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BALLISTIC RESEARCH LABORATORIES

REPORT NO. 686

The Infrared Spectra of Carbohydrates

LESTER D. KUHN

ABERDEEN PROVING GROUND, MARYLAND

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LESTER P. KUHN

PROJECT NO. TB3-0110P OF THE RESEARCH AND
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MAY 1949

ABERDEEN PROVING GROUND, MARYLAND

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DUPLICATING BRANCH
THE ORDNANCE SCHOOL
ABERDEEN PROVING GROUND
MARYLAND

BALLISTIC RESEARCH LABORATORIES
REPORT NO. 686

Lester P. Kuhn
Aberdeen Proving Ground, Md.
7 April 1949

THE INFRARED SPECTRA OF CARBOHYDRATES

ABSTRACT

In seeking improved methods for analyzing nitrate esters of carbohydrates, including nitrocellulose, which are helpful in propellant compositions and for identifying decomposition products, the possibility of using infrared spectroscopy for this purpose was investigated. The spectra of a number of carbohydrates and their derivatives were obtained. The changes in the infrared spectra that accompany the thermal decomposition and the burning at low pressure of nitrocellulose are shown. The interpretation of these spectra is in agreement with information obtained by chemical methods.

THE INFRARED SPECTRA OF CARBOHYDRATES

The relatively slow decomposition which nitrocellulose undergoes at temperatures of about 100°C, and the burning of nitrocellulose at low pressures have engaged the attention of this laboratory for some time. In each of these problems considerable difficulty has been experienced in devising analytical procedures to measure the chemical changes that ensue. Since infrared spectroscopy has been found to be a valuable analytical tool in other fields of chemistry, an attempt has been made to appraise its usefulness for studying sugars and cellulose derivatives. Although this report is concerned with problems of analysis and identification, information relating to the molecular structure of the compounds studied can also be obtained.

The spectra of a large number of carbohydrates and their derivatives have been obtained with a Baird infra-red spectrometer. In most cases the substances were purified by re-crystallization until the melting point was constant. Substances which did not crystallize were measured by squeezing the viscous liquid between two rock salt or silver chloride plates. The technique used to prepare the samples of the crystalline substances was to dissolve them in a solvent such as water or methanol, and to put about 2 ml of the solution on a silver chloride window and evaporate to dryness in a vacuum oven. Samples prepared in this manner frequently give curves containing very broad and poorly resolved bands. Much sharper and better resolved bands were obtained when the solid sample was finely ground in mineral oil by rubbing with a muller on a ground glass plate. A comparison of the curves obtained by these two methods of preparing the sample is shown in Curve 1. The mineral oil has strong C-H bands at about 3.5 and 7 microns, but in the regions more interesting to the carboydrate chemist, around 6 and beyond 7.2 microns it is quite transparent. Each curve is marked with a symbol which indicates how the sample was prepared; "g" indicates that it was ground in mineral oil and "e" indicates that it was evaporated on the window from solution. The letter in brackets indicates the solvent used: W - water, C - chloroform, A - methanol, and P - pyridine. Each curve is numbered and the index for the curves is given in Table II. Curve 2 of glucose is typical of the sugar spectrum. There is a very strong band at about 3.1 microns due to the O-H stretching frequency, and in the region of 9 to 10 microns there are several closely spaced bands which are probably due to C-C and C-O vibrations. The effect of various functional groups which the carbohydrate chemist frequently encounters upon the spectrum is illustrated in the next four curves. Glucuronic acid, Curve 3, shows a very strong band at 5.6 to 5.8 microns, which is due to the C-O of the carboxyl group. Closer examination shows that this is actually two bands and it may be that the 5.6 band is due to a lactone carbonyl and that the 5.8 band is due to the carbonyl in a free carboxyl group. Glucal triacetate, Curve 4, has no free hydroxyls, hence the O-H band at 3.1 microns is missing. The carbonyl band due to the acetate groups again shows up strongly and a new band at 6.05 microns appears, which is due to the ethylene group. The strong band at 8 to 8.2 microns is characteristic of esters. The oxidation of many sugars with periodate results in a cleavage of the carbon chain and the newly formed primary carbon atoms appear as aldehyde groups. Curve 5 is for the oxidation product of alpha methyl glucoside, which has the structure CH_2OH-CH(CHO)-O-$CHOCH_3$(CHO) and it shows the band due to the C-O of the aldehyde group at about 6.2 microns. The nitrate bands show up very clearly in Curve 6 at 5.9, 7.7, and 11.8 microns. The assignment of these bands to the various functional groups has been done empirically and is the result of the study of a large number of

curves. The treatment of this subject by Barnes and co-workers[1] has been extremely helpful.

Below about 8 microns the structurally isomeric sugars show the same absorption bands, hence we have omitted this portion of the spectrum in the subsequent curves. The differences between these closely related compounds appear beyond 8 microns.

An examination of the curves shows that each compound has its own characteristic set of absorption bands and, except in one special case which will be discussed shortly, no two curves are identical. In some cases the bands are not as strong and do not show up as clearly as might be desired. This is generally due to a large amount of scattering of the incident light by the crystals of the sample, which results in a lack of contrast between the light which is absorbed and that which is transmitted. In the glycoside series the difference between the anomeric forms shows up very nicely. Thus the alpha methyl glucoside can be readily distinguished from the beta methyl glucoside (Curves 36 and 37), and in the methyl galactosides the alpha is again different from the beta compound (Curves 34 and 35). In the series of esters there is an interesting group of compounds consisting of the alpha acetates of glucose, cellobiose, cellotriose, and cellotetrose. In this polymeric homologous series (Curves 38 - 41), the monomer, the dimer and the trimer can be distinguished from each other. However, between the trimer and the tetramer there is very little difference, and between the tetramer and pentamer, which is not shown, there is no distinguishable difference. Apparently it is impossible to resolve the broad bands of the tri- and higher saccharides with our spectrometer, and any difference in detail which may exist between these spectra is invisible. The difference between the spectra of anomeric forms is again seen from a comparison of the curves for the alpha and beta forms of glucose pentaacetate (Curves 38 and 42) and for the two forms of methyl glucoside tetraacetate (Curves 43 and 44).

For mono- and di-saccharides, and for their derivatives, infra-red spectra may serve as a very useful tool for the determination of the presence of various functional groups for establishing the identity of the compound and for determining the presence of impurities. It has the advantage over other physical measurements such as melting point or optical rotation in that the spectrum is unique for each compound. Only a small sample is required, 15 mg, for evaporated films, and somewhat more for grinding in mineral oil, and the sample may be recovered. For this method to be useful for identification purposes it is necessary that the worker have at his disposal as complete a collection of curves of pure sugars as possible, and it is with this in mind that the accompanying curves were reproduced. The spectrum of any sugar is strongly influenced by the introduction of substituents. A comparison of the spectra of a series of derivatives of a given sugar reveals no band or arrangement of bands which is characteristic of the particular sugar. For this reason it has not been found possible to identify a substituted sugar by a comparison of its spectrum with that of the corresponding unsubstituted sugar, nor does it seem likely that a polysaccharide can be identified by comparison of its spectrum with the spectra of the monosaccharides of which it is composed.

Curves 73 to 79 give the spectra of various cellulose derivatives. The bands in the short wavelength end of the spectrum characteristic of the various functional groups show up very nicely but the bands corresponding to the C-C and C-O frequencies in the region of about 9 - 10 microns are very broad, as was the case with the tetrasaccharide discussed above. Curve 73 for nitrocellulose shows a

[1] Barnes et al., Infra-red Spectroscopy, Reinhold Publishing Co, (1944)

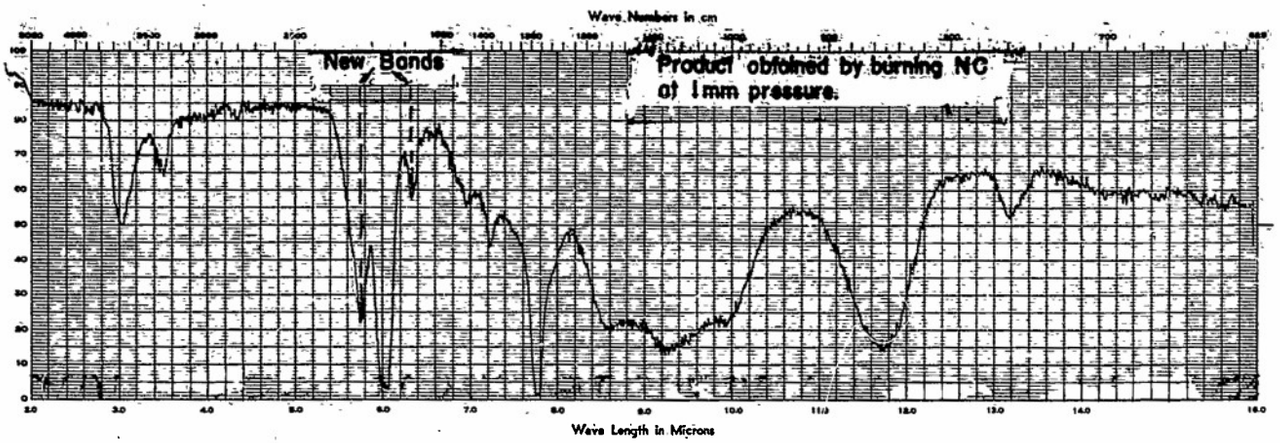
weak O-H band at 3 microns due to the fact that the cellulose is not completely nitrated, a C-H band at 3.45 and nitrate bands at 6.0, 7.8, 12, 13.3 and 14.3 microns. Cellulose was oxidized with NO_2 to polyglucuronic acid which was then nitrated. Curve 74 shows the spectrum of this nitrated polyglucuronic acid which has the new band due to the C=0 of the carboxyl group at 5.75 microns. A commercial sample of methyl cellulose is shown in Curve 75. It too shows a band of 5.7 microns indicating the presence of carboxyl groups which must have been formed by oxidation of the cellulose in the manufacturing process. In the curve for cellulose-6-trityl ether the aromatic C-H band appears at 3.3 microns, and several bands due to benzene rings at 6.3, 6.65 and in the region of 13 and 14 microns can be seen.

