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THE DETERMINATION
OF
SMALL AMOUNTS
OF
CERTAIN ESSENTIAL OILS

THESIS

Submitted by

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1914

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-:INTRODUCTION:-

Essential oils are used in making up flavoring extracts and table liquers. These preparations are nothing but alcoholic solutions of the essential oil to which a little glycerine or sugar may be added. The amount of oil actually present in these extracts is very small, often less than one percent. At present no satisfactory method exists for the accurate quantitative determination of the oil when the amount is less than two percent, especially in the case of peppermint, cinnamon, nutmeg or anise oils.

The determination of the amount of essential oil in a flavoring extract or table liquor has become necessary because of the adoption by the States and Federal authorities of standards for these food products. The standards fix the amount of oil which shall be present if the product is to be considered legal.

The main object of this thesis, then, is to devise a method whereby the amount of essential oil in a weak alcoholic solution may be determined with reasonable accuracy.

-:REVIEW OF LITERATURE:-

Little is to be found in chemical literature relating to the determination of essential oils in flavoring extracts, except lemon and orange extract. It is doubtful whether prior to 1899 any successful attempt had been made to devise methods of making these determinations.

The first fairly accurate method was proposed by A. S. Mitchell.⁽¹⁾ There are two steps in the determination; (1) the oil is precipitated by diluting some of the extract with water, and (2) the oil is separated from the resulting mixture by whirling in the centrifuge. The chief trouble with this method and its modifications which followed long after, is the slight solubility of certain of the oils in dilute alcohol. This error causes the loss of a large portion of the oil in the case of a very weak extract.

In 1908, the first important modification of Mitchell's method was made by Howard.⁽²⁾ He added chloroform to the mixture containing the precipitated oil, and after centrifuging, the chloroform was removed by rapid evaporation on the water bath. The chloroform is supposed to extract all the oil from the mixture. Apparently it does not, because results obtained by this method average low. There are two other reasons for loss of oil in this method; volatilization of the

oil during the evaporation of the chloroform, and the slight solubility of the oil in dilute alcohol. Modifications of this method using different solvents and vessels of different sizes and shapes have not been successful.

Hortvet and West⁽³⁾ have devised a method which is generally regarded as the best of those existing. The details and suitability of this method are discussed fully in the experimental part of this thesis.

Another procedure involving centrifuging has been suggested by R. S. Hiltner⁽⁴⁾ and is likewise discussed later in the thesis.

-:EXPERIMENTAL PART:-

(a) Study of the Hortvet and West Method.

The preliminary part of the experimental work comprised a study of the method. The details of the method have been thoroughly worked out by the authors for moderate quantities of oils. It was deemed advisable, however, to determine the accuracy of the method with the very small amounts considered in the thesis.

The method is based on the assumption that when an ethereal solution of an essential oil is rapidly evaporated no appreciable loss of oil occurs if the evaporation is stopped just as the last of the ether is removed.

The determination is carried out as follows:-
Ten cubic centimeters of the extract are transferred to a separatory funnel, one hundred cubic centimeters of water added, and the mixture extracted with three portions of ether using fifty, thirty and twenty cubic centimeters in succession. The combined ether extracts are washed twice with distilled water and then dried by standing over Ca Cl₂. The dried extract is filtered into a 150 cc. wide mouthed flask. The ether is evaporated rapidly by suspending the flask over a water bath and removing the vapors by suction. When about five cubic centimeters of ether remain, the solution is transferred to a 50 c.c. weighed flask and the evap-

oration is completed. The percent of oil is calculated by the following factor -

$$\frac{\text{Weight of oil} \times 100}{10 \times \text{Sp. Gr. of oil}} = \text{Percent of oil by volume.}$$

The above procedure require considerable care in manipulation and the details must be adhered to very closely. The critical part of the procedure is the evaporation of the ether. It must be stopped at exactly the right point or a marked loss of oil will occur. It is very difficult, however, to tell when the last drops of ether have disappeared.

A number of determinations were made by this method on alcoholic solutions of peppermint, nutmeg, cinnamon and anise oils, containing 0.4 percent of oil in each case. The following table shows the results obtained. The figures following the name of the oil give the percent found.

