S U M M A R Y

A STUDY OF VOLATILE CARBONYL COMPOUNDS OF COFFEE

by

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The purpose of this work was to examine the carbonyl compounds in the volatile constituents of coffee, if possible to assess their contribution to coffee aroma.

The work entailed mainly the collection of the carbonyl compounds and their subsequent separation, identification and quantitive estimation.

The volatile constituents of coffee were stripped by nitrogen in a modified Shipton apparatus and the carbonyl compounds converted to their hydrazones.

The carbonyl compounds were regenerated by a "flash-exchange" method which consisted essentially of heating a mixture of the hydrazones and α Ketoglutaric acid.

The regenerated carbonyls were examined by gas chromatography using an argon ionisation detector; the bulk of the work was done using dinonylphthalate as liquid phase and carbowax 1500 for some confirmatory work.

Both qualitative and quantitative determinations were made.

Identification and quantitative estimation of the constituent carbonyl compounds was further supported by thin-layer chromatography, paper chromatography, and by visible, ultra-violet and infra-red spectroscopy.

A tasting panel was used to determine the effect of storage and also to test the significance of the volatile carbonyl compounds in relation to the flavour of coffee.

The following carbonyl compounds were identified by gas chromatography and their quantities measured: acetaldehyde, propionaldehyde, acetone, iso-butyraldehyde, n-butyraldehyde, methylethylketone, diacetyl and iso-valeraldehyde; acetaldehyde and iso-valeraldehyde were present in approximately equal amounts forming the largest proportion of the total volatile carbonyl compounds.

Formaldehyde, which was not detected by gas chromatography, was identified and estimated by a chromotropic acid colorimetric method. Evidence is offered that, contrary to one published method, formaldehyde is not regenerated from its hydrazone.

The quantitative determination of diacetyls using the argon-ionization detector requires special consideration to avoid electron capture effects.

Experiments with stored samples of instant coffee showed that:

The quantity of volatile carbonyl compounds decrease with increasing time of storage. Tasting panel results suggest a relationship between this decrease and the palatability of the coffee.

It suggested that the quantity of volatile carbonyl compounds expressed as the weight of dry hydrazones/100 g. coffee could be used as an index of the age and storage treatment of the coffee.

The results also show that in the staling of instant coffee there is relative increase of acetaldehyde and an increase in the ratio of propionaldehyde to acetone.

Results are given for the composition of the fats extracted from green and roasted coffee.

The roasting of the extracted fat from the green beans gave no evidence for the production of volatile carbonyl compounds.

Experience gained in this work has shown the necessity for standard methods of collection and analysis. Comparison of the results from different workers is almost impossible since the variations caused by different methods seems to be greater than would be expected from different materials.

A STUDY OF VOLATILE CARBONYL COMPOUNDS OF COFFEE

by

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A B B R E V I A T I O N S

V.C.C.	Volatile Carbonyl Compounds.
KGKA	- Ketoglutaric Acid.
MEK	Methylethylketone.
T.B.A.	Thiobarbituric Acid.
V.R.S.	Volatile Reducing Substances.
CBI	Coffee Brewing Institute.
STD.	Standard.
PUB	Publication.
I.D.	Internal Diameter.
G.C.	Gas Chromatography.
D.V.	Detector Voltage.
G.F.	Gas Flow.

C O N T E N T S

P R E F A C E

The work described here was designed to examine the contribution of the volatile carbonyl compounds to the flavour of coffee.

This entailed the isolation and collection of these compounds and their subsequent separation, identification and quantitative estimation.

The carbonyl compounds were isolated by stripping with nitrogen and conversion to their 2, 4 - Dinitrophenyl-hydrazones. They were then regenerated by a system of "flash exchange," and separated by gas chromatography.

Identification and quantitative estimation of the constituent carbonyl compounds was further supported by thin-layer chromatography, paper chromatography, and by visible, ultra-violet and infra-red spectroscopy.

A testing panel was used to determine the effect of storage and also to test the significance of the volatile carbonyl compounds in relation to the flavour of coffee.

INTRODUCTION

Food flavour

The flavour of an attractive food serves not only to provide pleasurable sensations in eating but also to encourage the satisfaction of physiological needs.

The study of flavour chemistry is of intrinsic importance but the present interest in the development of non-conventional food sources to meet world shortages would encourage the view that a more detailed knowledge of this subject should help to make dull but nutrious foods more attractive.

Food flavouring substances are mainly volatile and are derived from traces of a wide range or organic compounds which may either be originally present in the food or which develop through cooking or commercial processing. Alcohols, ethers, esters, terpenes, aldehydes, ketones, thiols, and even hydrocarbons have been reported[1, 2] as contributing to flavour.[3]

Volatile flavour compounds are usually present in very small concentrations, but sufficient to create olfactory perception in higher animals as well as in insects. Pheromones,[4] the insects sex attractants could

be detected in the concentration of 10^{-20}g by the cockroach.[5] Man can perceive as low as 10^{-11}g of Ionone[76]. These concentrations could not be detected by any known instrument, and for this reason flavour work is usually assisted by organoleptic evaluations.

THEORIES of OLFACTION

Moncrieff[7] mentions 24 theories which have been put forward from 1870 to 1949 to explain the mechanism of olfaction. Many of these theories are frankly speculations and none are entirely satisfactory.

In 1963 Amoore[6] postulated his "Stereochemical theory of Olfaction" a refinement of his previous publication [75] in 1952.

This Theory is based on the ideas put forward by Moncrieff [7] who postulated that " ... the prerequisites for odour are (a) volatility; and (b) a molecular configuration or shape which permits the molecule to fit into receptor sites on the receptor system."

The stereochemical theory of olfaction stated that "the odour of a chemical is determined by the structure of the molecule particularly its size and shape."[6] It is assumed [75] that the olfactory epithelium contains receptor sites and if a chemical

is volatile and it's molecules have the appropriate configuration to fit closely into a receptor site, then a nervous impulse will be initiated possibly through a mechanism of depolarisation of the receptor membrane.

According to this theory chemicals of unrelated structure can exhibit very similar odours if their molecules have nearly the same shape and size, e.g. d - camphor, hexachloroethane and trinitroacetonitrile all have camphoraceous odour, and their molecules have nearly spherical shapes of about 7 A^{O} in diameter. Fig. (1).[75]

He further suggests that the human olfactory receptor sites may be classified into seven primary odours or Modalities of sense of smell; viz. ethereal, camphoraceous, musky, floral, peppermínty, pungent and putrid. Complex odours like almond are said to be caused by molecules which can fit into two or more primary odour receptor sites.

According to this theory one can predict the odour of new synthesised chemicals from their molecular models.

This theory still leaves many questions on olfaction unanswered e.g. why some substances have odour and some

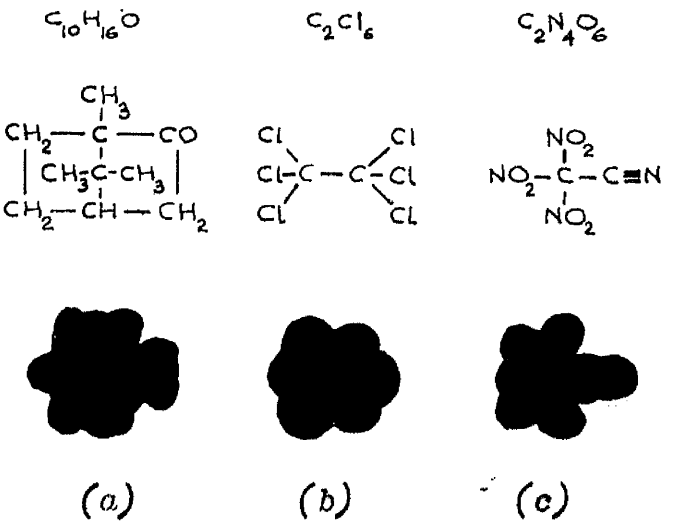


Fig. (1)

Empirical, structural & Stereochemical formulae of:-

a) d - camphor, b) Hexachloroethane,

c) Trinitro acetonitrile

have not, or why some chemicals can be perceived in concentrations even below their threshold in the presence of other compounds.(5)

THE ANALYSIS OF FOOD VOLATILES

The analysis of food volatiles follows the general sequence of sampling of the foodstuff, extraction, concentration, separation and identification of the volatiles, but the analysis may vary from one food to another in any of the noted steps, concerning the extracting solvent, the temperature, pressure, working under an atmosphere of air or in an inert medium. The choice of the conditions and the methods depends on the nature of the food and the problem under investigation.

Progress in the analysis of food volatiles has, until recently, been hindered by the fact that these volatile constituents are usually present in only small quantities. This has meant, that to recover reasonable quantities of material for examination, very large samples of food had to be treated, but recently new methods have been developed to overcome this difficulty.

When certain classes of organic compounds are of special interest, and when stable derivatives can be obtained, a quantitative recovery may be achieved by reacting the food flavourants with suitable reagents.

The available techniques for this sort of analysis are: gas chromatography, thin layer, column and paper

chromatography, spectrophotometry and Mass spectrometry.

The most notable advance has been the application of gas chromatography to flavour problems; apart from its value in the separation of the volatile constituents of food, its exceptionally high sensitivity permits the use of much smaller samples.

PREPARATION OF SAMPLES FOR ANALYSIS

This chapter is devoted to the methods used in preparing samples prior to injection into the gas chromatography column.

Cold-traps [39] The aroma producing constituents of foods are of a range of substances of widely different chemical constitution.

The only practical method of collecting the total volatile material of food is the use of a cold-trap. Some of these substances are highly volatile so that very cold traps, such as liquid nitrogen (- 194°C) or liquid oxygen (- 183°C) must be used. In some cases solid CO_2/acetone or alcohol baths may be used. Difficulties may arise from the presence of CO_2 and water in the atmosphere and in the case of liquid nitrogen even oxygen and argon [39] may be liquified. CO_2 and moisture can be trapped before entering the cold-trap by the use of caustic soda pellets followed by a desiccant.

The collected sample can be released into the chromatographic column by placing the trap into a hot water bath between 80 - 90°C. The timing of the chromatographic run usually being considered from the

amount of hot water immersion.

Enrichment column.(51)

This consists of a small U-tube, packed with liquid phase on Celite (e.g. 20% Dinonylphthalate) which is freed from volatile contaminants by heating and flushing with an inert gas. It is then sealed at one end with a rubber septum. A portion of the head-space vapour from the food container can be withdrawn by a syringe fitted with a hypodermic needle. This is discharged through the rubber septum into the enrichment-column where the volatiles are retained by the liquid phase. This operation can be repeated if necessary to build a sufficient concentration of the volatiles. The U-tube can then be reversed, taking off the rubber cap, and connected in series with the gas chromatographic column. The volatiles can then be released by placing the tube into a hot water-bath. The shape of the container which holds the food is preferred to be like the erlenmyer conical flask to have the volatiles collected in the neck of the container, which provides a reasonable sampling port.

Continuous gas-liquid extraction[13] Fig. (2)

The food sample (about 500 ml) is placed in a one litre round bottomed flask (A), equipped with a sintered glass dip (coarse) to aid in sweeping-gas dispersion. (B) is a tube packed with anhydrous K_2CO_3 to remove moisture from the sweeping gas, and has minimal adsorption for sample volatiles. (C) is a stainless steel U-tube which can be cooled with liquid nitrogen or other refrigerants. (D) is a pulsating pump for the recycling system, it permits a continuous gas-liquid extraction which ensures high recovery of volatiles as the system is a closed one.

Upon completion of collection, the U-tube can be disconnected and the collected sample injected into gas chromatography column.

Vacuum Sterilizing & Distillation apparatus[14] Fig. (3)

The apparatus is built on the principle of the "climbing-film" evaporator in the first stage, followed by condensation, fractionation and collection in the traps.

Steam Distillation

This is ideal for certain experiments and suits cooking vegetables, coffee, tea or any hot served food but should not be used for foods which do not normally

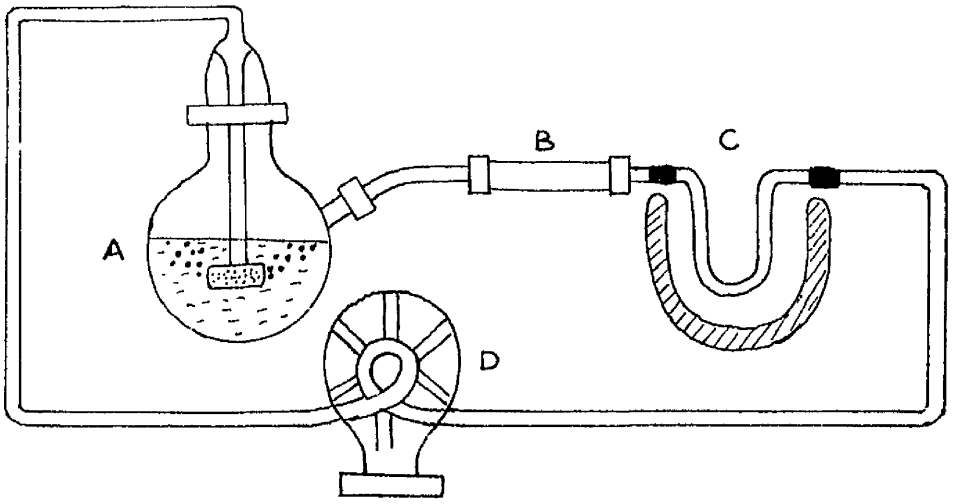


Fig. (2)

GAS - LIQUID EXTRACTION (13)

(A) 1l. Round Bottomed Flask

(B) Unhydrous K_2CO_3

(C) Cold Trap

(D) Pulsating Pump

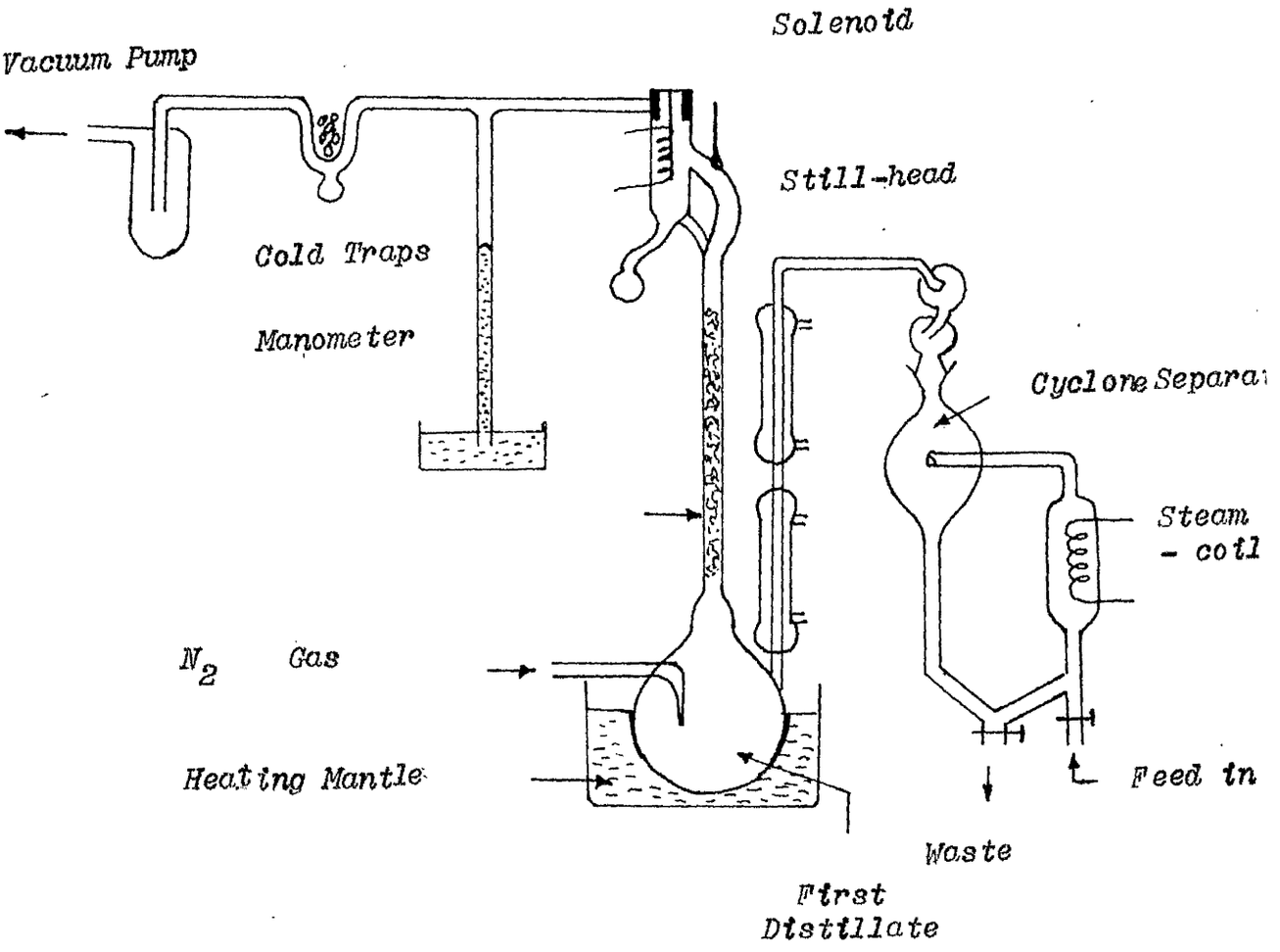


Fig. (3)

Vacuum Stripping and Distillation Apparatus.

receive a heat treatment at any stage of their preparation, such as fruits and fruit juices. Steam distillation of such foods may lead to the formation of volatile compounds which are not normally present in their aroma. Laurence [15] found that during distillation of cheese and butter a wide range of methyl ketones were formed in increasing concentrations as the steam distillation proceeds, and these compounds are not normally present in cheese but were formed as artifacts giving misleading results.

It is, therefore, important that such foods are treated by cold-extraction methods.

Continuous counter current liquid - liquid extractor [16] Fig. (4).

The flavourants of juices can be extracted by continuous counter-current methods.

The solvent flows upwards in the tube (a), penetrates through the ejected juice, and by doing so, the solvent takes with it the extractable compounds to the top, as the solvent is immiscible with aqueous solution and has lower specific gravity, then flows in the said arm (b) and back to the flask (C) and so by limited quantity of solvent a big sample of juice can be extracted.

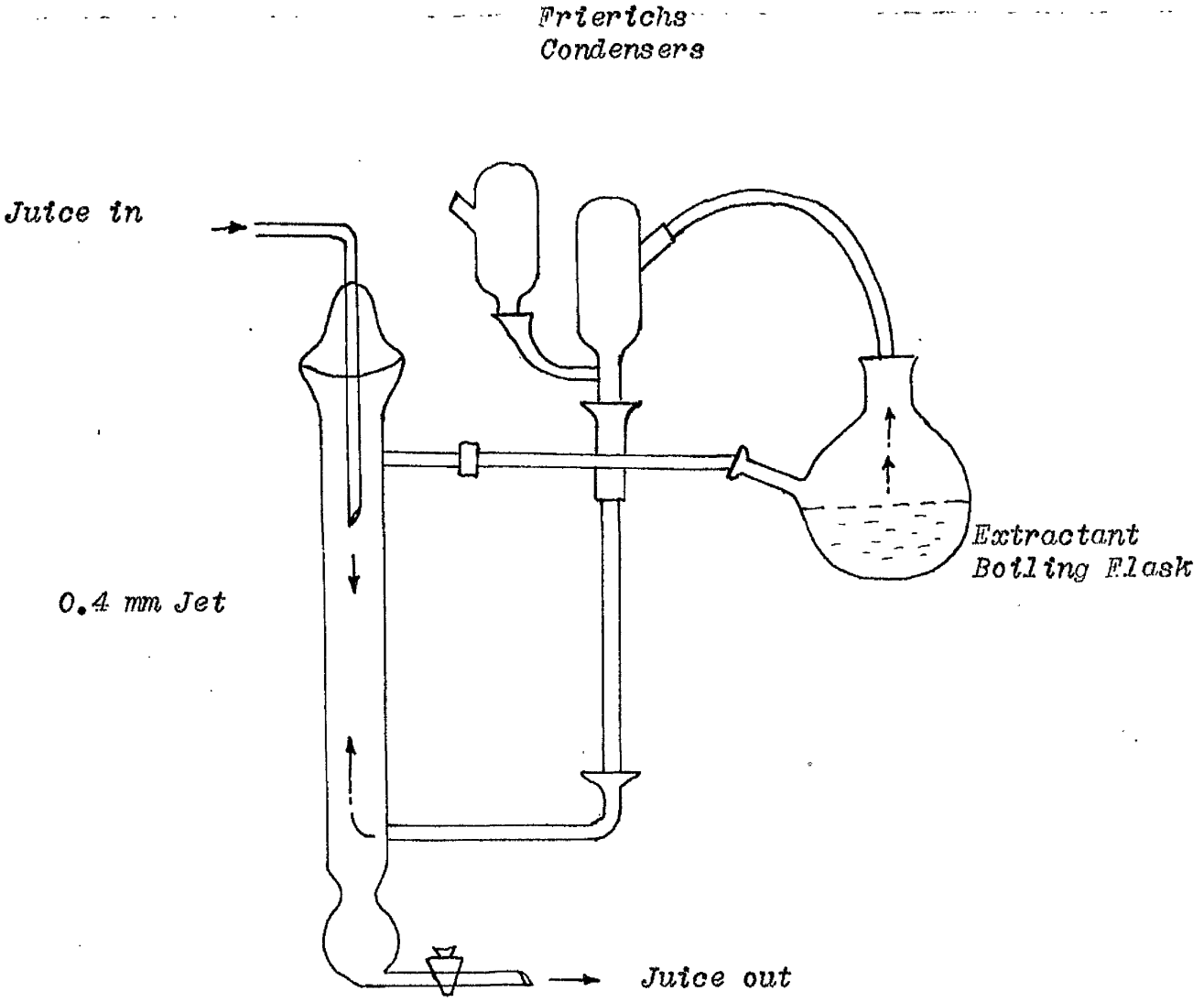


Fig. (4)

Continuous, countercurrent, Liquid-Liquid Extractor

Sampling of coffee volatiles[27] Fig. (5)

(A) Helium

(B) Flow throttle (glass capillary)

(C & M) Flow meters

(D_1 G_1 L & J) Three way Stopcock

(E) Coffee sampling apparatus (12 gms)

(F) Ice water condenser

(H) Preliminary column

(I) Three way stopcock, vent and vacuum line

(K) Trap

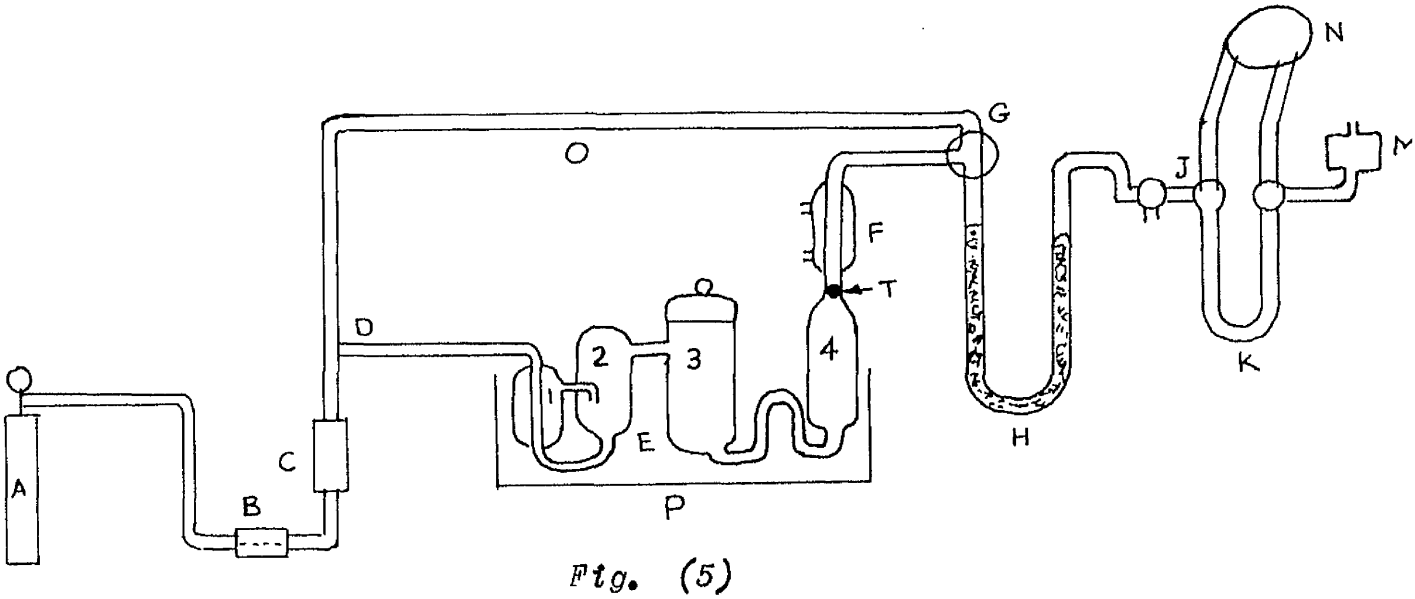
(N) Sampling Valve

(O) By-pass helium line

(P) 2 litre Beaker

(T) Silicone Seal (for injecting the internal standard) which is toluene

25 mls of distilled water to be placed in the bubbler tube F = 1 and 12 g of ground roasted coffee in E = 3. 0.05 ml of O = xylene solution containing 1% toluene to be injected at (T) by a Microsyringe. Volatiles can be collected first in trap (H) Carbowax precolumn in liquid nitrogen, by sweeping the volatiles with moistured helium. (H), will contain the volatiles including CO_2 and some water and by timing, the fraction of coffee volatiles can be released from (H) to (K) by removing the liquid nitrogen bath. Then trap (K) to be connected to the fractionator for separation by releasing K's contents, using water bath at 60°C.



Ftg. (5)

Schemattc Drawtng of the Coffee Volattles sampltng apparatus.

Trap (K) to be evacuated before the volatiles being eluted from the precolumn (H).

Rhoades[17] found that the water in B - I Fig. (5) which moistens the sweeping gas can give more than three times yield of collected volatiles than if dry gas is used, i.e. if water is excluded from K - L. (Table I).

The concentrations are expressed in Toluene equivalents which is used as an internal standard.

TABLE (I)

	3	8	9	5	4	6	7	8	9	10	11	12	13	14	15	16
	?	Acetaldehyde	Dimethyl-sulphide	?	?	Propion-aldehyde	?	Acetone	?	Non-identified v-small	Methyl ethyl Ketone	Non-identified v-small	Methyl Alcohol	Diacetyl	?	?
a	raw British mixed Gas	0,2	20,1	0,3	2,7	0,6	2,3	4,4	34,3	2,9	trace	2,3	trace	24,0	4,3	1,6	2,5
b	roast Indian mixed Gas	0,6	20,1	2,0	11,3	2,6	0,2	42,9	60,3	1,3	trace	11,0	trace	32,0	10,1	2,4	17,5

? = not identified

The aroma which is ordinarily smelt from served coffee is definitely associated with water vapour, and the application of moistened gas may not only be giving

a greater yield but possibly a more representative sample at ordinary consumption conditions.

Direct sampling

Sometimes it is possible to run samples on gas chromatography directly, i.e. without previous preparation such as trapping the volatiles or concentrating them.

This technique can be used with some liquid food materials which can be placed directly on to the column and in other cases samples of the head-space vapours from foodstuffs may be injected directly into the gas chromatographic column.

In some of these cases special precautions have to be taken to deal with water present in aqueous samples. The argon-ionisation detector is affected by the presence of water which tends to cause a fall in base line. This can be avoided by using a "back-flushing pre-column assembly"[9,10]. Fig. (6) which sits on the top of the ordinary gas chromatographic column. The pre-column consists essentially of a short column packed with diglycerol on Celite. It has the property of retarding the passage of water vapour until the desired volatile components have passed into the main column. The retained water vapour can then be flushed to atmosphere.

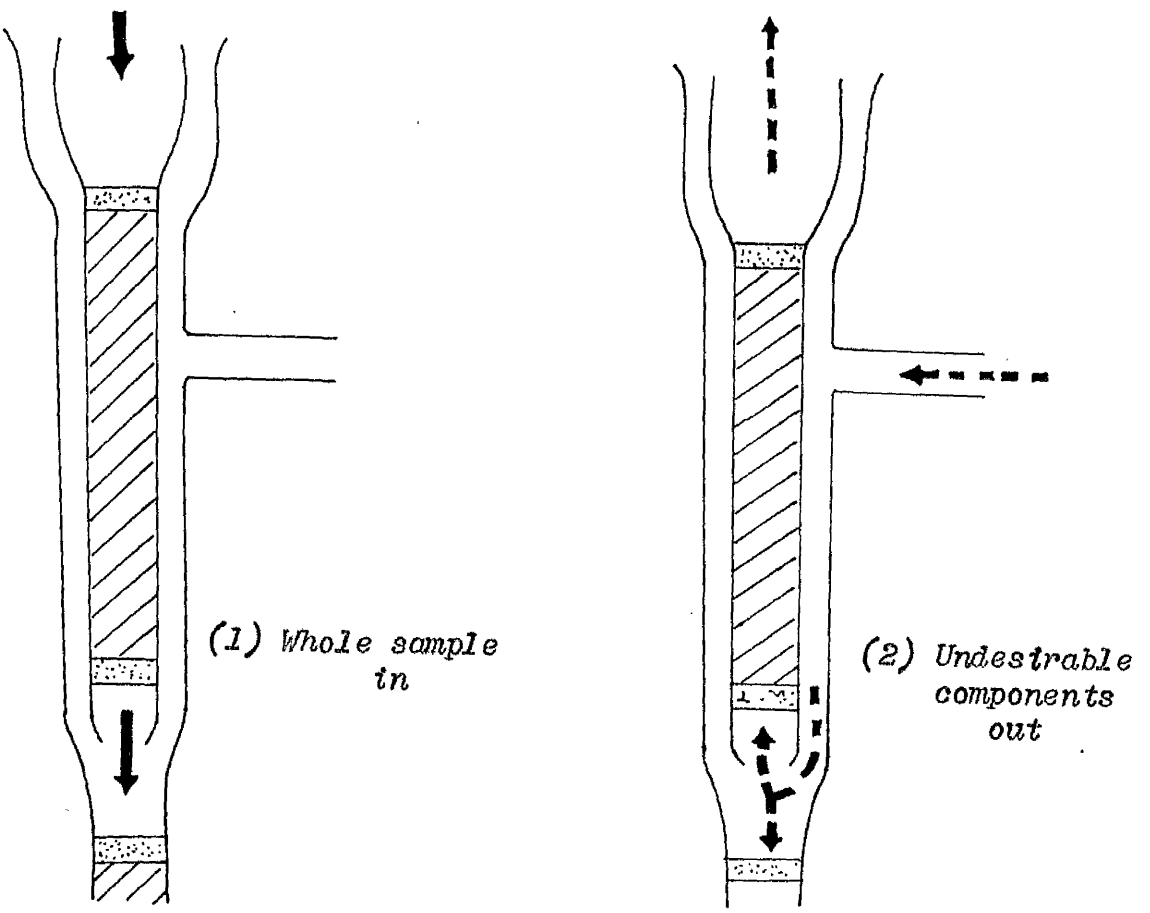


Fig. (6)

Back-flushing pre-column Assembly

The flame ionization-detector is insensitive to water vapour and can be used without modification of technique for vapour samples which contain appreciable quantities of water.

Samples of 1 m L - $\frac{1}{2}$ ml of volatiles collected by a syringe can be expelled directly into the chromatographic column associated with a high sensitive detector such as the micro-ionization.

An interesting experiment was made by direct sampling of natural and imitation Banana flavour,(12) which showed that, although it is thought to be the same by smell the composition of their vapours was found to be very different, but that might not be astonishing if interpreted according to the "Stereochemical theory of olfaction." (6)

THE EFFECT OF ROASTING AND PREPARATION PARAMETERS ON COFFEE FLAVOUR

Coffee is one of the world's most popular beverages, the annual consumption in the U.S.A. alone being more than 3.64 billion pounds of coffee beans.(18)

Coffee is not consumed for its nutrional value but as a pleasant beverage. For this reason the flavour and particularly the aroma is all important in coffee processing.

The aroma of prepared coffee beverage is affected by a large number of factors e.g. the variety of bean particularly as regards size and chemical composition, the degree of roast, the particle size of the ground coffee, the purity of the water used to make the beverage, the temperature and time of contact between the ground coffee and the water.

Uniformity of flavour is essential for marketing purposes and is achieved by blending coffee beans from different sources, control of time and temperature of roasting and the fineness of grinding.

Type of bean and blending

The selection of the coffee bean is designed to give the desired flavour in an economical manner.

Coffees from Brazil and South America are generally milder flavoured but rather expensive while those from Africa have a much stronger "roughee" flavour and are cheaper. There is no single ideal blend since the accepted standard varies from country to country. For example, in Europe, Germany uses mild coffee almost exclusively. France, on the other hand, uses mainly strongly flavoured coffees from Africa and the United Kingdom uses about two thirds African and one third mild coffees.

Methods and degree of roasting

The development of coffee flavour is influenced by the mode and degree of roasting, whether blending is done before or after roasting, temperature and time of roasting. These factors can lead to changes and losses of some of the palatability of the product. The choice of any set of roasting conditions depend on the properties desired in the final product. Flavour requirements vary in different parts of the world, for example, in Europe coffee is roasted to a greater degree than would be acceptable

in North America.

Grinding

Grinding is another important factor which requires control to maintain uniformity of particle size. The greatly increased surface area produced on grinding is important in the adsorption of aroma producing substances and aids in efficient extraction. But very fine grinding is undesirable as it results in loss of clarity of the coffee brew.

Preparation of the brew

Having established the control of all the desired factors, the volatile aromatics and CO_2 are released when the water is mixed with the roast, because of the selective preference of the fibre structure for water which destroys the adsorptive power of the dry coil surface.

To get fresh aroma, the water temperature should be between 85 - 95°c with the shortest contact time between ground roasted coffee and water consistent with the proper degree of extraction. Over extraction leads to bitterness and astringency.(24)

The sooner the prepared coffee is consumed the better.

"Instant" Coffee

In recent years there has been a very great increase in the production of "instant" coffee which is prepared usually by spray-drying a concentrated extract of ground roasted coffee. The quality of the product depends largely on the degree of extraction, the temperature of drying and the physical loss of volatile materials.

The best instant coffee is that produced from a good blend of good roasted coffee with an optimum extraction rate of 28 - 32%.[23] Over-extraction will add bitterness to the coffee. Spray-drying and cooling are likely to cause some changes and losses of the volatile constituents.

Some loss of flavour is inevitable in the manufacture of instant coffee.

Development of Coffee Flavour

Roasting for full flavour development can be reached at about 204°C by degradation and synthesis occurring simultaneously within the bean cells in the absence of air.[22]

Table II [22] shows the composition of coffee before and after roasting. The changes which occur in the proportions of some of the constituents might be an

indication of the contribution of these substances to the formation of coffee aroma. Sugar and other carbohydrates along with tannic acid show the greatest reduction.

TABLE XI

Composition of coffee before and after roasting (22)

	Raw %	Roasted %
Water	10.73	3.16
Sugar	8.03	0.75
Caffeine	1.07	1.20
Crude fibre	24.00	13.03
Ether extract	11.08	13.75 (pet. ether cold extract)
Aqueous extract	30.35	12.03
Ash	3.00	4.03
Nitrogenous substances	12.04	2.27 (Total nitrogen)
Other nitrogen free extractives	19.30	
Dextrin	6.86	-
Tannic acid	9.02	-

The acidity of coffee aqueous solutions increases to a maximum, and then decreases as roasting progresses. Lightly roasted coffees have the highest acidity.(25) When the expulsion of volatile acids reaches a maximum during roasting, this point might constitute an indication as to when to end the roast.(59) Moreover volatile acids exhibit characteristic flavours in addition to their acid properties.(26)

It is common knowledge that many of the flavouring substances used in industry are carbonyl compounds, and it is quite possible that these compounds play an important part in coffee aroma.(27)

Rhoades(28) showed by gas chromatographic analysis that an index for the degree of roasting was derived from the variations in these compounds with increasing roasting temperature, and that the ratio of diacetyl to acetyl propionyl increases with the increase in roasting temperature. Fig. (7).

Because of the importance of the carbonyl compounds particular attention was devoted to their analysis.

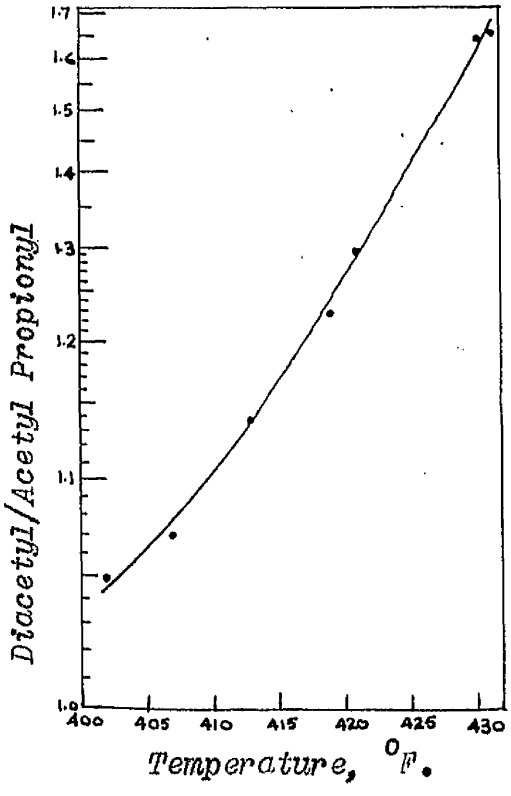


Fig. (7)

Relationship between degree of roast and the ratio of diacetyl to acetyl propionyl.

The Volatile Carbonyl compounds of Coffee Aroma

Coffee flavour is not present in the raw-beans but is formed during the roasting process, principally from the water soluble components.[19] An examination of coffee aroma volatiles shows that about 400 ppm of these substances is present in roasted coffee. They are discrete chemicals, mostly aldeydes and ketones. Sulphides, furans, esters and alcohols are also present. The aldehydes are predominant in taste and aroma.[20b]

Aldehydes and ketones are of special interest not only because they form the largest proportion of coffee aroma but also because of their susceptibility to changes according to their chemical behaviour, as relatively unstable reducing substances.

Table XIX[20a] shows the quantities of carbonyl compounds expressed as percentages of the total volatiles reported by three different workers. It will be seen that the carbonyls form from 71.0% to 81.9% of the total volatiles. The proportions of the individual carbonyls, however, show marked variations; e.g. iso-valeraldehyde varies from 15 to 18.2%, acetone from 0.5 to 25.6% and only one worker reports the presence of 2 - Methyl butyraldehyde.