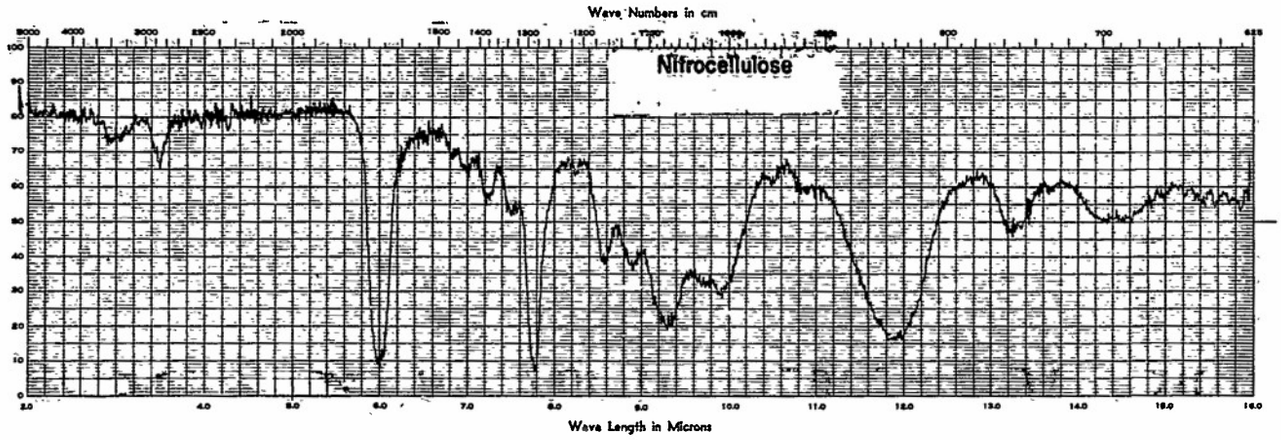
A comparison of the infrared spectra of nitrocellulose and two of its decomposition products is shown in Fig. 1. When nitrocellulose is heated at about 130 C a slow decomposition takes place and after about a day the material shows a new absorption band at about 5.7 microns which is due to the presence of the carboxyl group. This is the same band that appears in the polyglucuronic acid which is obtained when cellulose is oxidized with NO_2 (see Curve 74). It is also apparent that the ratio of the intensities of the OH to CH bands at about 3 and 3.5 microns respectively is greater in the heated material than in the original which indicates that new OH groups were formed or that CH groups have disappeared. The white solid decomposition product that is obtained when nitrocellulose is burned at a pressure of about 1 mm shows an infrared curve which has even stronger OH and C=0 bands than the heated material and which has a new band at about 6.2 microns. This band is tentatively assigned to the C=0 of an aldehyde group. As previously stated, such groups are produced in carbohydrates by oxidation with periodic acid, however the physical properties of the product obtained from the oxidation of cellulose with this reagent are such that no satisfactory infrared curve could be obtained. The evidence that this band is due to the carbonyl group was obtained by oxidizing alpha methyl glucoside with periodic acid to give a compound containing aldehyde groups and showing a new band at 6.2 microns (see Curve 5). These findings are in agreement with the results obtained by chemical methods by Professor Wolfrom and coworkers at Ohio State University.

To determine the possibility of using the infra-red spectrum for the quantitative determination of a functional group - in this case the nitrate group - we obtained the curves for several different nitrocelluloses of known nitrate content. Using the equation for Beer's law,

$$\log I_o/I = k\,c\,d$$

where I_o and I are the intensities of the incident and transmitted light respectively, k is the extinction coefficient, c is the concentration, and d the thickness of the sample, we attempted to see if k is constant. Since c has been determined by an independent method, d can be measured, and $\log I_o/I$ can be obtained from the height of the absorption band the equation can be solved for k. If k is known for a given absorption band, and has been found to be constant, then we can use this value of k for the analysis of an unknown and solve the equation for c. To secure good accuracy the absorption band should be of such strength that 30 to 70 percent of the light is absorbed. The nitrate band at 6 microns is so strong that even for very thin films it showed almost complete absorption. The O-H band at 3 microns is much weaker and more satisfactory for our purpose; furthermore, it has the advantage that for highly nitrated celluloses small differ-


Wave Numbers in cm
New Bands
Product obtained by burning NC at 1mm pressure.
Wave Length in Microns


Wave Numbers in cm
Nitrocellulose
Wave Length in Microns

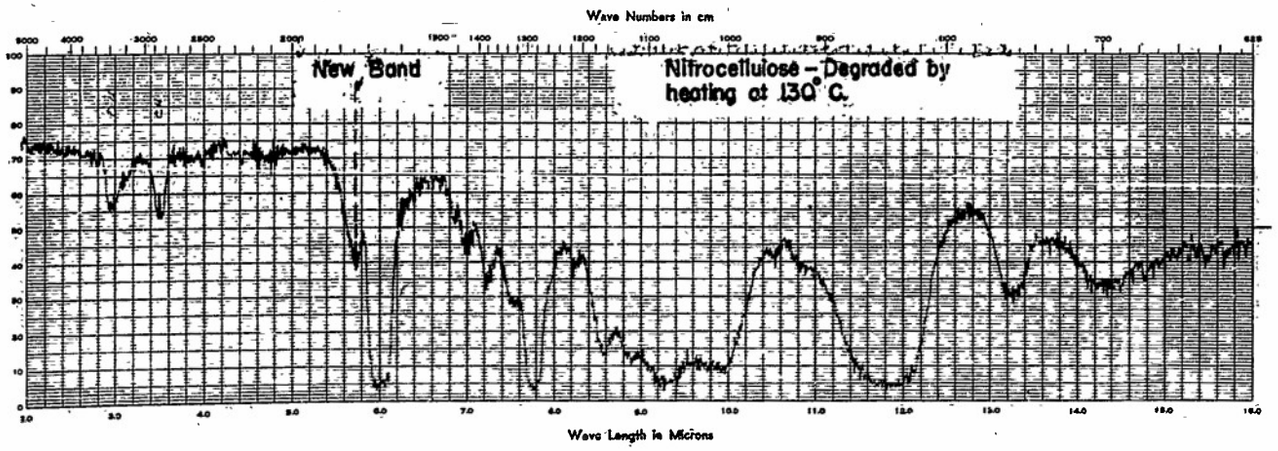

Wave Numbers in cm
New Band
Nitrocellulose – Degraded by heating at 130° C.
Wave Length in Microns

Figure 1

ences in nitrate content are accompanied by relatively large differences in percent of OH. Knowing the OH content, the nitrate content can of course be readily calculated. Films of such thickness as to give the OH band an absorption of about 50 percent were used. It was found that the value of k was not constant and one of the reasons for the lack of constancy is the difficulty of accurately measuring the film thickness, d, especially since the films are generally not of uniform thickness. To circumvent this difficulty we used the ratio of the OH and CH bands. If we write the Beer's law equation for the OH band and for the CH band

$$\frac{(\log I_o/I)_{OH}}{(\log I_o/I)_{CH}} = \frac{k_{OH}\, c_{OH}\, d}{k_{CH}\, c_{CH}\, d} \qquad \text{II}$$

$$\frac{(\log I_o/I)_{OH}}{(\log I_o/I)_{CH}} = k^* c_{OH} \quad \text{where } k^* = k_{OH}/k_{CH}\, c_{CH} \qquad \text{III}$$

and if we divide the equation for the OH band by the equation for the CH band, we get a new equation II in which the d's cancel, and which can be simplified to give equation III if we assume that c_{CH} is independent of the nitrate content which is probably not too bad an assumption since the number of CH bonds per molecule is independent of the number of nitrate groups. The scattered radiation was corrected for by taking as the value of I_o not 100 but the percent of light transmitted at a wavelength at which the nitrocellulose has no absorption band, namely 2.7 microns. The nitrocellulose films were made by evaporation of ethyl acetate solutions. To remove the solvent completely the films were dried for 48 hours in a vacuum oven at 50° C; however, the curves showed a strong band at 5.7 microns, indicating that solvent was still present. By soaking the films for several hours in ether or in water and then drying again, the curves obtained no longer showed the carbonyl band. It is quite possible that the ether and the water are also retained by the film but they are not readily detected from the infra-red curves. It was found that soaking the film in hexane and drying did not result in the removal of the ethyl acetate. The results from eight different samples are given in Table I. These films were soaked in ether. The variation in K* which amounts to about 8 percent is probably due to the variation in the amount of solvent retained by the films. The nitrate group can be determined quite conveniently by chemical methods, but for groups which cannot be determined conveniently by chemical methods the spectroscopic method may be advantageous.

SUMMARY

The infrared spectra of a number of carbohydrates and derivatives thereof have been obtained. Anomeric and epimeric forms are readily distinguishable. Functional groups are easily recognized. The appearance of new functional groups from the thermal decomposition and the burning at low pressure of nitrocellulose is demonstrated. A method for the quantitative determination of nitrate groups in nitrocellulose has been developed.