	Percent of Oil								Average
	Peppermint..	.52	.31	.03	.08	.20	-	-	-
Cinnamon....	.18	.17	.34	.20	-	.29	.31	.29	.27
Nutmeg.....	-	.19	.22	.13	-	.46	.11	.47	.26
Anise.....	-	.13	.37	.19	.20	.33	.33	.70	.32

The great variability of the first results is explained by lack of experience with the method. There were also some minor difficulties which had to be re-

moved.

Conclusions:- The Hortvet and West method is shown by these results to be unsatisfactory for the determination of essential oils in extracts containing less than one percent of oil. In the case of these weak extracts from one-fourth to one-half of the oil is lost, probably during the evaporation. This large fractional loss renders the results excessively low. If an extract, however, contained two or three percent of oil, the loss by weight would be about the same, but the percentage error would be much less. An example will illustrate this point. Ten cubic centimeters of a 0.5 percent solution is equivalent to .05 cubic centimeters of oil. Suppose .012 cubic centimeter is lost, then the result will be twenty-five percent low, giving .38 percent as the amount of oil. Ten cubic centimeters of three percent is equivalent to .3 cubic centimeters of oil. Here the loss of .012 cubic centimeters will give a result only four percent low, giving 2.8 percent as the amount of oil. Hence for extracts containing three percent or more of oil the method could be used with satisfactory results provided care is taken in the evaporation of the ether.

(b) Elaboration of Hiltner's Suggestion.

In Bulletin No. 152, Bureau of Chemistry, United States Department of Agriculture, Hiltner suggested "A Tentative Method for the Determination of Essential Oils in Alcoholic Solutions". The proposed method is as follows:

Prepare a quantity of hydrocarbon oil (boiling point 175 - 200 degrees centigrade) by fractional distillation of commercial kerosene. Pipette ten cubic centimeters of the extract into a Babcock milk bottle, add one cubic centimeter of the hydrocarbon oil, two drops of concentrated hydrochloric acid, and twenty-five cubic centimeters of hot water; shake vigorously, warm on the water-bath to about eighty degrees, whirl in a centrifuge, add water so as to bring the floating oil into the neck of the flask and again whirl. Determine the refractive index of the separated oil and of the hydrocarbon oil used at twenty degrees centigrade. The index of the essential oil must also be known. Hiltner gives no information as to the accuracy of the proposed method.

The method is based on a certain definite change in the refractive index of the hydrocarbon oil due to the mixing with it of the essential oil. On the addition of water, the hydrocarbon oil and essential oil are precipitated. During the whirling the two oils gather

at the surface of the diluted alcohol to form a homogeneous supernatant layer. The refractive index of this layer depends on the relative proportion of the two oils which compose it. This is illustrated by the following experiment. Ten cubic centimeters of peppermint oil and one cubic centimeter of hydrocarbon oil were mixed and the refractive index of the mixture was found to be 1.4449 at twenty-four degrees centigrade. According to the principle this should equal the mean of the refractive indices of the two oils which equals 1.4450

Refractive Index of pure peppermint oil	-	1.4582	at 24 C.
Refractive Index of hydrocarbon oil	-	<u>1.4318</u>	at 24 C.
		<u>2.8900</u>	
Mean	-	1.4450	

Thus, one cubic centimeter of peppermint mixed with one cubic centimeter of hydrocarbon oil causes an increase of $1.4450 - 1.4318 = .0132$ in the refractive index of the hydrocarbon oil. Then, 0.5, 0.3 or 0.1 of one cubic centimeter of essential oil mixed with one cubic centimeter of hydrocarbon oil would increase the index of the hydrocarbon oil by 0.5, 0.3 or 0.1 respectively of .0132. The last two sentences illustrate the principle very well. It is summarized in the following statement: The increase in the refractive index of the hydrocarbon oil due to the admixture of an essential of higher refractive index is inversely proportion-

al to the ratio of the volumes of hydrocarbon oil to the volume of essential oil.