TABLE XXX

Coffee Aroma Analysis[30a]

% of Carbonyl compounds in the whole volatiles[1]

	Coffee Roasted Aroma (2)	Coffee Roasted Aroma (3)	Coffee Roasted Aroma (4)	Brewed Coffee Aroma (4)
Acetaldehyde	17.9	19.9	25.0	22.8
Propionaldehyde	8.0	4.5	3.2	2.6
Butyraldehyde	-	0.7	0.3	0.1
Iso-butyraldehyde	-	3.0	6.8	5.2
2-Methyl butyraldehyde	-	6.8	-	-
Valeraldehyde	-	7.3	-	-
Iso-valeraldehyde	10.2	5.0	1.5	1.0
Acrolein	0.6	-	-	-
Dimethyl Acrolein	tr[5]	-	-	-
Methyl ethyl acrolein	1.4	-	-	-
Acetone	0.5	20.7	21.6	25.6
Methyl ethyl Ketone	14.2	3.3	8.3	6.4
Methyl vinyl Ketone	0.5	-	-	-
Diacetyl	10.3	7.5	6.4	8.4
2,3-Pentanedione	-	-	6.7	8.8
2,4-Pentanedione	9.2	-	-	-
	71.8	76.7	79.7	81.9

(1) Ribar (1960)
(2) Merritt, C. et al (1957)
(3) Flachs & Silvers (1960)
(4) Rhoades (1958 & 1960)
(5) Trace, tentatively identified

In all, sixteen different carbonyl compounds are reported here, falling into the following classes viz:-

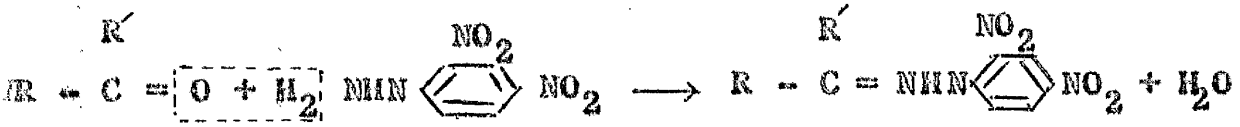
Unsaturated aldehydes	3
n-aldehydes	4
Iso-aldehydes	3
di-ketones	3
Unsaturated Ketones	1
Saturated Ketones	2
	16

EXPERIMENTAL

The approach to the work described here was to collect the coffee carbonyls in the form of their hydrazones, to liberate the carbonyls by a system of "flash-exchange" and to separate, identify and estimate them by means of gas chromatography.

Preparation of Hydrazones

Carbonyl compounds react with hydrazine to form the hydrazones as follows:



Carbonyl Compound

2,4 - Dinitrophenyl-hydrazine

Hydrazone

when (R) and/or (R´) is (H) the compound is an aldehyde otherwise it is a Ketone

Hydrazine Reagent

The reagent is prepared by dissolving 2 g. of 2,4 - Dinitrophenylhydrazine in a litre of 20% ethanolic 2 N - HCl. (i.e. 180 ml. Conc. HCl + 200 ml. C_2H_5OH + 2 g. 2,4 Dinitrophenylhydrazine, made up to

one litre with deionised water). The required quantity of this solution is filtered immediately before use. Because some of the carbonyl compounds are only sparingly soluble in aqueous solutions, the alcohol was added to facilitate the solution of these compounds, to bring them into phase with the reagent and to form their hydrazones in as short as possible time. Since, in this work, the carbonyl compounds are usually bubbled through the hydrazine reagent in a carrier gas, rapid reaction is essential to avoid loss. Some of the formed hydrazones might be partially dissolved in the alcohol, but this can be easily overcome by cooling down the mixture for recrystallization to take place.

When pure hydrazones have to be prepared as standards from the ordinary laboratory grade carbonyl compounds, the percentage of the alcohol in the hydrazine reagent can be raised to 30% or more so that a greater quantity of the hydrazones will dissolve in the alcohol and in this case the mixture is not cooled before filtration. This technique appears to give a purer product.

No. 1 Whatman filter paper was used for filtering

the hydrazones which were washed with sufficient 2N HCl followed by a thorough washing with deionised water.

Losses of hydrazones on filter paper are undesirable as that when very small quantities have to be prepared it is convenient to precipitate them in a centrifuge tube, then wash them as before, but by centrifuging and decantation.

The washed hydrazones were then dried in an oven at 105°C.

Purification of the hydrazones

For qualitative and quantitative work pure hydrazones are required; most of the standard hydrazones were found to give one distinct spot when they were tested for purity by thin layer chromatography but the hydrazones which needed further purification were dissolved in chloroform and passed through a short column of Santocel-kieselguhr, 4:1,(32) the hydrazones were eluted using chloroform as a solvent, leaving the crude material and the unreacted hydrazine adsorbed at the top of the column.

Stability of the hydrazones

Hydrazones are liable to slight decomposition on storage leading to the production of m - dinitrobenzene and to the liberation of carbonyl compounds.(31) Neither of these products is significant as far as qualitative work by the "flash-exchange" technique is concerned.

The hydrazones should be dried at temperatures below their melting points to avoid decomposition as well as to keep their crystalline form intact. This is important when infra-red work is involved as the different crystalline forms of the same substance may give rise to different spectra e.g. cortisone acetate has been obtained in five forms.

The collection of Cation Volatile Carbonyl compounds

Two devices were used for collecting these compounds for different purposes in different experiments:

(1) The "Shipton" apparatus, designed for the determination of SO_2 in foodstuffs was modified as shown Fig. (8).

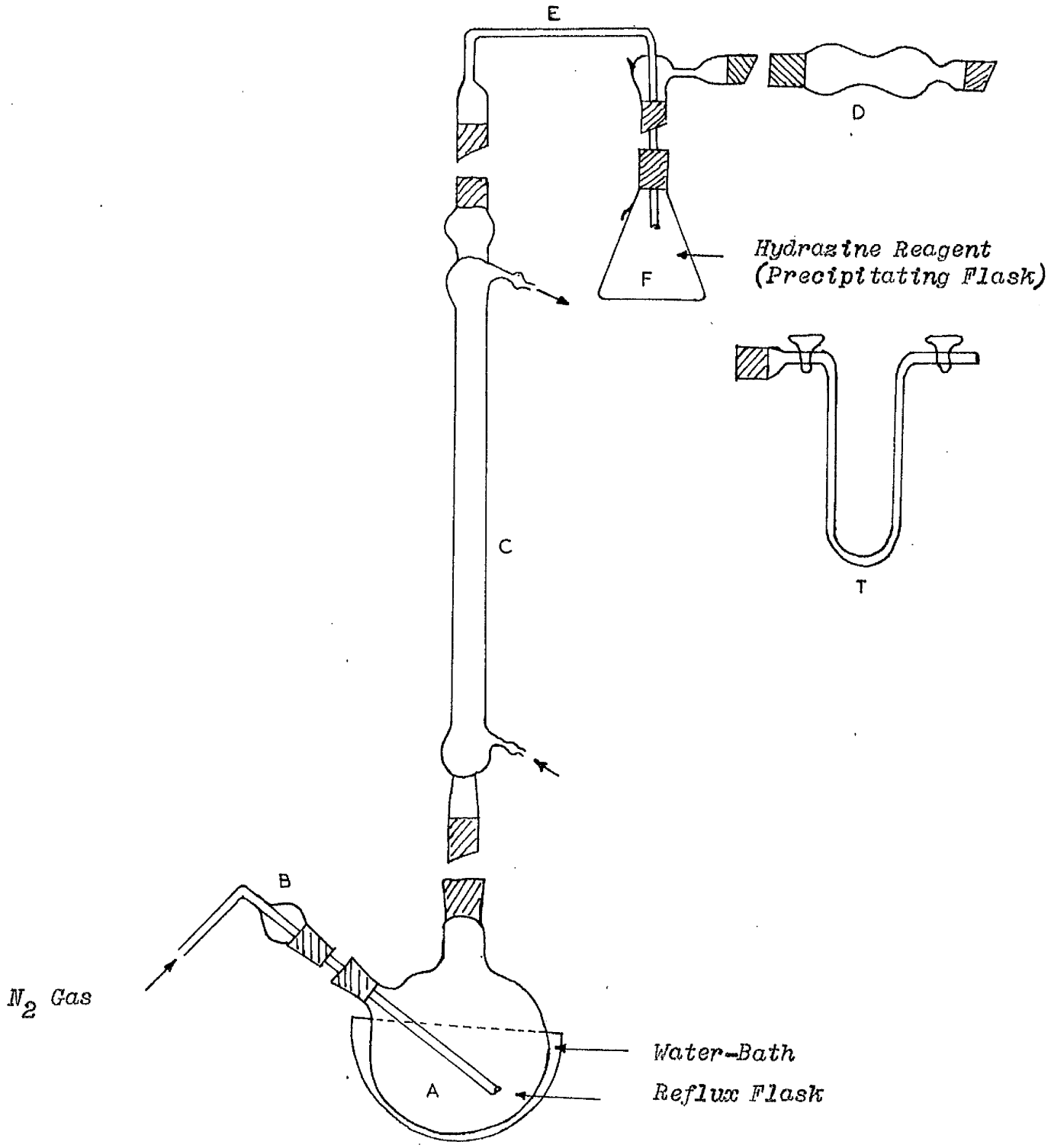


Fig. (8)

(Aroma collecting apparatus)

Slightly modified Shipton's Apparatus.

(2) Steam distillation Fig. (9) under atmospheric or reduced pressure.

The first was found to be very satisfactory and the collection was made as follows.

Procedure

50 g. of coffee sample was placed into the refluxing flask, followed by 500 ml deionized water at room temperature. The flask was connected immediately to the apparatus and immersed in an electrically heated water-bath at boiling condition. The hydrazine -reagent-flask had previously been connected to the top of the condenser. Nitrogen gas was then passed through the system to sweep the volatiles from the reflux flask and to bubble them through the reagent flask in which the V.C.C. were precipitated as hydrazones. The water and the compounds of about the same boiling point are refluxed into the coffee solution.

To get reproducible results the conditions were standardized as follows.

1. Coffee sample was 50 g.
2. Water added to the coffee was 500 ml. deionized water at room temperature.

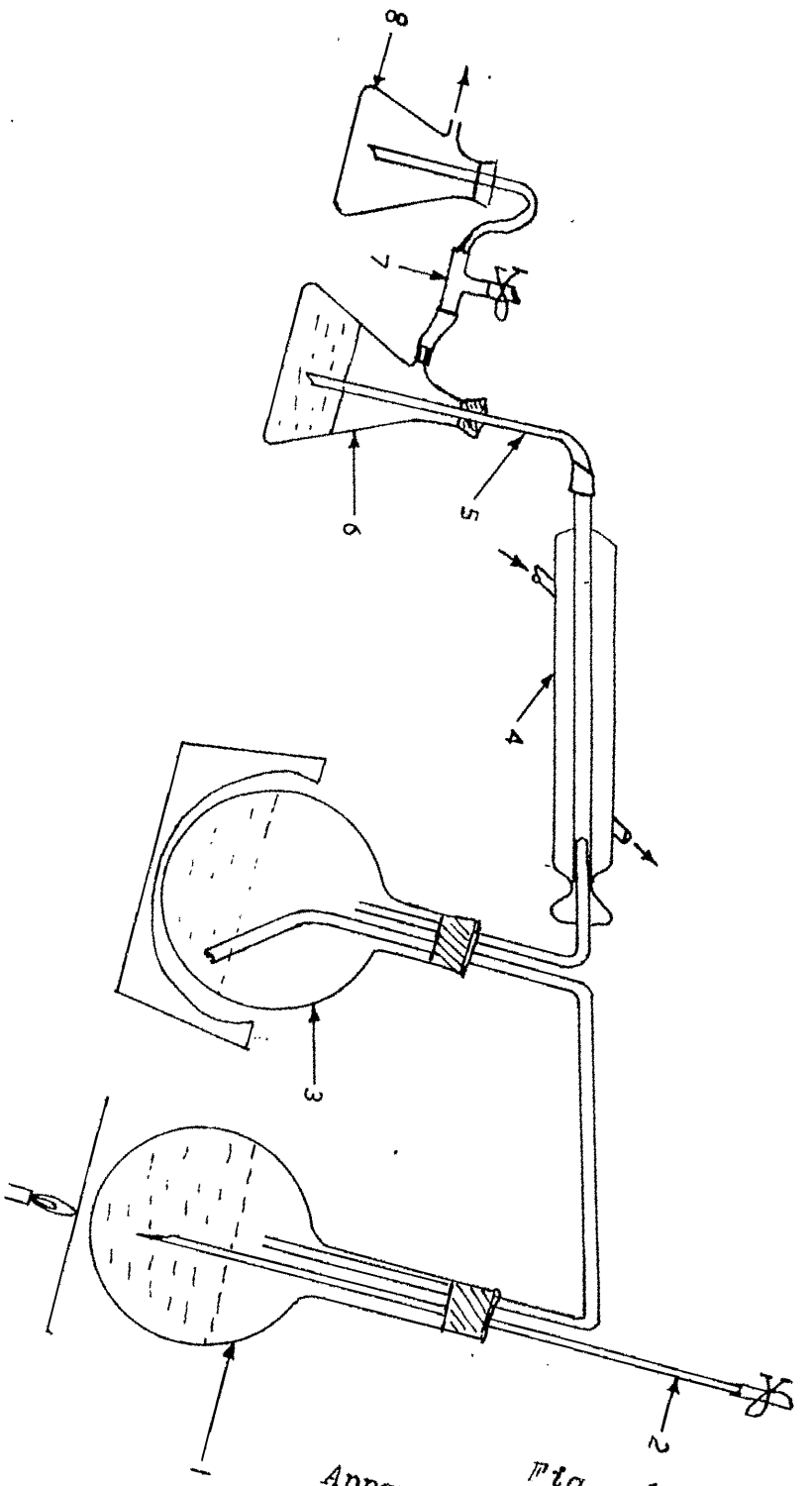


Fig. (9)
Apparatus for steam distillation under atmospheric or reduced pressure.

3. 200 ml of filtered hydrazine reagent was used.

4. Nitrogen bubbled through the aqueous coffee solution leaving the apparatus at a rate of 60 ml/min. (measured by a soap bubble flowmeter).

5. Outlet water from the condenser was of the rate flow of 600 ml/min.

6. Inlet condenser water temperature was between 8 - 10°c throughout the collection period.

7. Stripping time of the volatiles was two hours.

8. Each sample was taken from 2 oz. tin with a vacuum diaphragm cover (Nescafe tins).

This efficiency of capturing the V.C.C. quantitatively was tested by introducing a guard trap at the exit of the hydrazine reagent flask and this showed no sign of any escaping carbonyls.

After 2 hours, the apparatus was disconnected, starting by the precipitating flask to avoid back suction, then the bath off, gas off, followed by detaching the reflux flask from the condenser.

The collected hydrazones were filtered on No. 1 Whatman filter paper, washed with 2N HCl, then by water and dried as usual, and accurately weighed in the following way.

Two filter papers of equal weight were each placed in a funnel, the funnels were placed one above the other so that the filter and the washings from the upper passed through the lower.

The two filter papers were dried at the same temperature and the difference was that due to the hydrazones. This gave very constatant results, and the yield of dry coffee volatile hydrazones was reproducible, 65 ± 5 mg.

It was noticed that the aroma of the cuit gas from the precipitating flask was disagreeable and in no way resembled coffee aroma. It would appear that the carbonyl compounds are essential constituents of coffee aroma.

Steam-Distillation Fig. (9).

This method of collecting the coffee volatile compounds, was mainly used in qualitative experiments, particularly when the sample required a treatment which involved slower reactions compared with the time

of precipitating the hydrazones. For example in the oxidation of aldehydes using freshly precipitated Ag_2O, about one hour contact time is required. This contact time cannot be achieved in the Shipton apparatus where the aldehydes are bubbled through the Ag_2O reagent in a stream of nitrogen. Even if the volatiles were previously collected in a cold trap there are serious practical difficulties in avoiding loss.

In general, the hydrazones obtained from the steam distillation method were darker in colour and appeared to be of lesser purity than those collected from the Shipton apparatus.

The steam distillation was used by placing the coffee sample in Flask 3, the volatiles were driven by the steam generated from the Flask 1, then the condensed volatiles reacted with the reagent in the revolving Flask 6, which was kept in an ice-water bath.

THE ANALYSIS OF CERTAIN VOLATILE CARBONYL COMPOUNDS

Before embarking on the main part of the work, preliminary experiments were made to establish the best conditions for analysis, e.g. the choice of a liquid phase which would give adequate separations and the choice of the operating conditions of the gas chromatography equipment.

Selection of liquid phase

Tests carried out with mixtures of known carbonyls on a 6' column in a Griffin and George Apparatus fitted with a katharometer detector, showed that good separations could be obtained using 20% di-nonylphthalate on Celite. (Fig. 10.)

The tests were carried out with liquid samples and the components emerged in accordance with their boiling points. Table IV. This is to be expected as di-nonylphthalate is relatively non-polar.

TABLE IV.

Retention times obtained from direct tests (Fig. 10).

Carbonyl Compound	Acetaldehyde	Propion-aldehyde	Acetone	Iso-Butyr-aldehyde	Methyl Ethyl Ketone	Iso-Valer-aldehyde
° ("Boiling Point")	21	48.8	56.5	61.5	79.6	92.5
Retention time	1.00	2.53	3.05	4.37	7.50	10.00

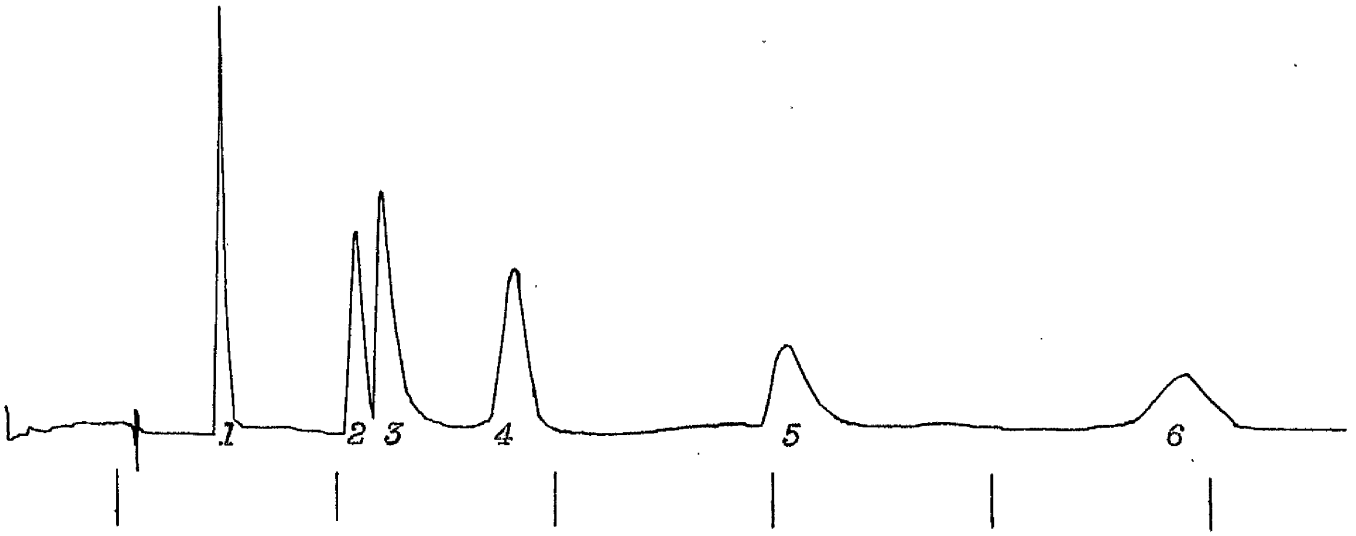
Fig. (10)

Reagents mixture of carbonyl compounds on

6', 20% Dinonylphthalate on Celite 80 - 100 mesh
Col. Temp. 35°C, G.F. 1.82/hr.
No. of theoretical plates $_{37(b)}$ *to Iso-Butyraldehyde = 610*

---

(1) Acetaldehyde *(2) Propionaldehyde*
(3) Acetone *(4) Iso-Butyraldehyde*
(5) Methylethyl Ketone *(6) Iso-Valeraldehyde*



While satisfactory separations could be obtained on this instrument its low sensitivity made it unsuitable for the small samples which would be necessary for "flash-exchange."

For this reason the remainder of the work was done on a Pye-Argon apparatus using an ionisation detector. This, however also has its limitations particularly as regards response to different classes of compounds and this problem is discussed below.

The response of the Ionisation Detector

The response of the argon detector to different classes of compounds is not linear over the range of detector voltages available,[8] but varies with the ability of the compound to capture electrons. This can seriously affect quantitative measurements and is particularly marked at the lower ranges of detector-voltage. While the electron capture effect is most marked at very low detector voltages e.g. 8 - 24 volts (which is the range at which electron-capture detectors operate) it is still appreciable with highly electrophoric compounds at the lower end of the normal voltage ranges used in the argon-ionisation detector viz. 750 - 1500 volts.

Lovelock (37), gave values for the electron affinity of different classes and of different compounds, e.g. aliphatic alcohols, ketones and aldehydes are given values 0.1 - 1 and di-ketones the value 10^3 - 10^4

These values are relative to chlorobenzene which was given an arbitrary value of 0.1.

Naturally occurring substances like the coffee V.C.C. contain compounds of different affinities for electron capture, so that the response to these highly electrophoric compounds will be smaller than their actual concentrations justify. This is due to the fact that these electrophores can cause a marked decrease in the electron-current flow.(39) No significant results can be obtained without a remedy to this problem, particularly when quantitative work is required. To overcome this, small samples and detector voltages of 1750 volts and above are necessary (36, 37, 38).

Electron Capture effect of Diacetyl

Of the coffee V.C.C., diacetyl is the most likely to cause difficulties in quantitative work because of its much greater electron affinity in

relation to the other carbonyl compounds present.

This effect was demonstrated by running a mixture of diacetyl and t-amylalcohol over the voltage range, 750, 1000, 1250 and 1750 volts. Amyl alcohol was chosen, in preference to the carbonyl compounds because of its convenient retention time and it was available in a purer form than the carbonyl reagents. In addition, it falls into the same group as the carbonyl compounds from the point of view of electron affinity as Fig. (12) shows. This gave a simple and convenient mixture for illustrating the effect of detector voltage.

Approximately 0.02 µl samples were injected into the gas chromatography column using a Hamilton micro-syringe, the detector voltage was raised from 750 1000 1250 1750 volts before injecting samples.

As a result the diacetyl peak was increased by increasing the detector voltage, compared with the alcohol peak area which was considered to be relatively unchanged.

Table (V) shows the ratio of the peak height of diacetyl to that of t-amyl alcohol at the different voltages used.

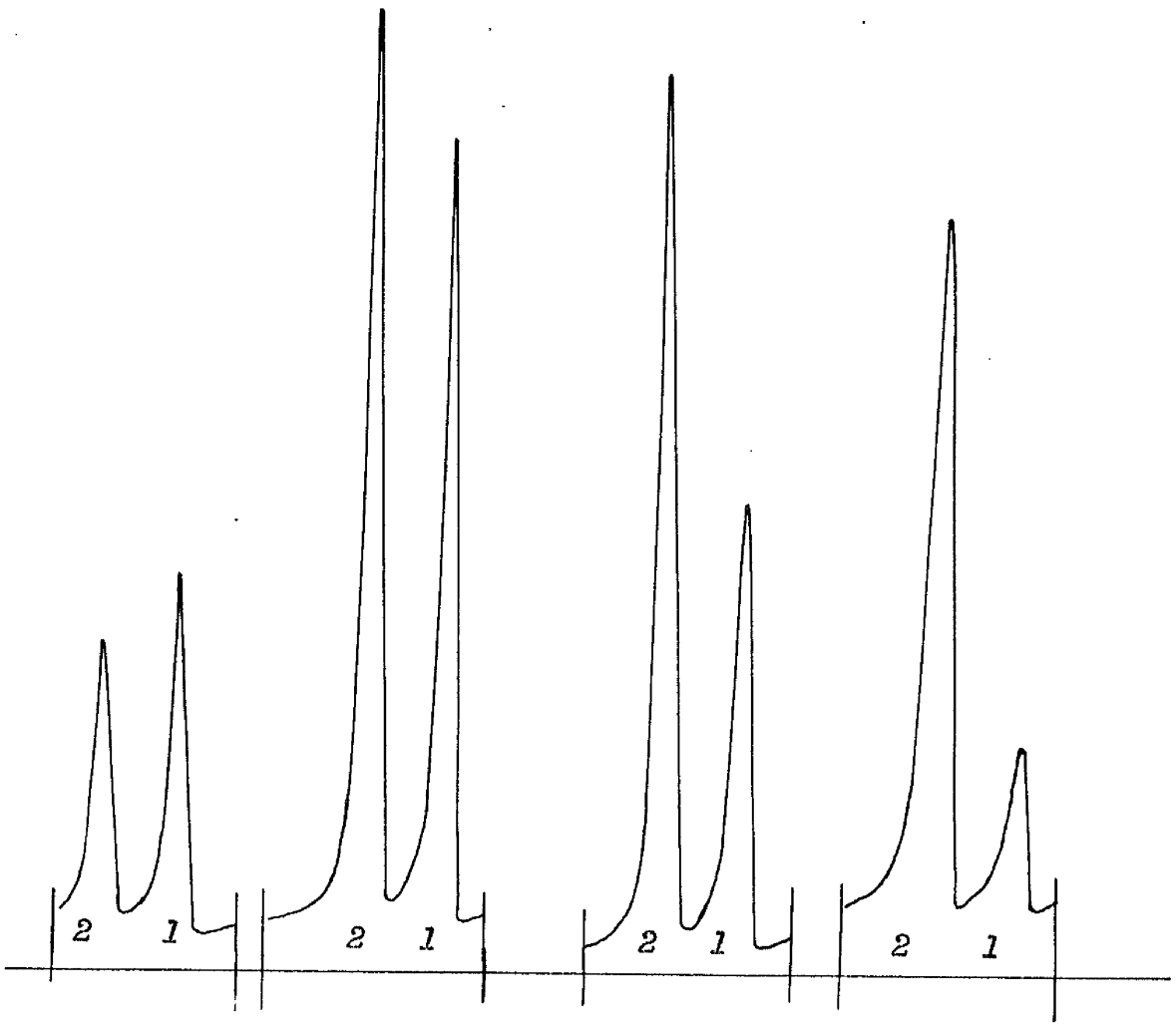


Fig. (11)

The effect of change of detector-voltage on detector response.

(1) Diacetyl (2) t-amyl alcohol

TABLE V.

Ratio of peak height of diacetyl/t-amyl alcohol

Detector Voltage	750	1000	1250	1750
Ratio of peak height, $\frac{\text{Diacetyl}}{\text{t-amyl alcohol}}$	0.244	0.52	0.90	1.24

The final conditions chosen for gas chromatography were as follows:

Gas Chromatograph	-	Pye Argon with Sr^{90} β - ionisation detector
Column Length	-	4 ft.
Column i.d.	-	4 mm.
Packing	-	20% dinonylphthalate on 100 - 120 mesh Celite
Detector Voltage	-	1750 Volts

Coffee samples: All coffee samples used in this work were from instant coffee, (2 - oz. Nescafe Tins), unless otherwise noted.

Flash Exchange gas chromatography

The method used was that of Ralls,[29] in which the carbonyl compounds were liberated from their hydrazones by mixing them with - Ketoglutaric

acid (∝KGA), and packing into a glass capillary tube. The tube is immersed in a hot oil-bath to liberate the volatile carbonyl compounds into the gas chromatography column through the injection system.

Procedure

A few mgs. of dry purified coffee hydrazones were mixed with three times their weight of ∝KGA, and six times their weight of dry Celite. (10 mg. mixture ≡ 1 mg. hydrazones).

Celite was added to form a porous packing to facilitate the escape of the evolved volatiles. The mixture was then finely ground with a nichrome spatula on a watch-glass until a homogeneous blend was obtained. Portions of this powder were then packed in capillary tubes pulled out of ordinary glass-tubing. The capillaries were within i.d. of 0.5 - 1.0 mm, and about six cm. in length and sealed at one end.

They were packed by introducing the mixture into the open end with a spatula, then tapped by letting the capillary fall in a long tube standing

vertically on the table, until the desired packing was accomplished. The capillary was then bent in a flame, and the open end was tightly inserted into the hole in a small cylindrical silicone rubber bung (4 mm diameter and 5 mm height), which acted as a connection between the capillary and the hypodermic needle.

The bending of the capillary should be done on a sharp flame in the shortest possible time to avoid decomposition of the adhering packing.

The sample is now ready for injection into the gas chromatograph. The needle is forced through the silicone rubber septum at the top of the column and the capillary immersed for 10 - 15 sec. in an electrically heated silicone-oil bath held at 250 ± 5°C.

The needle was then withdrawn from the column before the capillary was removed from the oil bath. If the bath is removed first, the capillary cools down rapidly creating a low pressure which draws back some of the volatiles if the needle is still in the top of the column.

Sample injection can be carried out with or without shutting off the gas, providing the end of the needle is below the gas inlet level as shown in Fig. (12).

The quantity of sample required to give an acceptable response in the gas chromatogrph is related to the sensitivity of the detector and the design of the flash exchange system.

Using a thermal-conductivity detector an average of 1.12 mg. hydrazones was required.[29]

In this work, using the B - ionisation detector and keeping all flash-exchange connections as short as possible, acceptable responses were obtained from as little as 0.05 mg. of a mixture of 8 - 9 hydrazones.

Results and Discussion

It was observed that in all chromatograms from the flash exchange reaction a peak No. 10 (Fig. 13) appeared after iso-valeraldehyde which could not be any of the carbonyls known to be present.

Experiments with a mixture of ∝ KGA and Celite showed that this peak was due to a breakdown product of ∝ KGA Fig. (14).

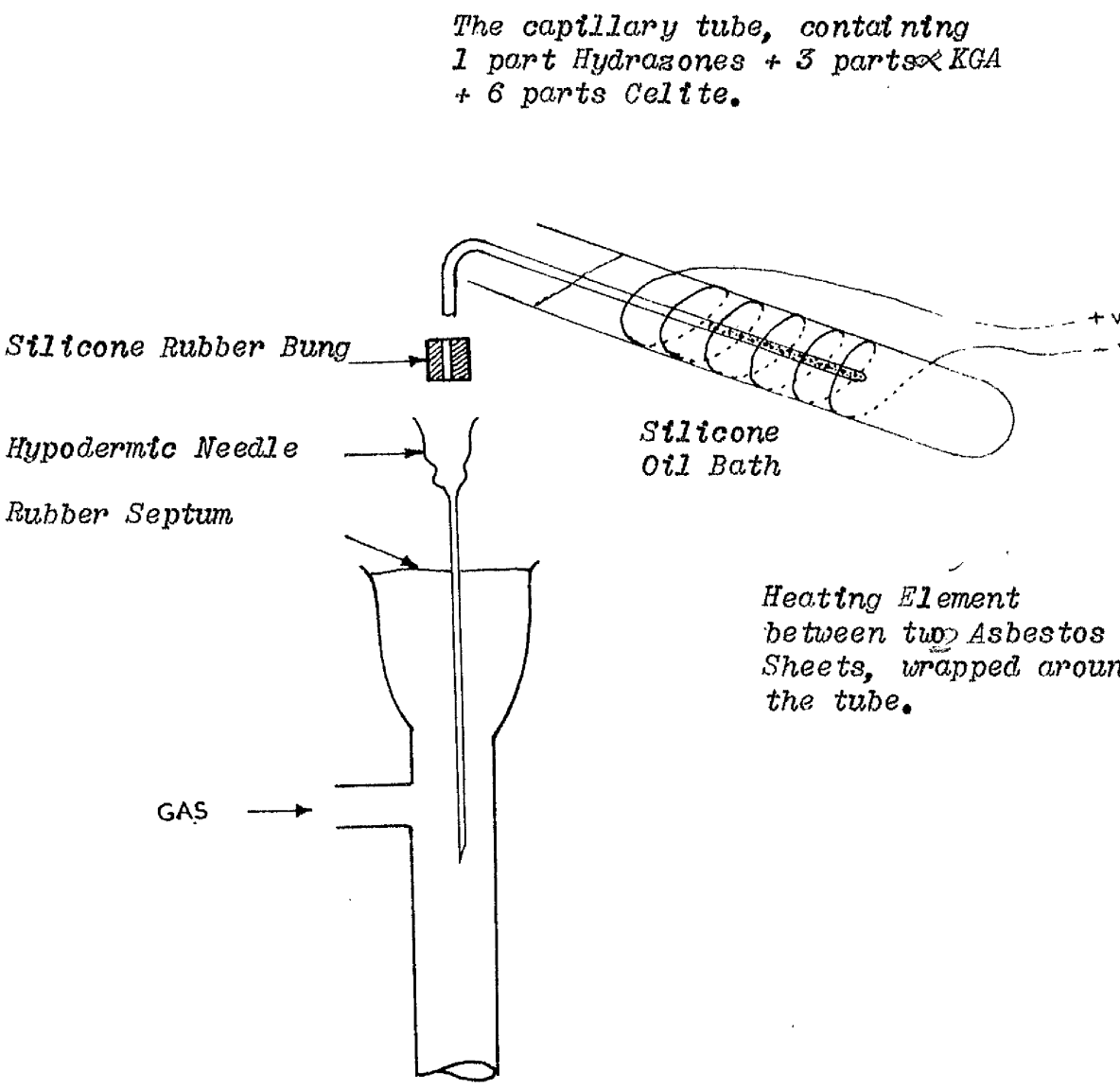


Fig. (12)

Flash-exchange injecting device.

Fig. (13)

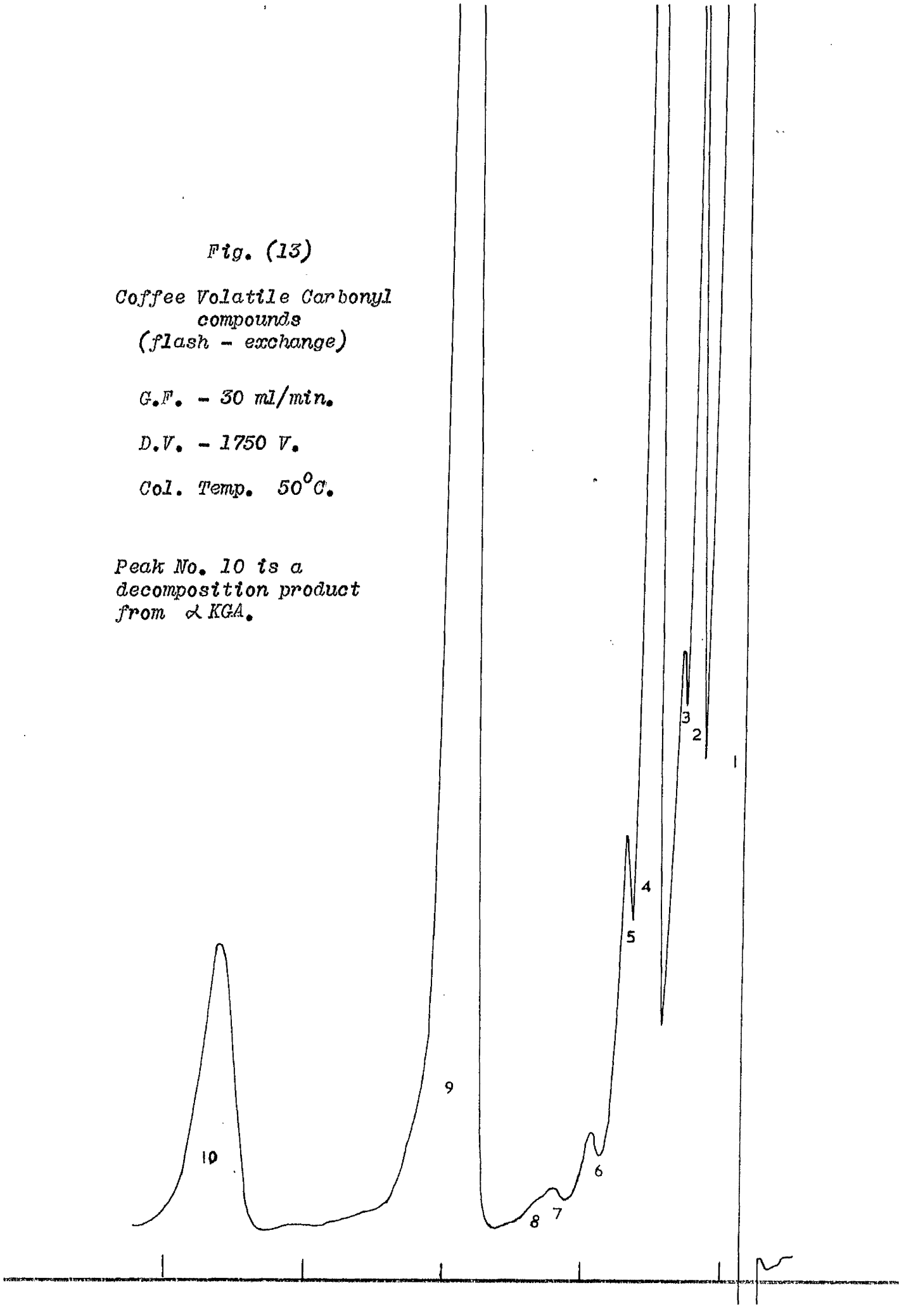
Coffee Volatile Carbonyl compounds (flash - exchange)

G.F. - 30 ml/min.

D.V. - 1750 V.

Col. Temp. 50°C.

Peak No. 10 is a decomposition product from α KGA.



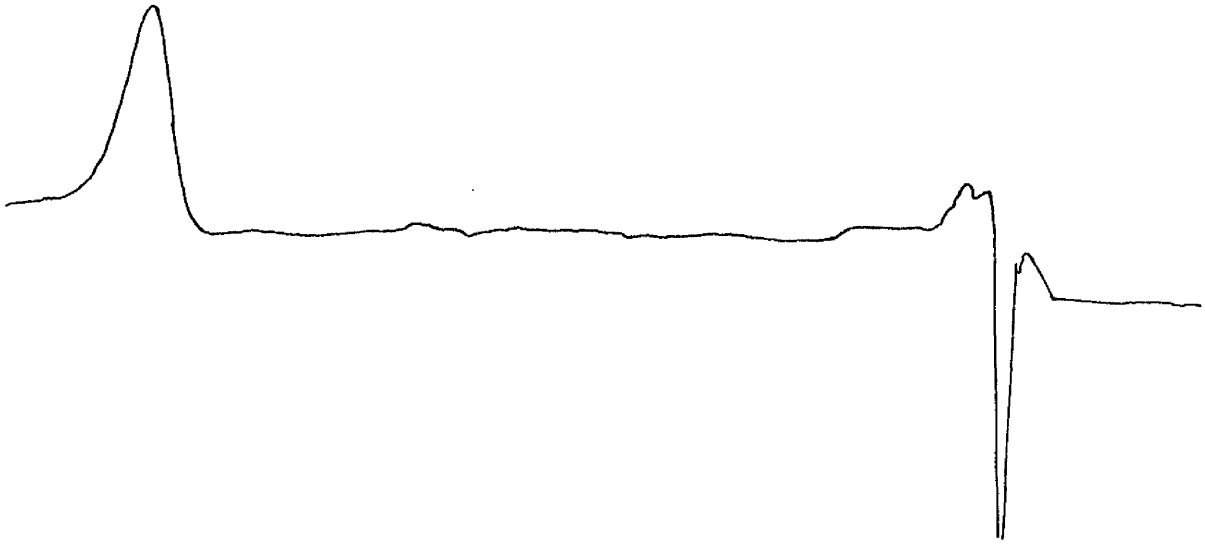


Fig. (14)

A break-down product peak of ∝ KGA.

G.F. - 30 ml/min.

D.V. - 1750 V.

Col. Temp. - 50°C.

The original paper[29] on the flash-exchange method did not report such a peak but Theodore etal[30] did.

An attempt was made to determine the nature of the compound responsible for this peak. A mixture of α KGA and Celite was heated in the flash-exchange apparatus and the vapours bubbled through a test-tube containing the hydrazine reagent. No precipitate of hydrazones was obtained even when quantities up to 8 - 10 mg. α KGA were used. When Schiff's reagent is substituted for the hydrazeine reagent there was no development of a violet colour to indicate aldehydes.

While it was shown that the decomposition was not a carbonyl compound its identity was not established. Its retention time is too short for it to be a fatty acid or an anhydride and too long to be an ester or ether of five carbon atoms or less. There is a probability of it being an alcohol of five carbon atoms e.g. Pentanol - 3 (B.P. 116^{o}C) would be expected to emerge about this point.