The author is indebted to Professor M. L. Wolfrom, of Ohio State University, for supplying most of the sugar samples, and to the DuPont Company for the nitrocellulose samples.

Lester P. Kuhn

Lester P. Kuhn

TABLE I

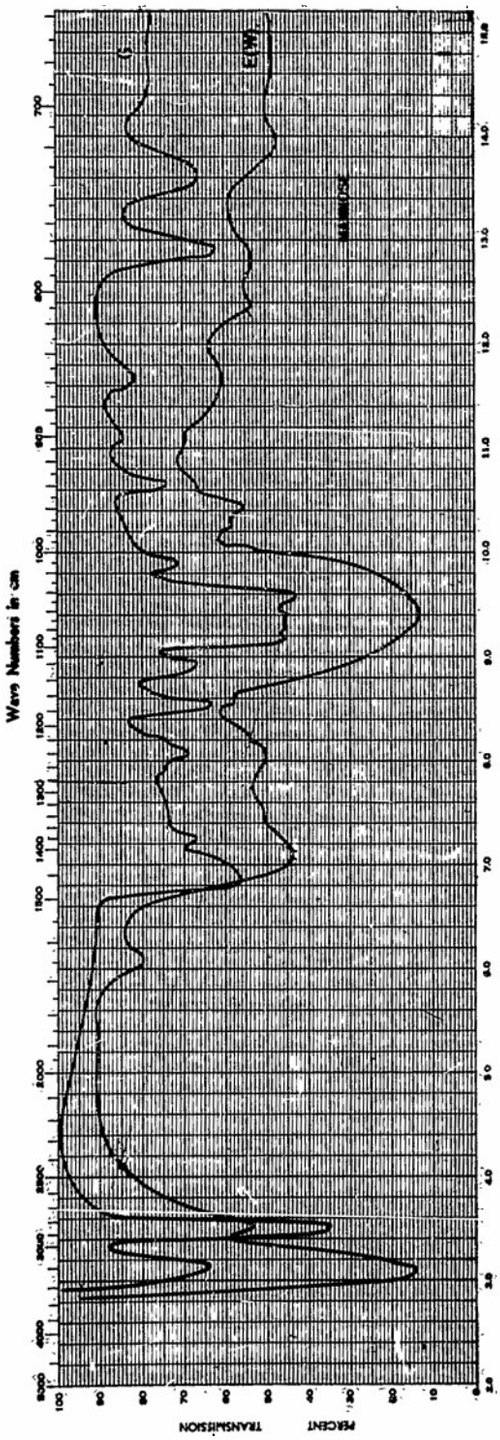
Determination of Extinction Coefficient

Sample	% N	# OH groups Per glucose Unit	$(I_o/I)_{OH}$	$(I_o/I)_{CH}$	$\log(I_o/I)_{OH}$	$\log(I_o/I)_{CH}$	$\frac{\log(I_o/I)_{OH}}{\log(I_o/I)_{CH}}$	k*
5	10.87	1.065	79/19.5	79/25	.607	.500	1.21	1.13
9	11.35	.925	80.5/22	80.5/25	.562	.505	1.11	1.05
3	11.88	.77	82/33	82/26	.398	.490	.814	1.07
4	11.96	.75	86/34.4	86/27.8	.394	.490	.805	1.07
6	12.60	.555	75/42.5	75/31.5	.267	.433	.617	1.11
10	12.66	.53	88/64.3	88/52	.137	.228	.603	1.13
2	13.4	.28	83/68	83/42.2	.086	.292	.294	1.05
8	13.4	.28	78.5/60	78.5/31.5	.117	.396	.295	1.05

TABLE II

Index of Absorption Curves.

Curve No.	Compound	Curve No.	Compound
1	D-Mannose	41	alpha Cellotetraose Quatradecaacetate
2	D-Glucose	42	beta D-Glucose Pentaacetate
3	D-Glucuronic Acid	43	Methyl alpha D-Glucoside Tetraacetate
4	D-Glucal 3, 4, 6-Triacetate	44	Methyl beta D-Glucoside Tetraacetate
5	Methoxy formyl methyl 1- formyl 2- hyroxy ethyl ether	45	Methyl beta D Galactoside Tetraacetate
6	Sucrose Octanitrate	46	Levoglucosan Triacetate
7	D-Ribose	47	Alpha L-Arabinose Tetraacetate
6	L-Arabinose	48	Sorbitol Hexaacetate
9	D-Xylose	49	Mannitol Hexaacetate
10	D-Galactose	50	3-Methyl D-Glucose Tetraacetate
11	alpha D-Glucose	51	Lactose Octaacetate
12	L-Sorbose	52	D-Galactose Dimethylacetal Pentaacetate
13	L-Rhamnose	53	D-Galactose Diethylmercaptal Pentaacetate
14	L-Fucose	54	L-Rhamnose Diethylmercaptal Tetraacetate
15	Maltose	55	D-Glucose Diethylmercaptal Pentabenzoate
16	Mellibiose H_2o	56	3-Methyl Glucose Tetrabenzoate
17	Cellobiose	57	3-Methyl D-Glucose
18	Trehalose	58	2, 3, 4, 6-Tetramethyl D-Galactose
19	D-Lactose	59	2, 3, 4, 6-Tetramethyl D-Glucose
20	Raffinose	60	2, 3, 4, 6-Tetramethyl D-Mannose
21	Erythritol	61	Methyl tetramethyl D-Glucoside
22	Xylitol	62	Methyl tetramethyl alpha D-Mannoside
23	Mannitol	63	Methyl 2, 3, 6-triethyl beta D-Glucoside
24	Sorbitol	64	Levoglucosan
25	Fucitol	65	D-Galactose Oxime
26	i-Inositol	66	D-Mannose Oxime
27	Dulcitol	67	L-Rhamnose Diethylmercaptal
28	Arabino Lactone	66	D-Galactose Diethylmercaptal
29	D-Glucono delta-Lactone	69	Diacetone D-Glucose
30	Methyl alpha Methyl D-Galacturonoside	70	Monoacetone D-Glucose
31	Alpha D-Gluconoleptonolactone	71	D-Glucosamine Hydrochloride
32	Methyl beta D-Xyloside	72	Kojic Acid
33	Methyl alpha D-Mannoside	73	Nitrocellulose
34	Methyl alpha D-Galactoside H_2o	74	Cellulose Oxidized with NO_2 and Nitrated
35	Methyl beta D-Galactoside	75	Methyl Cellulose
36	Methyl alpha D-Glucoside	76	Cellulose Acetate
37	Methyl beta D-Glucoside	77	Trityl Cellulose
36	alpha D-Glucose Pentaacetate	76	6-Trityl 2, 3-Diphenylurethane Cellulose
39	alpha Cellobiose Octaacetate	79	6-Trityl Cellulose Acetate
40	alpha Cellotriose Undecaactetate		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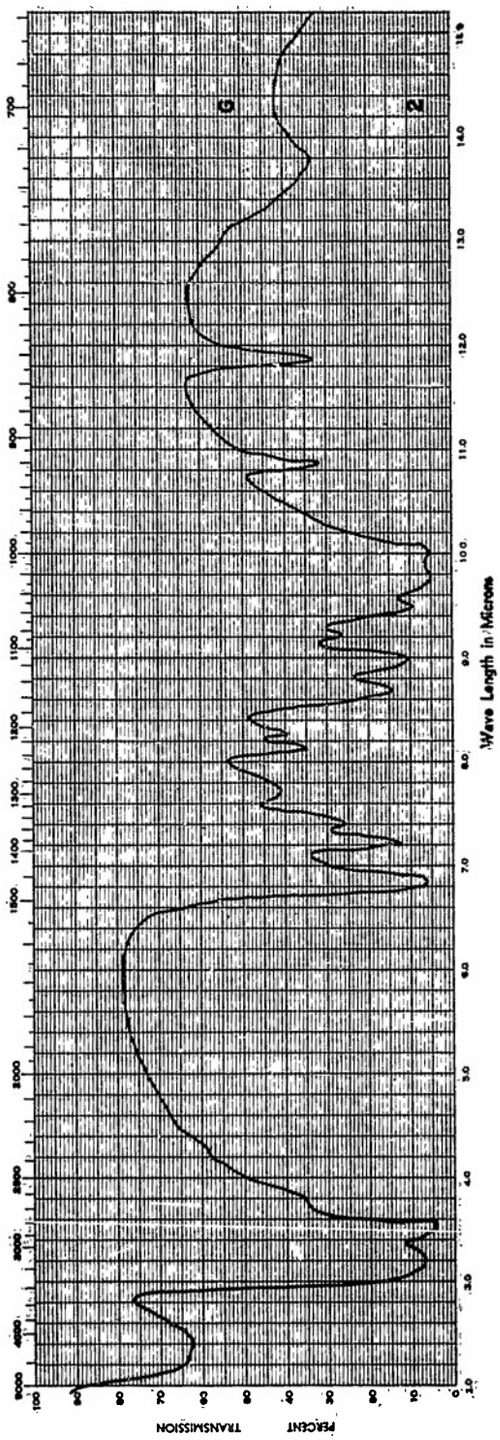
E(W)
G