There are three things which may hinder the successful application of this principle: (1) The refractive index of the essential oil may not be a constant for all samples; (2) The relative proportion of the oils in the supernatant layer may not be the same as the relative proportion of the oils in the mixture before centrifuging; (3) Some constituent of either oil may be retained by the dilute alcohol, thus causing a change in the refractive index of the separating oil. The first error may be removed by a preliminary extraction of the oil with ether as in the directions given in the Hortvet and West method and the determination of its refractive index. The other possible sources of error are discussed later.

The method was first tried on peppermint extract because the difference between the refractive index of peppermint oil and hydrocarbon oil is less than in the case of the other oils. Obviously, the greater the difference between the indices of the hydrocarbon oil and essential oil, the more delicate will be the method. This is the reason for choosing a hydrocarbon of oil of the boiling point given. Now, the index of peppermint, however, is not much greater than that of the hydrocarbon oil, hence a weak peppermint extract would be a more rigorous test of the delicacy of the method than

in the case of extracts of the other oils.

Some preliminary work was necessary before starting on the determinations. The hydrocarbon oil was prepared by distilling commercial kerosene; the fraction distilling between 175 - 200 degrees being used. This distillate was washed three times with concentrated sulphuric acid, once with dilute potassium hydroxide, and then re-distilled. All refractive indices were taken on an Abbe refractometer, over a range of temperatures between twenty degrees and thirty degrees. Plots were made showing the variation of the index with temperature, in the case of peppermint oil and anise oil and the two hydrocarbon oils used. In later work it was found best to make all determinations at room temperature, and so the refractive indices of nutmeg and cinnamon was taken only at room temperature.

The results of the first five determinations on peppermint oil were not good. The increase in the refractive index of the hydrocarbon oil due to the admixture of .125 cubic centimeters of peppermint oil varied from .0012 to .0027. It was felt that these unequal results might be due to slightly varying conditions in the precipitation and so great care was taken in the following determinations to have the various factors, such as amounts of water, time of whirling, volumes of extract and hydrocarbon oil, etc., strictly comparable.

The results agreed much better.

These results, however, were much higher than the theory required. The theoretical increase in the refractive index of the hydrocarbon oil due to the admixture of one cubic centimeter with .125 cubic centimeter of peppermin is .0015. Results found were .0025, .0026 and .0024 respectively, averaging .0010 above the theoretical. To learn if these high results were due to a change in the hydrocarbon oil itself, a "blank" run was made. The "blanks" were run under exactly the same conditions as the real determination except that alcohol instead of extract was added. The correction found from the "blanks" equalled the difference between the actual increase and the theoretical. The fact that the "blank" correction reduces the actual increase to the theoretical renders the method particularly applicable to peppermint extract.

The increase in the refractive index of the hydrocarbon oil in the "blank" run is due probably to the retention of some constituent by the dilute alcohol.

Two unknown peppermint extracts were determined by this method using the "blank" correction. The amount of oil was found to be .40 percent and .58 percent respectively. The actual amounts were .45 percent and .50 percent.

The determination of oil in anise extract was next

tried. The results obtained did not at all equal the theoretical values even after the "blank" correction was applied. It was not expected in the case of anise that the actual results could be reduced to the theoretical as easily as with peppermint because of the much greater difference in refractive index of the hydrocarbon and essential oil. The difference between the actual and theoretical increase, however, is so great here that there is not much hope of being able to reduce the actual to the theoretical by means of correction. In order to apply this method to anise extracts, an empirical relation between the amount of oil in the extract and the refractive index of the mixture of anise and hydrocarbon will have to be derived.

The difference between the actual and theoretical increase in the case of anise is due probably to the retention of a component of anise by the dilute alcohol. This could be ascertained by noting the difference between the index of the dilute alcohol formed in a run, and an ordinary similar dilute alcohol. Possibly a correction as useful as the "blank" in peppermint determination might be obtained in this way.

Determinations on cinnamon and nutmeg extracts also gave results which did not agree with the calculated values. Experiments were then tried to see if mixtures of cinnamon or nutmeg and hydrocarbon oil followed the principle of the method. It was found in these experi-

ments that cinnamon and nutmeg oil do not mix with the hydrocarbon in the cold. Cinnamon settles from the hydrocarbon oil almost immediately after shaking, while nutmeg forms a turbid mixture.