Limitations of the Flash-Exchange Technique

Ralls[29] showed in the original paper that carbonyl compounds from C_2 - C_6 gave rapid regeneration; bis - carbonyl compounds were not regenerated, and formaldehyde was either not regenerated or polymerised; unsaturated carbonyls were regenerated but to a lower degree than saturated; aromatic aldehydes (e.g. benzaldehyde) were not regenerated.

Haughes[40] reported that the unsaturated carbonyls were not regenerated in any appreciable quantity.

On the other hand, Stephens & Teszler[45,46] proposed a modification of the flash-exchange method in which the base of the capillary was packed with a mixture of the hydrazone of formaldehyde and KGA. The sample under test was packed on top of this. It was stated that the regenerated formaldehyde would sweep the regenerated test carbonyls out of the capillary and so give full recovery.

In view of the contradictory evidence, tests were made regarding the regeneration of formaldehyde, diacetyl as a di-carbonyl compound, crotonaldehyde an unsaturated carbonyl, furfural a heterocyclic carbonyl and acetophenone an aromatic carbonyl. The tests were

made by carrying out the flash-exchange in the usual manner but the evolved vapours were received in a test tube containing the hydrazine reagent.

The formation of a precipitate was taken as evidence of regeneration. These tests showed that formaldehyde was not regenerated even when large samples were used, while the remainder gave appreciable quantities of precipitate.

In addition, tests using gas-chromatography showed that reasonable responses could be obtained from unsaturated compounds e.g. crotonaldehyde and acrolein; from the bis - carbonyl compounds, diacetyl; the heterocyclic carbonyl furfural; and the aromatic carbonyl acetophenone which, incidentally, has eight carbon atoms. Fig. (15) shows the peak produced by regeneration of acetophenone.

<u>The qualitative analysis of the volatile carbonyls of coffee</u>

Fig. (16) shows the chromatogram obtained from a sample of coffee V.C.C. The peaks were indentified by separate experiments using the hydrazones of known carbonyl compounds.

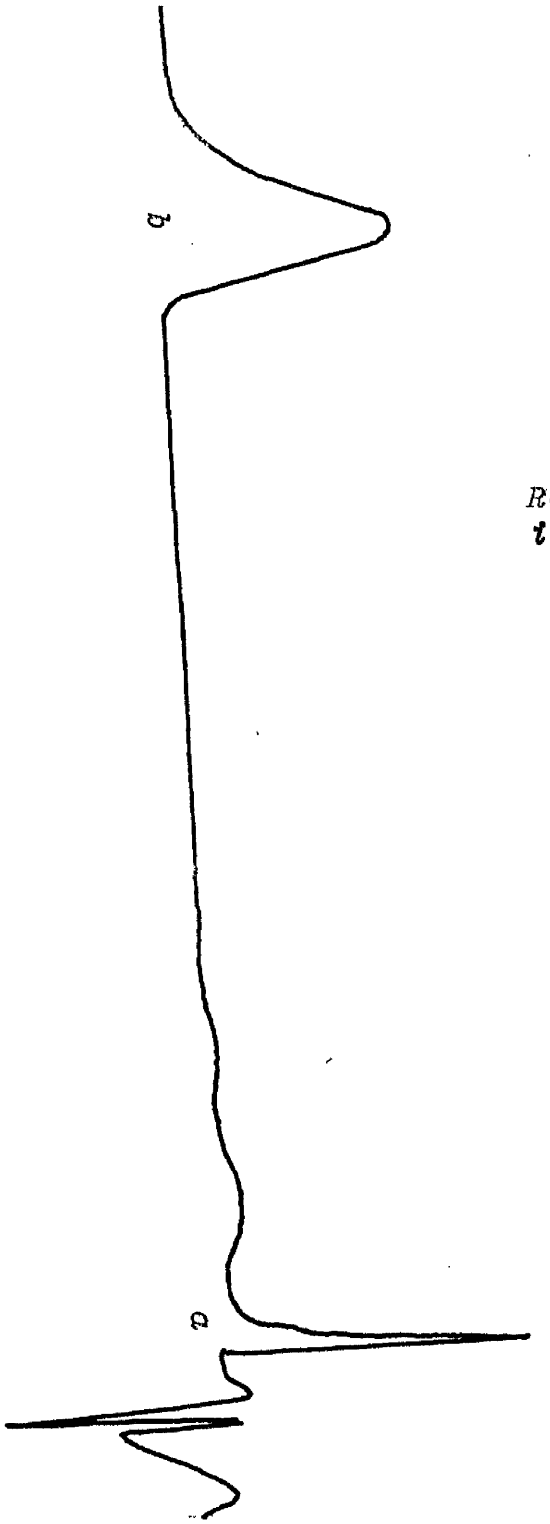


Fig. (15)

Regeneration of acetophenone, from its Hydrazone by flash-exchange method.

a) From ∝ KGA
b) Acetophenone

G.F. - 300 ml/min.
D.V. - 1500 V.
Col. Temp. - 122°C.

Fig. (16)

Fash-exchange of Coffee hydrazones collected by Shipton's apparatus from 50 g. Nescafe for two hours.

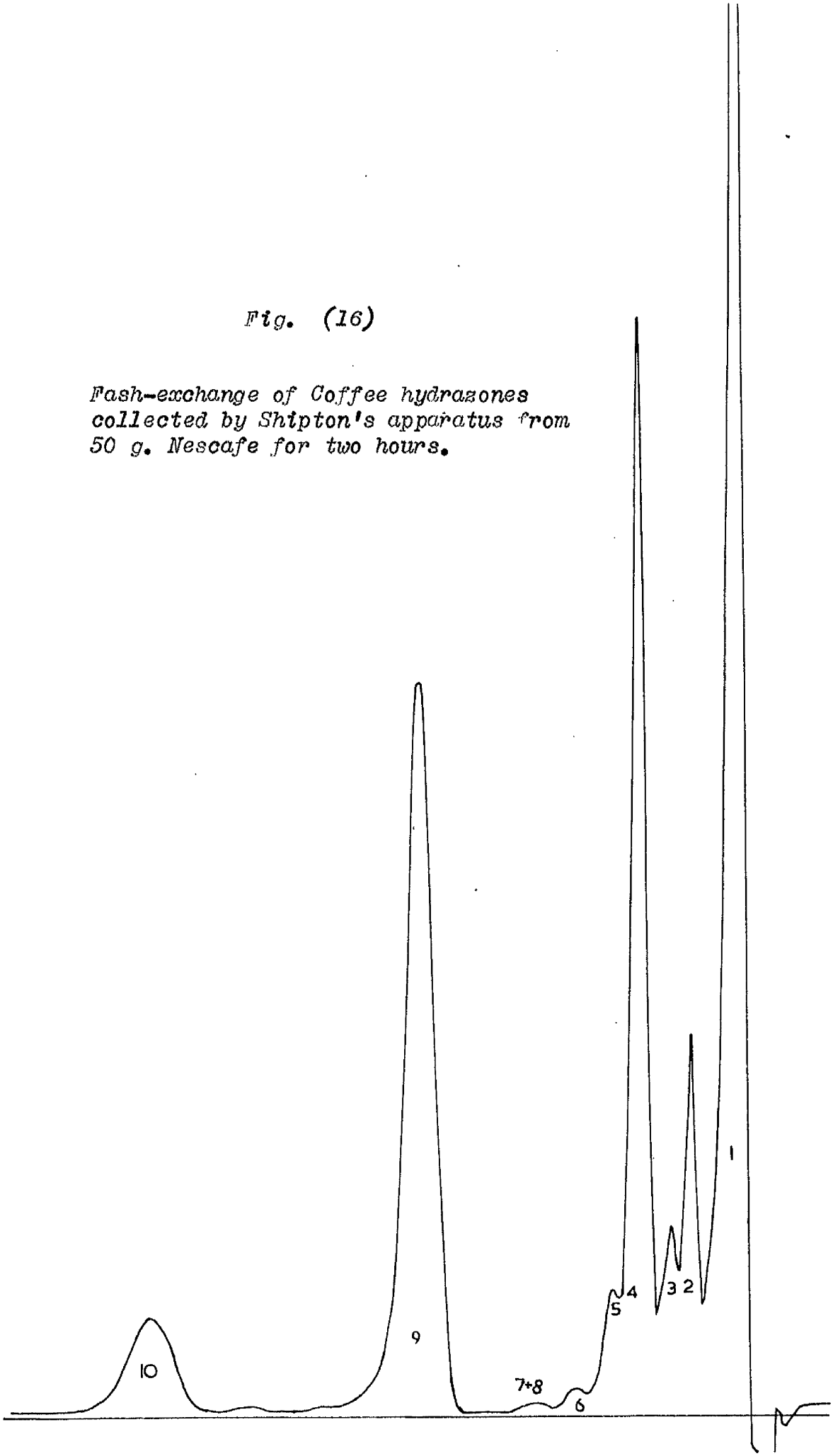


TABLE VI.

Peak No.	Compound	
1	Acetaldehyde	
2	Propionaldehyde	
3	Acetone	
4	Iso-butyraldehyde	
5	Not identified	(a)
6	n - butyraldehyde	
(7	methylethylketone)	(b)
(8	diacetyl)	
9	Iso-valeraldehyde	
10	Not identified	(c)

(a) This peak has been shown Fig. (17) to be associated with iso - butyraldehyde and appears even when an authentic standard of iso-butyraldehyde is used. As is shown in Fig. (18) it is not n - butyraldehyde. Pyruvic aldehyde (B.P. 72°C) would be expected to emerge about this point but this has not been confirmed.

(b) Peaks 7 and 8 were not well separated but tests showed that they occupied the positions of methylethylketone and diacetyl. Fig. (19) shows the chromatograms from a mixture of methylethylketone and diacetyl in ether solution. Some degree of separation was achieved. Fig. (20) shows the result of the

Fig. (17)

Flash-exchange of iso-Butyraldehyde hydrazone; shows an extra non-identified subsidiary peak.

(a) Iso-Butyraldehyde

(b) Non-identified.

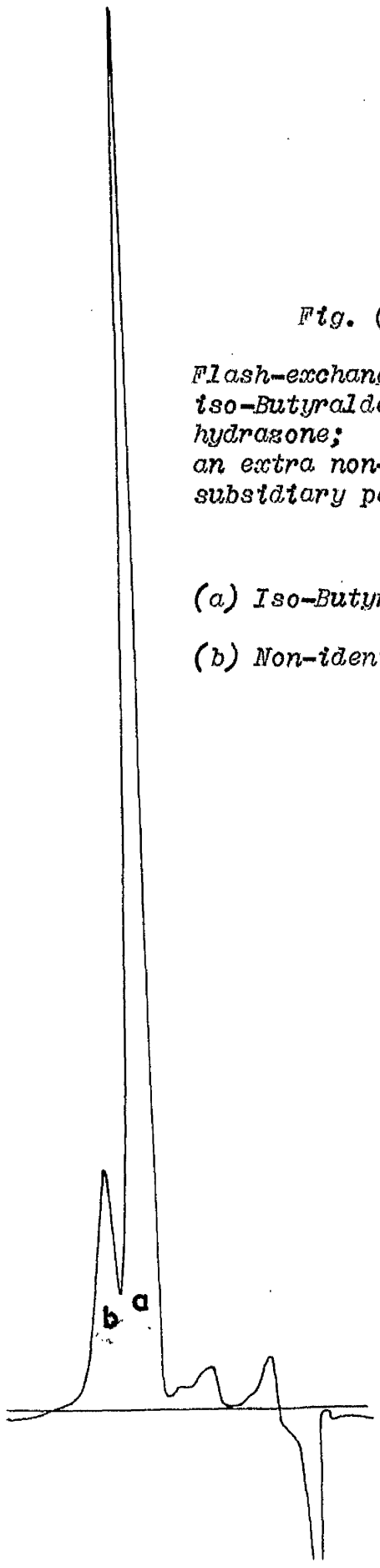


Fig. (18)

Flash-exchange of iso-Butyraldehyde and n-Butyraldehyde hydrazones shows that the subsidiary peak is not n-Butyraldehyde.

(a) iso-Butyraldehyde

(b) non-identified

(c) n-Butyraldehyde

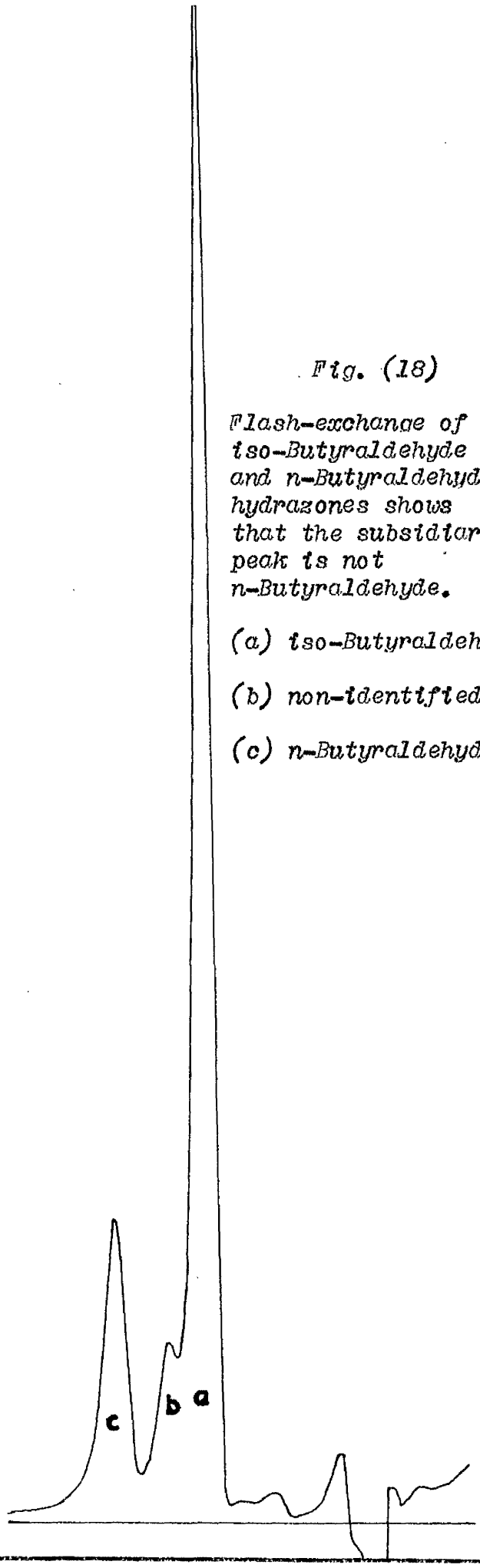


Fig. (20)

a) *Ethyl ether*
b) *MEK*
c) *On addition of extra Diacetyl to the mixture, this peak was enlarged.*

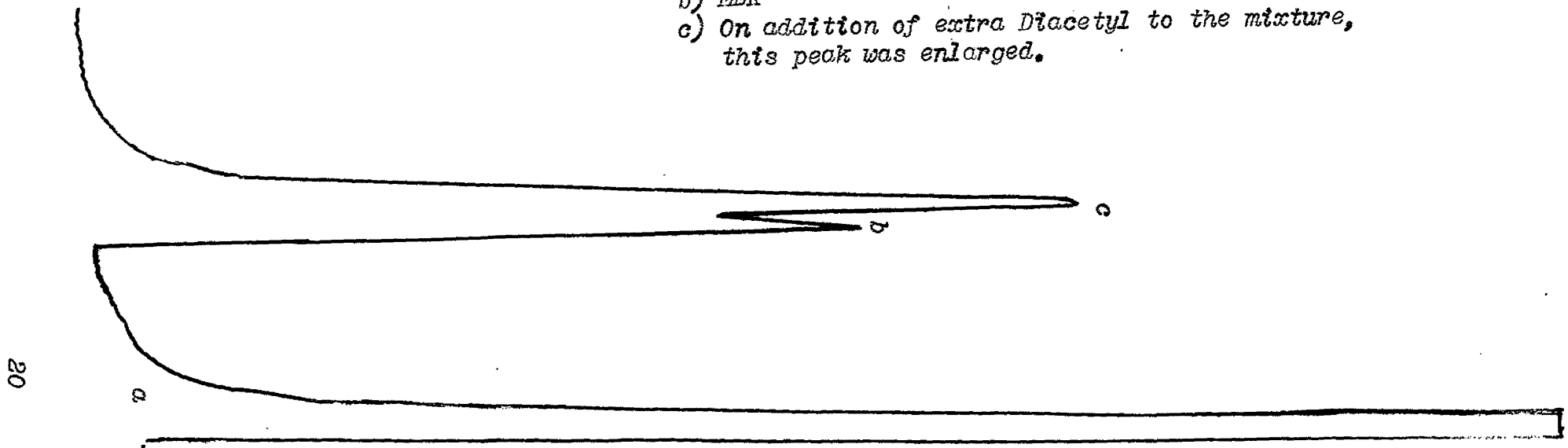
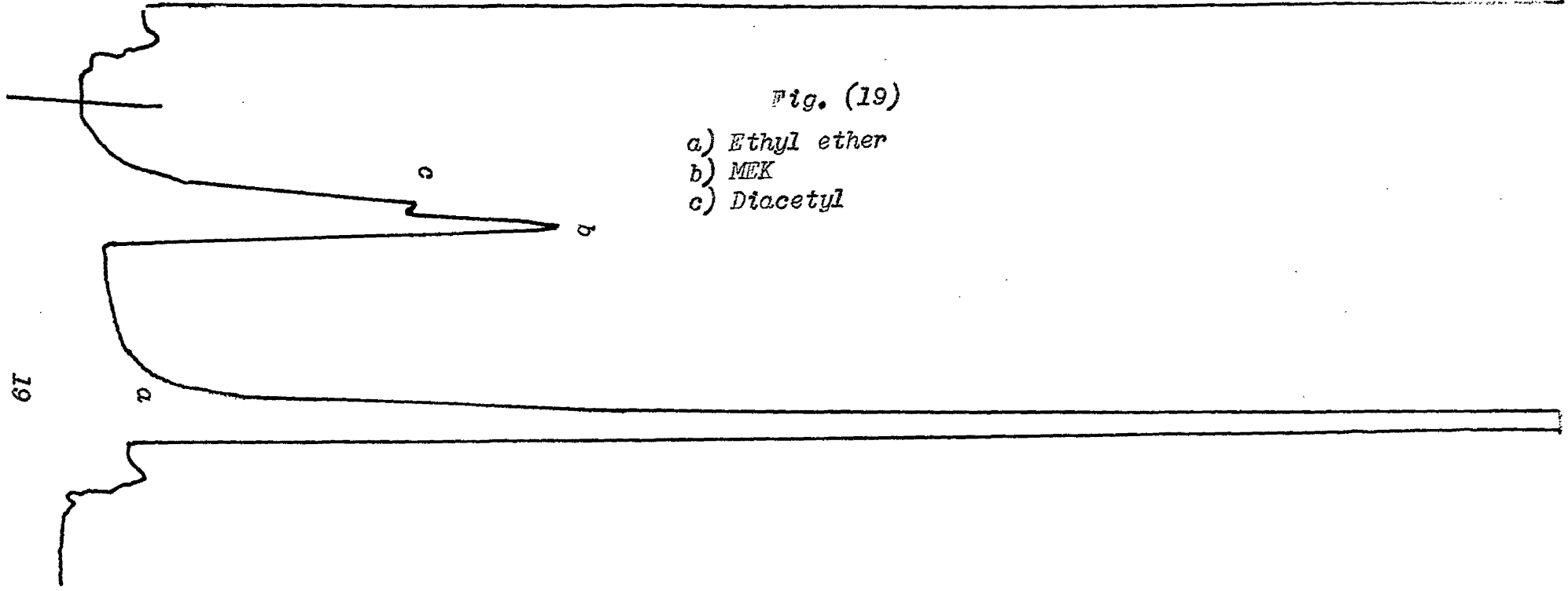


Fig. (19)

a) *Ethyl ether*
b) *MEK*
c) *Diacetyl*



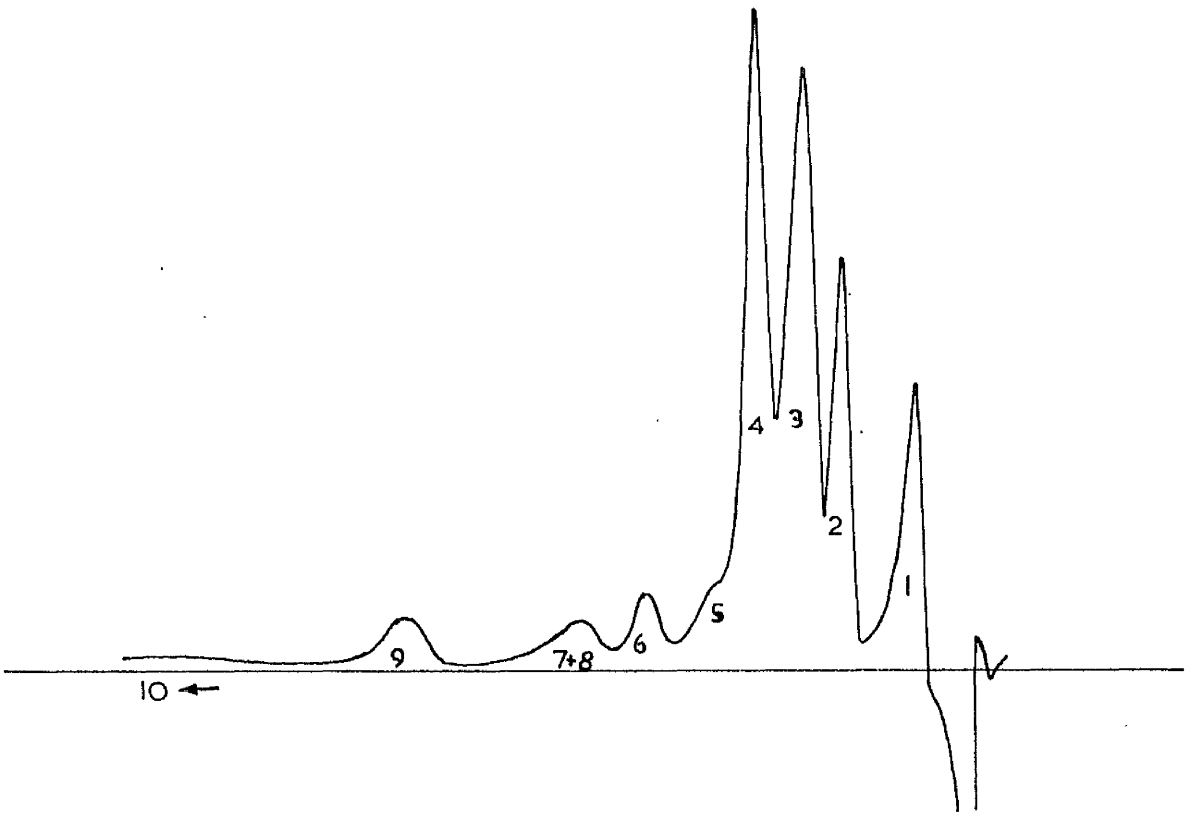
addition of more diacetyl to the previous mixture. It will be seen that the second peak of the pair is greatly enlarged.

(c) This has previously been shown to come from decomposition of the ∝KGA.

Fig. (21) shows a chromatogram of a mixture of eight known carbonyls for comparison with the coffee carbonyls.

Although many samples of the coffee volatile carbonyls were chromatographed in this way, none of them departed in any essential manner from the general picture given in Fig. (16).

Fig. (21)



Flash-exchange of eight authentic hydrazones.

(1) *Acetaldehyde*
(2) *Propionaldehyde*
(3) *Acetone*
(4) *Iso-Butyraldehyde*
(5) *Non-identified*
(6) *n-Butyraldehyde*
(7 & 8) *MEK + Diacetyl*
(9) *Iso-Valeraldehyde*

G.F. - 17 ml/min.
DV. - 1750 V.
Col. Temp. - 50°C.

CONFIRMATORY EVIDENCE OF THE COMPOSITION OF THE COFFEE CARBONYL COMPOUNDS

Identification by disproportion

One of the confirmatory methods used for identifying the peaks of the coffee V.C.C. was to add a small quantity of a known hydrazone to the mixture of hydrazones from coffee. If the added carbonyl was not one of these already present it would be expected to give rise to an extra peak - provided they were separated. Increase in size of any one peak was taken as further evidence of identity.

Formaldehyde

Since the presence or absence of formaldehyde could not be established under the conditions used in this work for gas chromatography, other methods of testing were considered.

It seemed reasonable to expect that, during the roasting process, some formaldehyde would be produced along with the other volatile compounds.

The presence of formaldehyde was established by the use of chromotropic acid and this is described in detail on page (63).

Test for Unsaturation

Since unsaturated aldehydes have been reported [20a] to be present in coffee aroma to the extent of about 2% of the coffee volatiles but have not been found in this work, the coffee volatiles were tested using the Thiobarbituric acid (T.B.A.) method of Fritz and Libergott.[48]

The method consists essentially of adding about 20 mg. of the (T.B.A.) to the substances under test, followed by two drops of syrupy H_3PO_4. The tubes are placed in a bath at 120°C and if unsaturated aldehydes are present an orange product appears at once or within 1 - 2 min. The method is claimed to detect e.g. 0.01 mg. crotonaldehyde.

When the method was applied to the total coffee volatiles from 50 g. coffee collected in the cold trap of the Shipton apparatus, no orange product was obtained.

It would appear that the unsaturated aldehydes are not present or present in such small quantities < .01 mg. below the sensitivity of the method.

Ketones

When a mixture of aldehydes and ketones are subjected to the action of a mild oxidising agent, the aldehydes are preferentially oxidised.

This principle was used to obtain confirmatory evidence of the presence of ketones in the coffee volatiles

Procedure:

The method used was that of Huelin.[49] 400 ml. of coffee distillate was collected by steam distillation Fig. (9). The receiving flask 6 was immersed in an ice-water bath, 50 ml. of 1N $AgNO_3$ was added to the distillate followed by 50 ml of 2N N OH. All reagents were cooled down to 0°C before mixing. The container was stoppered and kept at 0°C for one hour with occasional shaking after which the contents were filtered in a Buchmer funnel using a double thickness of Whatman No. 1 filter paper. The precipitate was washed with water and the filtrate plus washings were

acidified with 50 ml 2N HCl.

The hydrazine reagent was added in excess and the hydrazones collected in the usual way.

These were then examined by flash-exchange gas chromatography. As Fig. (22) shows only partial oxidation took place but the proportions of the ketone peaks a & b, are markedly increased.

The di-carbonyl compounds

The hydrazones of di-carbonyl compounds are less soluble in most solvents than those of monocarbonyls; so that by extracting a mixture of these hydrazones with a suitable solvent a partial separation can be achieved.

A sample of the mixed hydrazones obtained from the coffee carbonyls was extracted with hexane.

The residue was then examined by flash-exchange gas chromatography.

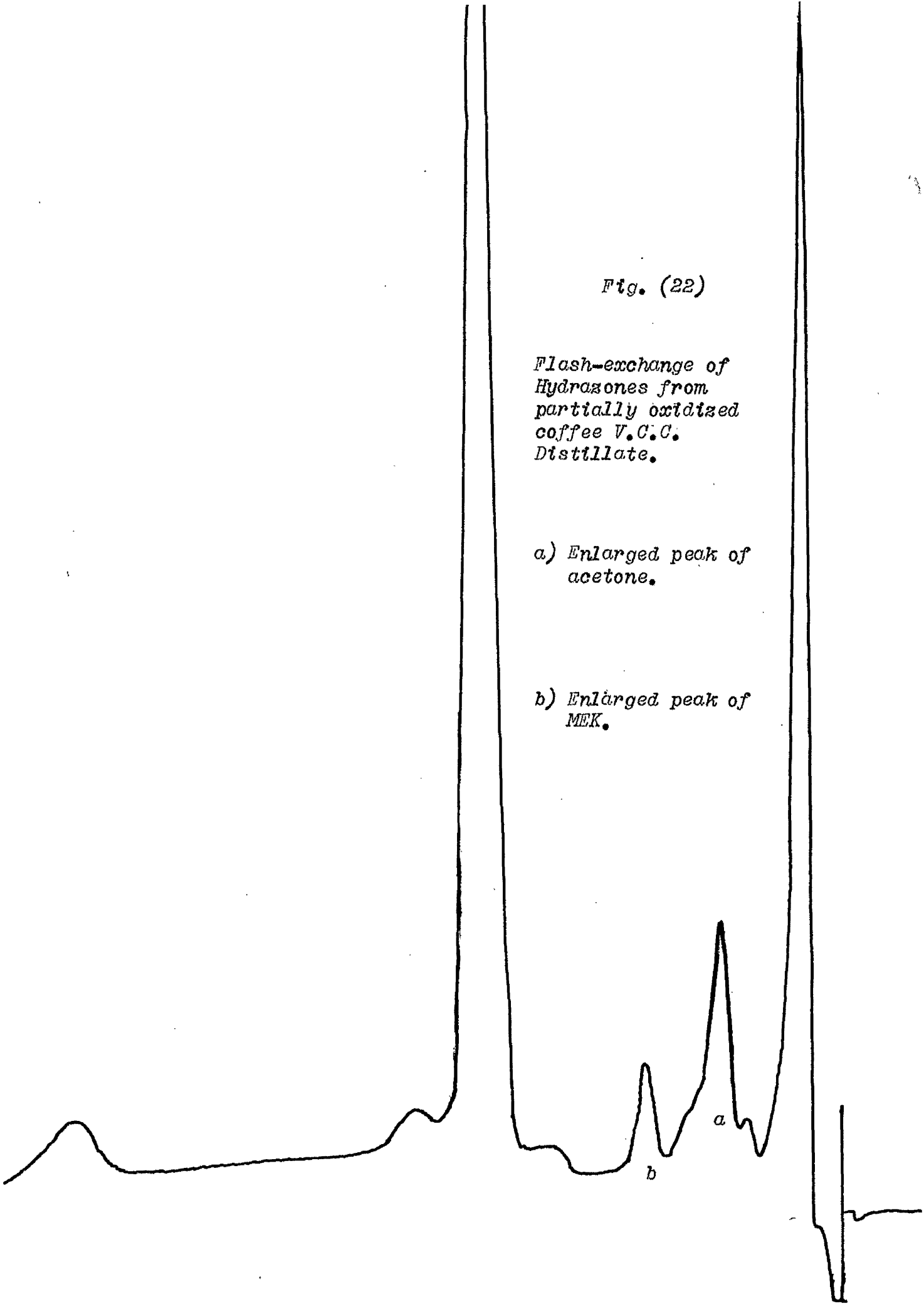
Fig. (23) shows that peak No. 7 (diacetyl) was increased in relation to the others.

Fig. (22)

Flash-exchange of Hydrazones from partially oxidized coffee V.C.C. Distillate.

a) Enlarged peak of acetone.

b) Enlarged peak of MEK.



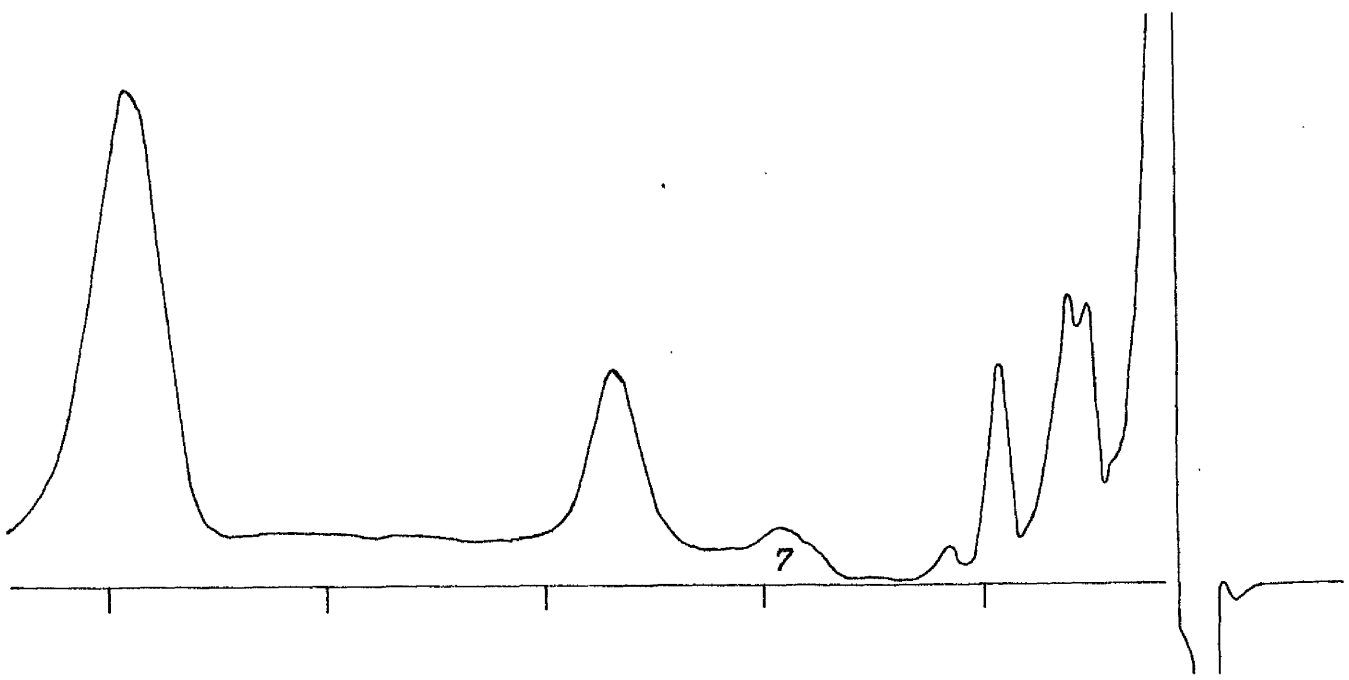


Fig. (23)

Flash-exchange of hexane insoluble hydrazones of Coffee V.C.C.

Enlarged proportion of Peak No. 7 (Diacetyl)

G.F. - 13 ml/min.
D.U. - 1750 V.
Col. temp. - 50°C.

Residual carbonyl compounds

Since it was unlikely that all the volatile carbonyls were removed from the coffee brew under the stripping conditions used, the residual coffee brew was itself examined.

The brew, after stripping, was extracted with diethyl ether, the extract reduced in volume on a water bath, hydrazine reagent added and the hydrazones collected in the usual way. These were then subjected to flash-exchange gas chromatography and the result is shown in Fig. (24).

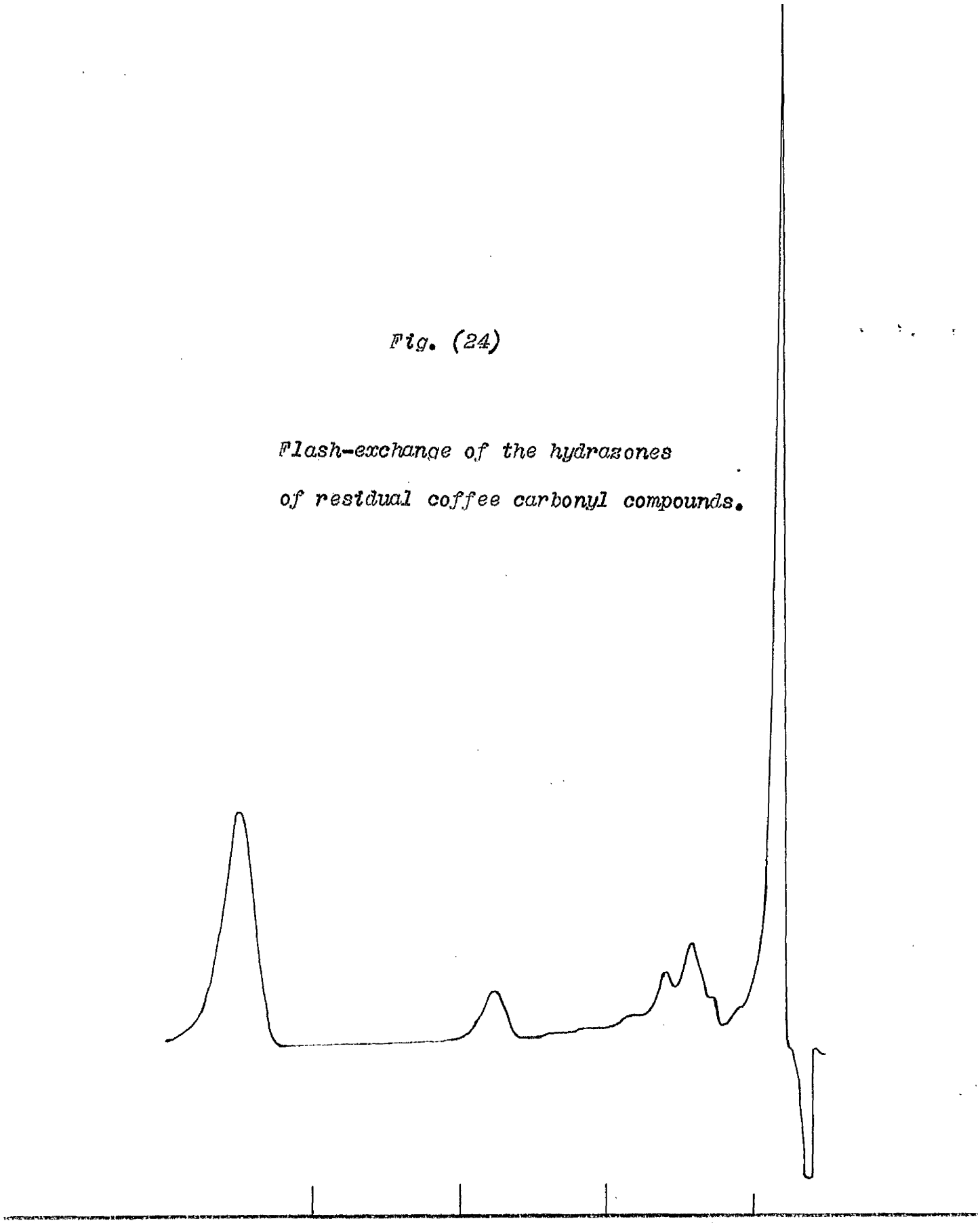
It will be seen that the proportions of the components present is different from that of the collected volatiles. In particular the proportion of iso-valeraldehyde in relation to that of acetaldehyde is much reduced.

This is surprising as it would be expected that the more volatile acetaldehyde would be lost more rapidly from the coffee brew. In addition there is a serious possible loss when the volume of the ether extract is reduced. It is possible that acetaldehyde is generated during the brewing process.

Fig. (24)

Flash-exchange of the hydrazones

of residual coffee carbonyl compounds.



QUANTITATIVE ANALYSIS of COFFEE VOLATILE CARBONYL COMPOUNDS

From the chromatograms, peak areas were calculated by multiplying peak height by the width at half-height.[41, 42] This method of calculation is preferred by most committees[42] laboratories to other methods such as multiplying peak height by retention time.[43, 44] The results from several methods of collection are given in Table VII.

The steam distillation was done under vacuum.

TABLE VII.

	Steam Distillation for 1 hr.		Shipton		
			1 hr.	2 hr.	
Coffee Samples	50 g.	25 g.	25 g.	50 g.	50 g.
Acetaldehyde	28.60	42.00	55.20	34.60	31.10
Propionaldehyde	5.94	3.53	7.00	6.90	5.60
Acetone	7.16	4.33	4.80	3.43	3.16
Iso-Butyraldehyde	21.46	16.02	16.10	20.20	25.03
n-Butyraldehyde	3.00	0.38	0.32	0.74	0.84
MEK + Diacetyl	3.14	1.24	0.18	0.31	0.47
Iso-Valeraldehyde	30.70	32.50	16.40	33.82	33.80

The quantity of hydrazones collected from 50 g. coffee after two hours in the Shipton apparatus was $65^{\pm}$ 5 mg. Taking the average molecular weight of the identified carbonyls, the collected hydrazones would contain about 17 mg. of carbonyl compounds i.e. 34 mg. of carbony compounds would be collected from 100 g. coffee (340 ppm).

