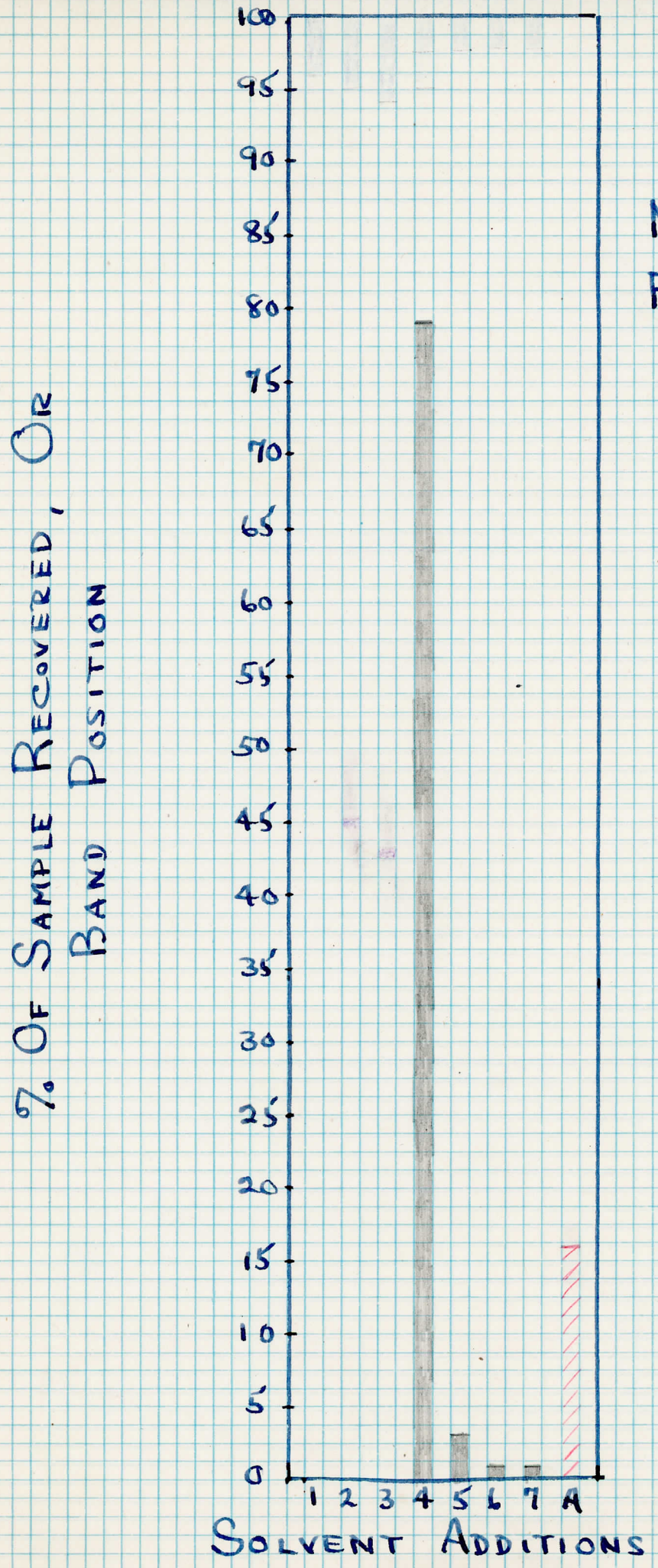


Fig 18

Neatsfoot Oil From Preceding Mixture (Fig 16)

V. Results and discussion

A. Elution characteristics of individual oils.

1. Mineral oils

a. White mineral oil (Fig. 1)

White mineral oil is completely eluted by the three volumns of petroleum ether.

b. Naphthenic mineral oil (Fig. 3)

Naphthenic mineral oil is completely eluted by the three volumns of petroleum ether plus the first diethyl ether development.

c. Paraffinic mineral oil (Fig. 2)

Paraffinic mineral oil behaves similarly to naphthenic mineral oil.

2. Fatty oils

a. Glycerol esters (Fig. 4-13)

All glycerol ester fatty oils were partially eluted by diethyl ether and acetone but not at all by petroleum ether. The per cent of sample recovered after diethyl ether and acetone treatment varied between 57% in the case of menhaden oil and 98% for castor oil.

b. Alcohol esters

Sperm oil (Fig. 14)

7% of the sperm oil was eluted by the second petroleum ether addition and 83% by the first and second diethyl ether washings. 10% was unrecovered.

Methyl oleate (Fig. 15)

36% of the methyl oleate was eluted by the first and second petroleum ether additions and 49% by the first diethyl ether addition. 15% was unrecovered.

B. Separation of fatty oils from mineral oils.

1. Separation of glycerol ester fatty oils from white mineral oil.

Since this latter oil is eluted 100% by the petroleum ether developments (Fig. 1) while glycerol ester fatty oils are not eluted by this solvent (Fig. 4-13), a glycerol ester fatty oil may thus be readily separated from white mineral oil by use of this chromatographic method.