Wave Length in Microns
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G
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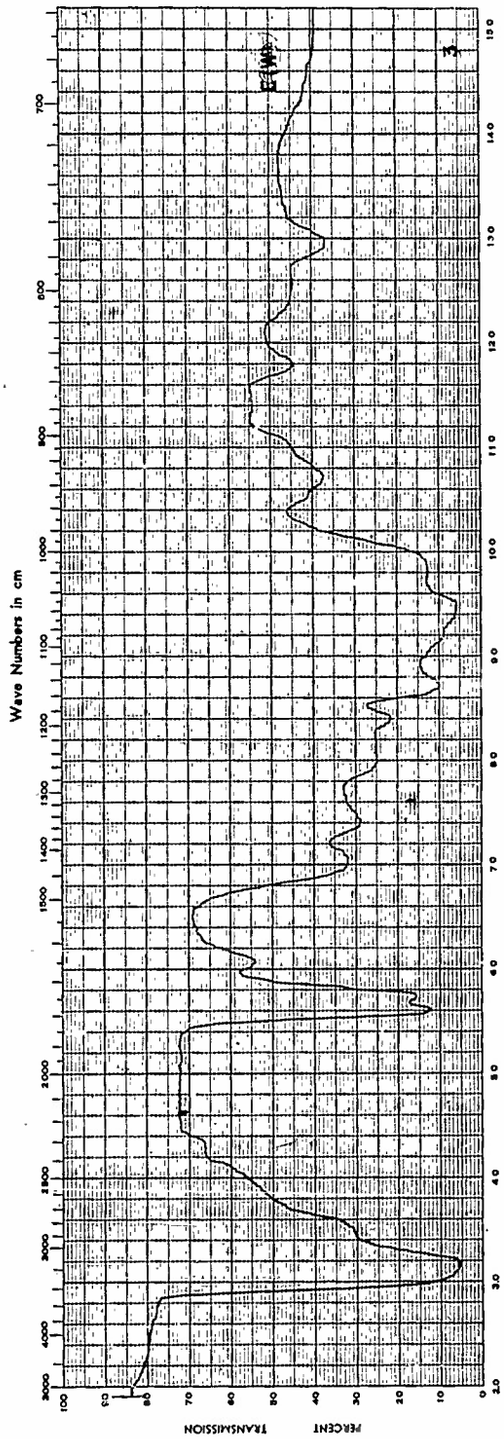
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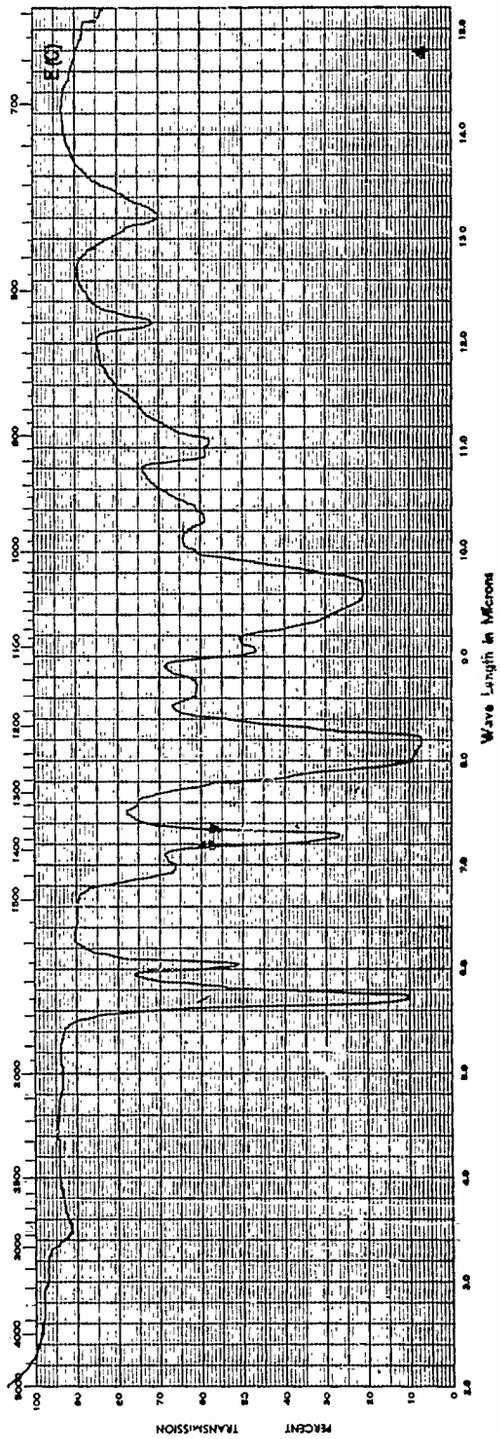
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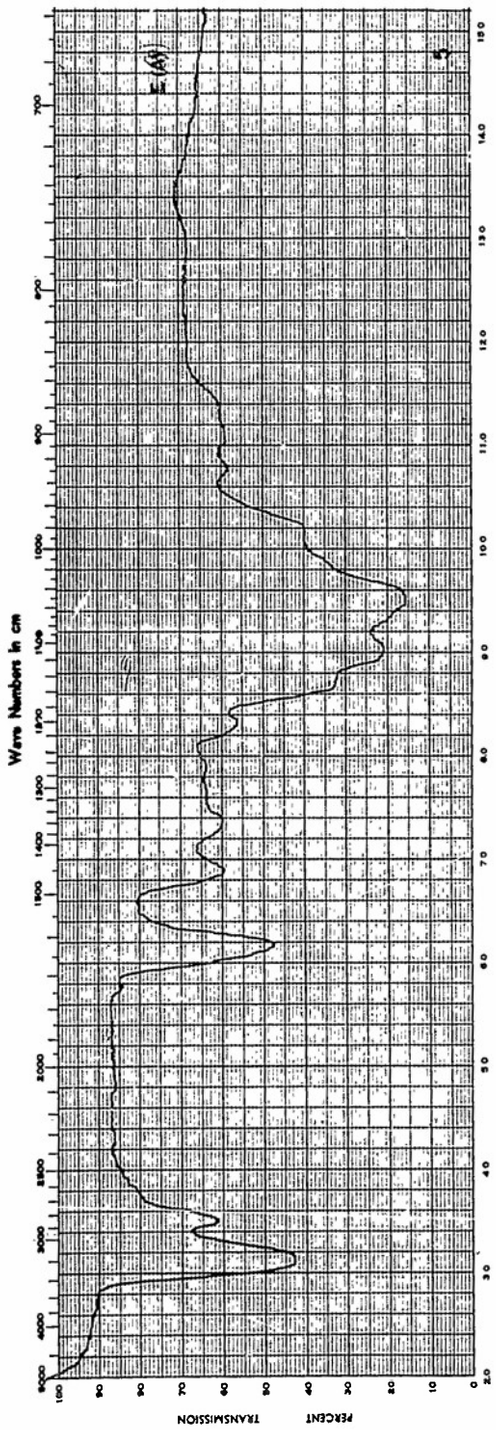
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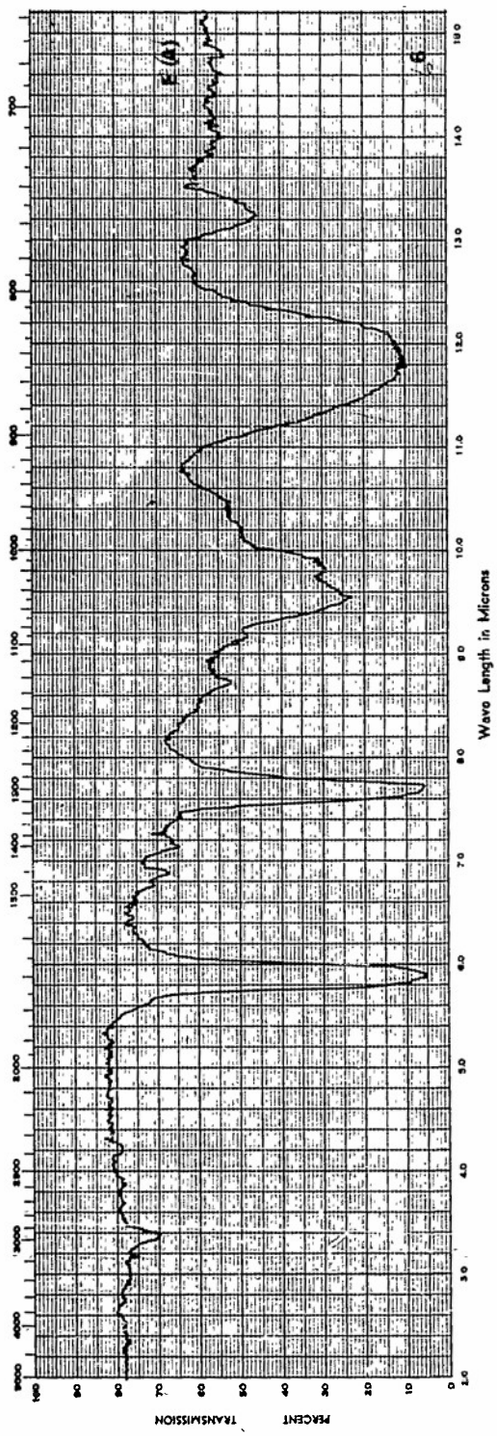
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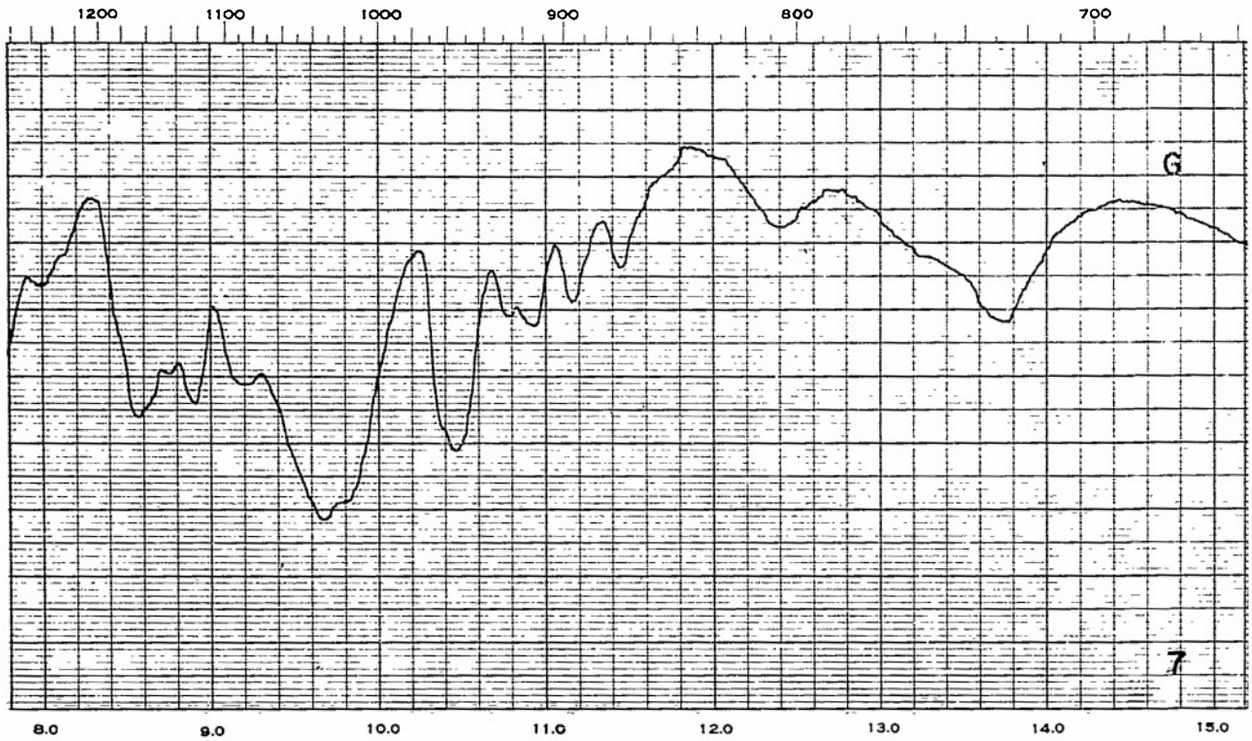