From the average of the values given in columns 4 & 5, Table VII, the weights of the various components has been calculated and expressed in part per million of coffee. Table VIII.

TABLE VIII.

Carbonyl compounds	Collected % carbonyls	p-p-m.
Acetaldehyde	32.8	111.7
Propionaldehyde	6.3	21.3
Acetone	3.3	11.2
Iso-butyraldehyde	22.6	76.8
n-butyraldehyde	0.8	2.7
MEK + Diacetyl	0.4	1.4
Iso-valeraldehyde	33.8	114.9

All the peaks of the liberated carbonyl compounds were within the scope of the chart except that of acetaldehyde as e.g. is shown by the sample collected from 50 g. coffee by steam distillation under vacuum Fig. (25).

If sufficiently small samples were used to bring the acetaldehyde peak into the chart the smaller peaks would be lost. The area of the acetaldehyde peak was measured by extrapolation and also checked by running a smaller sample and taking the proportion of the acetaldehyde peak relative to that of iso-valeraldehyde, both being within the chart.

With the exception of columns 4 & 5 the results given in Table VII are not strictly comparable but they do indicate the importance of standard methods of collection.

In the determination of coffee v.c.c. there are two main considerations. The first is the identification of the constituent carbonyls.

Accurate identification is, in fact, the lesser problem and improvement in methods will enable this to be achieved.

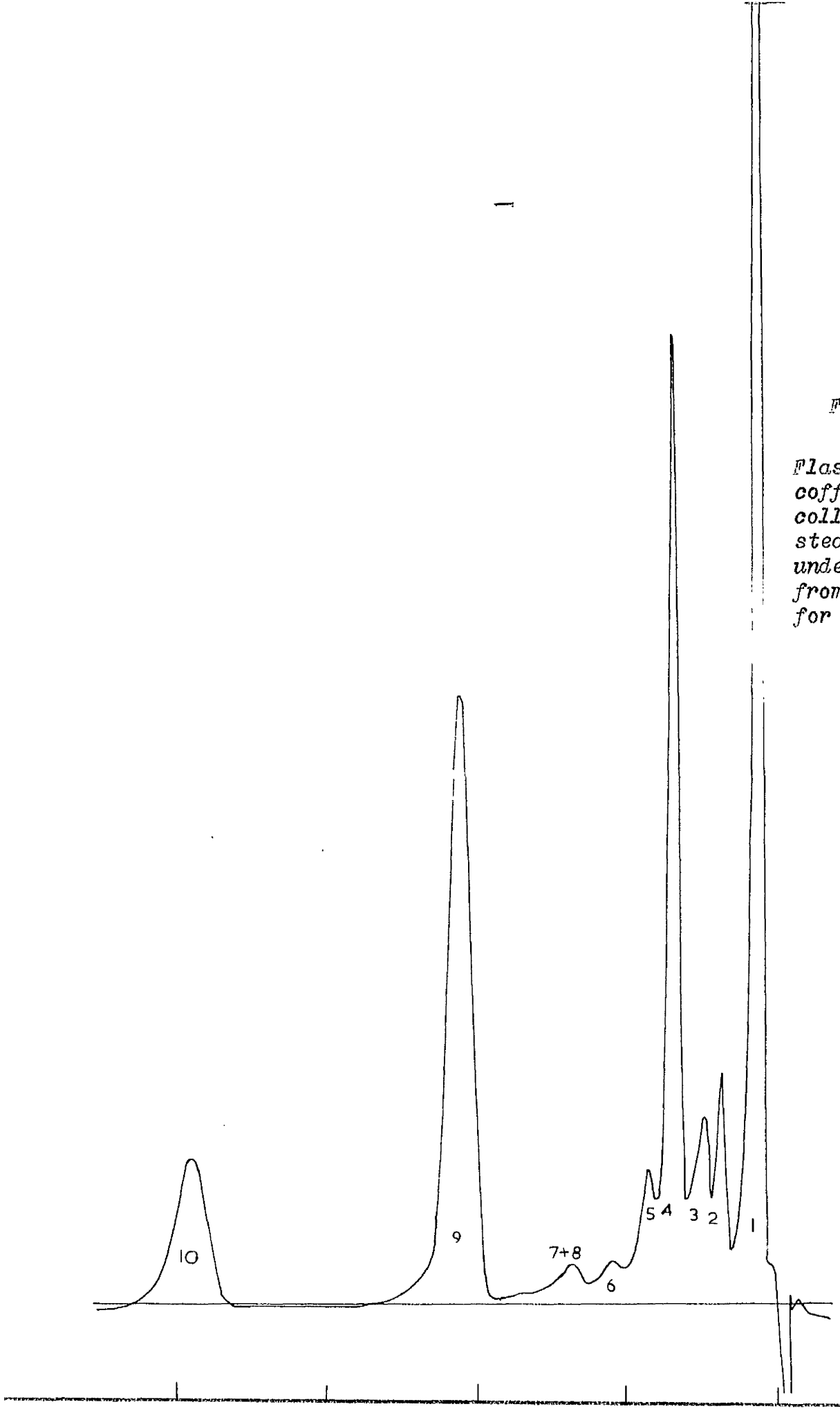


Fig. (25)

Flash-exchange c
coffee hydrazone
collected by
steam-distillati
under vacuum,
from 50 g. Nesca
for one hour.

The second consideration - the quantitative estimation of the proportions and absolute values of their components - is much more difficult. It is obvious from the results given here and from those of other workers that there are serious discrepancies and that these are caused largely by variations in the method of collection; comparison of the results of different workers is not possible.

This point would require careful consideration when comparative work is being done e.g. the effect of different processing conditions on any one given blend of coffee. Unless the conditions for the collection of the volatile carbonyls were rigidly standardised, the differences caused by this would probably be greater than the differences caused by any reasonable variations in the processing conditions.

As columns 4 & 5 (Table VII) show, some agreement can be obtained under standard conditions.

TABLE IX.

COFFEE AROMA ANALYSIS (per cent) 20a.

	Roasted Coffee Aroma	Roasted Coffee Aroma	Roasted Coffee Aroma
Aldehydes			
Acetaldehyde	17.9	19.9	25.6
Propionaldehyde	8.0	4.5	3.2
Butyraldehyde	-	0.7	0.3
Iso-Butyraldehyde	-	3.0	6.8
2-Methyl Butyraldehyde	-	6.8	-
Valeraldehyde	-	7.3	-
Iso-Valeraldehyde	18.2	5.0	1.5
Acrolein	0.6	-	-
Dimethyl acrolein	T	-	-
Methyl ethyl acrolein	1.4	-	-
Ketones			
Acetone	0.5	18.7	21.0
Methyl ethyl ketone	14.2	2.3	8.2
Methyl vinyl ketone	0.5	-	-
Diacetyl	10.3	7.5	6.4
2,3 - Pentanedione	-	-	6.7
2,4 - Pentanedione	0.2	-	-
Heterocyclo Compounds			
Furan	2.5	3.2	1.2
2-Methyl Furan	5.1	4.7	3.0
2,5-Dimethyl furan	0.3	-	-
Propyl furan	T	-	-
Butyl furan	T	-	-
Pyrol	0.5	-	-
N-Methyl pyrol	T	-	-
Dimethyl Pyrol	T	-	-

	Roasted Coffee Aroma	Roasted Coffee Aroma	Roasted Coffee Aroma
Sulphur Compounds			
Hydrogen sulphide	-	-	1.5
Carbon disulphide	0.9	0.2	-
Dimethyl sulphide	0.6	1.0	1.2
Methyl ethyl sulphide	0.3	-	-
Dimethyl disulphide	3.1	-	-
Methyl ethyl disulphide	T	-	-
Methyl mercaptan	-	0.1	1.2
Thiophene	-	0.1	-
Esters			
Methyl formate	4.9	4.0	3.4
Methyl acetate	5.7	1.7	-
Ethyl formate	-	0.3	-
Nitriles			
Acrylonitrile	0.5	-	-
Allylcyanide	1.1	-	-
Alcohols			
Methanol	0.9	0.2	8.2
Ethanol	0.3	-	0.3
Hydrocarbons			
Isoprene	-	3.0	0.3
C_4-C_4 Paraffins	-	2.0	-
Oxides			
Carbon dioxide	-	3.8	-

Table IX shows the results of three workers for the components of roasted coffee aroma. It will be seen that there are considerable differences and it is doubtful if these are caused entirely by differences in raw materials. Identification of the components of the total aroma volatiles must be difficult when so many are present and also when different classes of compounds are involved.

The merit of the method used in this work is that classes of compounds other than the carbonyls are excluded. This simplifies the identification and the estimation of the components.

It is possible that this approach could be extended e.g. the non-carbonyl components could be collected after they leave the hydrazine-reagent trap.

The greatest discrepancy among the results reported for the carbonyls lies with the amounts of methyl ethyl ketone and iso-valeraldehyde e.g. in the above table iso-valeraldehyde varies from 1.5% to 18.2% of the total volatiles.

Most of the work on coffee aroma by gas chromatography was made using Carbowax 1500 as the liquid phase (1, 17, 28).

Fig. (26) is a chromatogram obtained by Rhoades[17] for the total volatiles. Peak No. 11 has been ascribed to methyl ethyl ketone but its shape would indicate that it is not a single component; iso-valeraldehyde was not reported. The liquid-phase used was Carbowax 1500.

An attempt was made to confirm this, and a column was prepared using 15% Carbowax 1500 as the liquid phase. Fig. (27a) shows the peak obtained from methyl ethyl ketone by flash exchange of its hydrazone and Fig. (27b) the peak from a mixture of the hydrazones of methyl ethyl ketone and iso-valeraldehyde. No separation was achieved.

When considering the contribution made by the several carbonyl components to the aroma of coffee, one must consider not only the proportion of each component present but also its olfactory threshold.

For example, the threshold value[47] of propionaldehyde is 9.6 parts per 10^9 and that of butyraldehyde 9 parts per 10^9 so that these two substances can be detected at approximately equal levels. Should they be present at different levels their contribution to

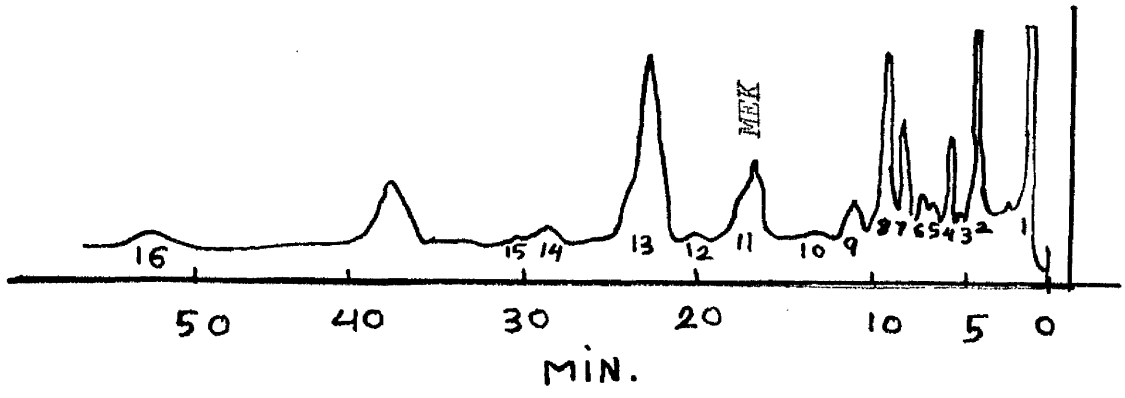


Fig. (26)

Total coffee volatiles[17]

Fig. (27)

a) *Flash-exchange of the hydrazone of MEK.*

b) *Flash-exchange of mixed hydrazones of MEK and iso-valeraldehyde.*

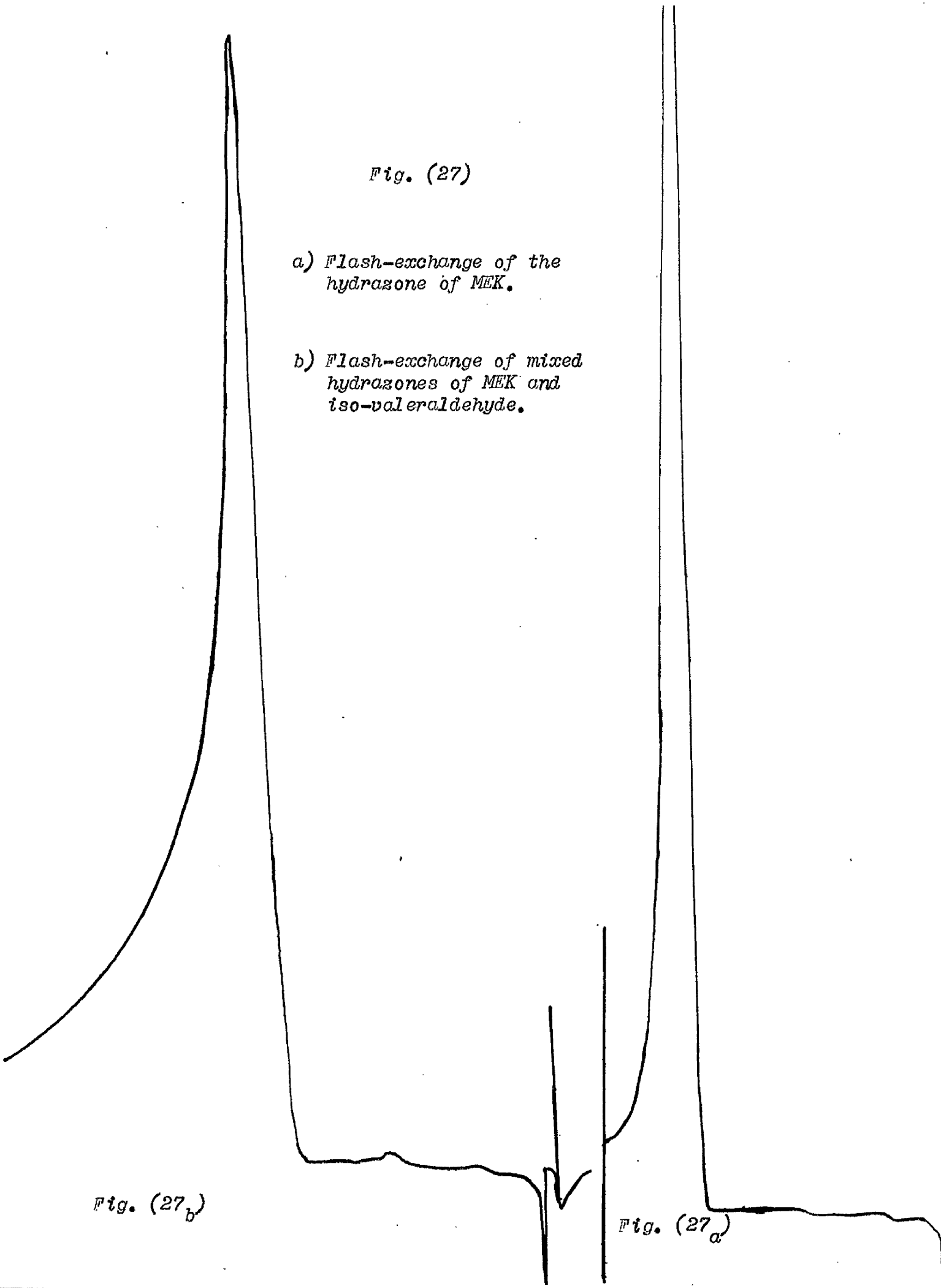


Fig. (27$_b$)

Fig. (27$_a$)

the aroma would be proportional to their respective amounts.

On the other hand, heptaldehyde has a threshold value of 3 parts per 10^9 so that it would be effective at 1/3 the concentration of butyraldehyde.

SPECTROSCOPY

1 - Ultra violet, 2-visible and 3-infra-red spectroscopy were also used for identification and for quantitative estimation of the coffee V.C.C.

Instruments and Reagents

Unicam SP 700 automatic recording - spectrophotometer for U.V. and visible ranges; Hilger Uvispek spectrophotometer for U.V., and Unicam Sp. 200 for the infra-red region.

Chloroform, methanol, Schiff's reagent, chromatropic-acid and alcoholic NaOH.

Experimental

(1) An attempt was made to estimate the quantities of the various carbonyl compounds present in the collected hydrazones by a combination of thin layer chromatography and ultra-violet spectroscopy.

Procedure

5 mg. of the coffee hydrazones were spotted on Silica-gel plates and separated into five groups Fig. (28) using 3 ; 1 benzene / pet. ether 60 / 80°C as solvent. (The details of the thin layer technique are given later). Appendix (A_1)

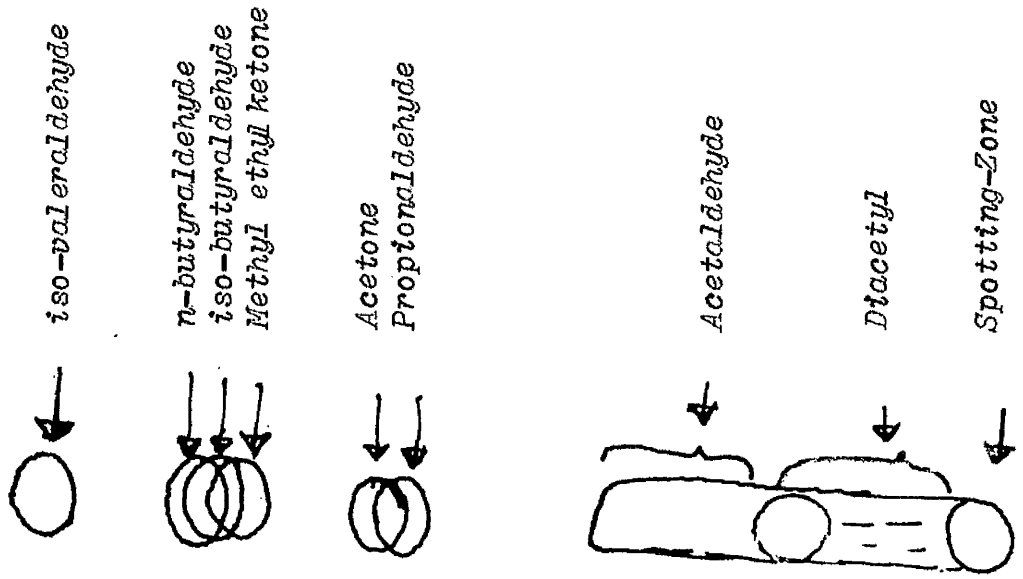


Fig. (28)

Schematic drawing of the separation of Coffee V.C.C. hydrazones by thin layer chromatography.

The spots were scratched off the plates and eluted with chloroform. The chloroform solutions of the hydrazones were made up to standard volumes and their optical density measured at 350 mμ in the Hilger Unispek spectrophotometer.

The corresponding concentrations were calculated from a calibration curve of the hydrazone of diacetyl in chloroform Fig. (29); a blank was prepared by eluting with chloroform a fraction of the Silicagel from the same plate. From the quantities of hydrazones allocated to each spot and from a knowledge of the components present in each spot, the proportions of the carbonyls present in the mixture were calculated. Table X.

TABLE X.

Carbonyl compounds	Percentage
Iso-valeraldehyde	17.80
Iso-butyraldehyde n-butyraldehyde Methyl ethyl ketone	12.90
Acetone Propionaldehyde Acetaldehyde	68.10
Diacetyl	0.72
Some diacetyl plus formaldehyde	0.48

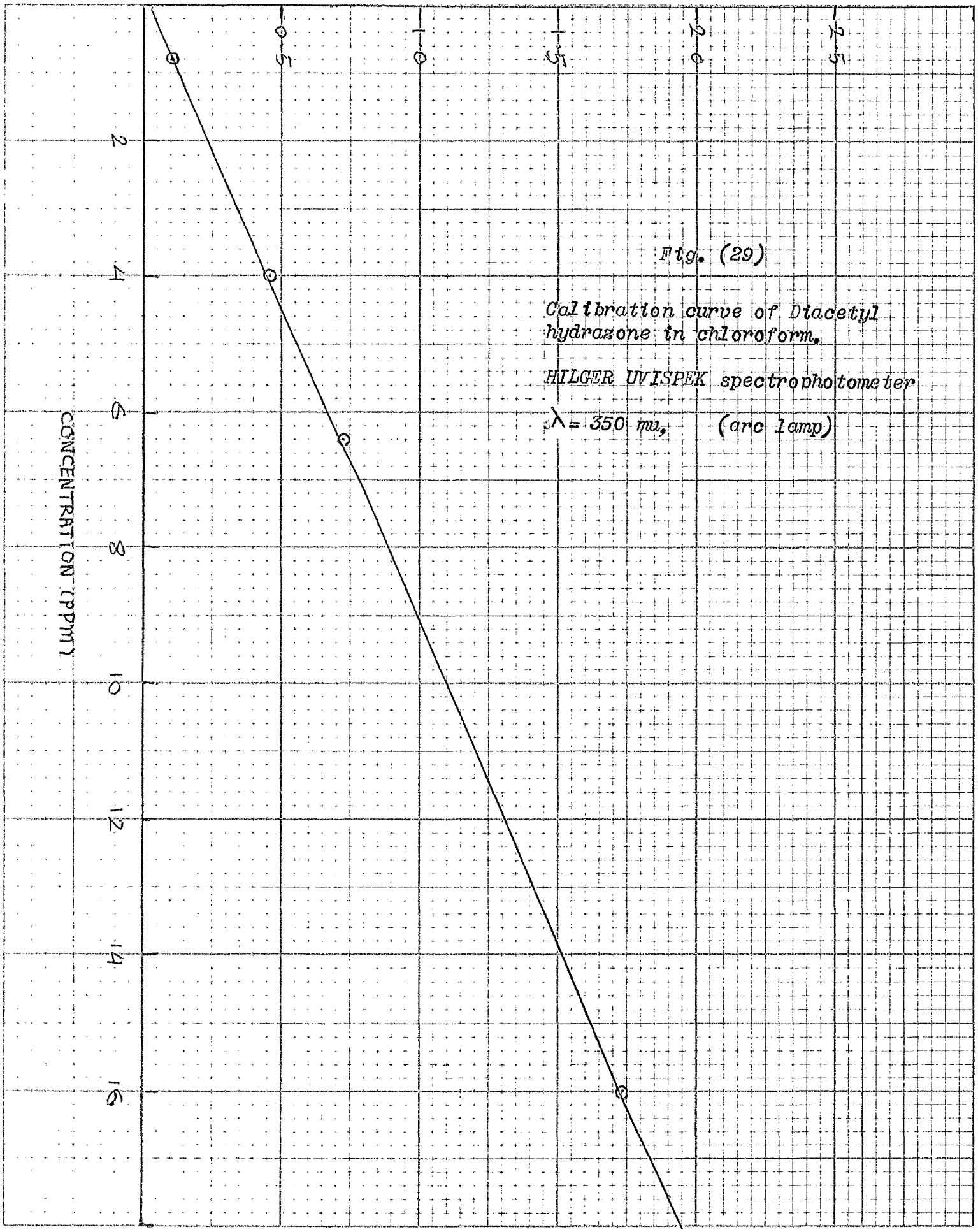


Fig. (29)

Calibration curve of Diacetyl hydrazone in chloroform.

HILGER UVISPEK spectrophotometer

λ = 350 mμ, (arc lamp)

Discussion

The results do not agree with those obtained by gas chromatogrphy but it is felt that with further experience this general method could be useful. Improvement is required in the degree of separation achieved by the thin layer method and recovery experiments using known hydrazones are required.

It has been suggested (63, 66) that ultra-violet spectrophotometry could be used as an aid to the identification of the various hydrazones. Table XI shows the absorption maxima of the hydrazones of various carbonyl compounds in 95% ethyl alcohol.(66)

TABLE XI.67

Hydrazones of:	Absorption maxima (mμ)
Formaldehyde	350
Acetaldehyde	336
Acetone	361
Propionaldehyde	358
Iso-butyraldehyde	358
Methyl ethyl ketone	360
Iso-valeraldehyde	358
n-valeraldehyde	358
n-Hexaldehyde	358

It would appear that since the absorption maxima are in the main either the same or only slightly different, the method is unlikely to be of much value.

Identifications are much more likely to be achieved by improvement in thin-layer techniques and ultra-violet spectrophotometry could be used for quantitative work.

Identification and determination of formaldehyde

The identification was based on the production of a magenta colour on reaction with chromotropic acid (1,8 - dihydroxynaphthalene - 3,6 - disulphonic acid).[70]

This is specific for formaldehyde.

Apparatus: Unicam Sp. 600

The Shipton apparatus as in Fig. (8) but instead of the usual reagent flask a tube containing anhydrous K_2CO_3 followed by a cold trap and a collecting tube containing the chromotropic acid reagent.

Reagents

Chromotropic acid:- 10% aqueous solution.

Concentrated H_2SO_4

Methanol freed from carbonyl compounds.

Procedure

The volatiles from 50 g. of coffee were passed through the drying tube, the cold trap and finally bubbled through the collecting tube containing the chromotropic acid. (1 m. 10% chromotropic acid and 10 ml. conc. H_2SO_4). The collection period was two

hours (the standard time for collection of the carbonyl-compounds as hydrazones). During this period the cold trap was immersed in a bath of ethanol and solid CO_2 at a temperature of approximately - 79°C.

The valves of the trap were closed before disconnection from the drying tube. The contents of the trap were washed carefully with methanol into the chromotropic acid reagent tube.

The reagent tube was stoppered and placed in a boiling water bath for 30 minutes to develop the colour. It was then cooled to room temperature, the contents transferred to a volumetric flask and made up to 100 ml. with water.

A blank was prepared using methanol, chromotropic acid reagent and water.

The optical density was measured at 570 mμ using the blue photocell on filter No. 2. The optical density was 0.192 and from the calibration curve (Fig. 30) the concentration of formaldehyde was found to be 2 ppm.

Note: The calibration curve was prepared from a series of dilutions of fresh sample of B.D.H. (Analar)

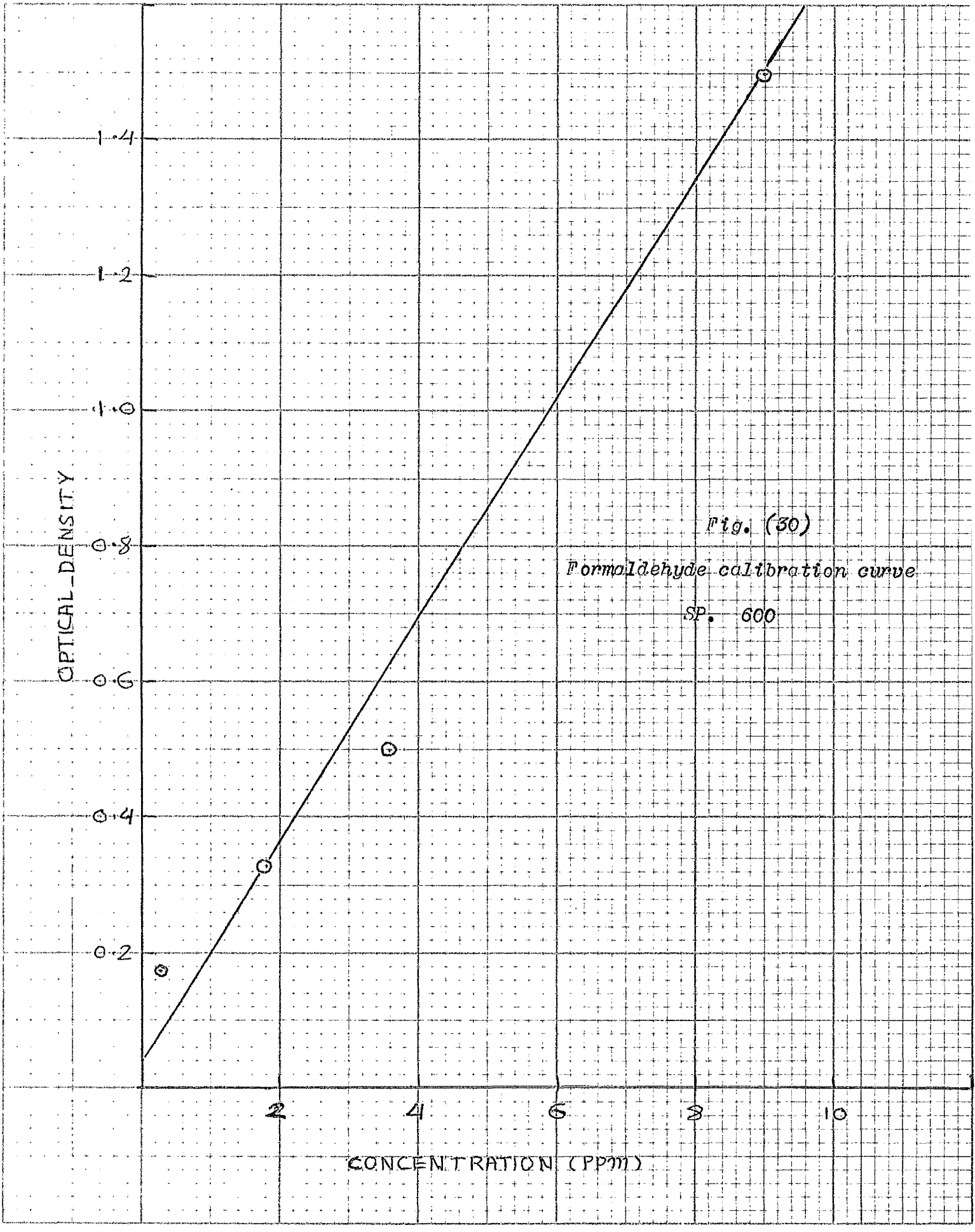


Fig. (30)

Formaldehyde calibration curve

SP. 600

formaldehyde stated to contain not less than 36% W/V H.CHO. This figure was accepted for the purpose of the experiment.

Colorimetric estimation of the volatile aldehydes of coffee

Preliminary experiments (described in detail in Appendix C) suggested a technique which might be of value particularly when comparative work being done.

The method consisted essentially of reacting the aldehydes in the distillate from the steam distillation of 50 g. coffee with Schiffs reagent followed by measurement of the developed colour. The total aldehydes were then determined in terms of acetaldehyde with the aid of a calibration curve Fig. (C6) and corrected to the average molecular weight of the coffee volatile aldehydes. The value obtained by this method was 150 ppm. of the volatile aldehydes which is lower than expected.

The method, while it has possibilities, needs further investigations particularly the effect of the pH of the reagent on colour intensity.

TABLE (XII)

Characteristic Absorption Bands of Hydrazones in $CH Cl_3$

Compound	Wave number cm^{-1}	Appendix
Aliphatic aldehydes		
n - } iso - }	1138	B_1
unsaturated	980-996, 1138	B_3
Aliphatic Ketones		
n -	1110 - 1118, 1138	B_2
iso -	1138	B_7
unsaturated	980-996, 1110-1118, 1138, 1710 (weak)	B_4
Dicarbonyls	1690-1720, 1100, 1138, 1710 (weak) *	B_5 & B_6

* Agrees with STARK & FORSS (11)

TABLE (XIII)

Characteristic Absorption Bands of hydrazones in solid KCl discs.

Compound	wave number cm^{-1}	Appendix
Aliphatic aldehydes		
n - , iso -	1118, 1138	B_8
unsaturated	980-1000, 1110, 1138	B_9
Aliphatic ketones		
n - , iso -	1110, 1138	B_{10}
unsaturated	996-1000, 1100, 1138	B_{11}
Dicarbonyls	1690 - 1720	B_{14} & B_{15}
Acetophenone	1000, 1110, 1138	B_{13}
furfural	1138	B_{12}

It was observed that the typical absorption bands for certain groups was shifted slightly with different compounds. This may be accounted for by hydrogen bonding effects.(70)

Hydrazones of the coffee volatile carbonyl compounds

The mixed hydrazones of the collected coffee V.C.C. were examined in chloroform solutions and in KCl discs. Both methods gave the typical spectra of aldehydes which might be expected since about 95% consists of aldehydes (B16 and B17).

Two 20 x 20 cm plates of silica-gel were heavily loaded with about 2.5 mg hydrazones of coffee V.C.C. and developed with benzene/pet. ether 80/60°C 3 : 1. Four bands were obtained. The bands were scraped off but bands 2, 3 & 4 were each devided horizontally into two fractions as indicated Fig. (31).

The hydrazones were eluted with chloroform and their spectra obtained.

Fractions 1, 2, 3, 4, 5 and 6 gave typical aldehyde absorption bands.

Fraction 7 showed the characteristic band for dicarbonyl at 1720 cm^{-1}. (B_{19}).

The hexane-insoluble hydrazones from the coffee volatiles were also examined by infra-red. They showed the characteristic band of dicarbonyl which is an indication of the presence of diacetyl (B_{20}).

In general, it would appear that infra-red spectroscopy would be of more value than ultra-violet as an aid to identification of the hydrazones of the carbonyl compounds.

FRACTIONS

SEPARATION

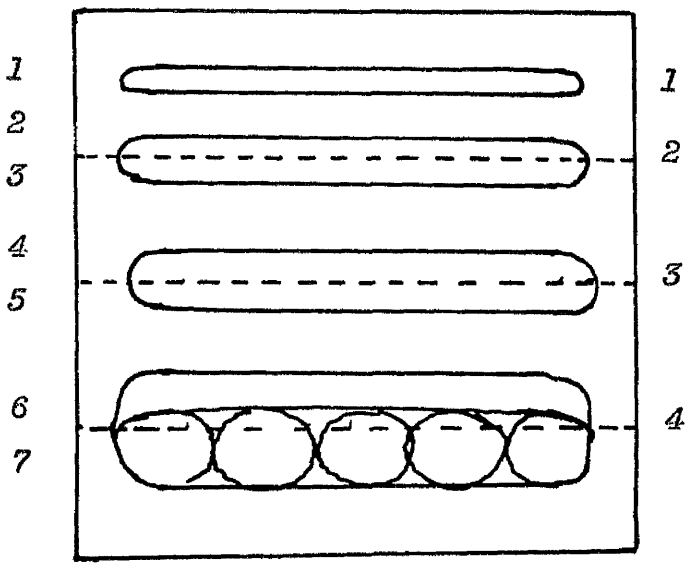


Fig. (31)

Thin-layer chromatography of coffee volatile carbonyl compounds hydrazones.

Paper Chromatography

Keigh [55] proposed a reverse phase method for the separation of the hydrazones of the lower molecular weight carbonyls. The paper was dipped in methanol saturated with heptane and the trough at the top of the tank contained heptane saturated with methanol. Methanol saturated with heptane was placed in the bottom of the tank.

The paper was air dried, spotted with the hydrazones and subjected to descending chromatography methanol acted as the stationary phase.

The separations were incomplete and in fact were similar to those shown Fig. (28) by thin-layer.

The method of Lynn, etal [56] was tried. This employs Whatman No. 7 paper impregnated with 10% 2-phenoxyethanol in acetone as stationary phase and n-heptane as the mobile phase. Whatman No. 7 is a thick paper and allows the application of large samples - up to 250 µg. on each spot. It was hoped that if satisfactory separations were achieved this would enable fractions to be eluted in sufficient concentrations for further determinations. In fact only four fractions

were obtained although in this case the spots were more compact and distinct.

Thin-layer chromatography of the carbonyl compounds

Thin-layer chromatography has a number of advantages over paper particularly with regard to the shorter development period, more compact spots, the greater possible sample size and the variety of treatments available for identification of the spots.

The problem of the separation of the hydrazones of the coffee V.C.C., however, was not solved.

A number of methods has been made on the separation of carbonyl compounds on thin-layer e.g. Marcus [60] reported on the separation of the carbonyl compounds containing eight carbon atoms or more without their conversion to hydrazones.

This cannot be done with the lower members of this group because of evaportion -

Dhont [65] has reported work on the separation of the hydrazones of normal saturated aliphatic aldehydes from C_2 - C_{12} Fig. (32). These could be separated using as solvent a mixture of benzene and light petroleum.

The problem with the separation of the hydrazones of the coffee V.C.C. lies in the fact that these compounds are with the rnage C_2 - C_5. In addition they contain a

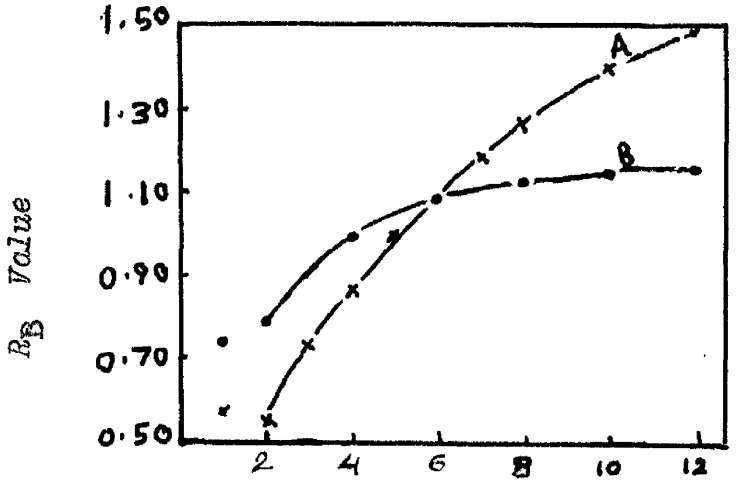


Fig. 32.

R_B values for 2,4-dinitrophenylhydrazones as a function of chain length: curve A, benezene - light petroleum mixture (3 + 1) as solvent; curve B, benzene containing 5 per cent. of ethyl acetate as solvent.

number of isomers and of course both aldehydes and ketones.

Some confirmatory evidence, however, can be obtained by spraying the plates with reagents which can characterise the components or by scraping the spots from the plates and eluting the components. For example colours can be developed by spraying the hydrazone spots with alcoholic or aqueous NaOH or KOH.(68)

Ketones give purple spots and aldehydes reddish-brown spots. Diacetyl (mono) give a rose-red colour and diacetyl (bis) an intense yellow centre with violet surrounding zones.(52)

The absorption maxima of these coloured compounds after elution of the hydrazones and treatment with alcoholic NaOH.

Aliphatic aldehydes have absorption maxima between 400 and 450 mμ; aliphatic ketones betwen 500 - 550 mμ.(49,67)

This technique can be used for both qualitative and quantitative work.

Column Chromatography

A column of a mixture of Bentonite on kieselguhr has been recommended (32) for the separation of the hydrazones of the lower molecular weight carbonyl compounds.

Other methods suggested include the use of silicagel saturated with nitromethane as stationary phase,[62] for the separation of the hydrazones of n-aliphatic aldehydes and methyl ketones from C_1 to C_{18}; for carbonyl compounds C_5 and upwards, Day[63] has suggested a column of celite with hexane as stationary phase.

Attempts to use column methods for the separation of the hydrazones of the Coffee V.C.C. failed, but the Bentonite-kieselguhr column was found to be useful for purifying the crude hydrazones. For rapid purification of solvents from carbonyls a reaction column of celite with H_3PO_4 and hydrazine [61] was prepared. This was preferred to any distillation method such as that of Gaddis[64] who refluxed the solvent with trichloroacetic acid followed by distillation.

Effect of Processing on the Coffee Volatile Carbonyl Compound

Series of coffee samples were supplied by R. Paterson & Sons Ltd. Glasgow.