Above fifty degrees a homogeneous liquid is formed. The refractive index of the mixtures of either one of these oils with the hydrocarbon do not agree with the indices calculated according to the principle of the method. Consequently, if this method is to be used for determinations of oil in extract of cinnamon or nutmeg an empirical formula will have to be derived.

The following table shows the summary of results on peppermint, anise, cinnamon and nutmeg extracts. The determinations were carried out on .5 % solutions. The figures are averages of two or more determinations.

Extract	No. of cc. of extract used.	Increase in refr. ind. of hydrocarbon "Blank" correction applied.	Calculated increase.	Difference.
Peppermint	25	.0015	.0015	.0000
	10	.0006	.0005	.0001
	20	.0012	.00011	.0001
	5	.0003	.0003	.0000
Anise	25	.0073	.00112	.0039
	10	.0029	.0054	.0025
Cinnamon	10	.0040	.0027	.0013
Nutmeg	10	.0013	.0015	.0002
	25	.0031	.0047	.0016

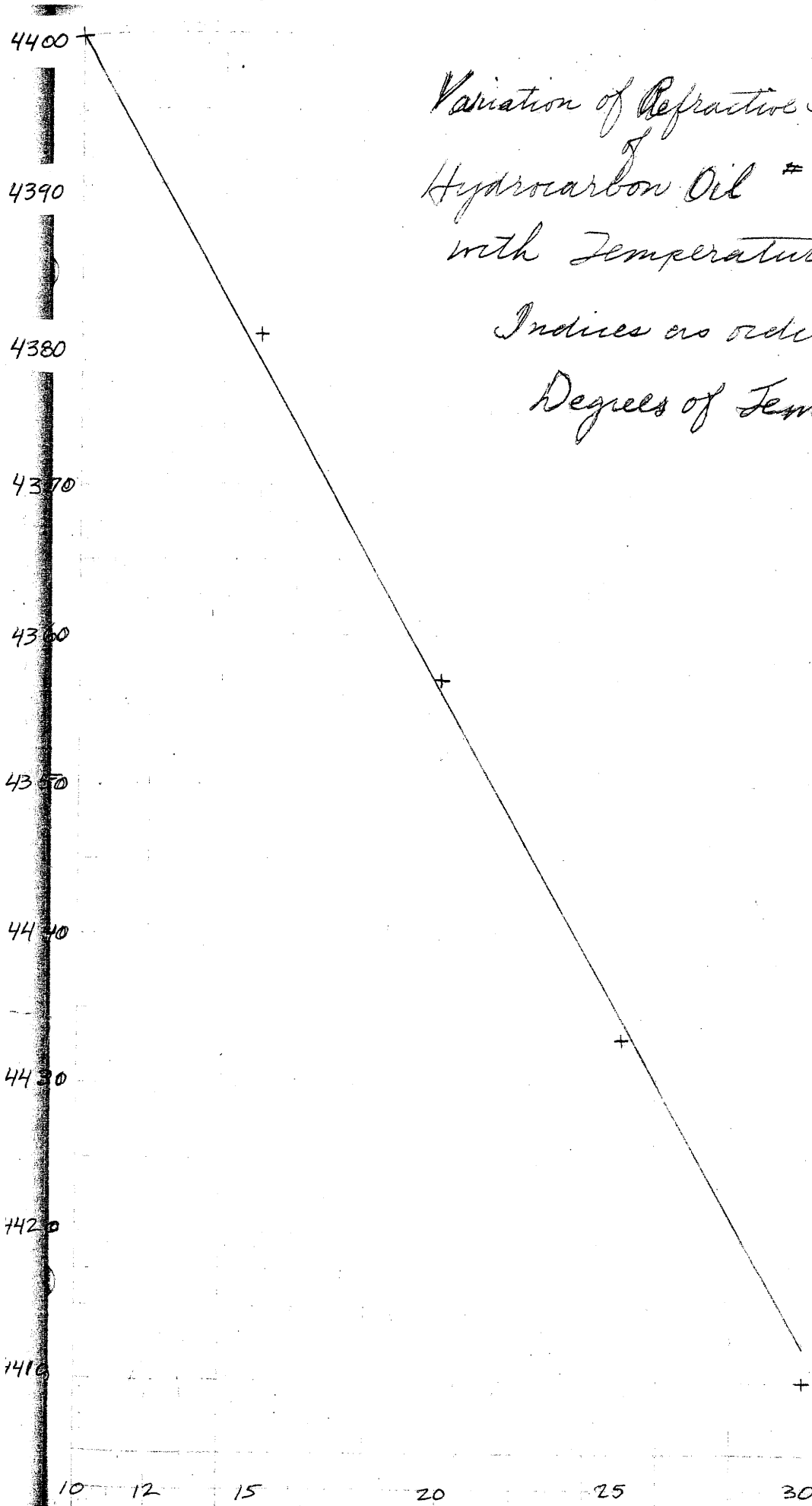
-:GENERAL CONCLUSIONS REGARDING THE METHOD:-

Hiltner's method is suitable for the determination of oil in weak peppermint extracts, but not in weak nutmeg, anise or cinnamon extract. It might be made satisfactory for the last oils by derivation of empirical formulae. Further study of the means of applying the method to these latter oils would be highly desirable because ease of manipulation, and rapidity.

Variation of Refractive Index
of
Hydrocarbon Oil # 1.
with Temperature.

Indices as ordinates

Degrees of Temp as abscissae.