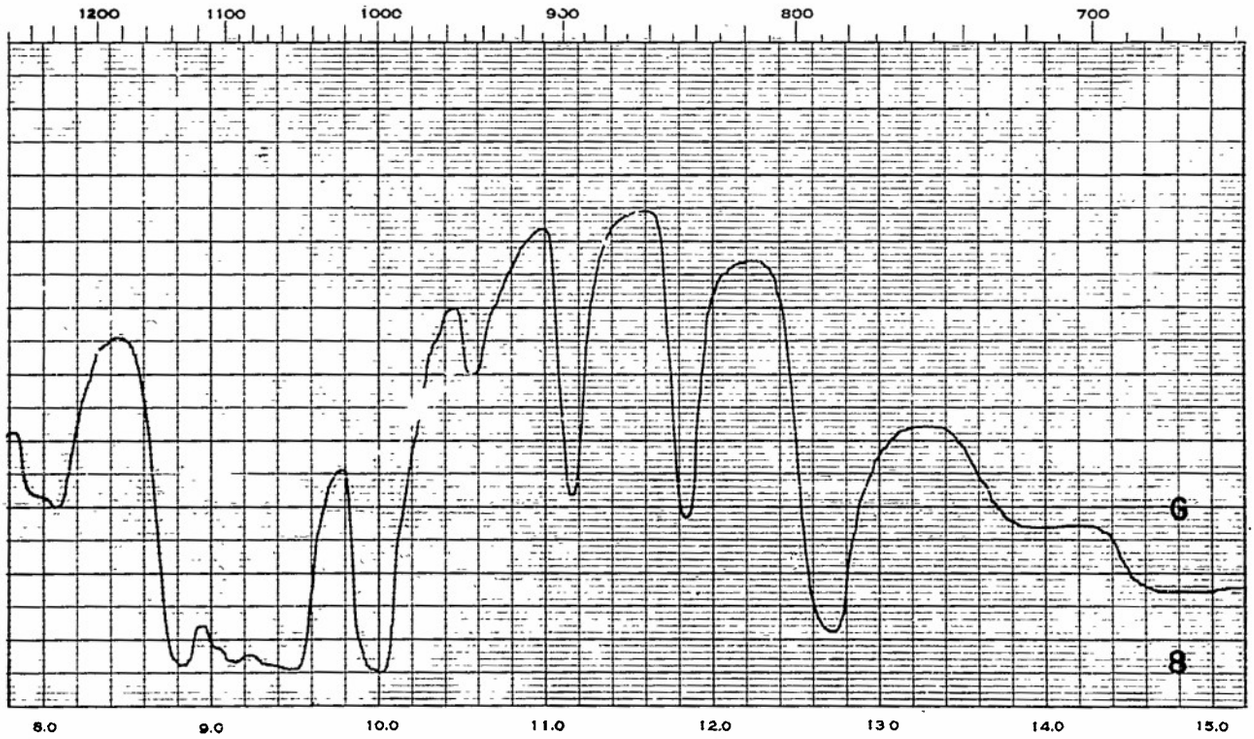

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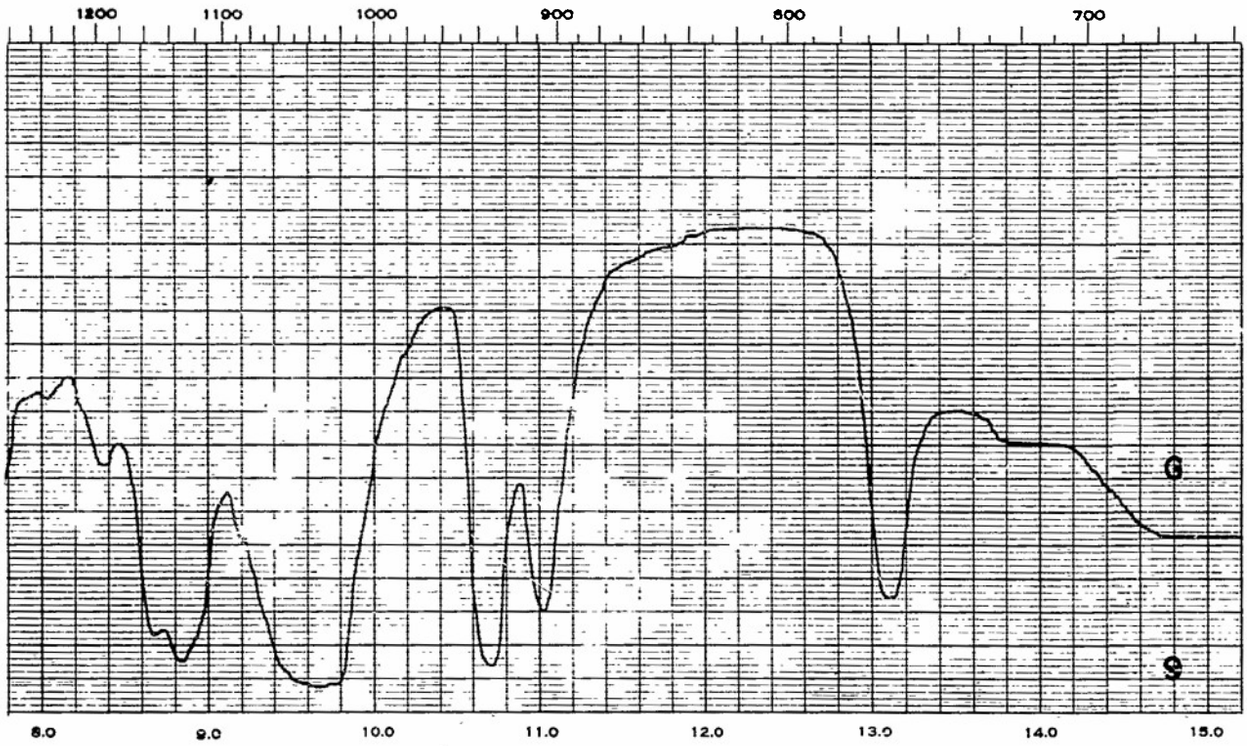