The following procedure further supports this conclusion. When a mixture of 51% by weight of neatsfoot oil and 49% by weight of white mineral oil is chromatographed, it is apparent that the elutions produced by the first and second washings represent mineral oil while the elutions produced by the fourth thru seventh represent neatsfoot oil components.*

Altho mineral oil has been almost 100% recovered by elution, only 84% of the neatsfoot oil has been eluted from the column. That fraction of the 16% of neatsfoot oil unrecovered which represents glycerol esters could possibly be recovered by treating the column with a more effective solvent. However, it has been shown (2), (3), (4) that the more highly adsorbed glycerol esters can be recovered from an alumina column by extruding the column and washing the collected adsorbent with diethyl ether whereupon the adsorbed glycerides are dissolved in the solvent from which they are easily recovered.

Since it has been determined (5) that fatty acids react with active alumina, it is doubtful that slight part of the unrecovered sample made up of free (non-esterified) fatty acids could be recovered by the above method.

*Fig. 16 shows the elution characteristics of the mixture. Assuming the separation of the two oils to be complete, Figs. 17 and 18 show the elution characteristics of mineral oil and neatsfoot oil, respectively. The similarity of these two figures with Figs. 1 and 5, respectively, is evident.

2. Separation of alcohol ester fatty oils (sperm oil and methyl oleate) from white mineral oil.

a. Sperm oil

An examination of the elution characteristics of sperm oil (Fig. 14) shows 7% of the sample eluted by the second addition of petroleum ether, 77% by the first diethyl ether addition and 6% by the second. While mineral oil, on the other hand, is 99% eluted by the two petroleum ether treatments (Fig. 1). A ready separation of these two oils by the above method is indicated.

The recovery of that portion of the sperm oil remaining in the column would proceed as described under VB1, Separation of glycerol ester fatty oils from white mineral oil.

b. Methyl oleate

An examination of the elution characteristics of this oil (Fig. 15) reveals that 17% of the sample is eluted by the first and 19% by the second petroleum ether addition, and 49% by the first diethyl ether treatment. Since white mineral oil is also eluted by two petroleum ether treatments (Fig. 1), a complete separation of these oils using the solvent-adsorbent method as outlined is doubtful.

3. Separation of fatty oils from naphthenic and paraffinic mineral oils.

Unlike white mineral oil, these two oils attain 100% elution only upon addition of the first portion of diethyl ether (Figs. 2 and 3). Since all fatty oils give elutions under the solvent action of this first diethyl ether treatment (Figs. 4-13), it is doubtful that a complete separation of the two oil types could be made by use of a 10 cm. column of alumina.

C. Identification of a fatty oil by the appearance of its chromatogram.

1. In ultraviolet light

Each sample examined gave a highly distinctive chromatogram in ultraviolet light. It is, therefore, possible to identify a fatty oil by means of its ultraviolet chromatogram.

In addition it is to be observed that all fish oils tested gave a brown band (Fig. 6-8) while no other oils showed this characteristic.

2. In visible light

As seen from the following table, the chromatograms observed in visible light are less numerous and less distinctive in color than the ultraviolet chromatograms. It is therefore impossible to identify a fatty oil by means of its chromatogram as observed in visible light.

It is to be observed that all fish oils give a yellow band in visible light as do certain other oils, butthis band when produced by a fish oil is not to be observed under ultraviolet light although when produced by another oil is present in ultraviolet light as well as visible light.

APPEARANCE OF CHROMATOGRAMS IN VISIBLE LIGHT

Oil	Color of Band	Position of Band *1	Developments During Which Band Present *2	Color of Band In Ultraviolet Light
Herring	Olive	90-100	1,2,3,4,5,6	Olive
Herring	Olive	90-99	7	Olive
Herring	Yellow	99-100	7	Not Present
Menhaden	Brown	73-82	1,2,3	Brown
Menhaden	Yellow	99-100	7	Not Present
Menhaden	White	84-90	1,2,3	White
Salmon	Tan	91-100	1,2,3,4,5,6	Tan
Salmon	Tan	91-99	7	Tan
Salmon	Yellow	99-100	7	Not Present
Linseed	Yellow	52-54	1	Blue
Linseed	Yellow	35-39	2	Blue
Linseed	Yellow	31-34	3	Blue
Sperm	Yellow	98-100	1	Yellow
Sperm	Yellow	97-100	2	Yellow
Sperm	Yellow	96-100	3	Yellow
Naphthenic	Brown	99-100	1	Yellow
Paraffinic	Yellow	99-100	1	Gray

*1 The scheme for positioning bands is given in IV, Explanation of Data Charts.

*2 The scheme of designating developments in this table is similar to that used to designate them in the charts. For an explanation of this scheme see IV, Explanation of Data Charts.

D. Future work.

Since separation of chromatographic adsorption bands depends upon differences in the rate of their descent thru the column, this separation increases as the length of the column increases.

In certain cases the ultraviolet chromatogram bands of an oil were poorly separated and thus hard to define. Increase of the column length over the 10 cm. column length used in this experiment should correct this difficulty.

Increase of the column length would also give more informative data concerning fatty oil--mineral oil separations.

Future work extending the preliminary work presented in this paper should therefore be done on a column greater in length than 10 cm.

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