Two jars of instant coffee had been prepared from the same raw coffee blend;

No. 8 was from coffee roasted in a "Probat" roaster and cooled with water,

No. 9 was roasted in the R.P. & S. Whitmer roaster and not water cooled.

Both ground, roasted coffees were extracted to the same rate (39% total solids) before spray-drying.

Hydrazones of the Coffee V.C.C. were collected as usual in the Shipton apparatus then analysed by the flash-exchange method. The chromatograms are shown in Figs. (33, 34,).

In both samples the proportions of acetone to the other components is smaller than that found for Nescafe samples.

The main difference between the samples (8 & 9) was the ratios of iso-valeraldehyde to that of propionaldehyde. This was 4.24 and 2.95 for jars No. 8 & 9 respectively.

The Effect of Storage on the Coffee Volatile Carbonyl Compounds

2 oz. tins from the same batch of Nescafe instant coffee were stored at 55°C. This high temperature was chosen to accelerate the changes which take place during storage, although it is recognised that the changes may be different when coffee is stored at lower

Fig. (33)

Flash-exchange of hydrazones of Coffee V.C.C.

Coffee sample roasted in (Probat) roaster and cooled with water

x = iso-valeraldehyde

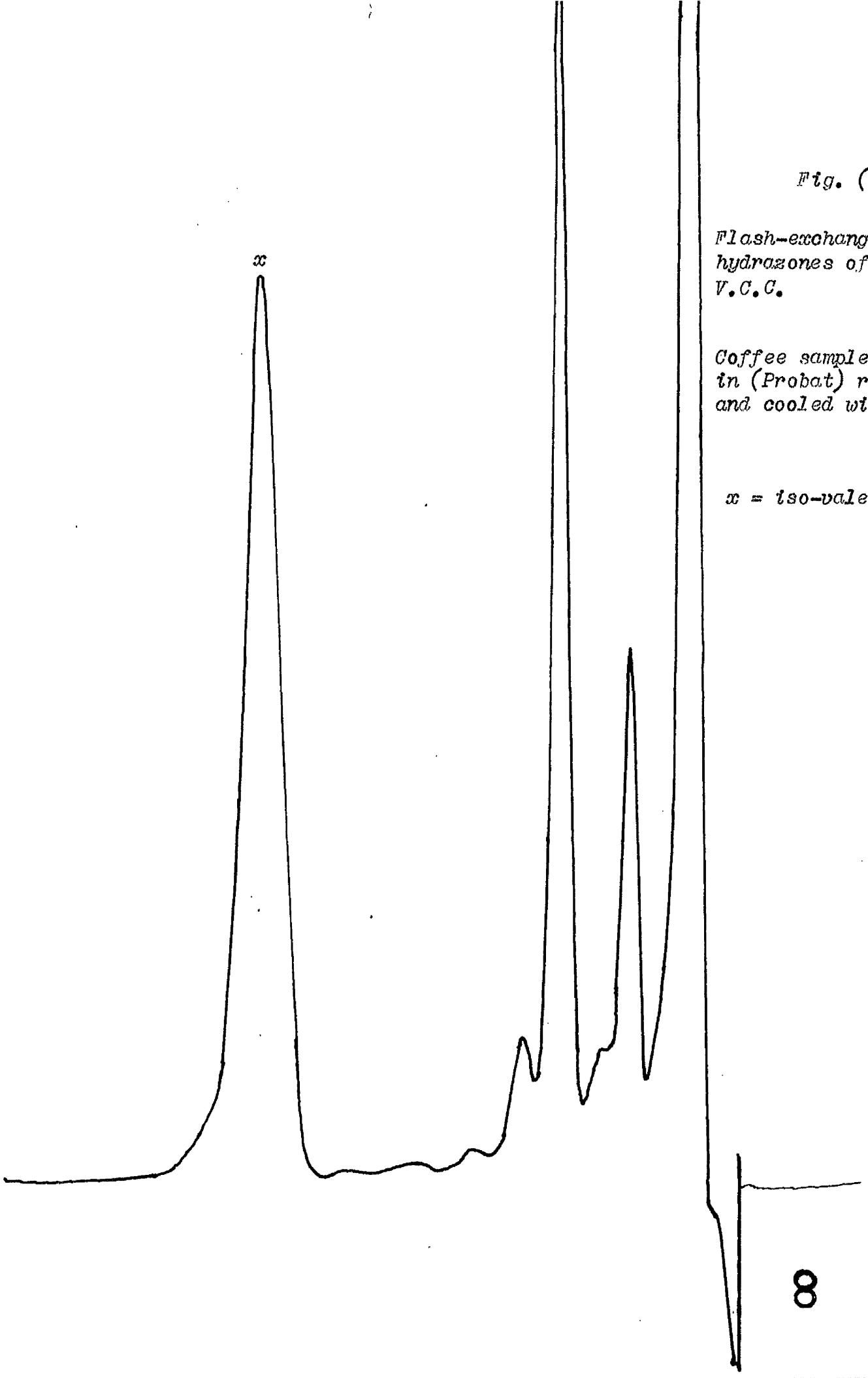
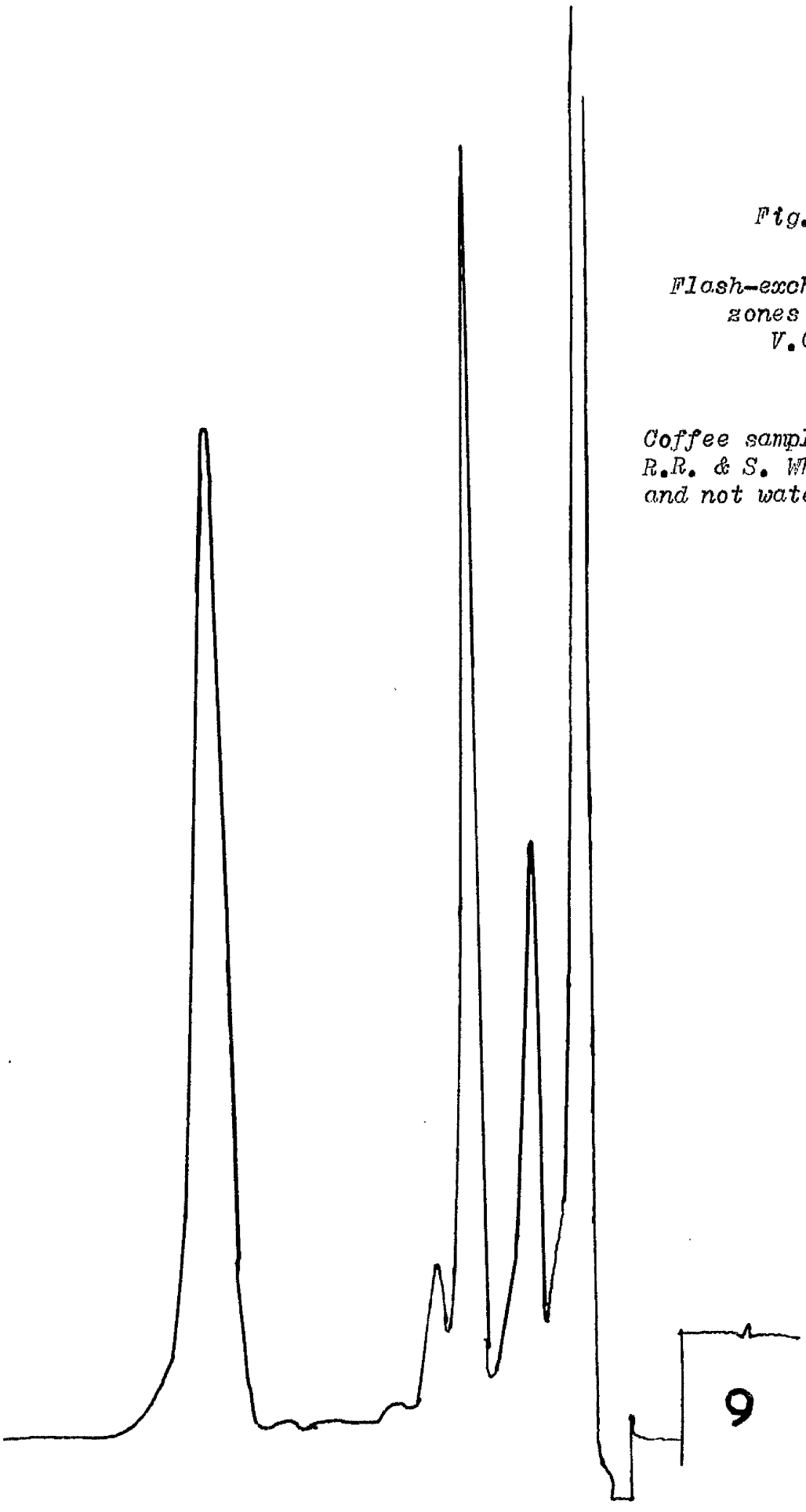


Fig. (34)

Flash-exchange of hydrazones of Coffee V.C.C.

Coffee sample roasted in R.R. & S. Whitmer roaster and not water cooled.



temperatures for longer periods.

After one, two and three months a tin was removed for examination. The hydrazones of the Coffee V.C.C. were collected and then examined by gas chromatography. The chromatograms are shown Figs. (35, 36, 37).

The main changes were:-

1 - The quantity of the collected hydrazones was found to decrease with increase in storage time Fig. (38).

2 - The ratio of propionaldehyde to acetone increased with storage, Fig. (39).

3 - There is an increase in the quantity of acetaldehyde in relation to the other components.

It is possible that the quantity of hydrazones could be used as an index of storage conditions and might be related to the deterioration of aroma which occurs in stored coffee.

Fig. (35)

Flash exchange of hydrazones of coffee V.C.C.

Coffee stored at 55°C for one month

a) Proptonaldehyde

b) Acetone

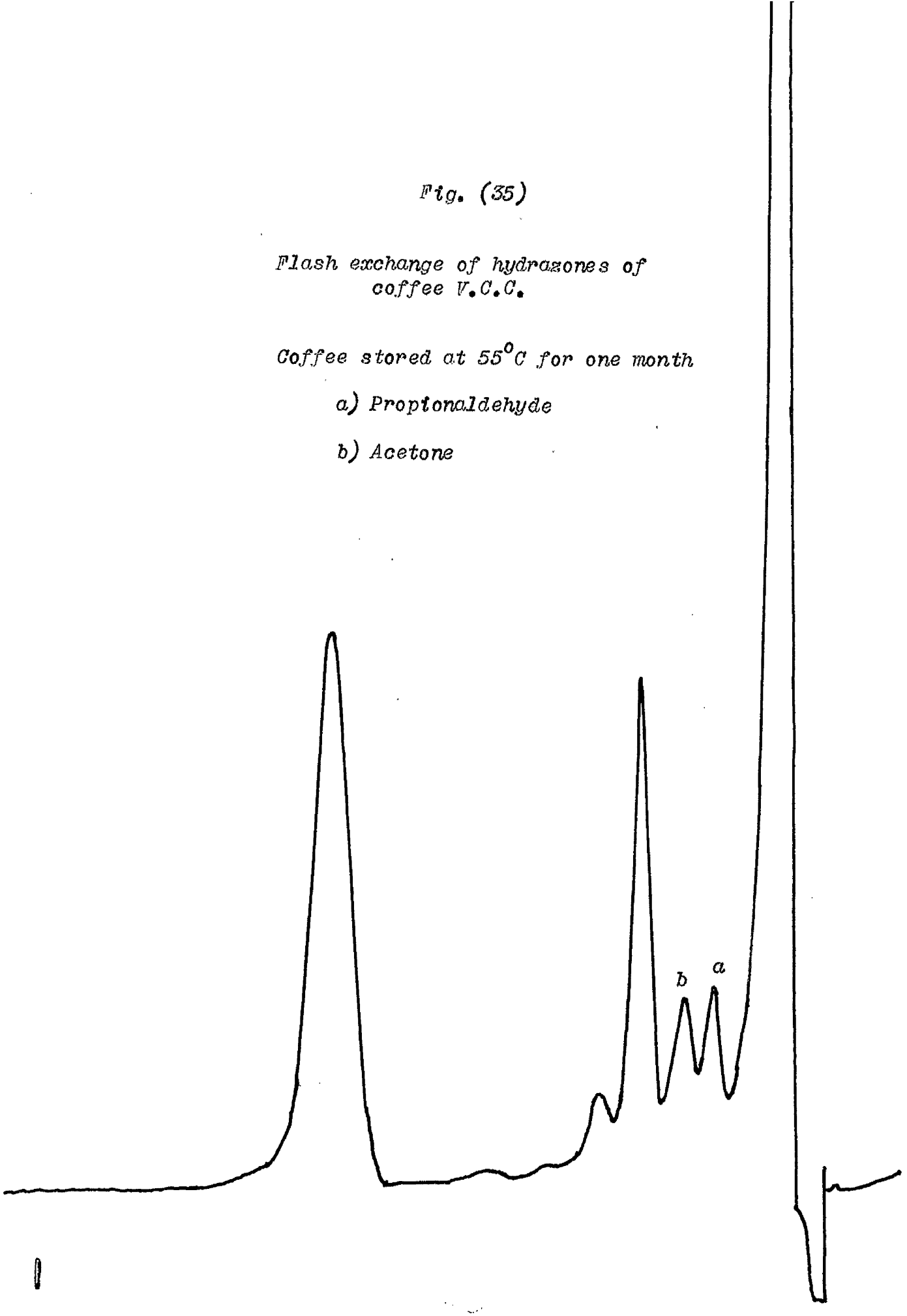


Fig. (36)

Flash-exchange of hydrazones of Coffee V.C.C.

Coffee stored at 55°C for two months

a) Propionaldehyde

b) Acetone

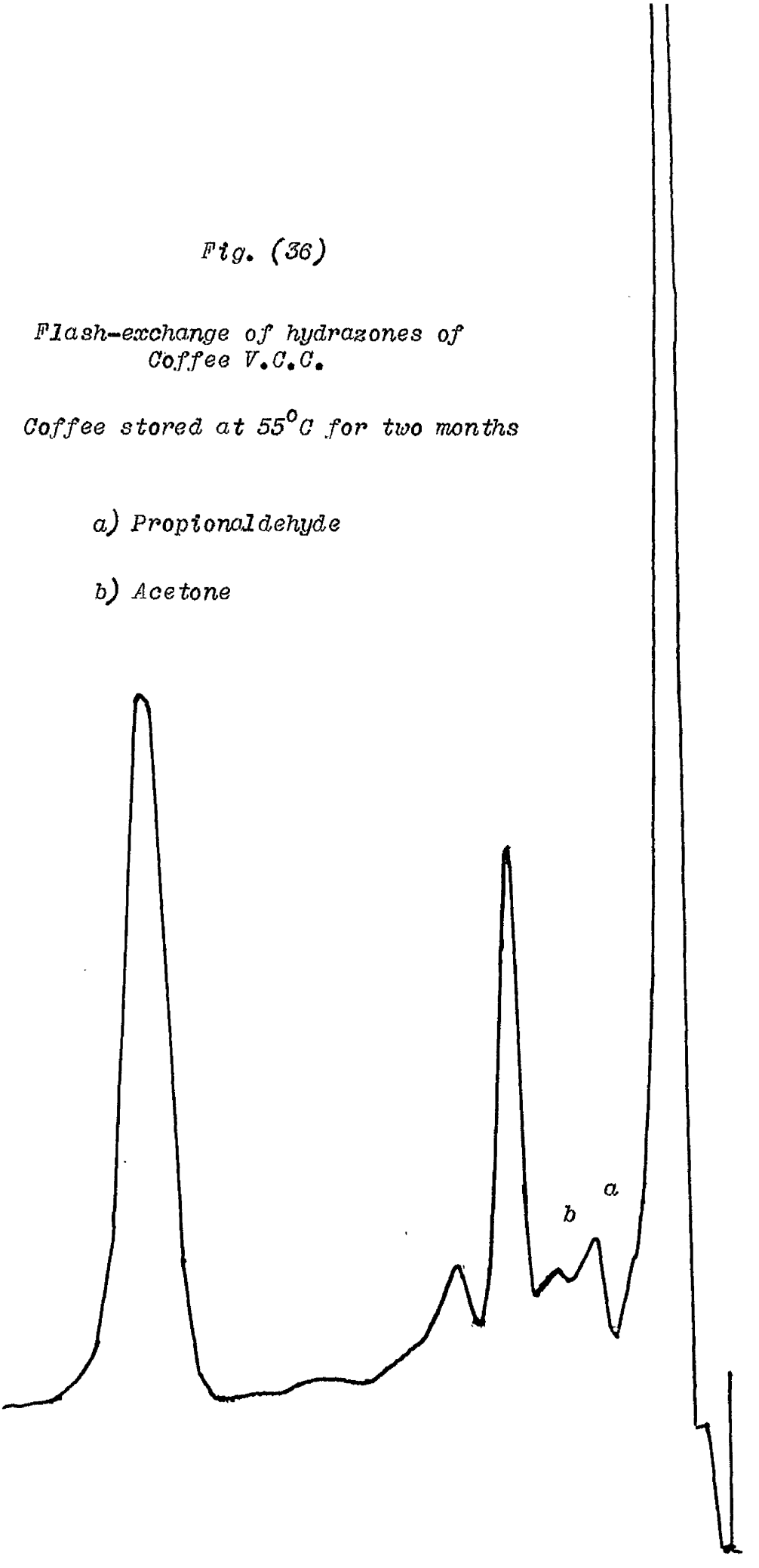


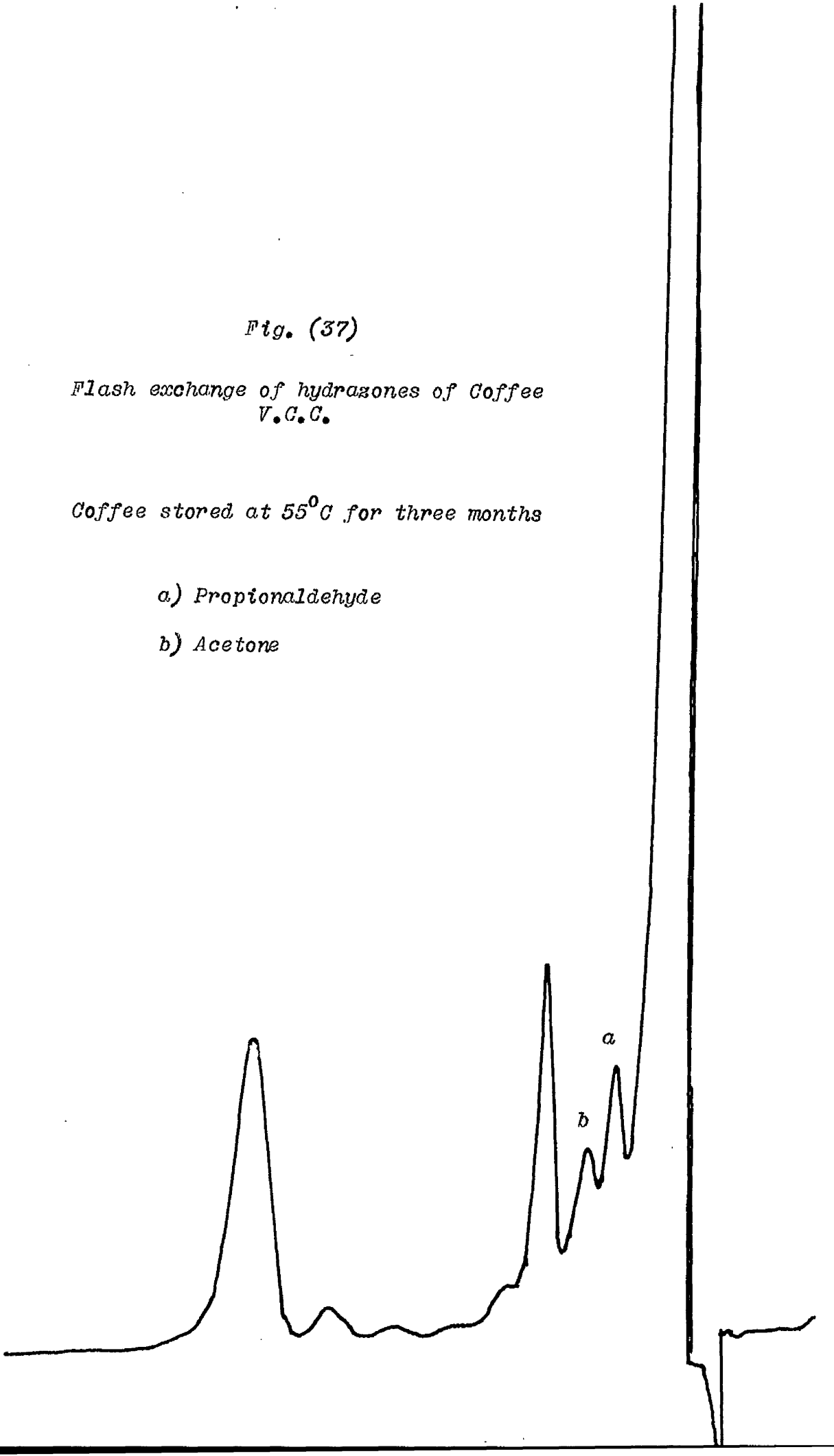
Fig. (37)

Flash exchange of hydrazones of Coffee V.C.C.

Coffee stored at 55°C for three months

a) Propionaldehyde

b) Acetone



PERIOD OF STORAGE (DAYS)
20
40
60
80
100
10
20
30
40
50
60
70
Mg COEFEE V.e.s. DNPHO

Fig. (38)

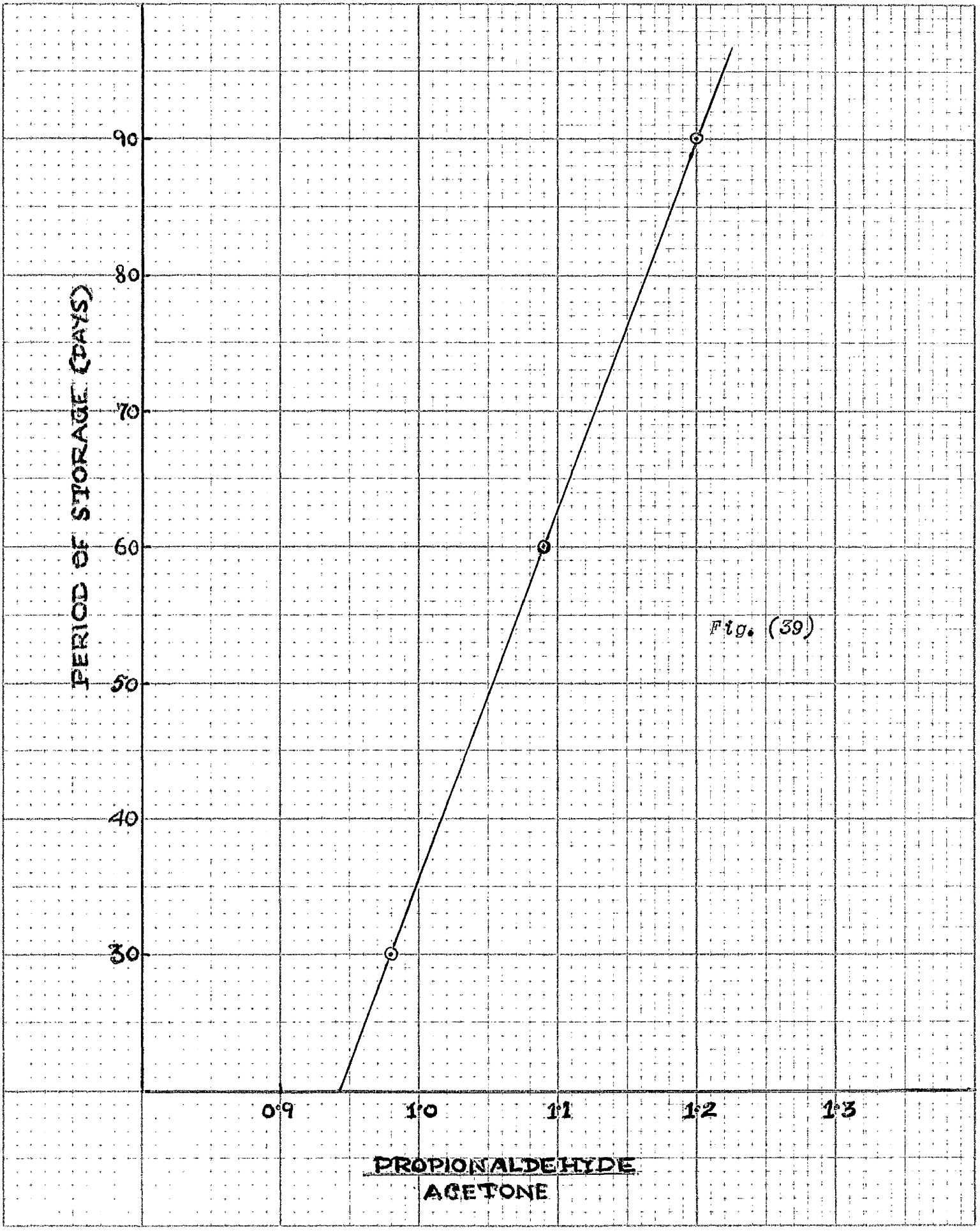

PERIOD OF STORAGE (DAYS)
90
80
70
60
50
40
30
0·9
1·0
1·1
1·2
1·3
PROPIONALDEHYDE
ACETONE

Fig. (39)

Coffee Volatile Reducing Substances (V.R.S.)

By the (VRS) is meant those substances oxidisable by permanganate or dichromate reagents.

Stanley [27] reported that the coffee VRS in brewed coffee were sensitive to the length of time the coffee brew was held, and concluded that the quantity present could be used as an indication of the age and treatment of the coffee brew.

His measure of the VRS was the "oxidation" value which was defined as the number of ml 0.1N dichromate or permanganate required to oxidise the steam distillable materials from 1 g. coffee.[50] He found that the VRS decreased with the increase in holding time and temperature. Table (XIV).

TABLE XIV

Recovery of the Volatile Reducing substances (VRS) from standard coffee brew.(50)

TIME OF HOLDING (hours)	TEMPERATURE OF HOLDING (°C)							
	69		79		89		93	
	VRS/100 ml std. brew	% VRS of "0"	VRS/100 ml std. brew	% VRS of "0"	VRS/100 ml std. brew	% VRS "0"	VRS/100 ml std. brew	% VRS "0"
0	250	----	250	----	250	----	250	----
1	195.0	78.0	193.5	77.4	159.0	61.6	118.0	47.2
3	125.5	48.6	119.5	47.8	137.5	55.0	82.5	33.0
6	71.9	28.8	69.7	27.9	42.0	16.8	42.0	16.8
24	46.5	18.6	43.2	17.3	32.4	13.0	11.3	4.5

VRS = Microequivalents of Reduction.

Stanley[27] also showed that these losses were largely due to the rapid loss of the V.C.C. over the first hour followed by a slower loss up to 24 hours.

Possible Mechanism of the formation of Volatile carbonyl -compounds

In an experiment to collect hydrazones from the green coffee V.C.C., 50 g. of green beans was stripped in Shiptons apparatus in the standard way and after two hours no hydrazones could be collected. This showed that green coffee contains no volatile carbonyl compounds and that these are formed in the process of roasting which causes a considerable fall in bean constituents particularly the sugar, other carbohydrates and tannic acid.(22)

A portential aroma-producing mechanism is the so called Streeker degradation(5) where by an amino acid is oxidatively deaminated and decarboxylated to yield the aldehyde with one carbon atom less.

By cooking the foodstuff, autoxidation, lypolysis, dehydration and decarboxylation may give rise to odourous aldehydes, lower fatty acids, lactones and ketones from fat.(5)

It was suggested(5) that cooked foods will produce a similar pattern of low boiling volatiles varying only in the relative quantities present. This suggests that these common volatiles are produced by degradation of

metabolites which are normally present in all biological material.

It was suggested[52] that branched chain aldehydes might be formed according to the so called "alkali hypothesis" and if this is correct, the oxidation of the amino acids, glycine, alanine, valine and lucine will produce, formaldehyde, acetaldehyde, iso-butyraldehyde and iso-valeraldehyde respectively.

$$R - \underset{NH_2}{\overset{H}{C}} - COOH \xrightarrow{ox.} R - \overset{H}{C} = O$$

n - nonal and n - hexanal may be products of exidative degradation of oleic and linoleic acid respectively:

$$CH_3(CH_2)_7 \; CH = CH(CH_2)_7 \; COOH \longrightarrow CH_3(CH_2)_7 \; CHO$$

$$CH_3(CH_2)_4 \; CH = CHCH_2 \; CH = CH(CH_2)_7 \; COOH \longrightarrow CH_3(CH_2)_4 \; CHO$$

Lawerence[15] suggested as a possible mechanism for the formation of methyl ketones that traces of keto-acids from acetates become incorporated into the triglyceride molecule and breakdown on heating to give a range of methyl ketones with an odd number of carbon atoms.

The simplest unsaturated aldehyde acrolein ($CH_2 = CH\ CHO$) is formed in small quantities when fats are heated to a high temperature as in frying. This may be caused by the dehydration of glycerol.[33]

$$C_3H_8O_3 \xrightarrow{-\ 2\ H_2O} CH_2 = CH\ CHO$$

Another view[49] is that aldehydes may be intermediates in the interconversion of acids and alcohols. Acetaldehyde probably arises also from decarboxylation of pyruvic acid. Acetone is probably a product of fatty acid metabolism, and could rise from decarboxylation of acetoacetic acid, an intermediate in the B-oxidation of fatty acids.

An interesting experiment was that of Reichstein etal[19] who showed that coffee aroma can be obtained by heating tannic acid recovered from coffee beans in the presence of sugar and caffein. They observed also that the peroxidose extracted from horse radish develops a coffee aroma upon heating.

Raw coffee also includes peroxidases which may possibly play their part when coffee flavour is developed in the roasting process.

The fatty acid composition of green and roasted Coffee fat

A sample of green beans and roasted beans of the following blend was used:

40% UGANDA)
30% CONGO) (ROBUSTA)

20% COLOMBIA (ARABICA)

10% INDONESIAN (ROBUSTA)

The beans were coarsely ground and defatted with diethyl ether in a soxhlet extractor under nitrogen.

Methyl esters were prepared from fat extracted from the green and roasted coffee by BF_3/MeOH method[53] Appendix (A_3) The methyl esterers were run on a PYE Angon gas-chromatograph. Chromatograms are shown in Figs. (40 and 41).

The results obtained for the green and roasted coffee were very close so that no significant conclusions could be drawn.

Table XV shows the percentage composition of fatty acids of green and roasted coffee beans along with Hildish results [54] for green beans.

Fig. (40)

Coffee green beans fatty-acid -methyl esters.

G.F. - 40 ml/min.

D.V. - 1250 V.

Col. Temp. - 170°C.

On LAC 2R 446 Col. packing

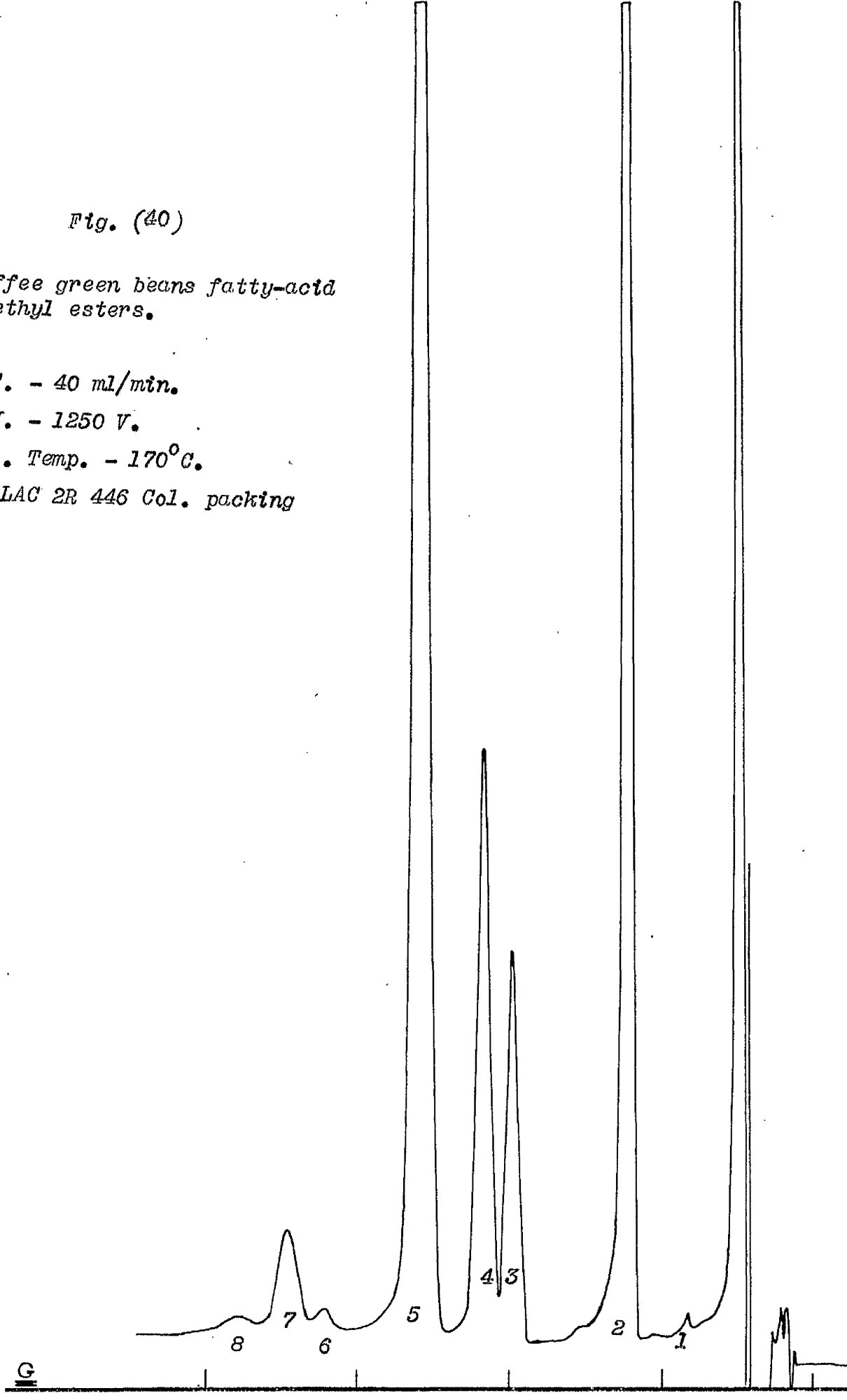


Fig. (41)

Roasted coffee beans fatty-acid-methyl esters.

G.F. - 40 ml/min.

D.V. - 1250 V.

Col. Temp. - 170°C.

On LAC 2R 446 Col. packing

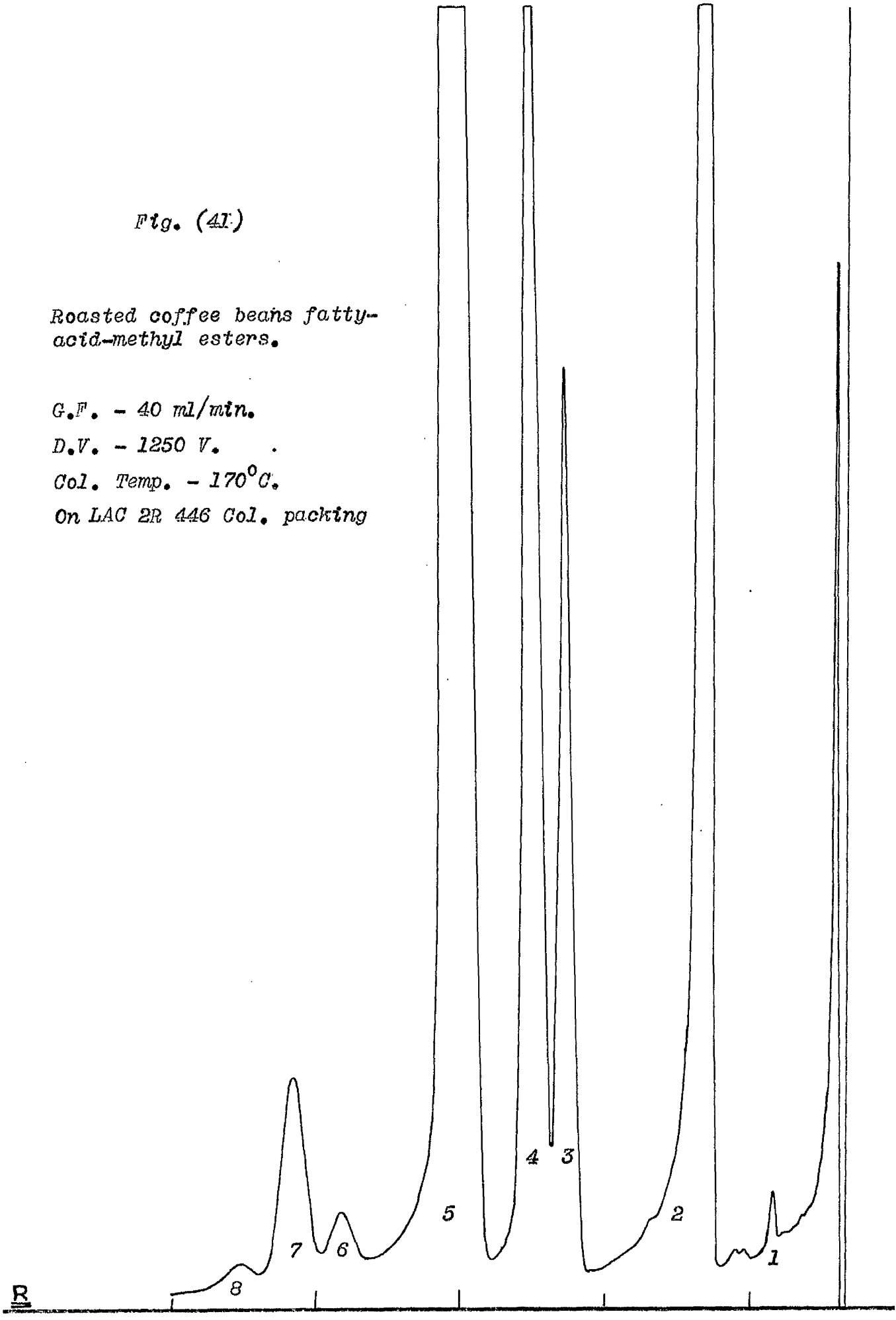


TABLE XV

Percentage composition of coffee beans fatty acids.

	Fatty acids	Hildich[54]	Green Coffee	Roasted Coffee
1	Myristic	-	0.13	0.2
2	Palmitic	28.2	33.00	31.0
3	Stearic	12.7	5.65	7.6
4	Oleic	17.3	19.00	13.0
5	Linoleic	35.8	38.50	45.0
6	Linolenic	-	0.65	0.4
7	Cis-linoleic ?	-	2.50	2.6
8	Arachidic	-	0.57	0.2

Another experiment was done on the fat extracted from green coffee beans which was subjected to roasting temperature about 220^{o}C in an oil bath with nitrogen gas bubbling through. The exit gas was bubbled into hydrazine reagent as shown in Fig. (42).