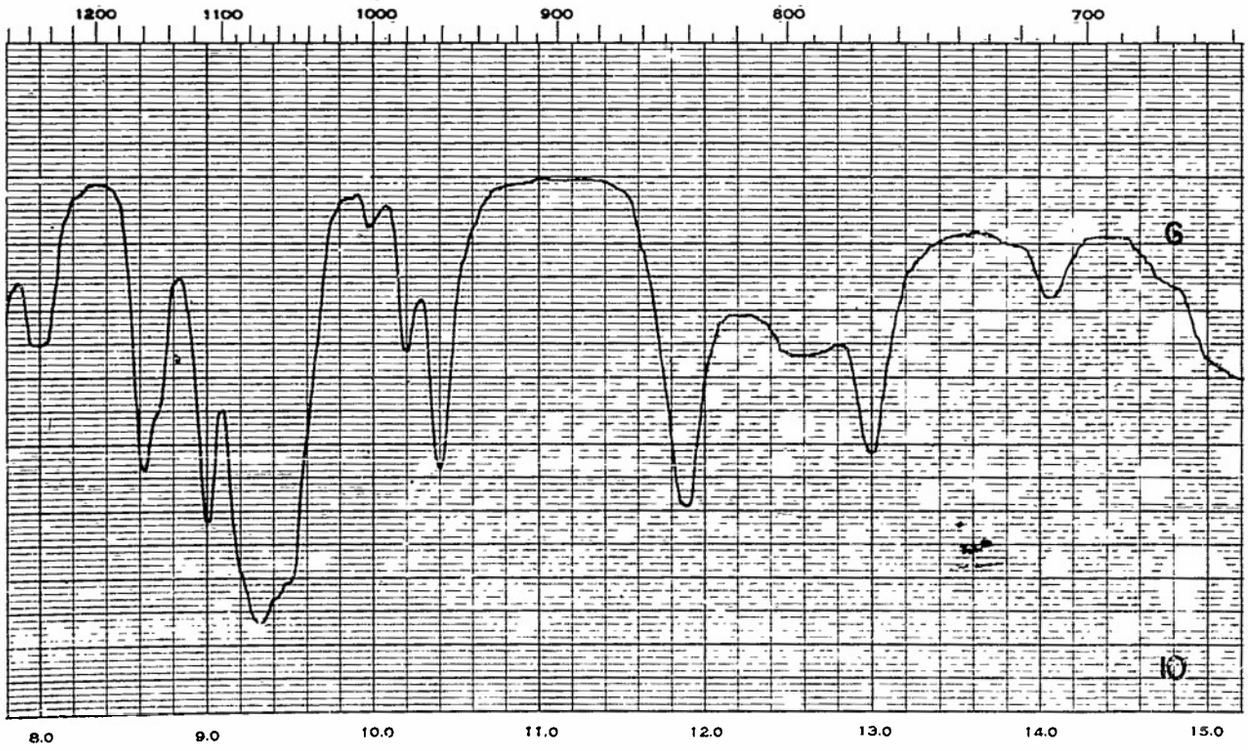

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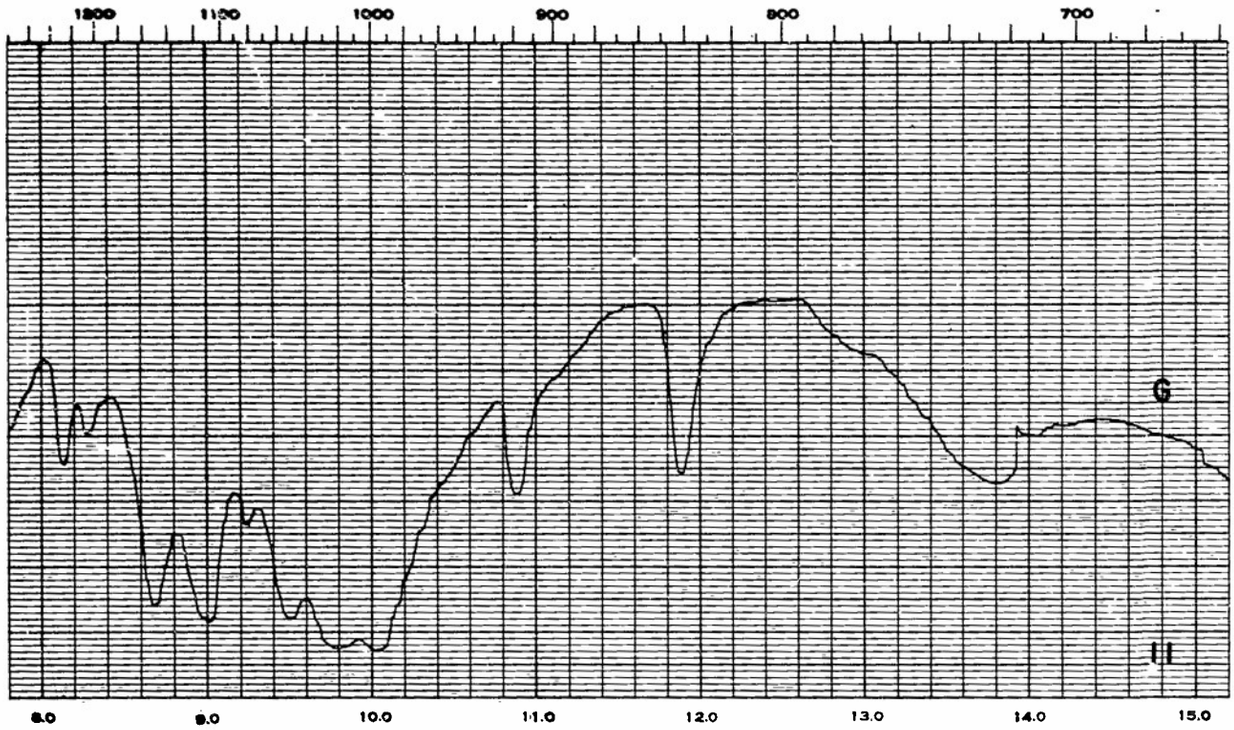

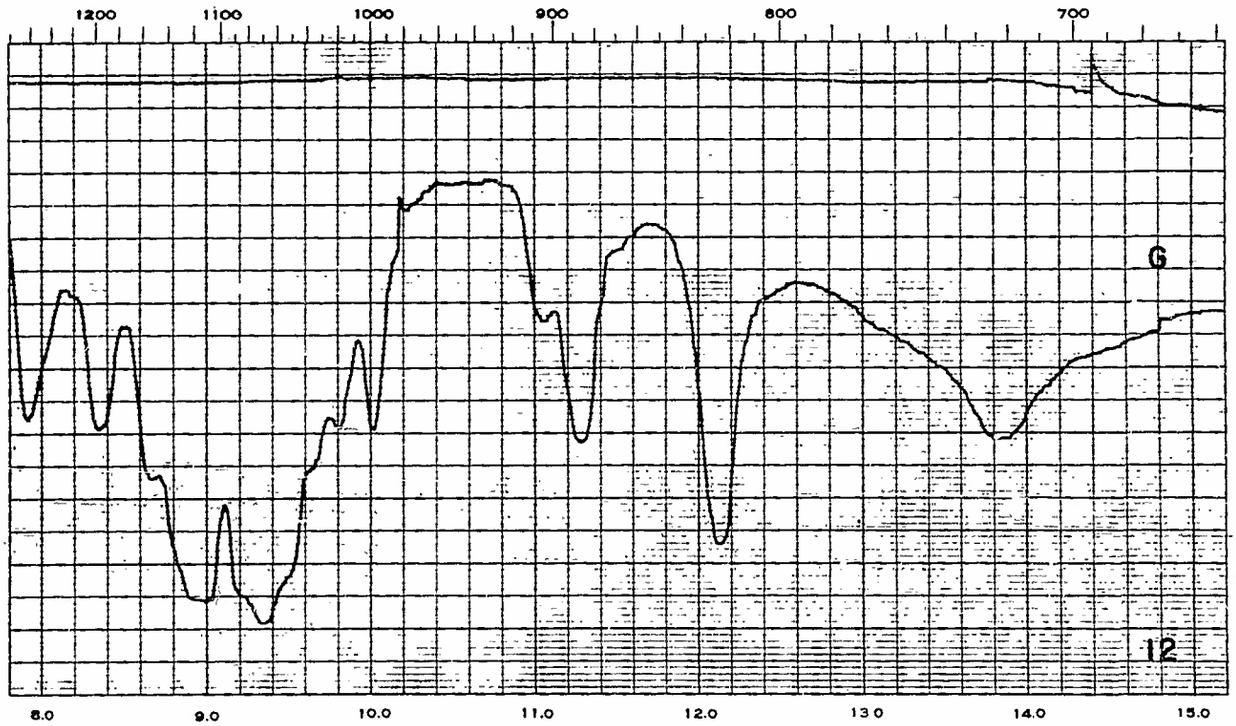

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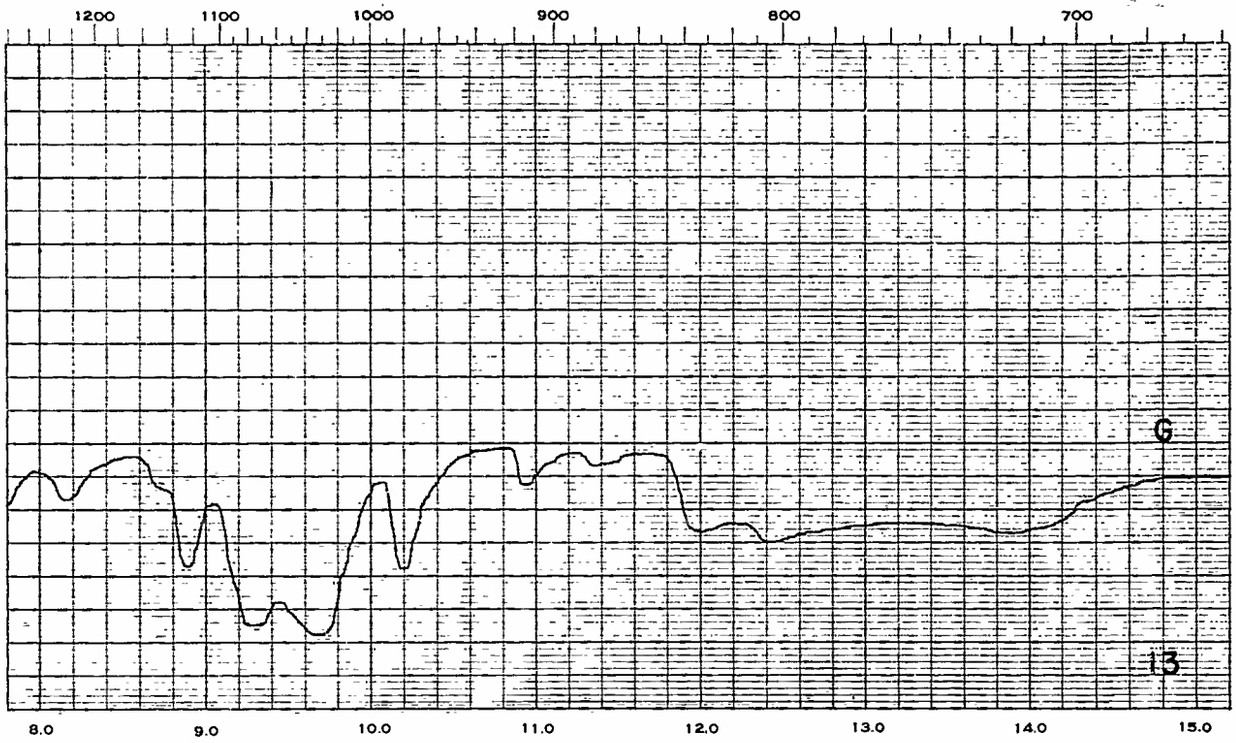