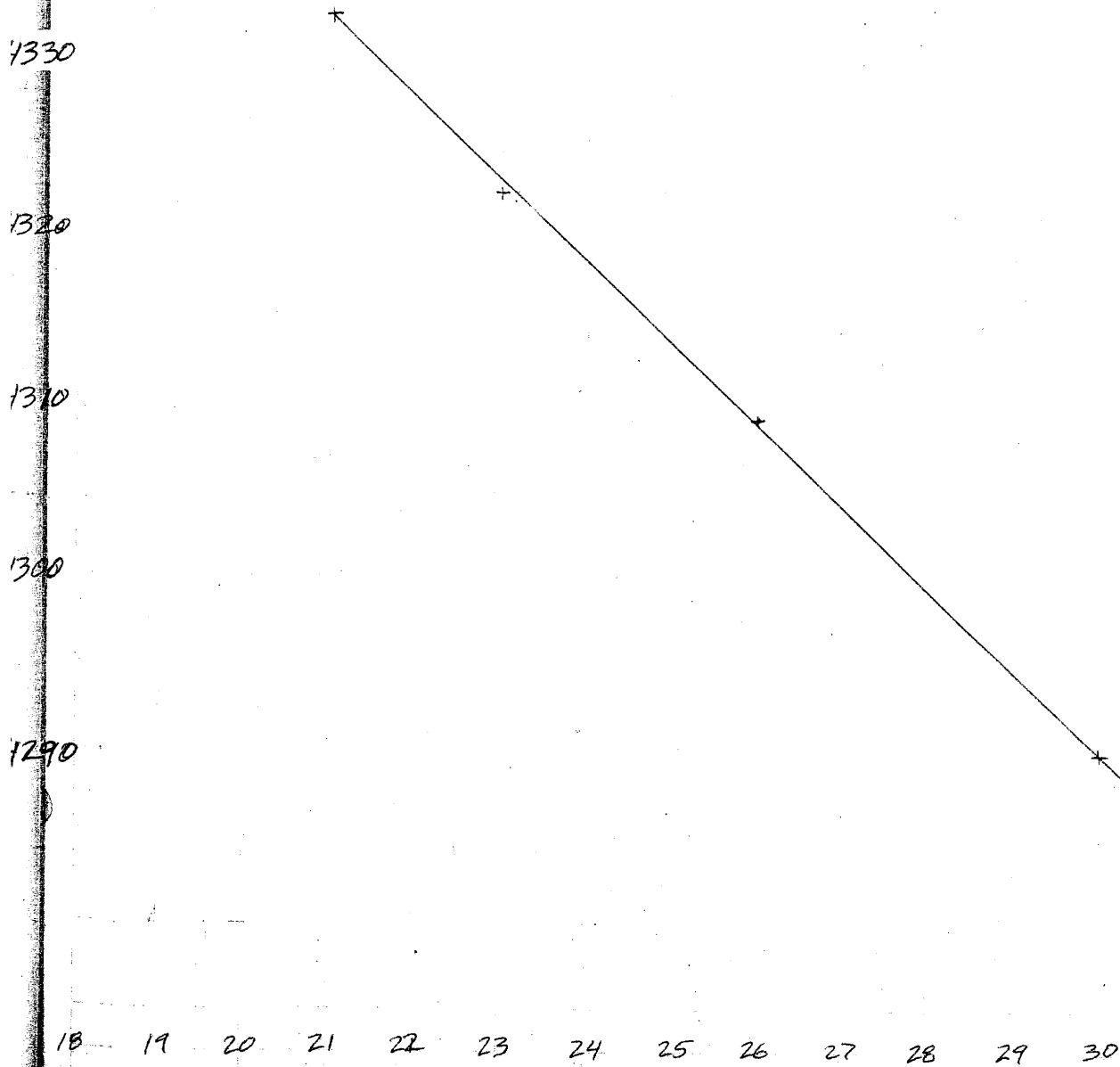
Variation of Index of Refraction
of

Hydrocarbon Oil # 2

with Temperature

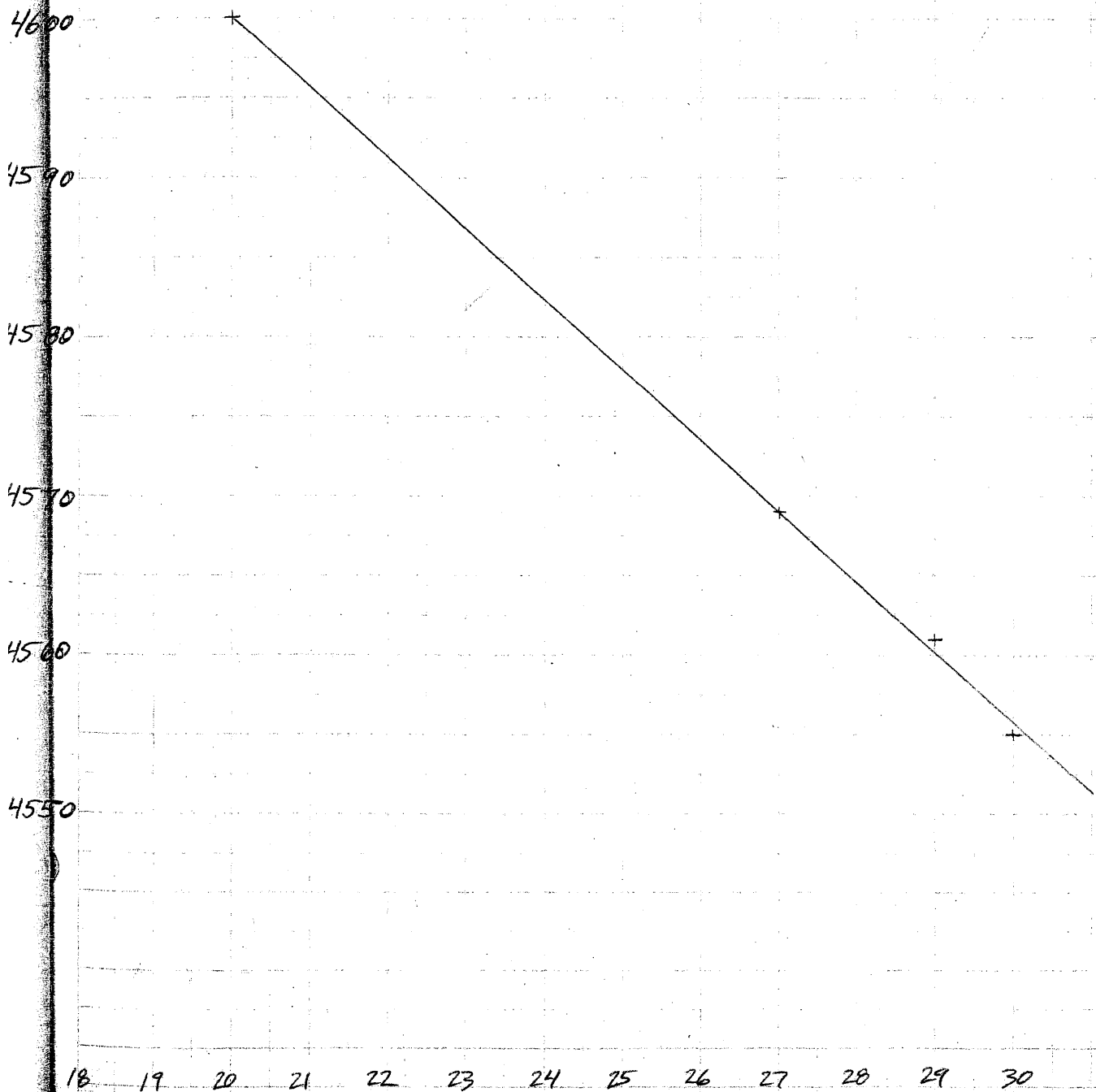
Indices as Ordinates

Temperature Degrees as Abscissae

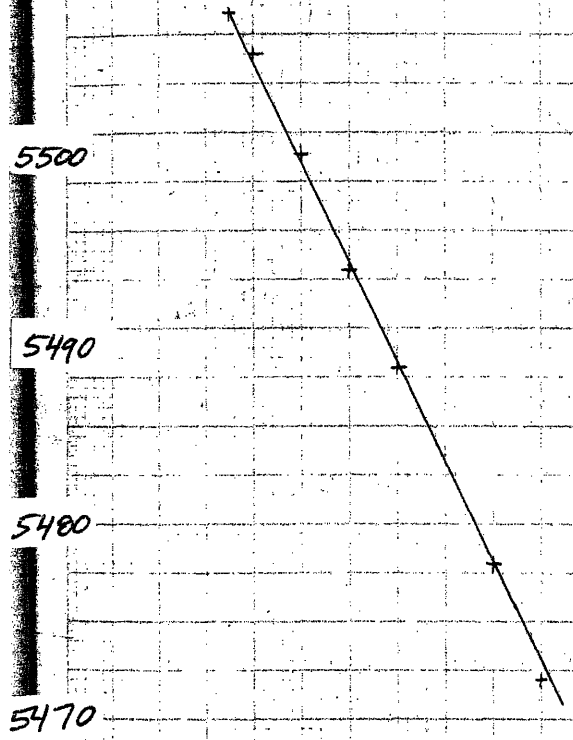


Variations of Refractive Index
of Peppermint Oil
with Temperature.

Degrees of Temperature as Abscissae
Indices as Ordinates.



Variation of Refractive Index
of
Anise Oil with Temperature
Indices as Ordinates
Temperature Degrees as Abscissae



(c) Nephelometric Measurements.

The turbidity produced when an extract is diluted with water suggested the use of the nephelometer for the determination of the oil. The nephelometer is used for detecting small differences in weakly turbid solutions. The turbidity produced by diluting a very weak extract of an essential oil with water is usually slight and its degree is supposedly dependent upon the amount of oil present. Hence, it was thought that the nephelometer would be particularly suitable for detecting differences in such turbidities.

Before using the nephelometer, however, experiments were tried with the Jackson Candle Turbidimeter. In this instrument a lighted candle is placed below a graduated glass cylinder. The turbidity of a liquid is measured by pouring it slowly into the glass cylinder until the candle flame just disappears. This instrument measures satisfactorily a turbidity produced by the presence of very small opaque particles in a liquid, as, for example, that due to a suspension of barium sulphate^{but}/in these experiments it is not found suitable for the measuring of a turbidity produced by the suspension of minute, translucent glöbules, as is the case with an insoluble oil.

Measurement of turbid liquids by a nephelometer is based on the fact that a turbid liquid reflects upward

some of the light which strikes it horizontally. Distilled water will reflect no light upward. The greater the turbidity, the greater is the percent of light sent upward.