A trace of reddish-orange precipitate was formed in the hydrazine reagent flask. This was run on PYE Argon gas chromatograph by the "flash-exchange" technique "

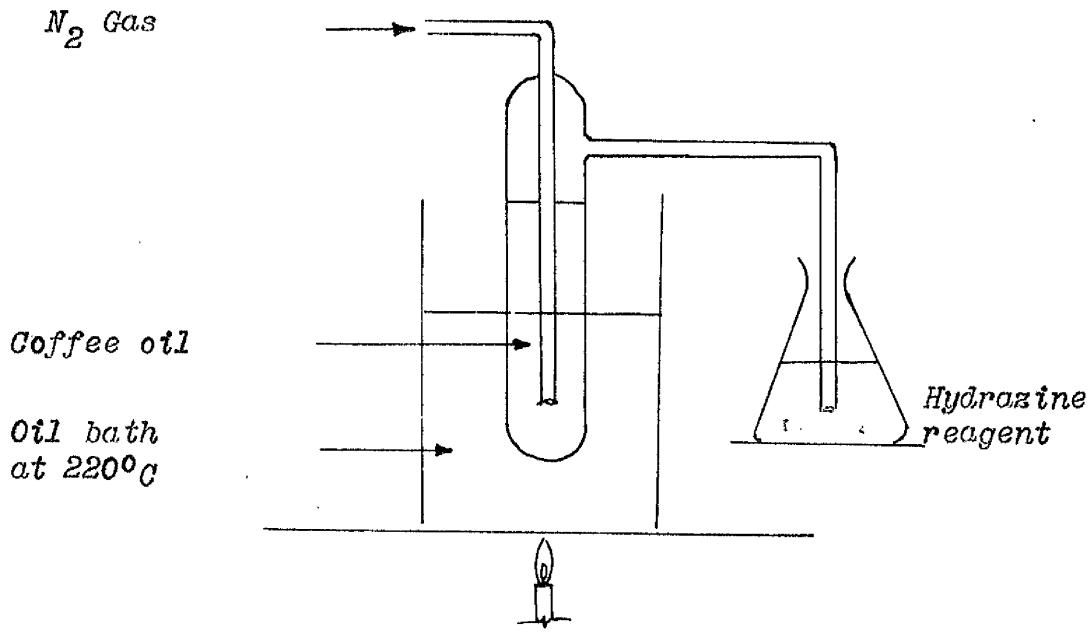


Fig. (42). *Roasting of coffee green beans fat at 220°C under nitrogen gas.*

but gave no sign of any volatile carbonyl compound.

It is not easy to generalise that the same would happen to the fat by roasting the coffee bean as a whole.

It has been reported[19] that fats do not participate in the aroma formation but that they have the capacity of absorbing the aroma once it has been formed and of protecting it from evaporation, oxidation and deterioration.

TASTING PANEL:

1. Role of volatile carbonyl compounds in coffee aroma. A sample of instant coffee was stripped of its volatiles in the Shipton apparatus. The exit gas from the hydrazine reagent flask was passed through a cold trap immersed in a bath of ethanol and solid CO_2. In this way two fractions were obtained - the volatile carbonyl compounds (as hydrazones) and the non-carbonyl volatiles.

The stripped coffee brew was then divided into two parts; to one part (x) the carbonyl compounds were added back by regeneration.

The non-carbonyl volatiles were added to the second part (Y).

These two samples were then presented to a tasting panel of 9 members using:

(a) Paired comparison test for difference,

(b) Paired comparison test for preference.

These represented a simple analytical and consumer test respectively.(71)

(a) Difference: 9 out of 9 found the samples to be different

(b) Preference: 7 out of 9 preferred (Y).

2. Effect of processing on coffee volatile carbonyl compound:

Coffee was prepared from jars 8 and 9 (Patersons Ltd.) 8 had been water cooled after roasting and 9 air cooled.

Previous work had shown that sample No. 8 contained a greater proportion of iso-valeraldehyde. These were presented to the panel and the same questions asked.

Difference: 8 out of 9 found the samples to be different.

Preference: 5 out of 9 preferred sample No. 8 (water cooled).

3. Effect of Storage:

Ranking Test

Coffee prepared from three 2 oz. tins of instant coffee (Nescafe) stored at 55°C for one, two and three months were presented to the panel along with a control sample (unstored fresh Nescafe).

The following numbers were given to the presented samples:

No. 3 Control

No. 1 Stored for one month

No. 4 Stored for two months

No. 2 Stored for three months

The Panel gave the following Rankings.

Table XVI.

Coffee samples	1	2	3	4
Ranked by Judge	1	2	4	1	3
Ranked by Judge	2	4	3	1	2
Ranked by Judge	3	3	4	1	2
Ranked by Judge	4	4	2	1	3
Ranked by Judge	5	3	4	1	2
Ranked by Judge	6	1	4	3	2
Ranked by Judge	7	2	4	1	3
Ranked by Judge	8	2	3	1	4
Ranked by Judge	9	2	4	1	3
Total of Ranks	23	28	31	24	9

Calculations (72)

$W = \frac{S}{S_{max.}} =$

On the hypothesis that the Judges have no agreement at all, $\frac{n(n+1)}{2} = \frac{9 \times (4+1)}{2} = 22.5$

Total ranks	33.0	32.0	11.0	24.0
	22.5	22.5	22.5	22.5
	+ 0.5	32.0 / 22.5 9.5	- 11.5	+ 1.5

S = The sum of the squared differences between observed and expected rank totals.

$= (0.5)^2 + (9.5)^2 + (-11.5)^2 + (1.5)^2 =$

$= 0.25 + 90.25 + 132.25 + 2.25 = 225$

(S) corrected = 225 + 1 = 224

$S_{max.}$ = The maximum possible sum of squares, when the Judges are in complete agreement

$= \frac{n^2(n^3-n)}{12} = \frac{9^2 \times (4^3-4)}{12} = \frac{81 \times (64-4)}{12} = 405$

($S_{max.}$) corrected = 405 + 1 = 407

The coefficient of concordance (W) which is the measure of degree of agreement between Judges.

$W = \frac{S}{S_{max.}} = \frac{224}{407} = 0.55$

(W) is tested for significance, using Snedecor's distribution for (F), as follows:

$$F = \frac{(n-1)W}{1-W} = \frac{(9-1) \times 0.55}{1-0.55} = \frac{8 \times 0.55}{0.45} = \underline{9.77}$$

Now entering tables of (F) with degrees of freedom of greater estimate = $(n-1) - \frac{2}{n}$ =

$$= (4-1) - \frac{2}{9} = \frac{24}{9} = \underline{2.77}$$

And degrees of freedom for the lesser estimate =

$$= (n-1)\left[(n-1) - \frac{2}{n}\right] = (9-1)\left[(4-1) - \frac{2}{9}\right] = \underline{22.2}$$

The approximate value of (F) from the tables (58)

is 3.05 for 5% level

and 4.82 for 1% level

The high value of (F) shows a significant degree of agreement in ranking stored coffee samples according to their palatability, which means that instant coffee samples in tightly closed cans can deteriorate markedly if stored for long periods before consumption.

The degree of staling is in direct relation to the period of storage.

Summary and Conclusions

1. The development of sensitive techniques enabled the flavour work to progress rapidly, but unless the perception of taste and smell with relation to the chemical structure of the perceived substance is well understood, the applications of the obtained results will be of much less value.

2. The flash exchange G.C. technique found to be very unsatisfactory to the study of coffee V.C.C.

3. Spectroscopy of all ranges, thin film and paper chromatography are necessary to confirm the results obtained by G.C.

4. Infra-red spectroscopy showed two absorption bands; one at about 1110 - 1118 cm^{-1} and another at 1138 cm^{-1} for the hydrazones of ketones in chloroform; An absorption band at 1137 - 1140 cm^{-1} for the hydrazones of aldehydes.

   Unsaturated aldehydes and ketones hydrazones in chloroform have characteristic absorption band at

980 - 996 cm^{-1} with a weak band at about 1710 cm^{-1}. Dicarbonyl compound hydrazones show an absorption band between 1690 and 1730 cm^{-1}. No differences could be noticed among spectra of n-aldehydes and iso-aldehydes or iso-ketones in chloroform, but all show an absorption band at about 1138 cm^{-1}.

5. If the ionization detector is used in the analysis of V.C.C., a high detector voltage (1750 V and above) is required if some of the volatile constituents have high electron-capture affinity.

6. Beside applying high detector voltage, when compounds like diacetyl are present in the V.C.C., the reduction of the sample also is advisable.

7. Formaldehyde was identified in coffee volatiles by a chromotropic acid method and its concentration found to be around 3 ppm.

8. Acetaldehyde, propionaldehyde, acetone, iso-butyraldehyde, n-butyraldehyde, methylethylketone, ,

diacetyl and iso-valeraldehyde were separated and identified by flash-exchange gas chromatography, supported by spectroscopy and different chromatography techniques.

9. The coffee brew remaining after stripping the volatiles was found to contain little remaining carbonyl compounds which suggest the stripping by the standard procedure used was efficient. A sample run by flash-exchange showed that the proportions of the carbonyl compounds were not the same; i.e. all aldehydes were present in smaller proportion compared to diacetyl which increased with methyl ethyl ketone.

10. More than 95% of the coffee carbonyl compounds is composed of aldehydes.

11. The method developed for measuring the total aldehydes in the presence of other reducing substances could be made more efficient with further refinement of the procedure.

12. Steam distillation is required for some experiments which cannot be done in Midgley's apparatus, e.g. the oxidation of coffee aldehydes in an alkaline solution of $AgNO_3$.

13. Acetaldehyde and iso-valeraldehyde, which occur in approximately equal amounts, form the largest proportion of the total V.C.C.

14. Carbonyl compounds content measured as the weight of dried hydrazones of the coffee volatiles may be taken as an index of the degree of heat treatment of coffee.

15. The ratio of propionaldehyde to acetone increases with increasing periods of storage of the coffee at 55°C. The ratio may be of value in studying coffee staling providing a standard method is employed.

16. Coffee fat seems not to contribute to the creation of coffee V.C.C. in the roasting process.

17. There would appear to be a relationship between the decrease in the amount of V.C.C. with storage and the palatability of the brew.

18. Nescafe samples stored at 55°C for 1, 2 and 3 months plus a control (i.e. fresh unstored Nescafe), were presented to the panel for ranking according to their merit in palatability. The panel was in a good agreement in their judgments. They assigned the highest palatability to the control sample. The shorter the period of storage the more palatable the sample was.

19. No volatile carbonyl compounds could be detected from 50 g. ground green coffee beans when subjected to the standard methods of collecting the coffee hydrocarbon by Shipton's apparatus.

A P P E N D I X (A)

P R O C E D U R E S

(A_1)

Thin - layer Chromatography [57]

Apparatus

Spreader: - Desago - This to be adjusted to give layers of different thicknesses.

Plates - Glass, 20 x 20 cm and 20 x 5 cm.

Silicagel G - Contains 15% 2 $CaSO_4.H_2O$ to act as a binder.

Procedure: The thoroughly cleaned plates are placed in position on the template which holds five 20 x 20 cm plates.

30 g. Silicagel G are mixed with 60 ml distilled water in a mortar, care being taken to avoid the formation of bubbles. A smooth thin paste is desired free from lumps. The mixing must be done in the shortest possible time to avoid "setting" of the calcium sulphate. The mixture is rapidly transferred to the spreader and the plates covered with a smooth and continuous movement. Uniformity of the film-thickness over the area of each plate is important. The plates are air dried then activated for 30 min. at 120°C. They should then be stored in a desiccator and

protected from laboratory fumes. When used, the plates and the solvents should be at the same temperature.

(A_2)

Preparation and packing of the gas chromatographic column

Solids: 100 - 120 mesh celite was heated in a muffle-furnace at 300 - 350°C for five hours, cooled to room temperature, transferred to a 1 litre beaker, covered with conc-HCl and left overnight.

The acid was then decanted and the celite washed with distilled water until acid free. The purpose of the acid washing was to remove metallic impurities particularly iron. The fine dust particles were decanted off and the celite dried at 125°C for 4 hours.

The required amount of liquid phase (dinonylphthalate) was dissolved in chloroform and made into a slurry with the celite. The chloroform was evaporated on a water bath with careful stirring. The last traces of chloroform were removed by heating in an oven at 115°C.

The column was packed by adding small quantities at a time followed by vibration using an electrical vibrator. The packed column was placed in position in the apparatus and kept at 115°C with a slow stream of argon passing through until a stable base line was obtained.

(A_3)

Preparation of Methyl Ketone

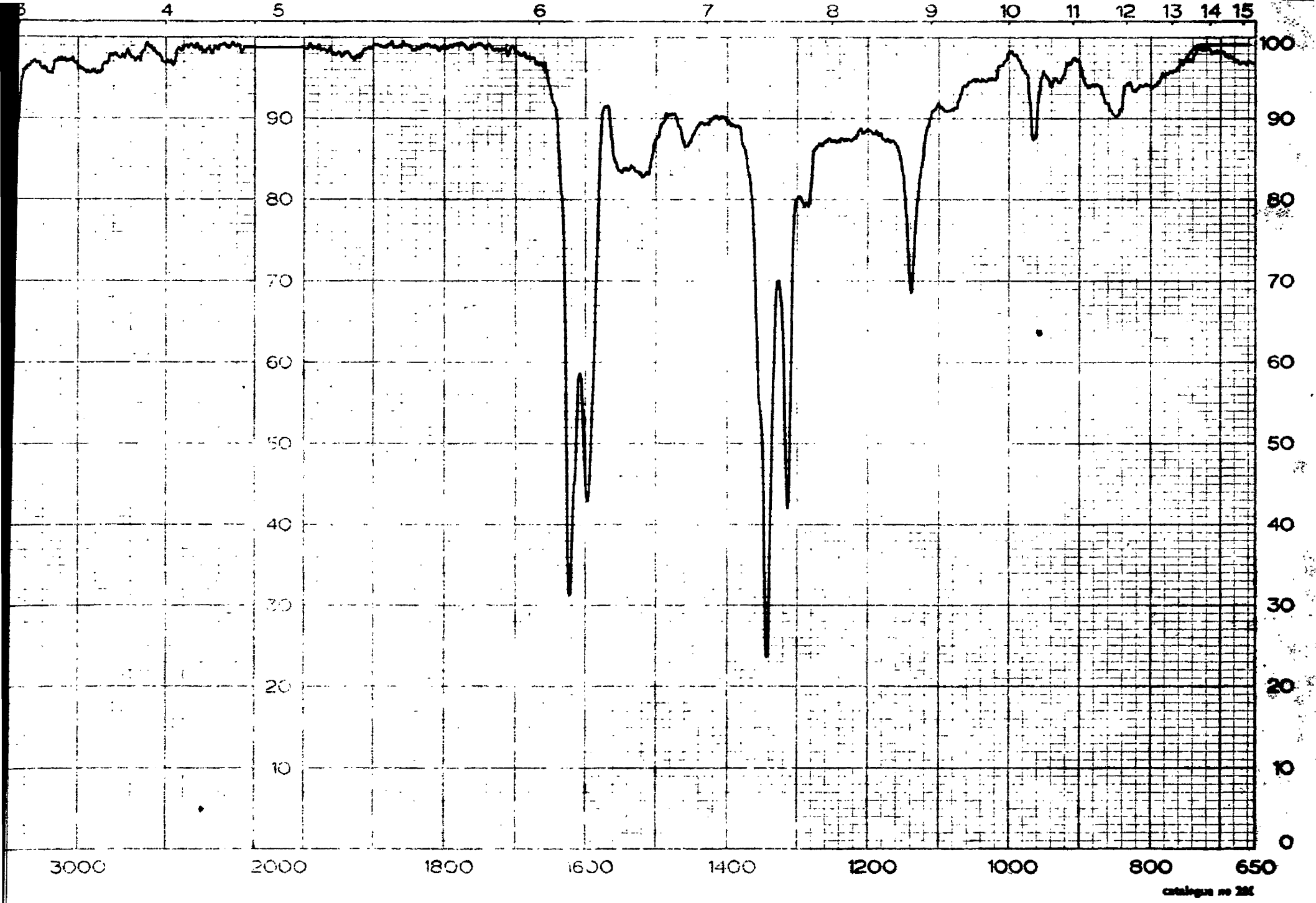
Saponify approximately 0.1 g. oil for 30 min., under nitrogen, with N/2 alcoholic KOH (20 ml) under reflux. Add an equal volume of water and acidify with moderately strong HCl.

Extract in a separatory funnel with 20 ml diethyl ether followed by 20 ml. petroleum ether. Repeat the extraction and combine all the ether extracts. Dry with anhyrous Na_2SO_4 and decant. Evaporate the ether extracts under nitrogen and cover the residue at once with one ml of the BF_3/Methanol reagent.

A 1f.oz. screw capped bottle is convenient for this purpose. Place for exactly 2 min. in a boiling water bath then add immediately 10-15 ml water. Transfer to a separating funnel and extract with 20 ml petroleum ether until the aqueous phase is vertically clear. Dry the solution of the ethers with anhydrous Na_2SO_4, evaporate the ether under nitrogen and vacuum distil the esters which are then ready for gas chromatography.

APPENDIX (D)

INFRA-RED SPECTRA



catalogue no 28C

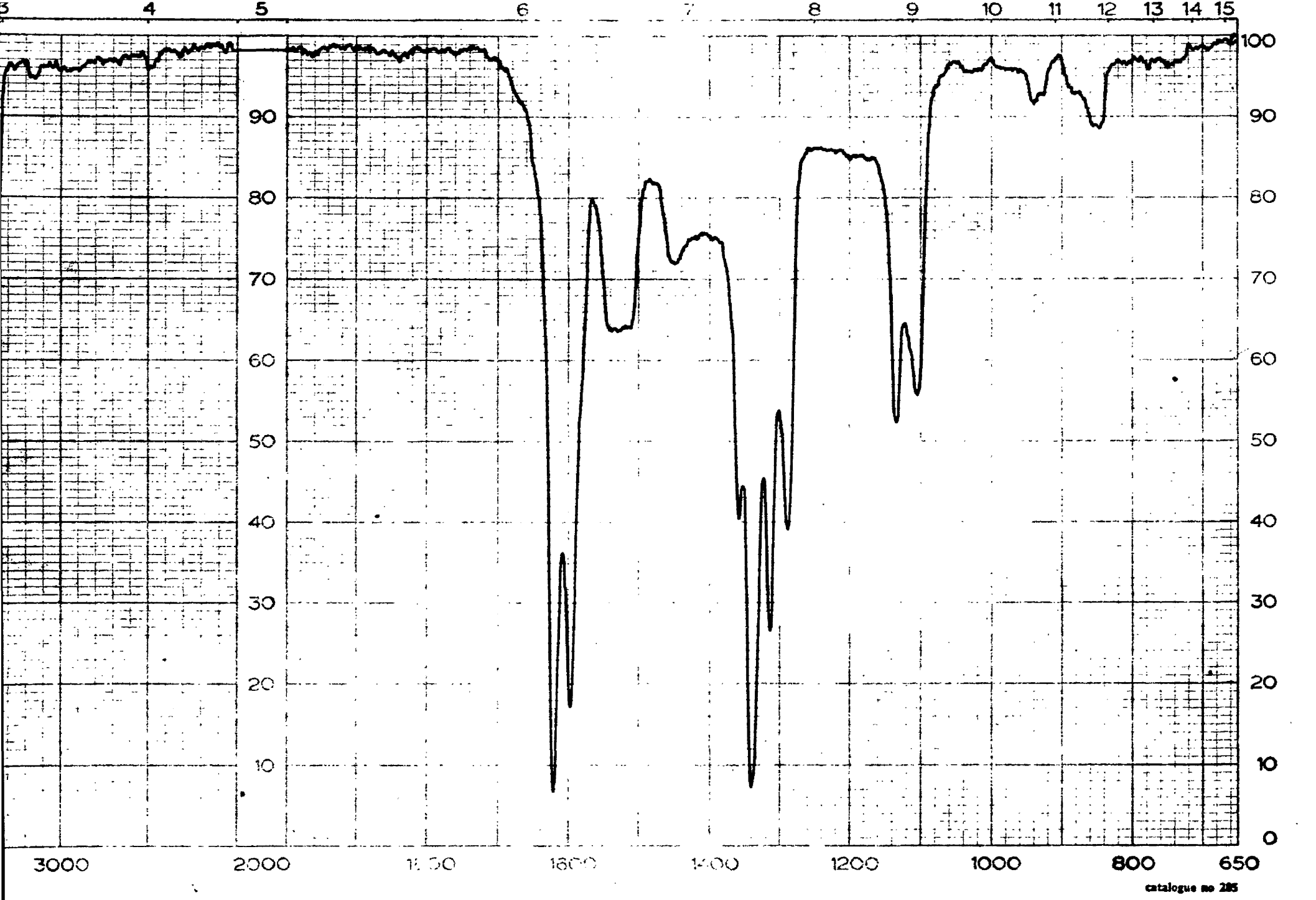
FORMALDEHYDE
DNPH

FORMULA

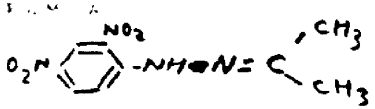
O_2N (C6H3, NO_2) $-NH-N=CH_2$

PHASE
THICKNESS
REMARKS Bl.

NUMBER
DATE 17/12/63
OPERATOR



ACETONE
DNPH



$CHCl_3$

1 min

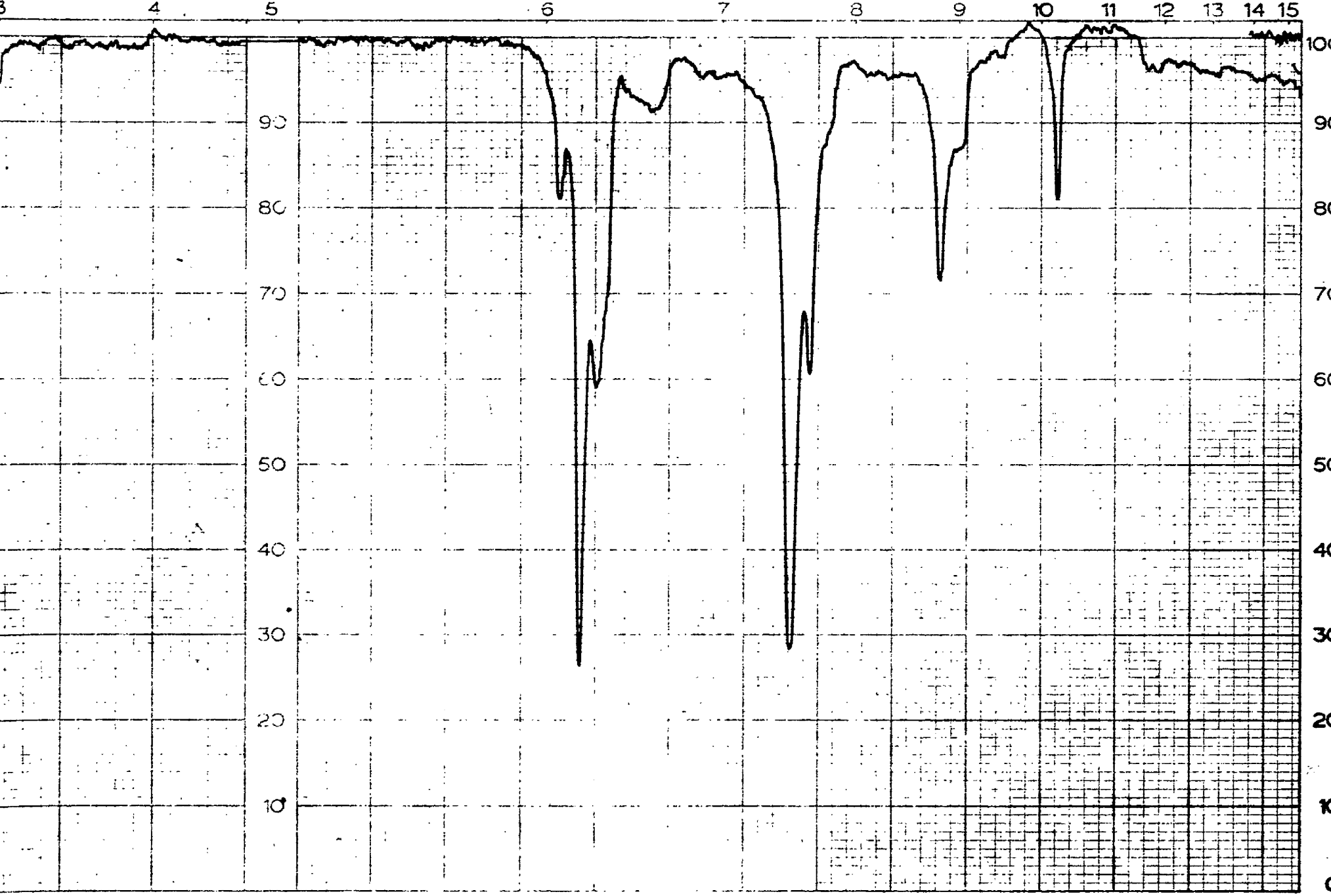
$B_{0.2}$

NUMBER 8

DATE 17.12-63

OPERATOR

REFERENCE



CROTONALDEHYDE DNPH

FORMULA O_2N (NO_2) $-NH-N=NC-CH=CH-CH_3$

PHASE

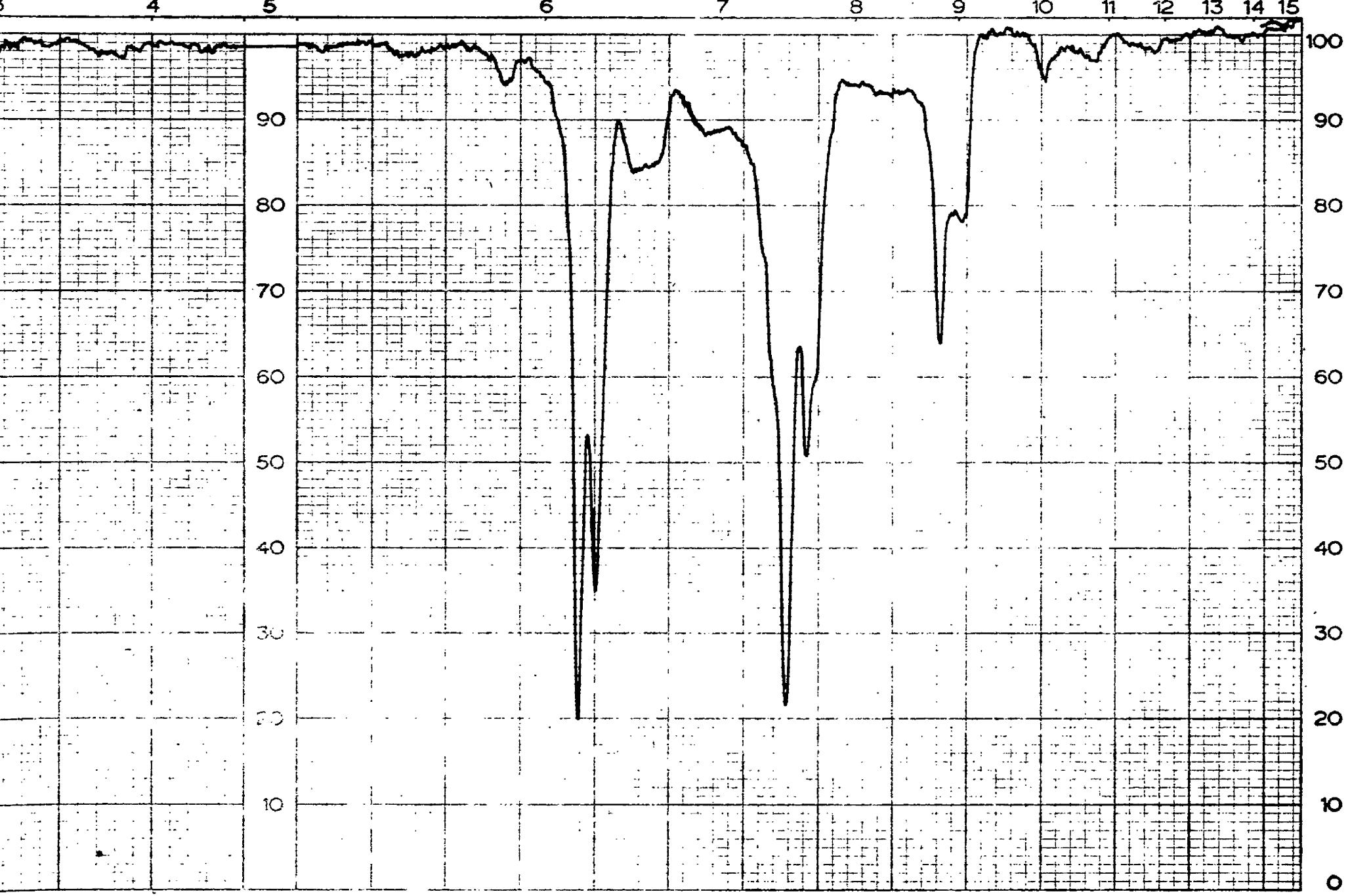
THICKNESS 1 mm

REMARKS B.3.

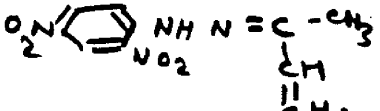
NUMBER (18)

DATE 13-1-64

OPERATOR



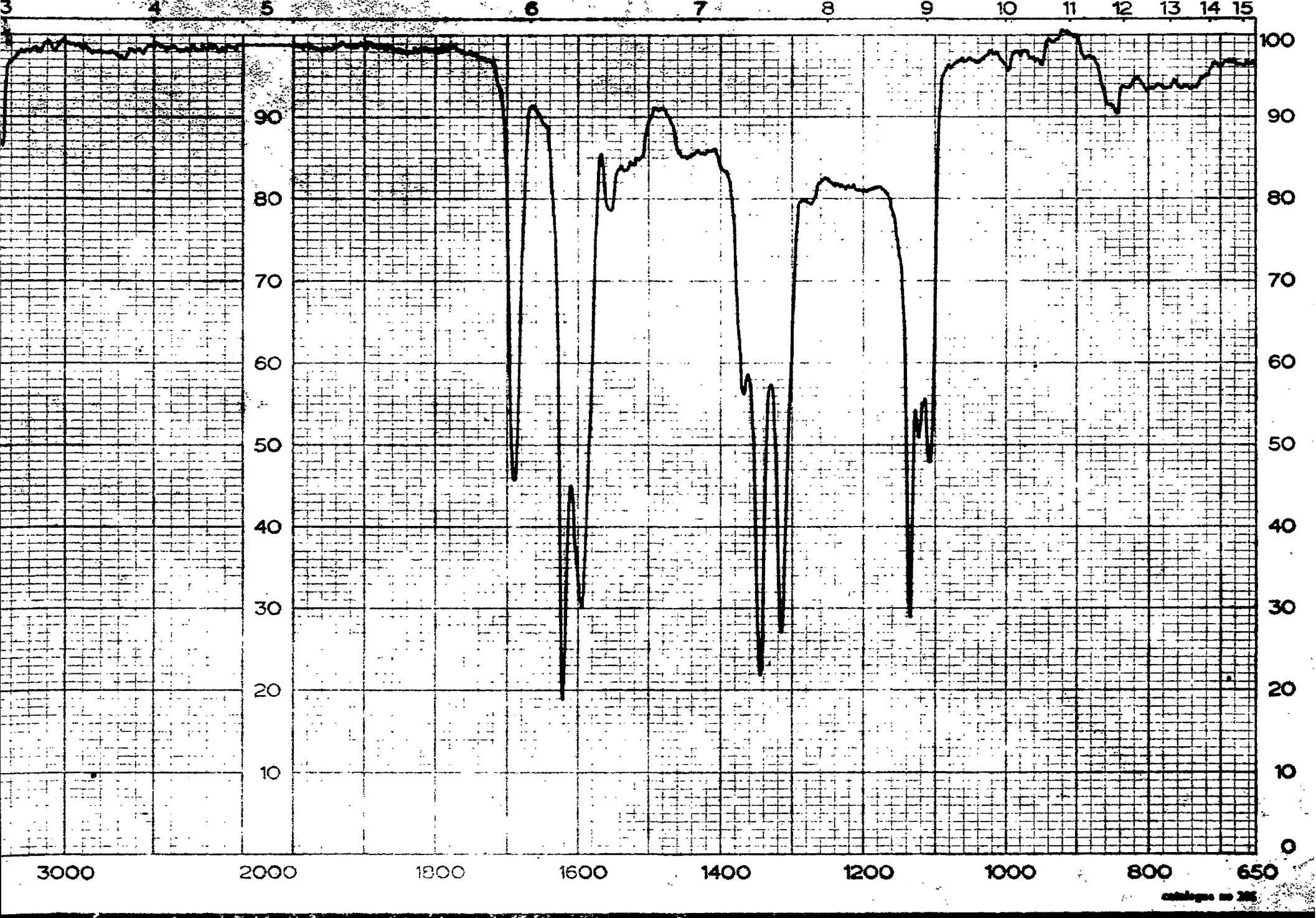
ETHYL VINYL KETONE
DNPH



PHASE liquid solvent $CHCl_3$
THICKNESS 1 —.—
REMARKS B.4.

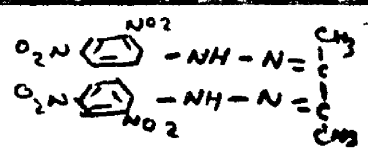
NUMBER 20
DATE 22-1-64
OPERATOR

REFERENCE



DIACETYL DNPH

FORMULA



PHASE $CHCl_3$

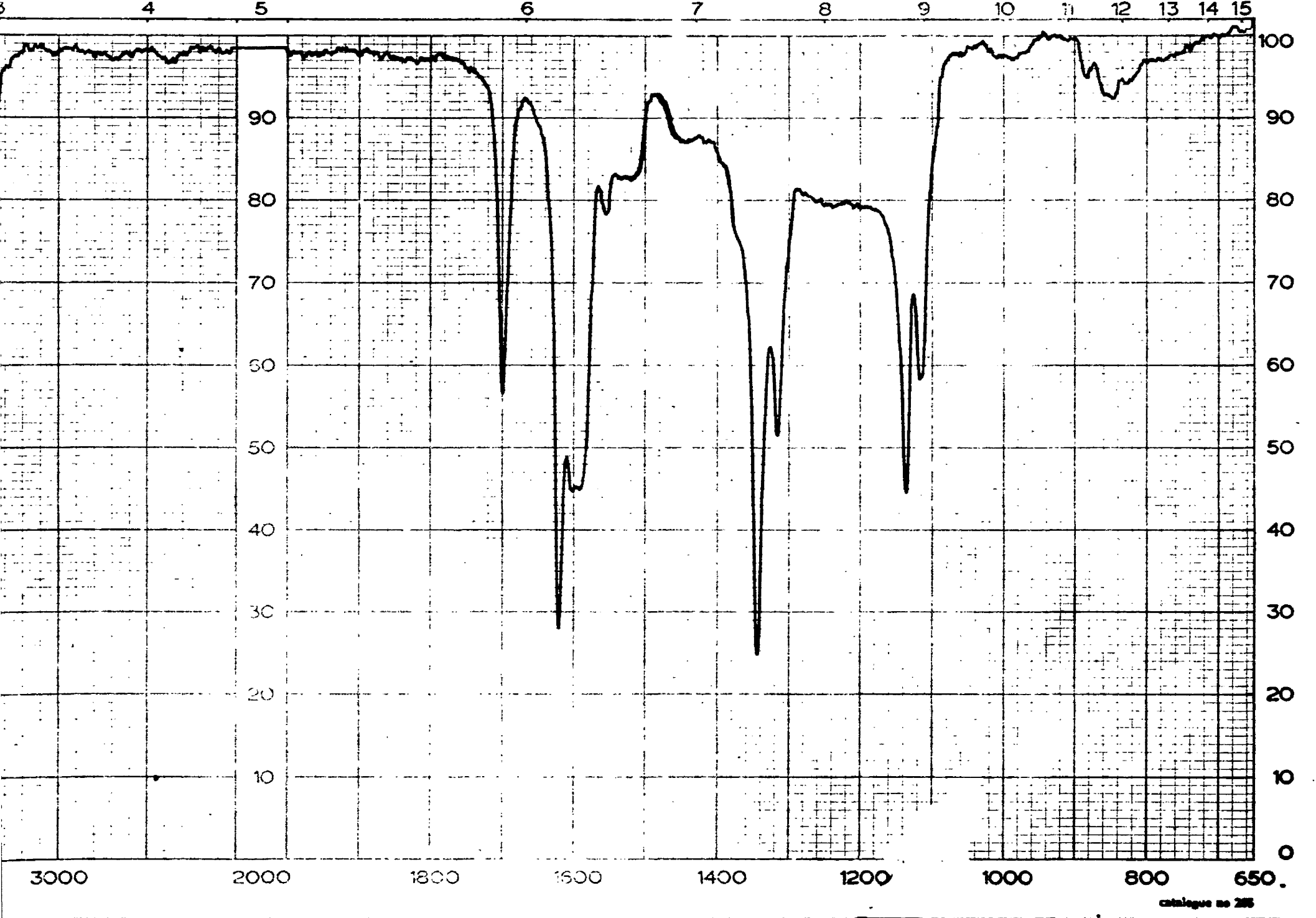
THICKNESS 1 mm

REMARKS B.5.

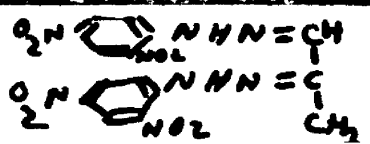
NUMBER 11

DATE 7-1-64

OPERATOR

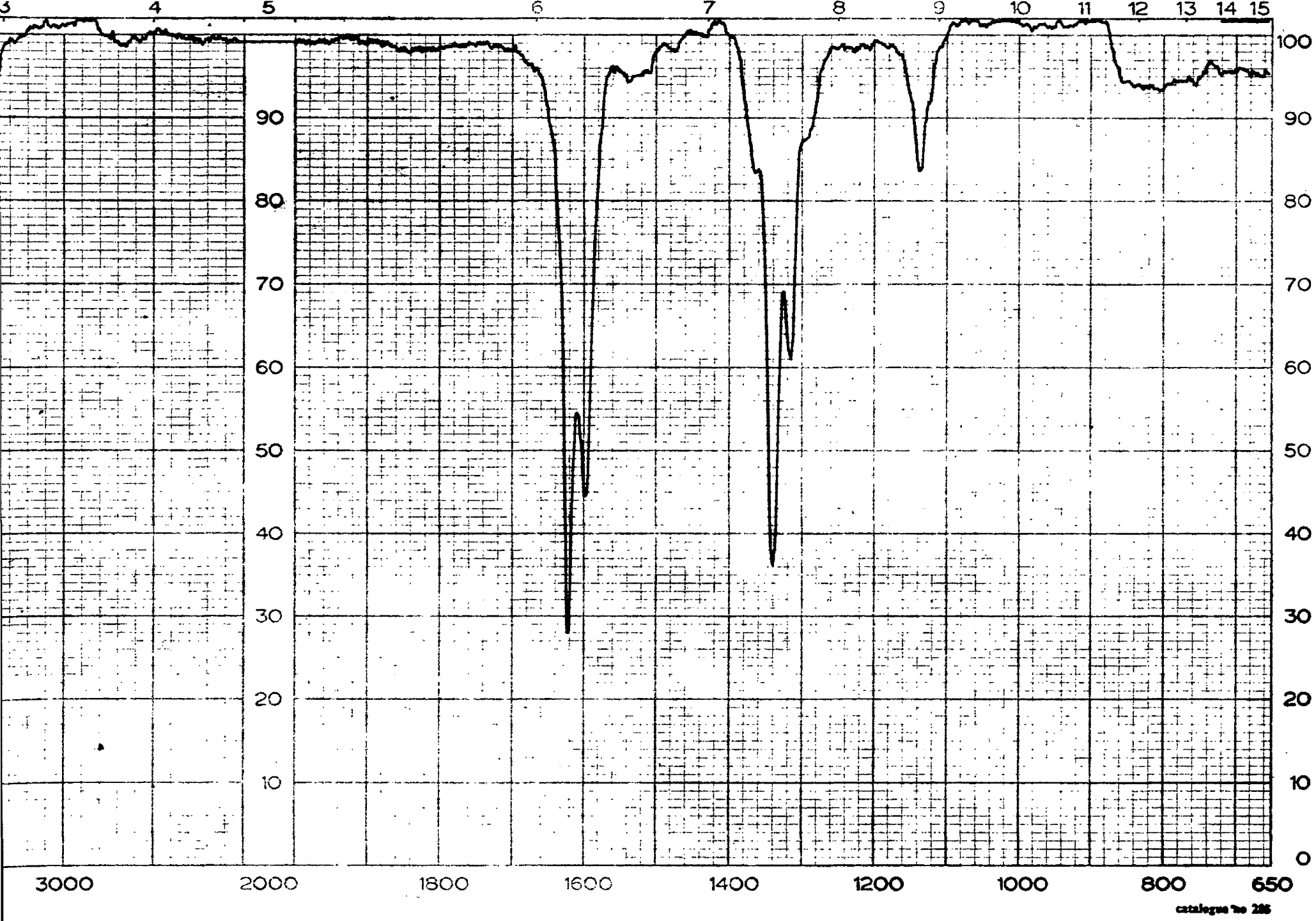


PYRUVICALDEHYDE
DNPH



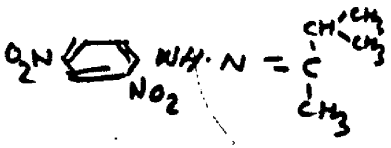
PHASE liquid chloroform
THICKNESS 1 —
REMARKS B.6.