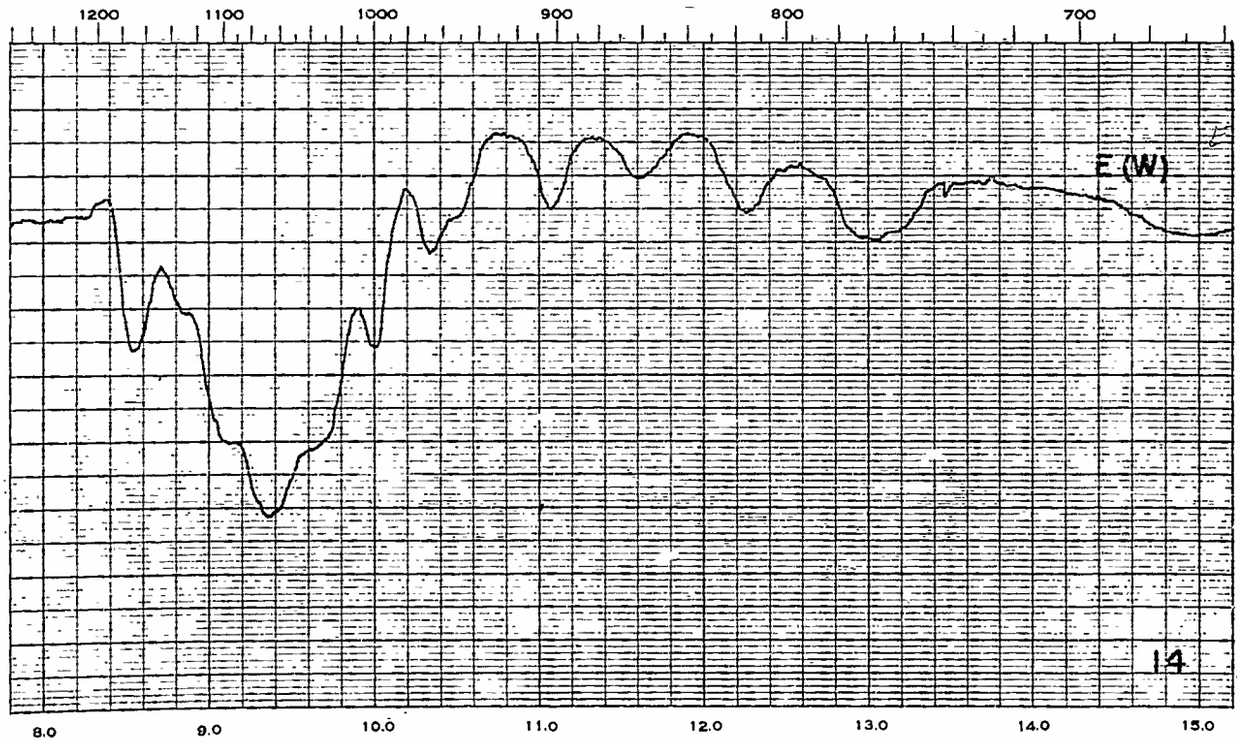

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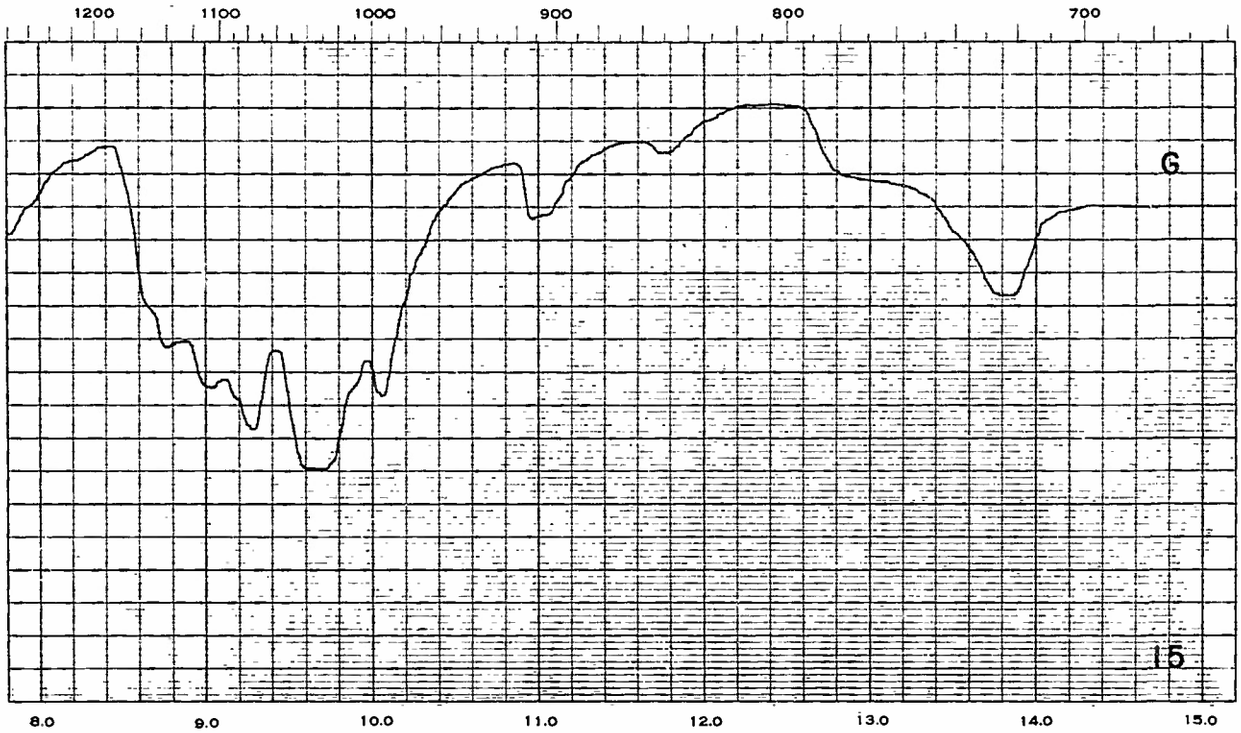
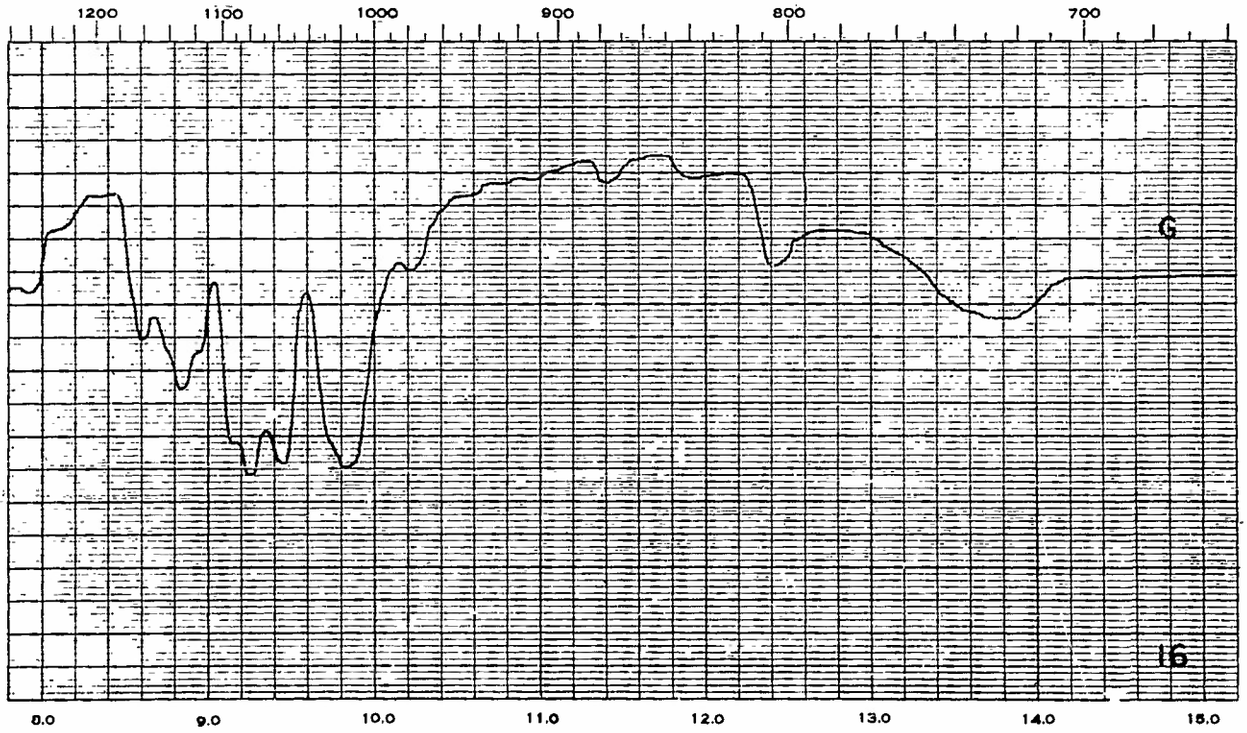