The form of nephelometer employed in this work was one proposed by Kober⁽⁵⁾ and attached to the well known Dubusq colorimeter. The liquids to be measured are put into two glass tubes, the top, bottom and three sides of which are fully protected from all light by dull black casings. The fourth side of the tubes is exposed to the light from a powerful Mazda lamp placed about eighteen inches from the instrument. The light reflected upward by the liquid passes up through the bottom of a glass plunger, the sides of which are painted black, into the eyepiece of a telescope placed on top of the instrument. The plungers in the tubes are movable up and down and their movement is followed on a scale by an indicator. On looking through the telescope of the instrument, the observer sees two half-fields differently illuminated, one half-field being produced by the light reflected up from one tube, and the other half-field by the light from the other tube. The amount of light sent by the liquids can be varied by moving the plunger up and down. When the light reflected by both tubes is equal, the field of the telescope appears uniform. Then a very slight shifting of one of the plungers should produce two differently illuminated fields.

The delicacy of the measurements of this instrument is dependent upon the distance through which one of the plungers can be moved without destroying a uniform field.

Quantitative measurements of unknowns are made by comparison with standards. If a standard solution is always used in the same way, that is, the same amount of precipitated oil, the same height of liquid in tubes, equal lighting conditions, etc., then the readings obtained with unknown solutions plotted against the ratios of the solutions will follow a definite and regular curve. This curve is expressed in the following equation -

$$Y = \frac{S}{X} - \frac{(1-X) K}{X^2}$$

where Y equals height of unknown, X equals ratio of solutions, and S equals height of standard solution, K is the constant obtained by substitution of values obtained by standardization of a standard with known solutions. Therefore, if once the value of K is derived for any given standard solution and height of standard solution, the nephelometric readings when the standard is compared with an unknown will give at once on calculation the ratio of the solutions. It is assumed that solutions which differ very much from the standard solution, as may be observable by a macroscopical examination of turbidities, will require as in

colorimetric work, a more suitable standard.

Nephelometric measurements were made on weak anise and cinnamon extracts. These two extracts are very different in regard to the degree of turbidity caused by addition of water. The former gives a heavy, opaque turbidity on dilution with water, while the latter under the same conditions gives only a slight translucent cloudiness. For example, ten cubic centimeters of a 0.5% solution of anise added to one hundred cubic centimeters of water forms an opaque liquid; but ten cubic centimeters of a 0.5% solution of cinnamon added to the same amount of water, will give only a barely visible cloudiness, cinnamon oil, being much more soluble in dilute alcohol than is anise oil.

0.5% solutions of anise and cinnamon were standardized by comparing with 0.4% solutions. In the case of the anise extracts the turbid liquids to be compared were prepared by adding five cubic centimeters of the extract to one hundred cubic centimeters of water, and with the cinnamon extract these liquids were produced by adding ten cubic centimeters to fifty cubic centimeters of water. From the heights of liquids giving equal illumination in the nephelometer and the known ratios of the solutions, the constant for the standards was derived by substituting these values in the equation given above.

The amount of essential oil in an unknown anise extract was successfully determined by comparison with standardized 0.5% anise solution. The turbid liquids compared were obtained in the same manner as those used in the standardization. From the data in the nephelometric measurement and the constant of the standard, the ratios of the standard of the unknown are calculated according to the equation given above. In the measurement of unknowns great care must be taken to reproduce exactly the conditions which obtain in the standardizations. An unknown cinnamon extract was also successfully determined in an analogous manner.

The following table shows the results obtained in the examination of these unknowns.

Extract	True percent of oil.	Percent found by nephelometric measurement.
Anise	.45	.43
Cinnamon	.53	.51

-:GENERAL CONCLUSIONS:-

These results indicate that the nephelometer can be successfully used for the determination of amounts of essential oils too small to be determined by hardly any other method. It is unfortunate that the nephelometer became available at so late a date that more work could not be done on this method.

-:SUMMARY OF EXPERIMENTAL WORK:-

(1) The Hortvet and West method for the determination of small amounts of essential oil in flavoring extracts was found to be unsatisfactory because of excessive loss of oil.

(2) A procedure developed from Hiltner's suggestion proved satisfactory for the determination of peppermint oil in weak peppermint extract. The procedure was not successfully applied to dilute anise, cinnamon, or nutmeg extracts.

(3) The results obtained with the nephelometer indicate that this instrument can be successfully applied to the determination of small amounts of essential oils.

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