NUMBER 26
DATE 18-2-64
OPERATOR



METHYL ISO PROPYLKETONE
DMPH

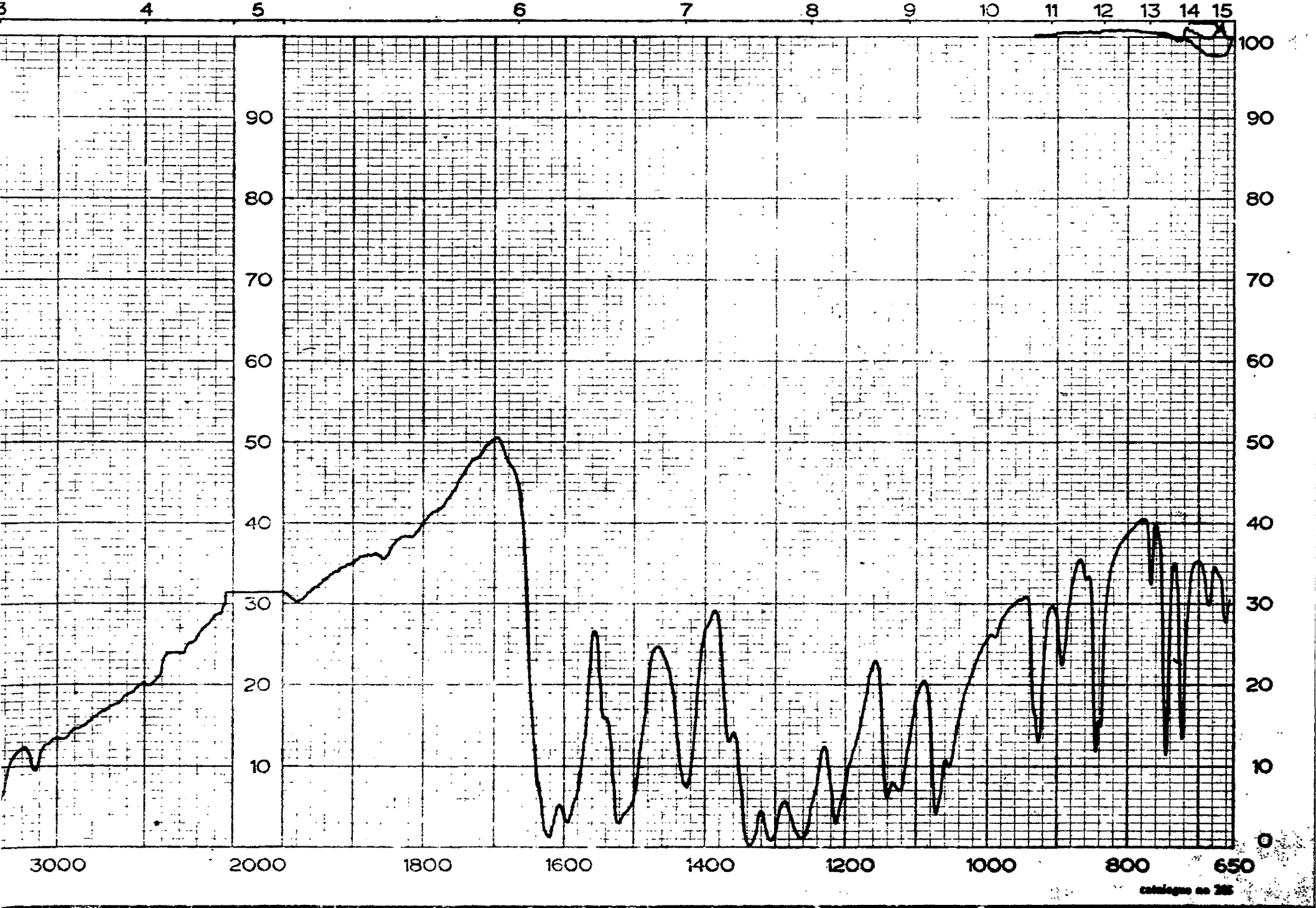
FORMULA



PHASE Liquid $CHCl_3$
THICKNESS
REMARKS B.7.

NUMBER 22
DATE 28.1.64
OPERATOR

REFERENCE



FORMALDEHYDE
DNPH

FORMULA O_2N NO_2 $NH\,N=CH\,CH_3$

PHASE Solid KCl

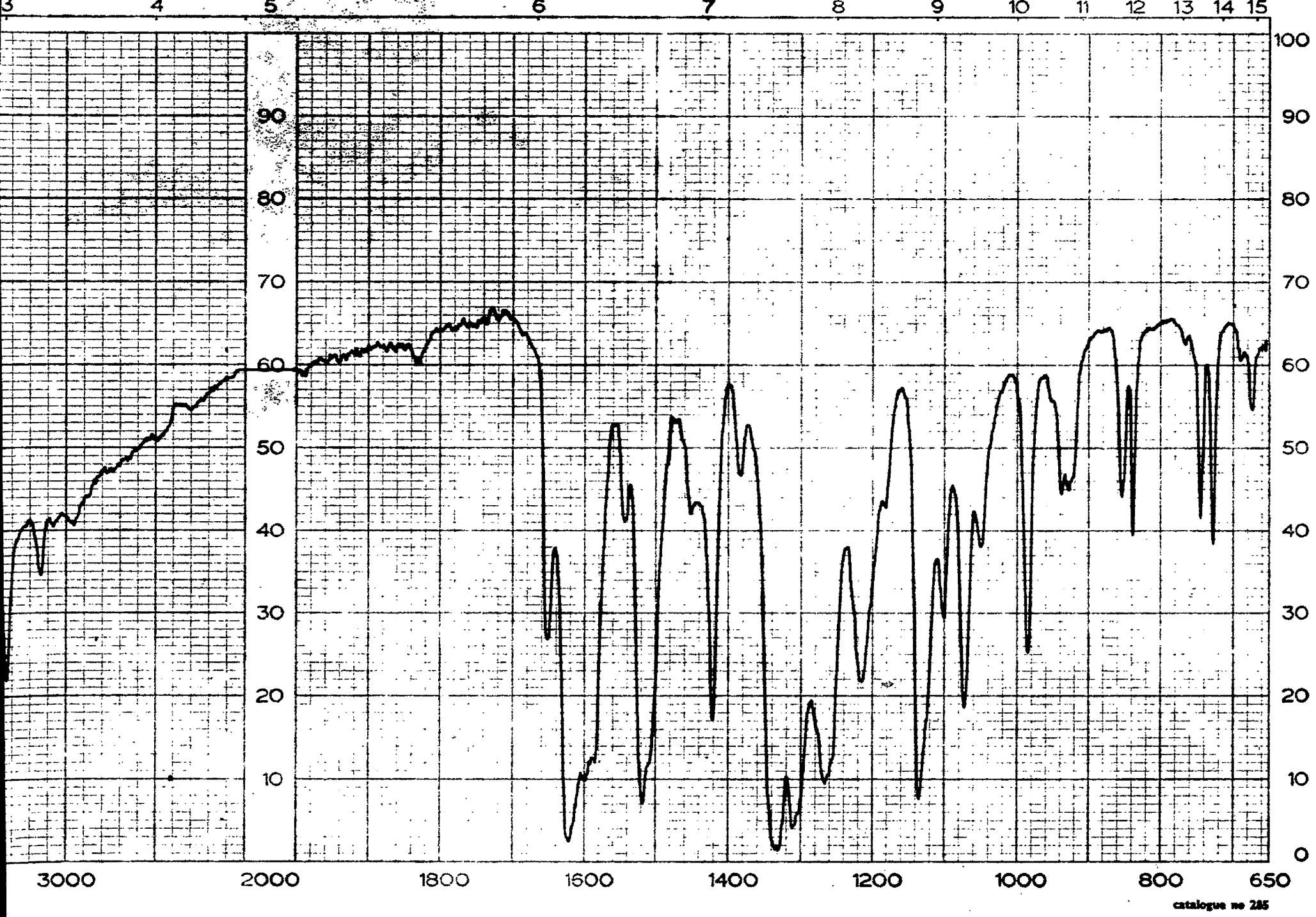
THICKNESS

REMARKS Scan Mode 3/5 B.S.

NUMBER 2

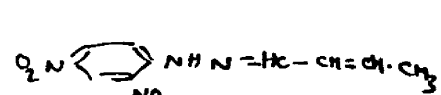
DATE 29-1-64

OPERATOR



CROTONALDEHYDE DNPH

FORMULA



PHASE Solid, KCl

THICKNESS

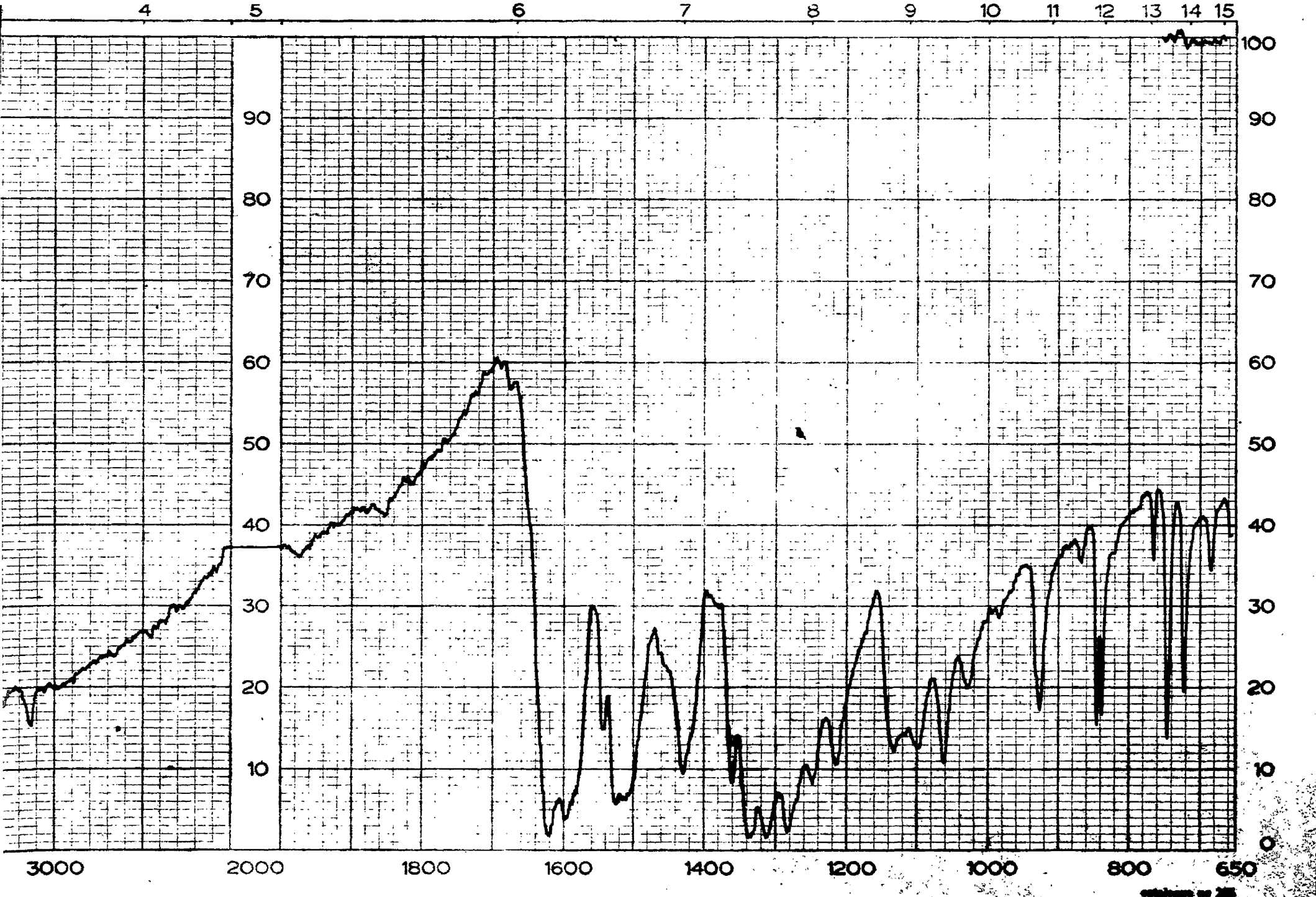
REMARKS B.9.

NUMBER 18

DATE 4-2-64

OPERATOR

REFERENCE



ACETONE
DNPH

FORMULA $O_2N(NO_2)C_6H_3NHN=C(CH_3)_2$

PHASE solid KCl

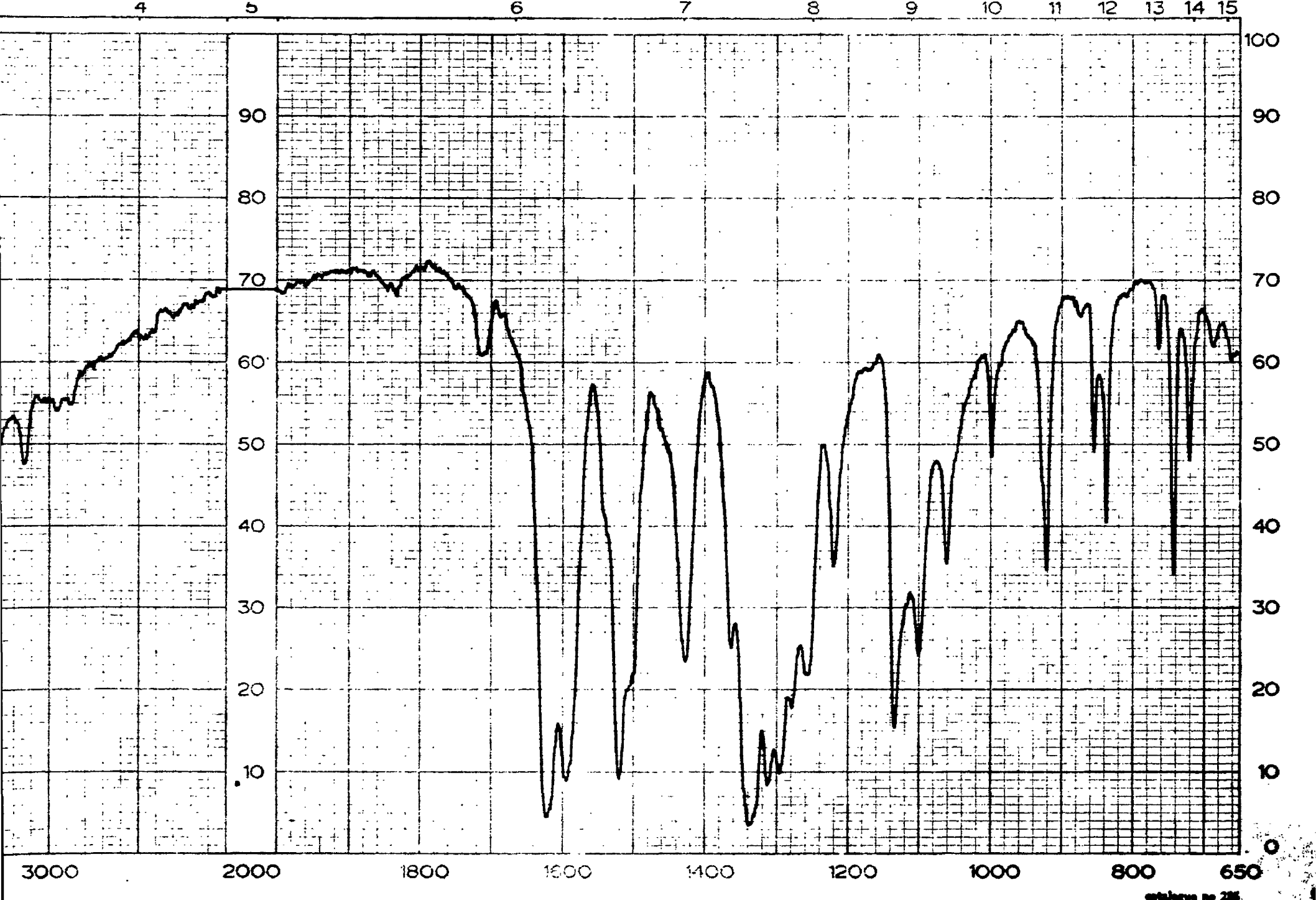
THICKNESS

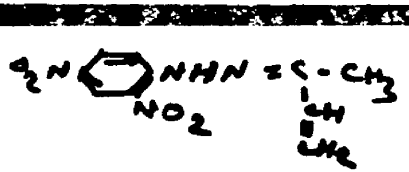
REMARKS B.10.

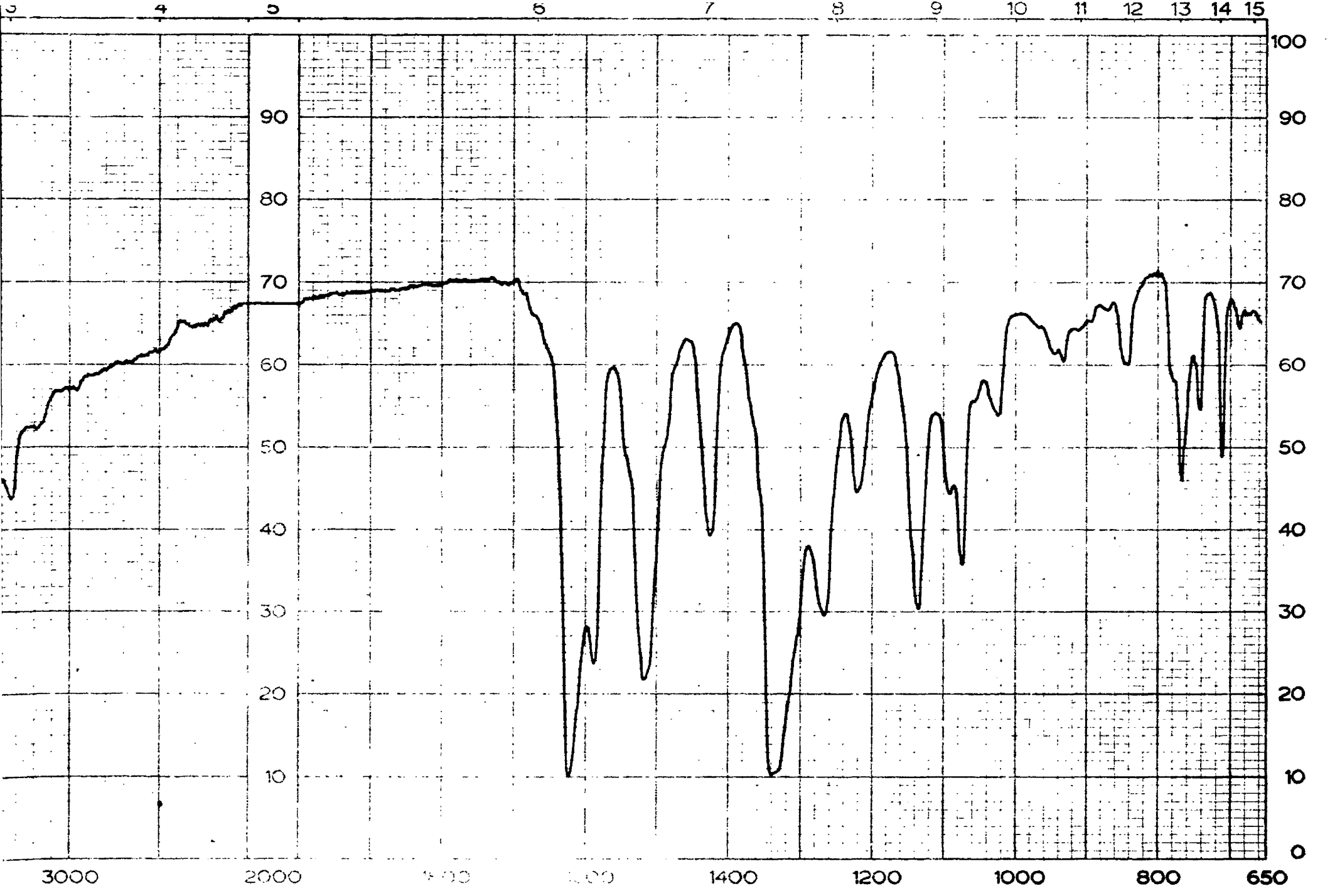
NUMBER 8

DATE 3-2-64

OPERATOR

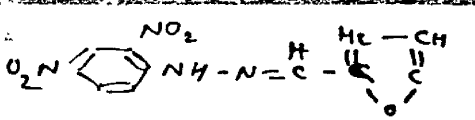


THYL VINYL KETONE DNPH	FORMULA 	PHASE Solid, KCl THICKNESS REMARKS B.11.	NUMBER 20 DATE 4.2.64 OPERATOR



catalogue no 288

FURFURALDEHYDE DNPH



PHASE solid, KO

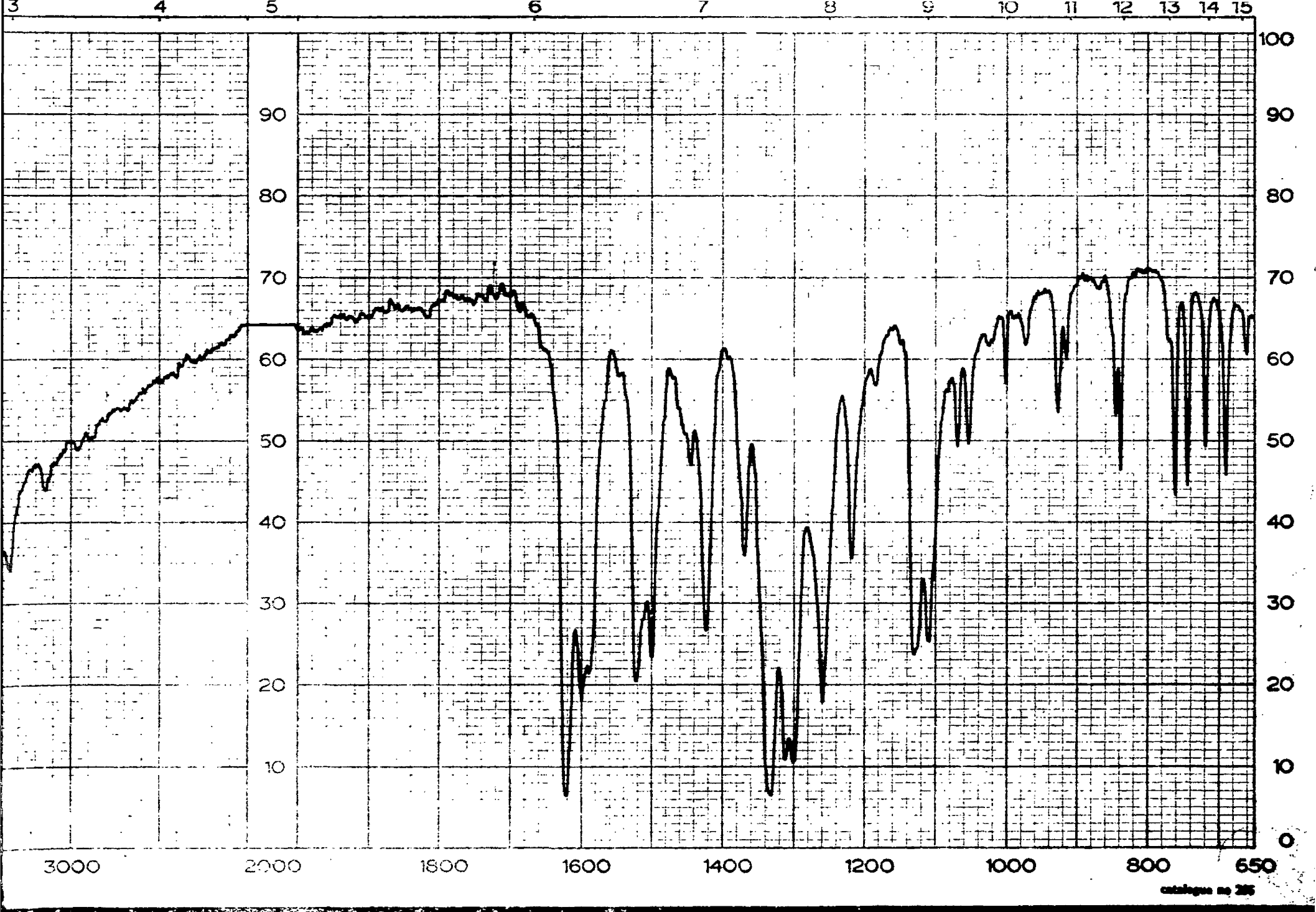
THICKNESS

REMARKS B.12.

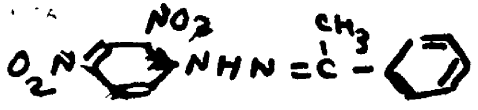
NUMBER 15

DATE 3-2-64

OPERATOR

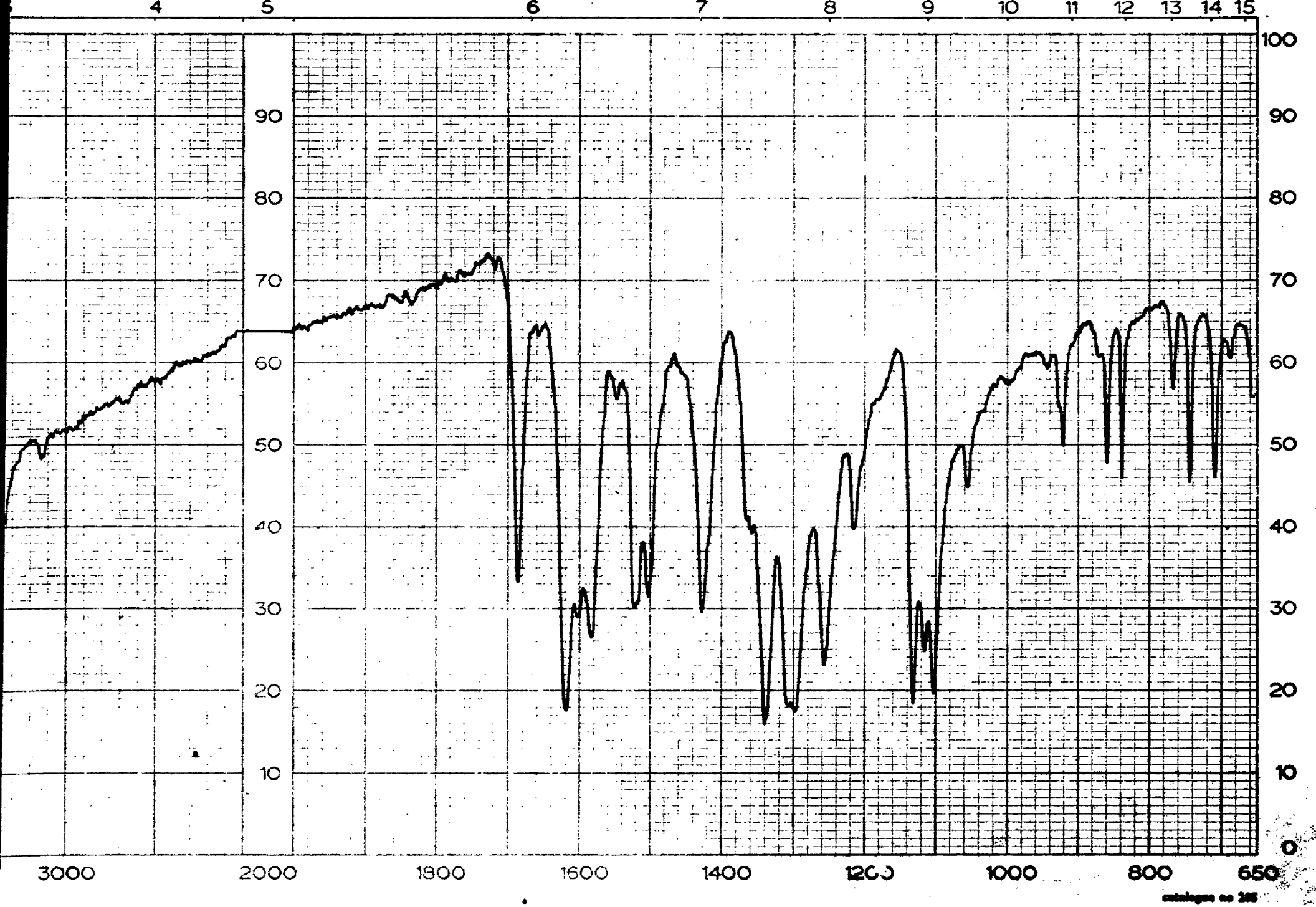


ACETOPHENONE
DNPH



PHASE solid, KCl
THICKNESS
REMARKS B.13.

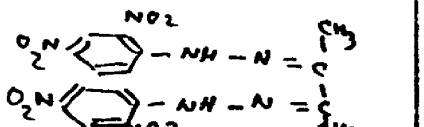
NUMBER 12
DATE 3-2-64
OPERATOR



catalogue no 208

DIACETYL DNPH

FORMULA



PHASE Solid, KCl

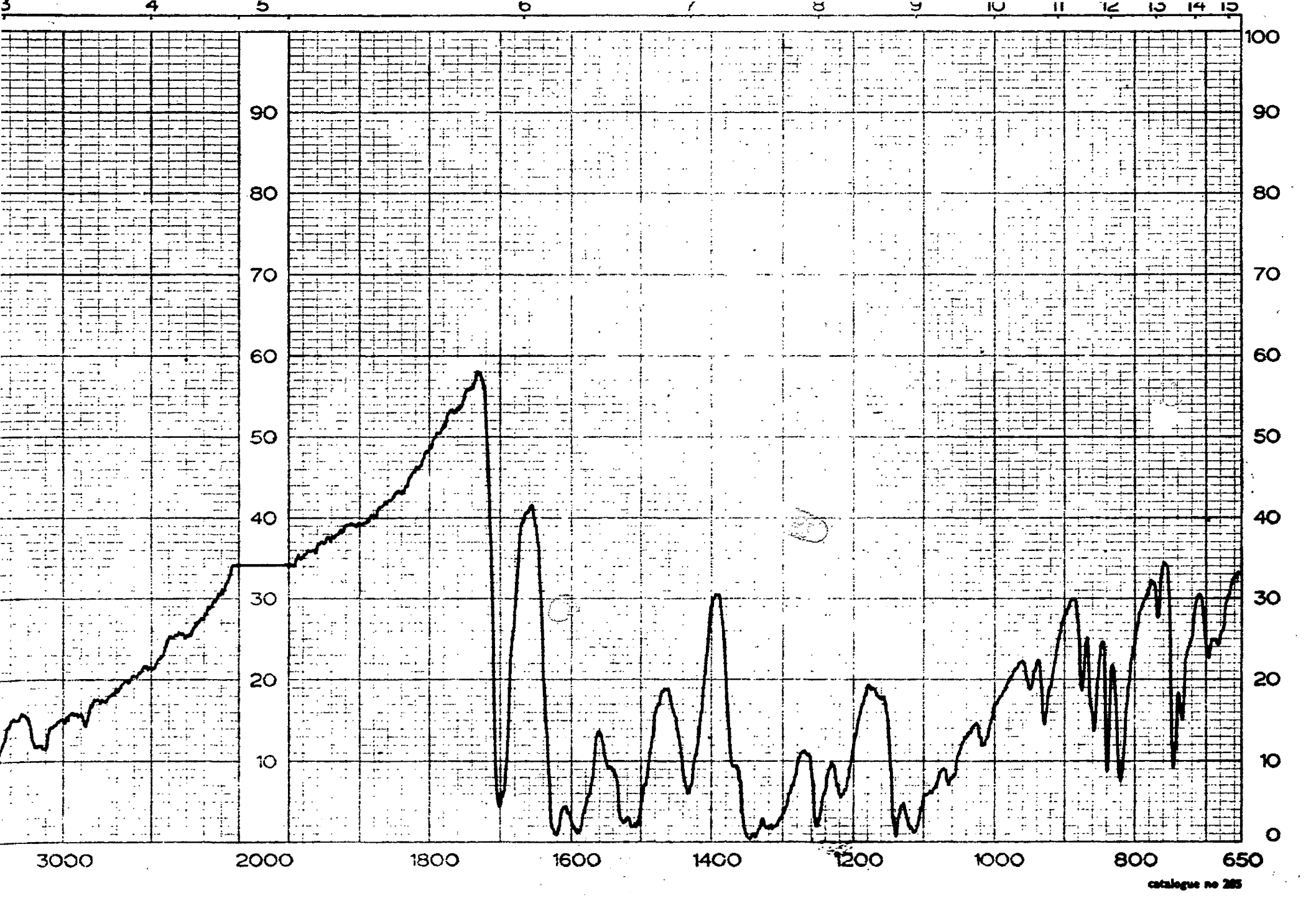
THICKNESS

REMARKS B.14.

NUMBER 11

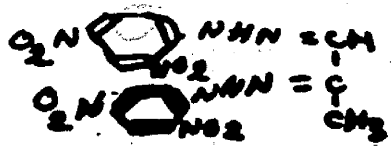
DATE 5·2·64

OPERATOR



PYRUVICALDEHYDE DNPH

FORMULA



PHASE Solid, KCl

THICKNESS

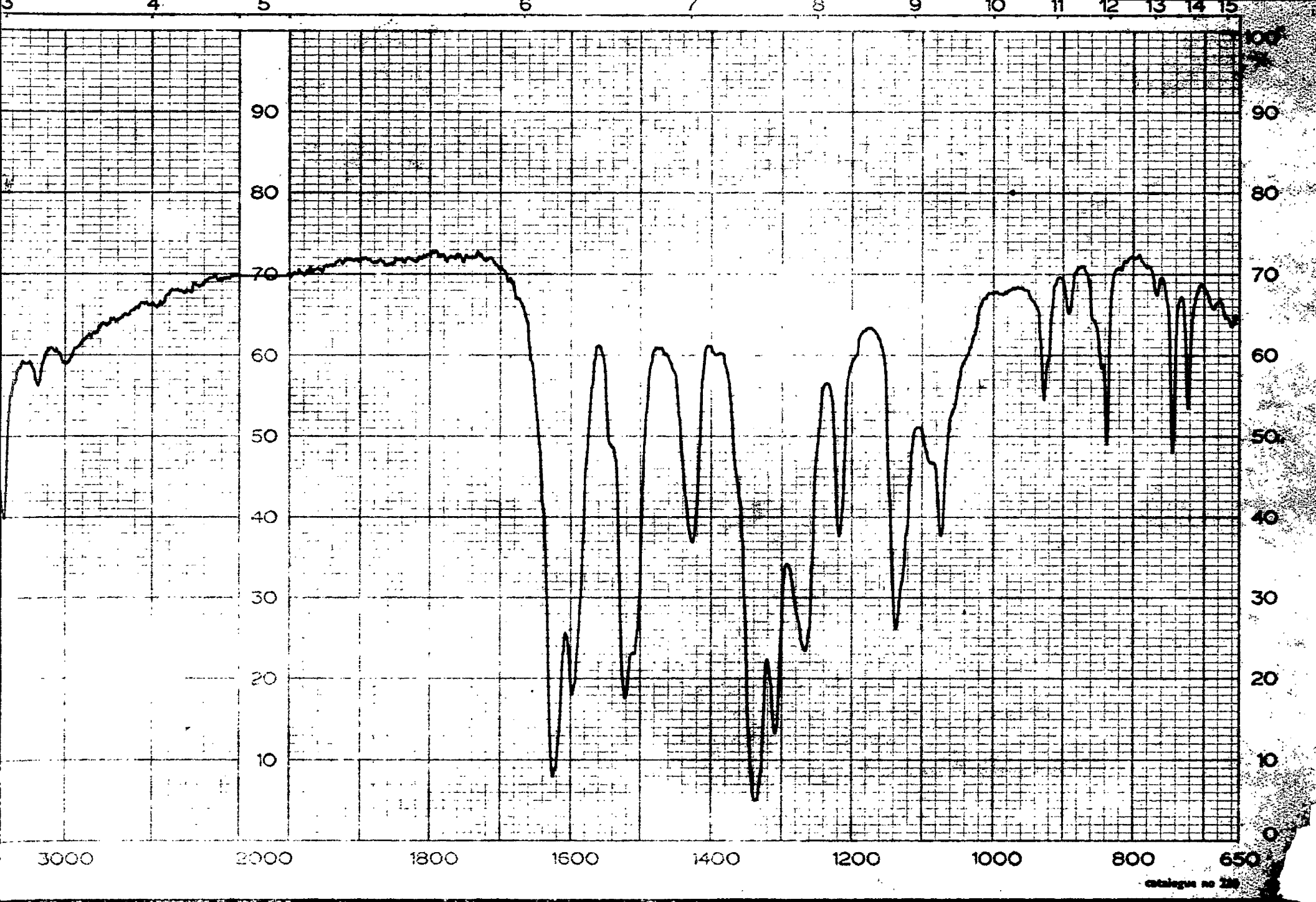
REMARKS B.15.

NUMBER 26

DATE 19.2.64

OPERATOR

REFERENCE

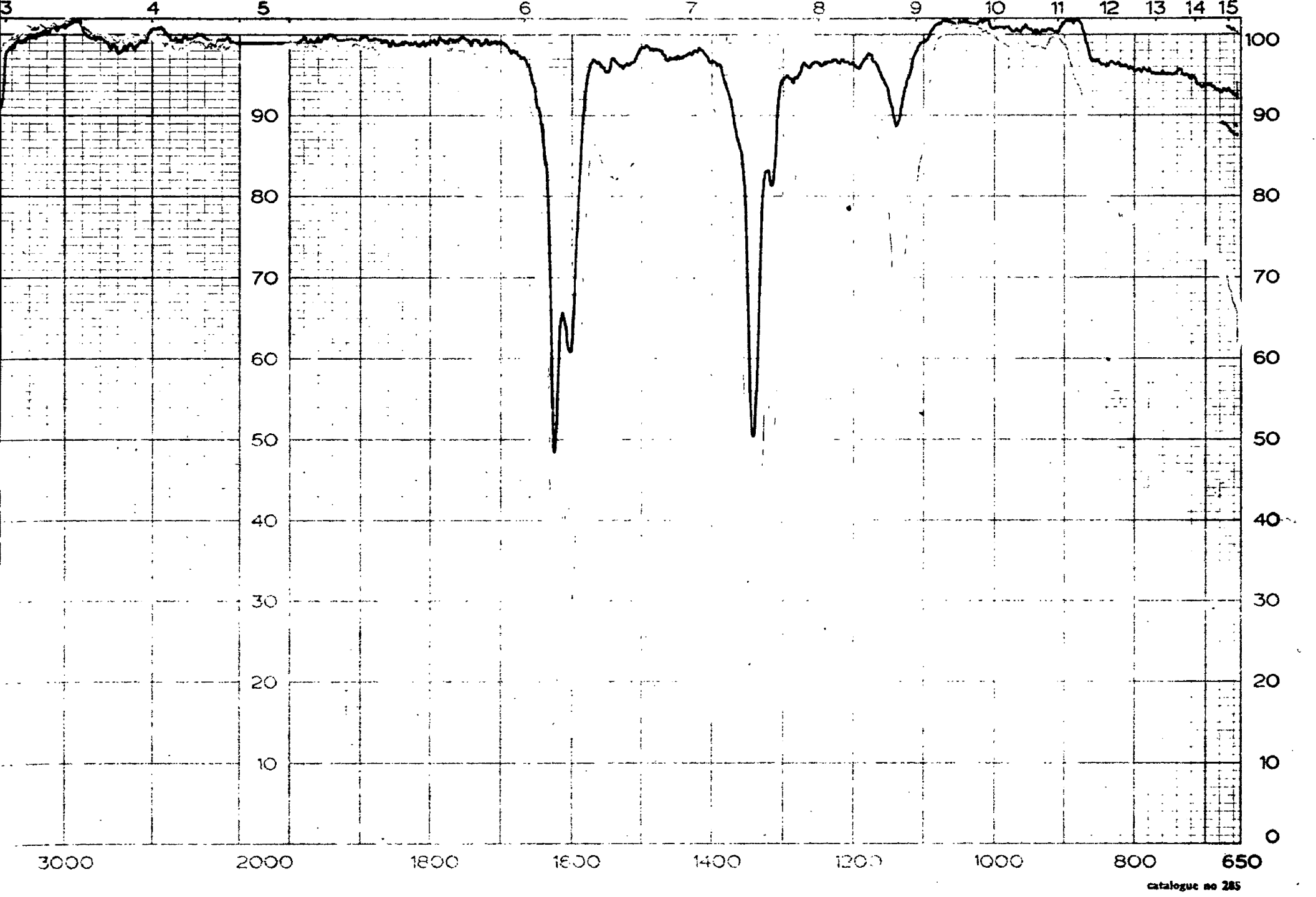


COFFEE V.C.C.
DMPH

FORMULA

PHASE solid, KCl
THICKNESS
REMARKS B.16.

NUMBER
DATE 25-2-64
OPERATOR



catalogue no 285

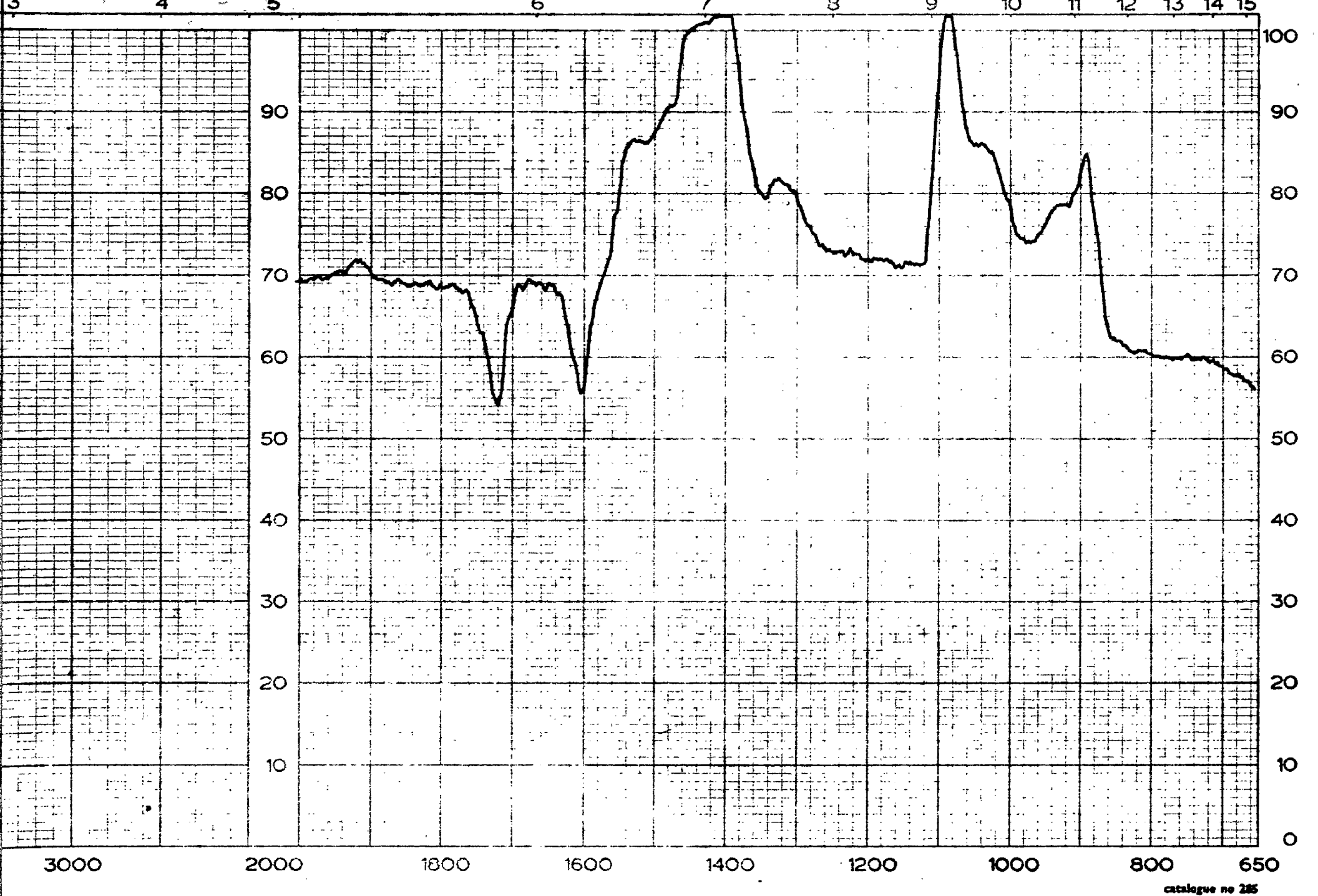
COFFEE V.C.C.
DNPH

FORMULA

PHASE liquid, $CHCl_3$
THICKNESS 1 mm.
REMARKS B.17.

NUMBER
DATE 25·2·64
OPERATOR

REFERENCE



catalogue no 285

FRACTION (7) DNPH THIN-LAYER.

FORMULA /

PHASE liquid, $CHCl_3$

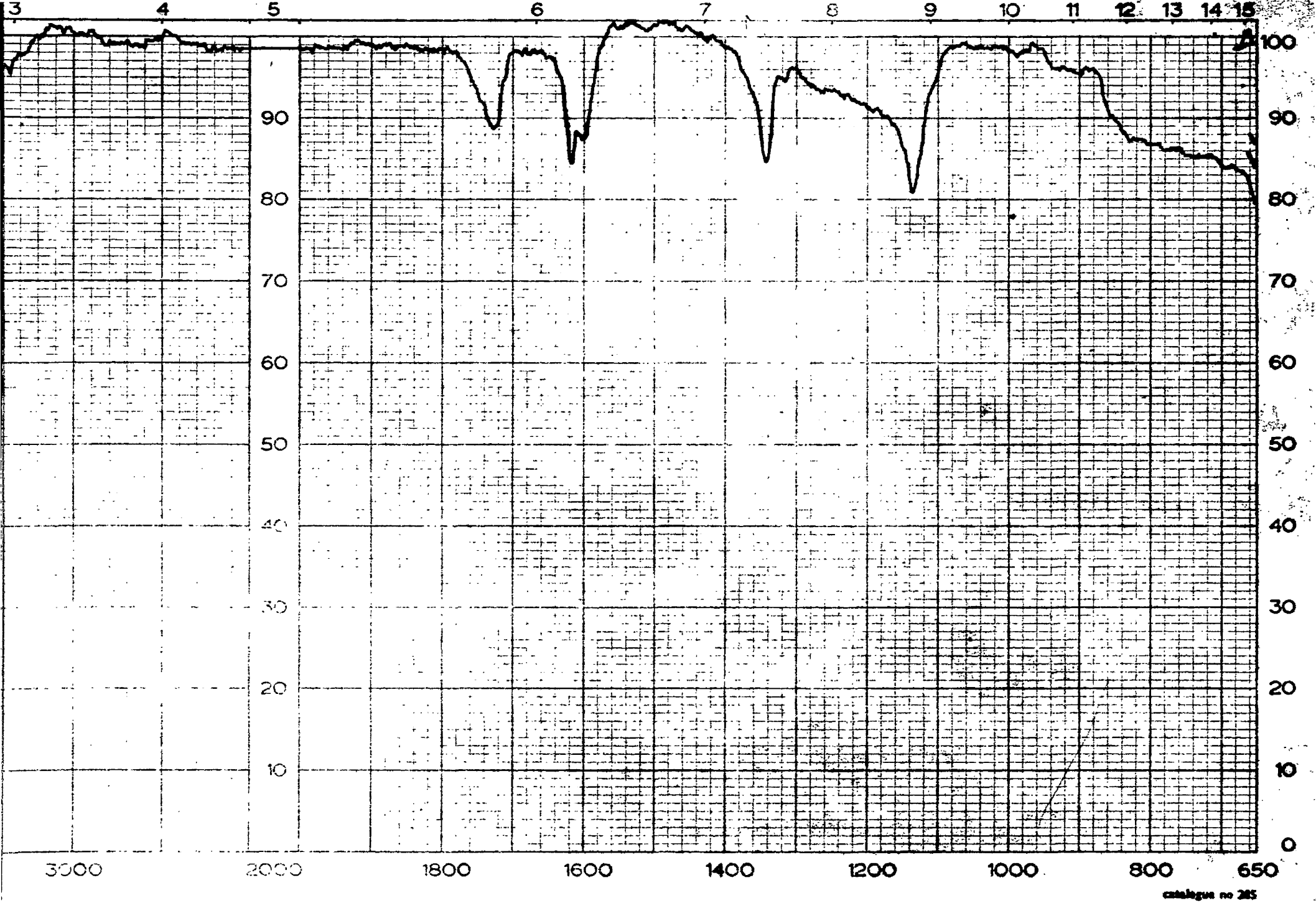
THICKNESS 1 cm.

REMARKS B.19.

NUMBER 7

DATE 23-4-64

OPERATOR



HEXANE INSOLUBLE
COFFEE DNPH

FORMULA

PHASE $CH Cl_3$
THICKNESS 1 mm
REMARKS B.20.

NUMBER
DATE 17-4-64
OPERATOR

A P P E N D E X (C)

DETERMINATION OF COMBINED VOLATILE ALDEHYDES.

ESTIMATION OF COFFEE VOLATILE ALDEHYDES

The available chemical determinations are either of more scale or are methods for specific aldehydes.[73] They consists of oxidation reactions, where the quantitative measurements are based on the amount of the consumed oxidising agent. Schiff's reagent has been suggested as a basis for rough quantitative work by measuring the colour in Nessler tubes.[35b]

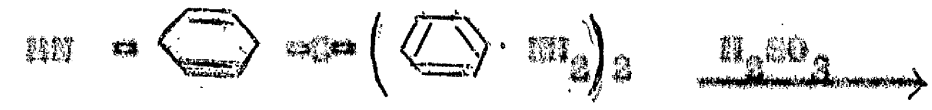
It was thought that the reaction of the coffee volatile aldehydes with Schiff's reagent might form the basis of an accurate and sensitive test.

Schiff's Reagent

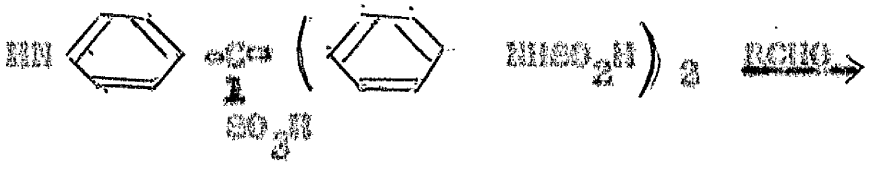
Was prepared by the ordinary method of decolorising the basic fuchsin with sulphur-dioxide,[33a] which may be prepared by using anhydrous sodium sulphite[34] or sodium hydrogen sulphite.[35a]

The sulphurous acid of the evolved SO_2 in aqueous solution, decolorise the dye, but the mechanism of the decolorisation and/ or of the restoring the colour when the aldehydes are added is not well known.

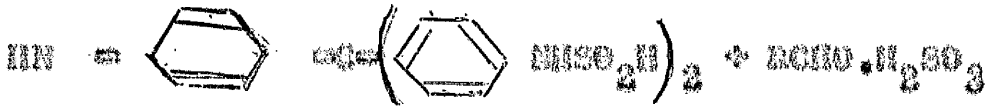
The following reactions are an approximation to the problem.[74]



(RED)



(colourless)



(PINK)

From here we see that the aldehydes restore the quinoid structure of the dye, and hence give the pink characteristic colour. Formaldehyde is exceptional in requiring up to six hours to develop the maximum intensity of colour.

Preparation of Schiff's Reagent

Dissolve 0.2 g. basic fuschin in 120 ml. hot water; cool and decolorize with a solution of 2 g. anhydrous sodium sulphite in 20 ml water; add a little charcoal and then 1 ml. conc. HCl. Store in a well stoppered amber glass bottle. This reagent can keep months for qualitative purposes, but fresh reagent is necessary when quantitative estimations are required.

Measuring "λ_0" at Max. absorption

A small quantity of acetaldehyde is mixed with 2 ml of Schiff's reagent in a 100 ml volumetric flask. The colour is allowed to develop (about 2 min) and the contents made up to volume with water.

The extinction was measured at different wave-lengths in a Unicam Sp. 700 spectrometer, using 1 cm Cell. The maximum absorption was found to be within 560 ± 5 mμ. Fig. (G_1).

The absorption maxima of different aldehydes

The following aldehydes were treated in the same way and all gave the same maximum Fig. (G_2); Formaldehyde, acetaldehyde, propionaldehyde, n-butyraldehyde and iso-butyraldehyde.

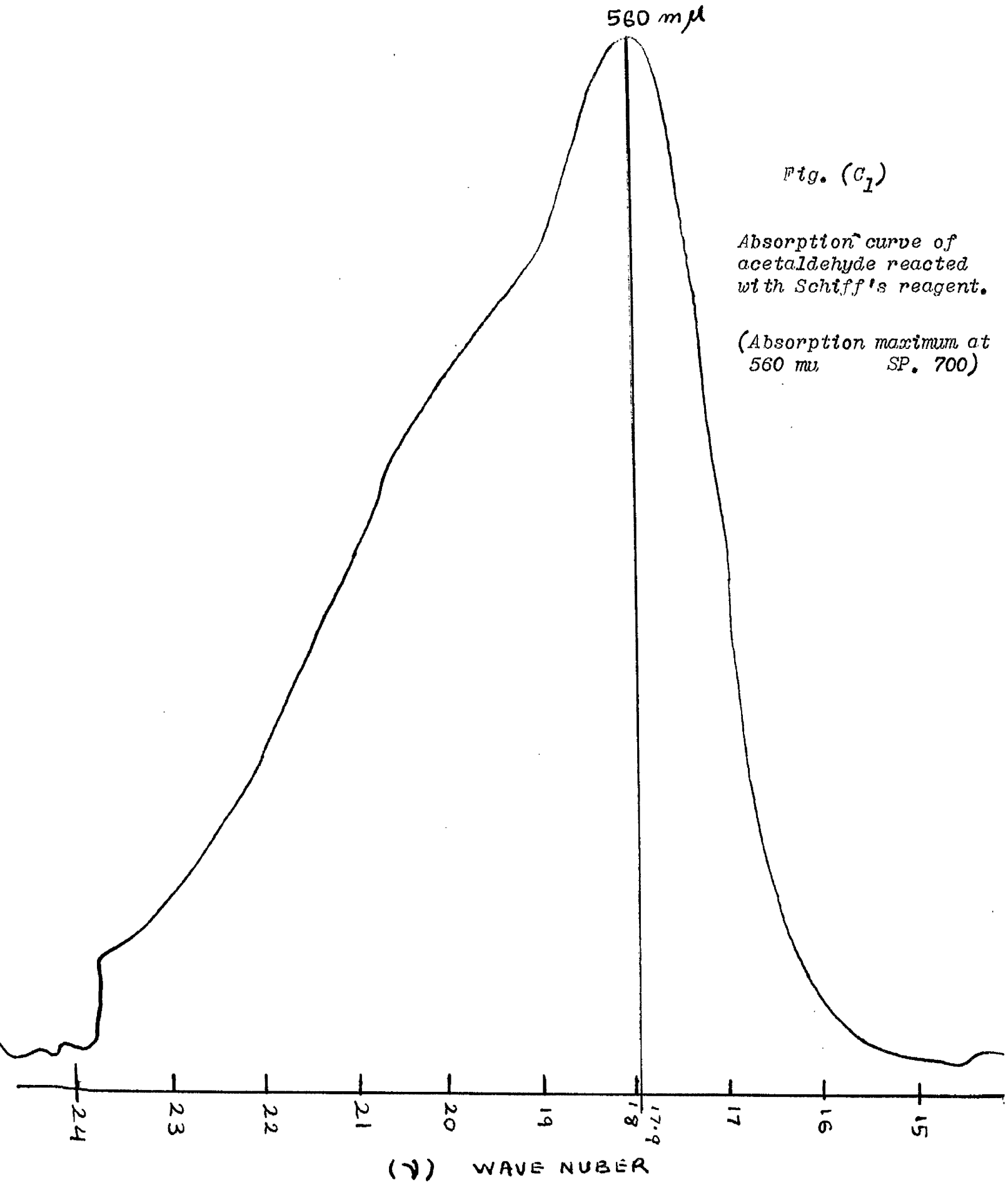
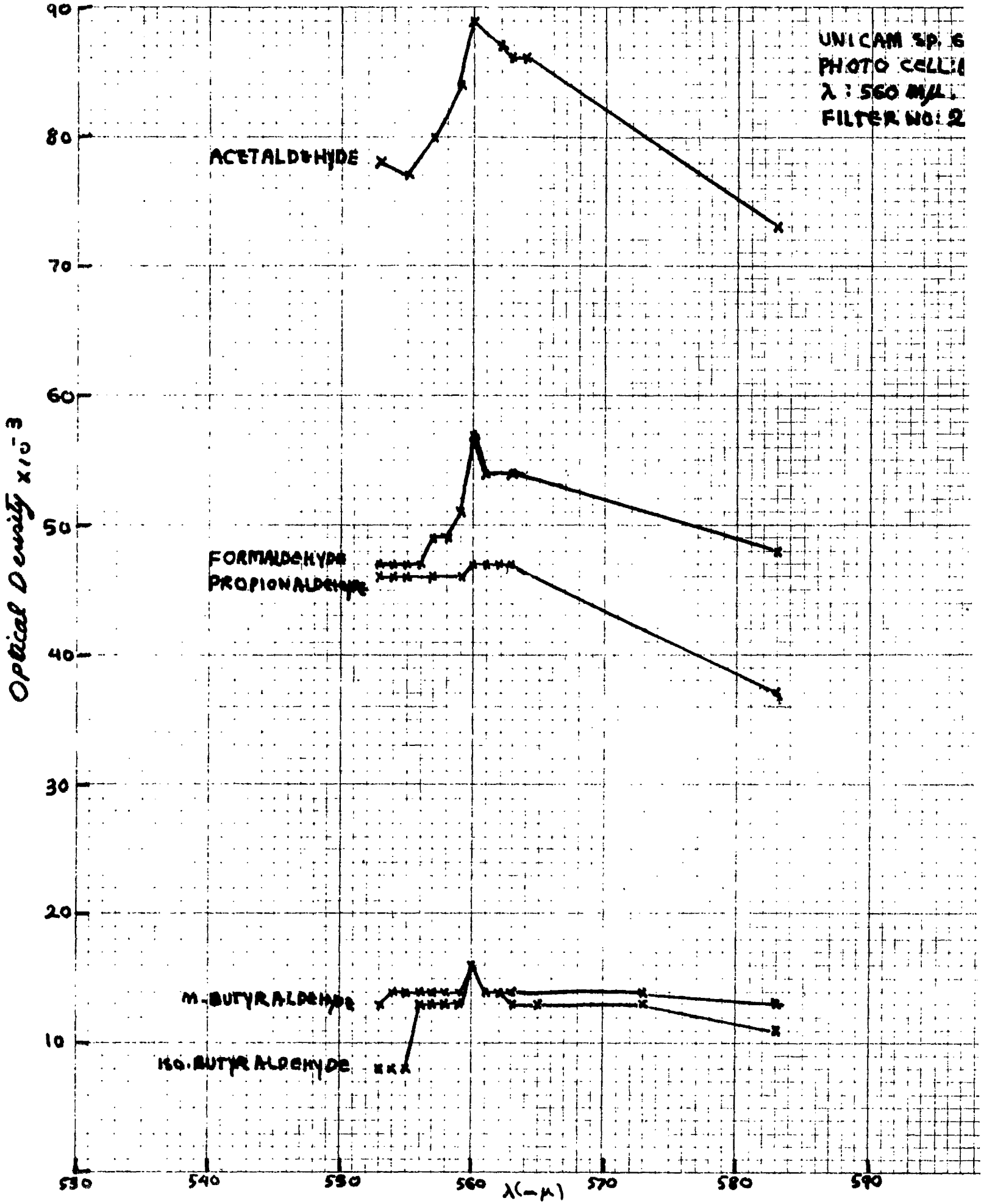


Fig. (C_1)

Absorption curve of acetaldehyde reacted with Schiff's reagent.

(Absorption maximum at 560 mμ SP. 700)

FIG. (C_2)


UNICAM SP. 6
PHOTO CELL:
λ : 560 mμ
FILTER NO: 2
ACETALDEHYDE
FORMALDEHYDE
PROPIONALDEHYDE
n-BUTYRALDEHYDE
Iso-BUTYRALDEHYDE
Optical Density x10⁻³
λ(—μ)
90
80
70
60
50
40
30
20
10
530
540
550
560
570
580
590

The Effect of the quantity of Schiff's reagent on the colour intensity

The quantity of Schiff's reagent varied from 0 - 30 ml, acetaldehyde being constant at 0.02 ml (lab. reagent). The results are shown in Table (1).

TABLE (1)

ml Schiff's reagent	Extinction x 10^{-3}
0	-
1	57
2	89
5	47
10	15
20	15

The optimum quantity of Schiff's reagent for maximum colour development is in the region of 2 ml. The reduction in intensity of colour using quantities greater than 2 ml is probably due to the excess of acid present.

Effect of p^H

Schiff's reagent was prepared and made neutral; 2 ml quantities were added to each of a series of 100 ml flasks and a constant quantity of acetaldehyde added to each. The contents of each flask was adjusted to give different p^H values, the volume made up to 100 ml, the optical density measured (Fig. C_3). The greatest intensity of colour at 560 ± 5 mμ was at $p^H 3$.

The Effect of Light

A sample in which the colour had been developed was divided into two, one portion placed in a clear flask and the other in an amber flask. The flasks were stoppered and left on the laboratory bench for 24 hours after which the depth of colour was measured. Fig. (C_4) shows that a greater degree of fading took place in the sample in the clear flask.

Time of Colour development

Fig. (5) shows that maximum colour development was reached after 45 - 65 min.

Fig. C3

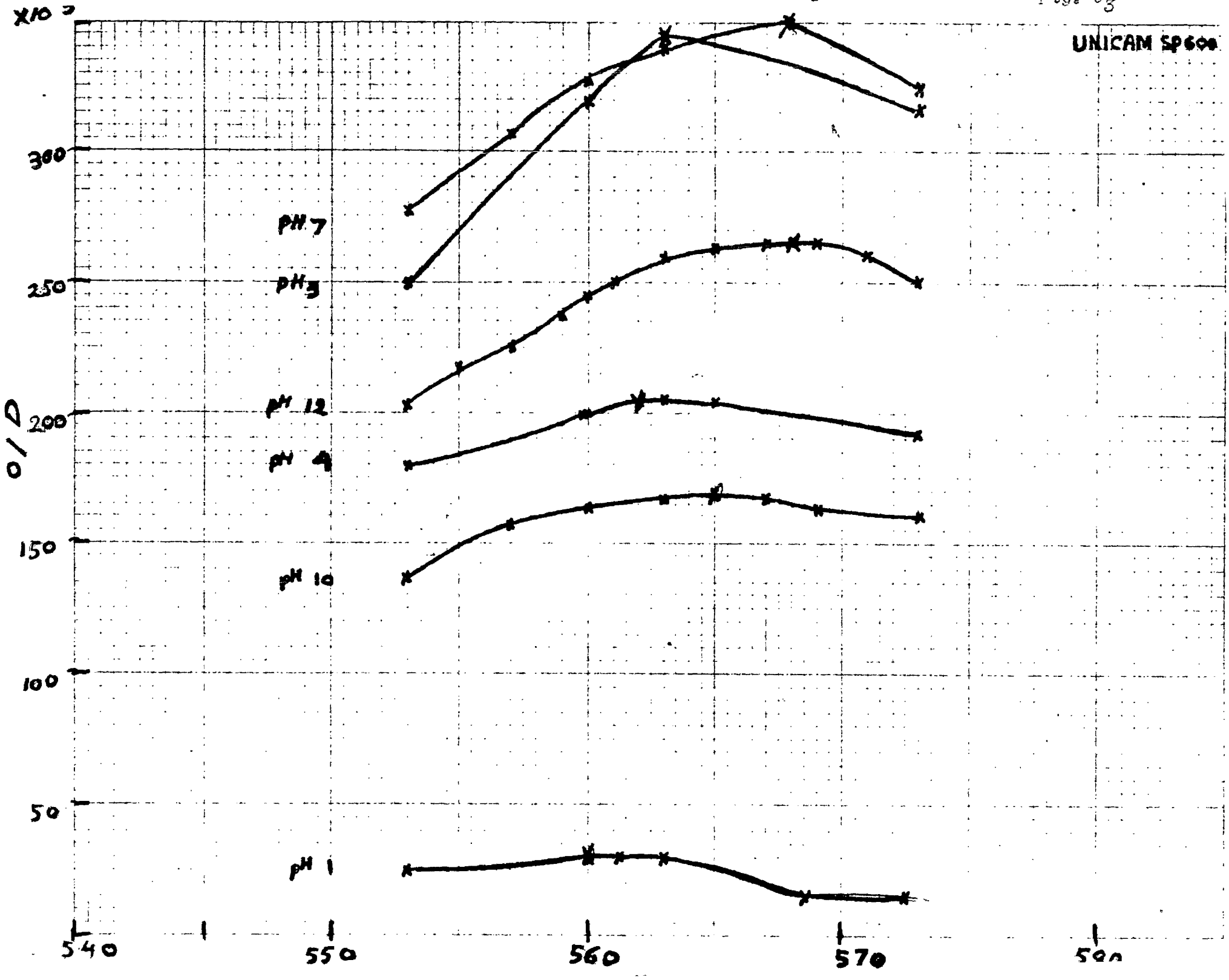

X10 -3
UNICAM SP600
300
250
200
150
100
50
O/D
pH 7
pH 3
pH 12
pH 4
pH 10
pH 1
540
550
560
570

Fig. (C_4)

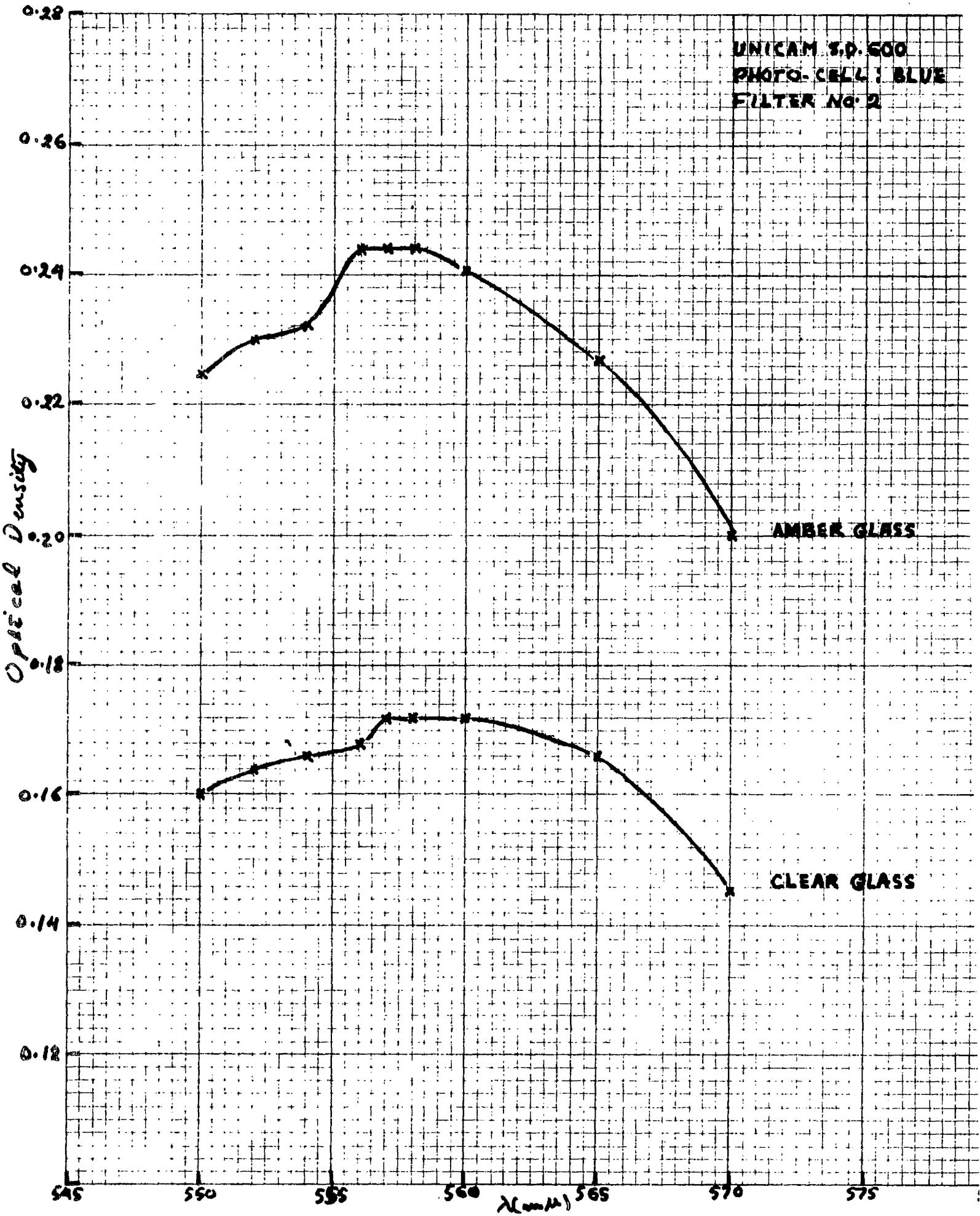
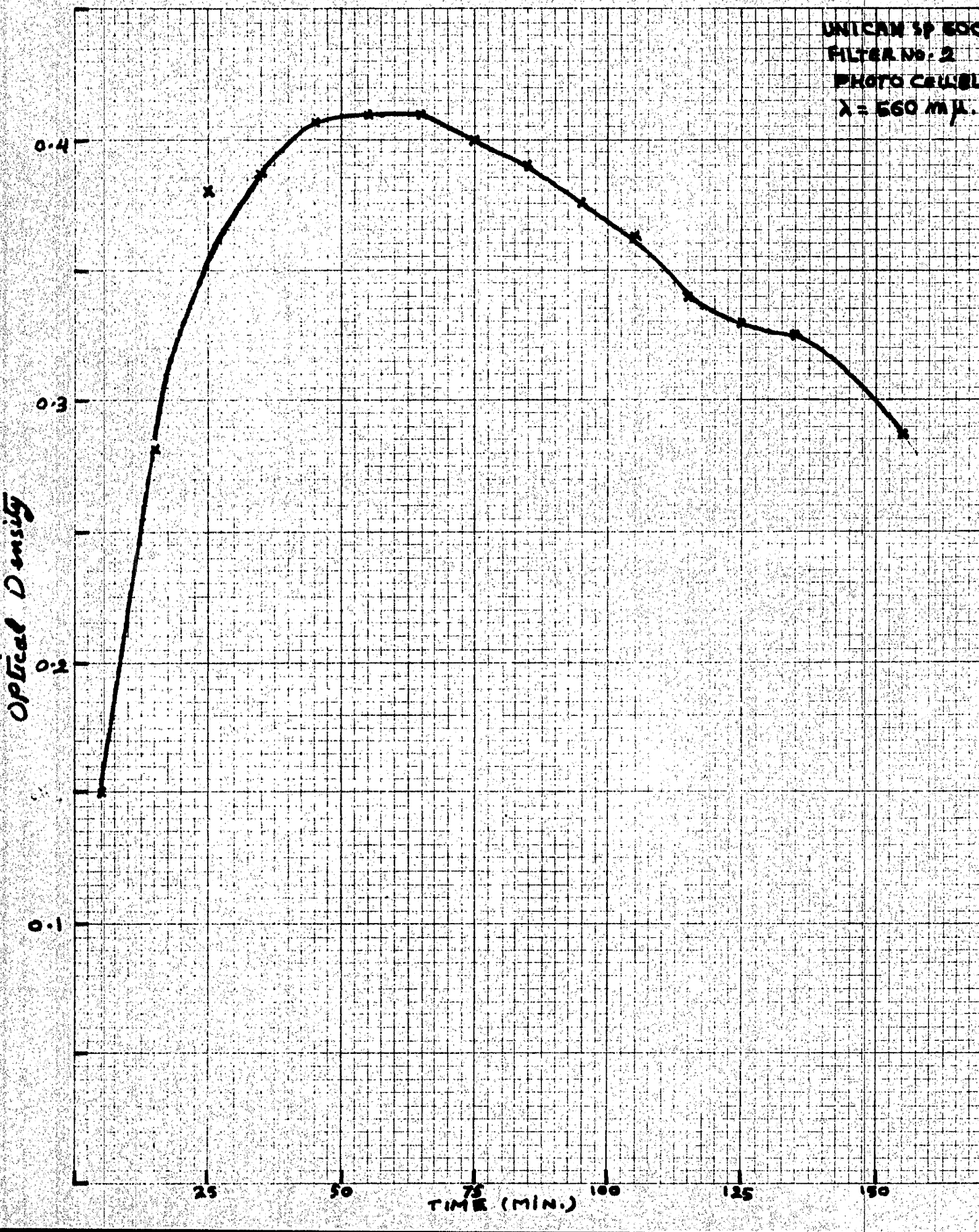

UNICAM S.P. 500
PHOTO-CELL : BLUE
FILTER No. 2
AMBER GLASS
CLEAR GLASS
Optical Density
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
545
550
555
560
565
570
575
λ(mμ)

Fig. (C_5)


UNICAM SP 500
FILTER No. 2
PHOTO CELL
λ = 560 mμ.
0.4
0.3
0.2
0.1
Optical Density
25
50
75
100
125
150
TIME (MIN.)

Suggested Procedure

On the basis of this preliminary work, the following procedure for the stimulation of aldehydes using Schiff's reagent is suggested.

To the aldehyde solution (sample of 1 - 100 ppm) add 2 ml of Schiff's reagent; make up to 100 ml with water in an amber volumetric flask stopper and leave to stand for 55 min; then take readings at = 560 m with the required filter and photo-cells, as recommended for that instrument.

A calibration curve for acetaldehyde was prepared in this way is shown in Fig. (C_6).

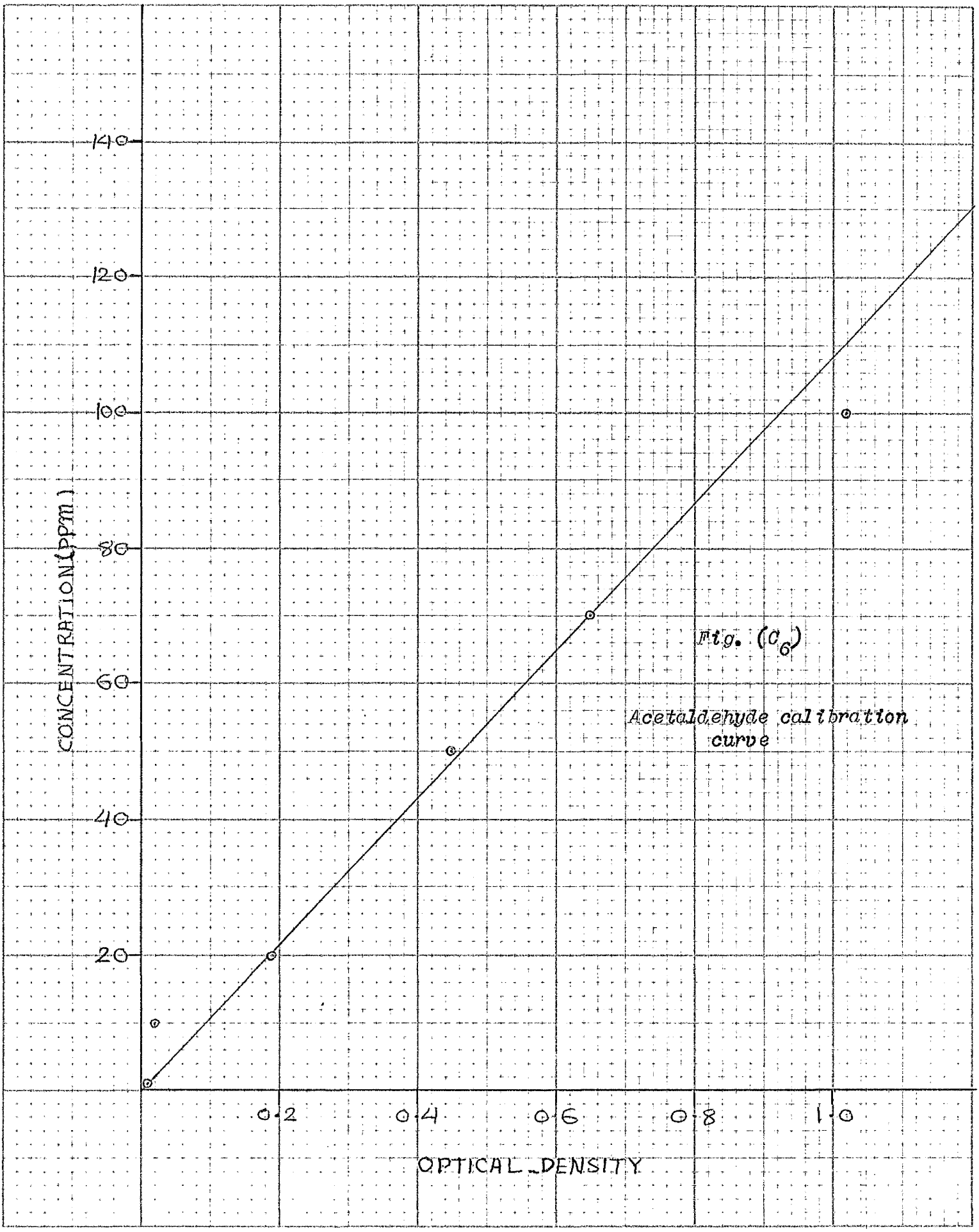


Fig. (C_6)

Acetaldehyde calibration curve

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