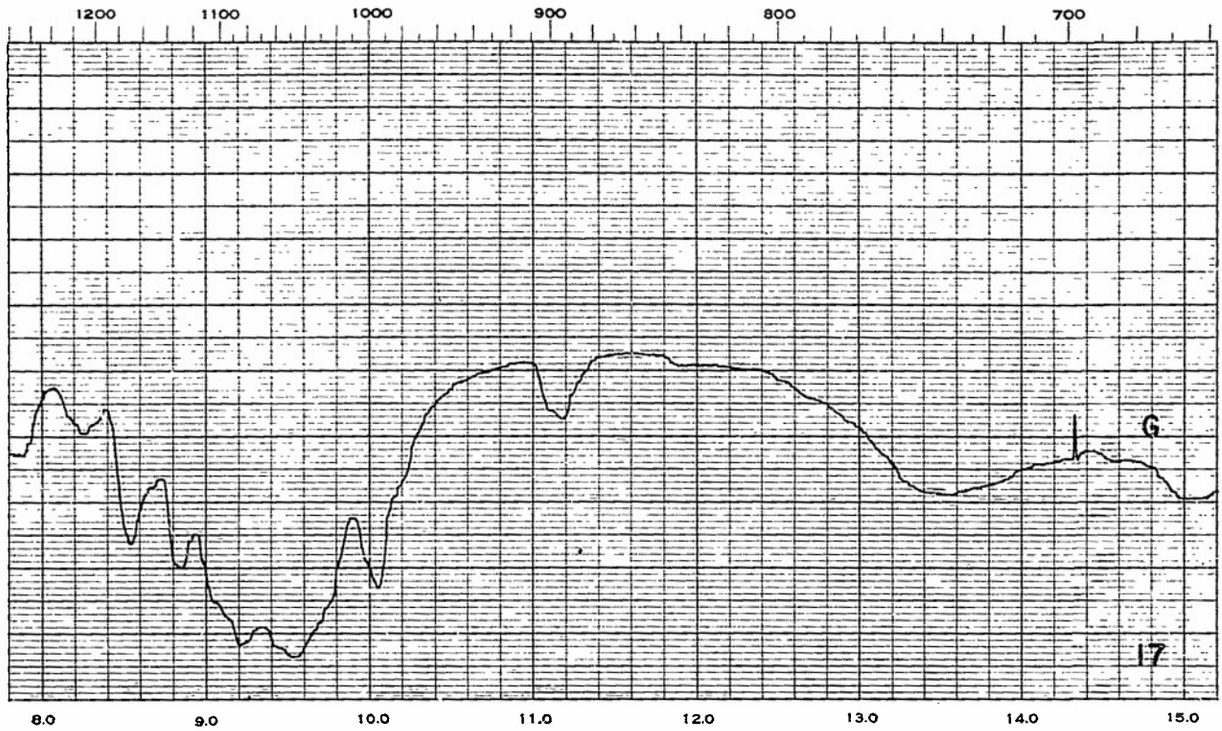
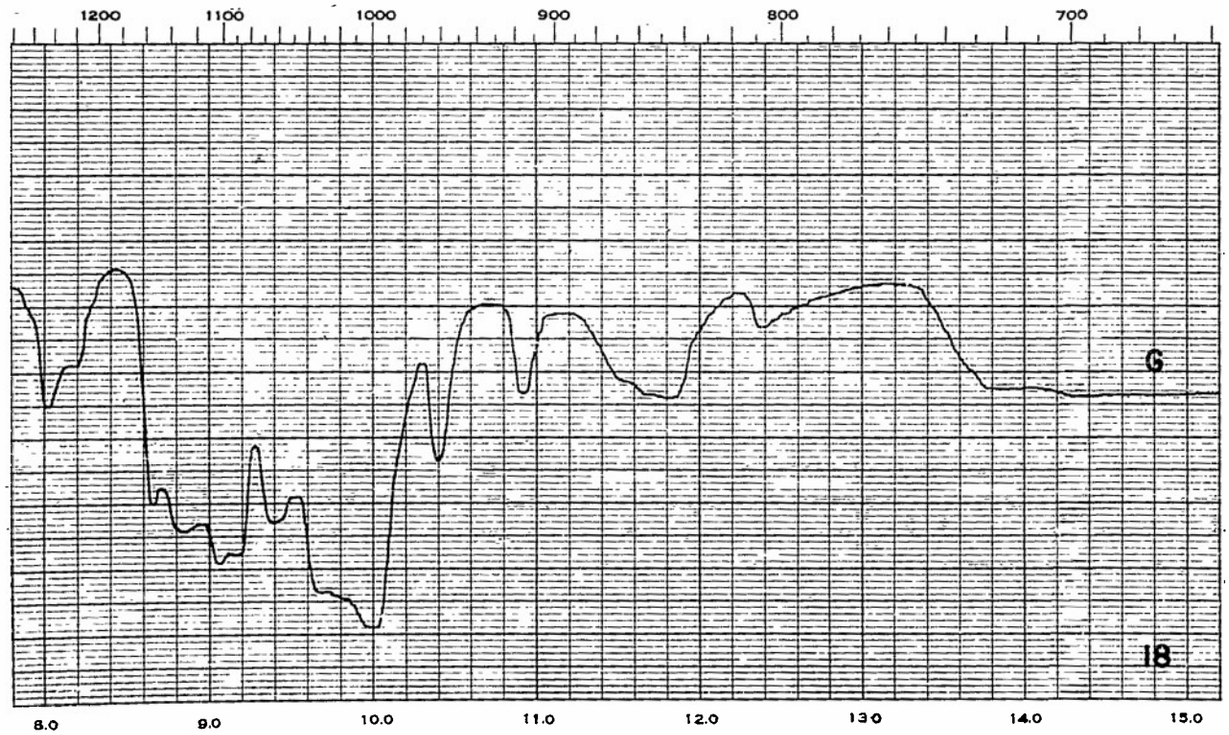




Wave Length in Microns

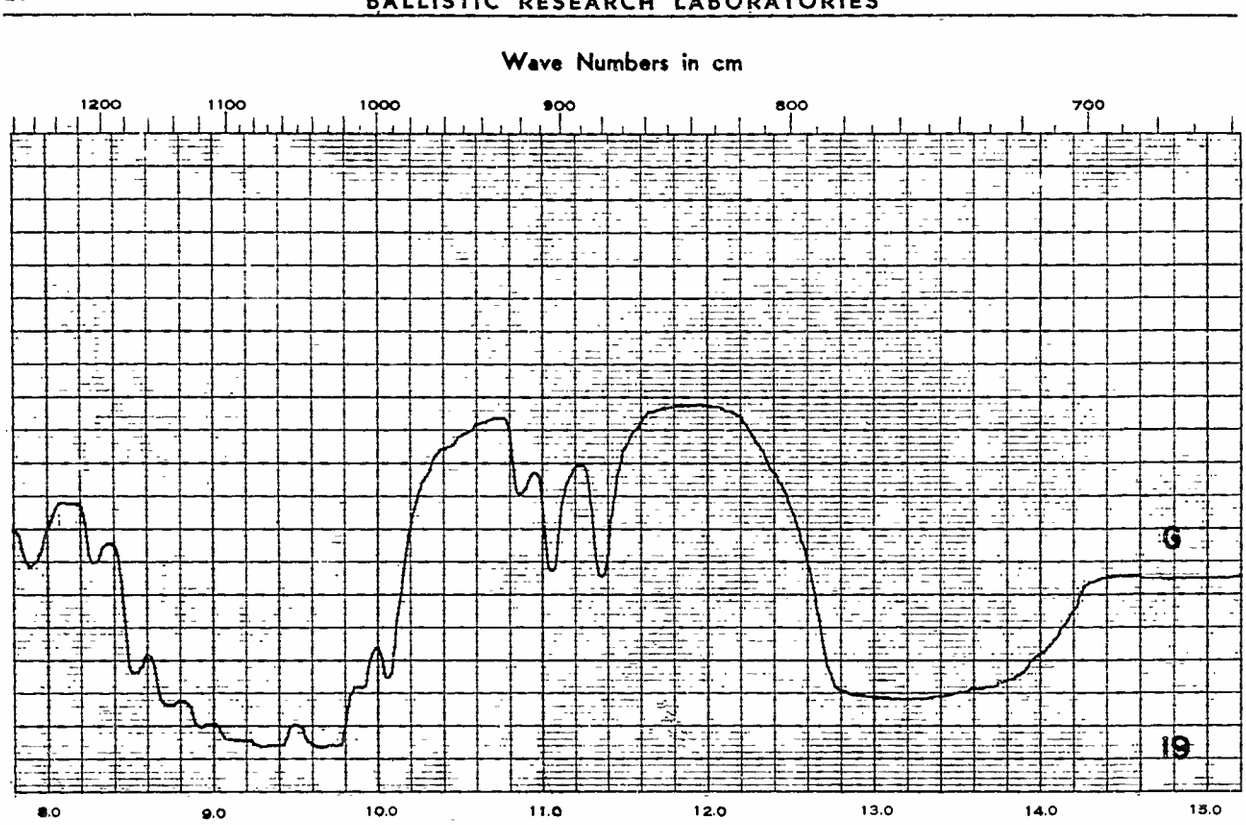
Wave Numbers in cm

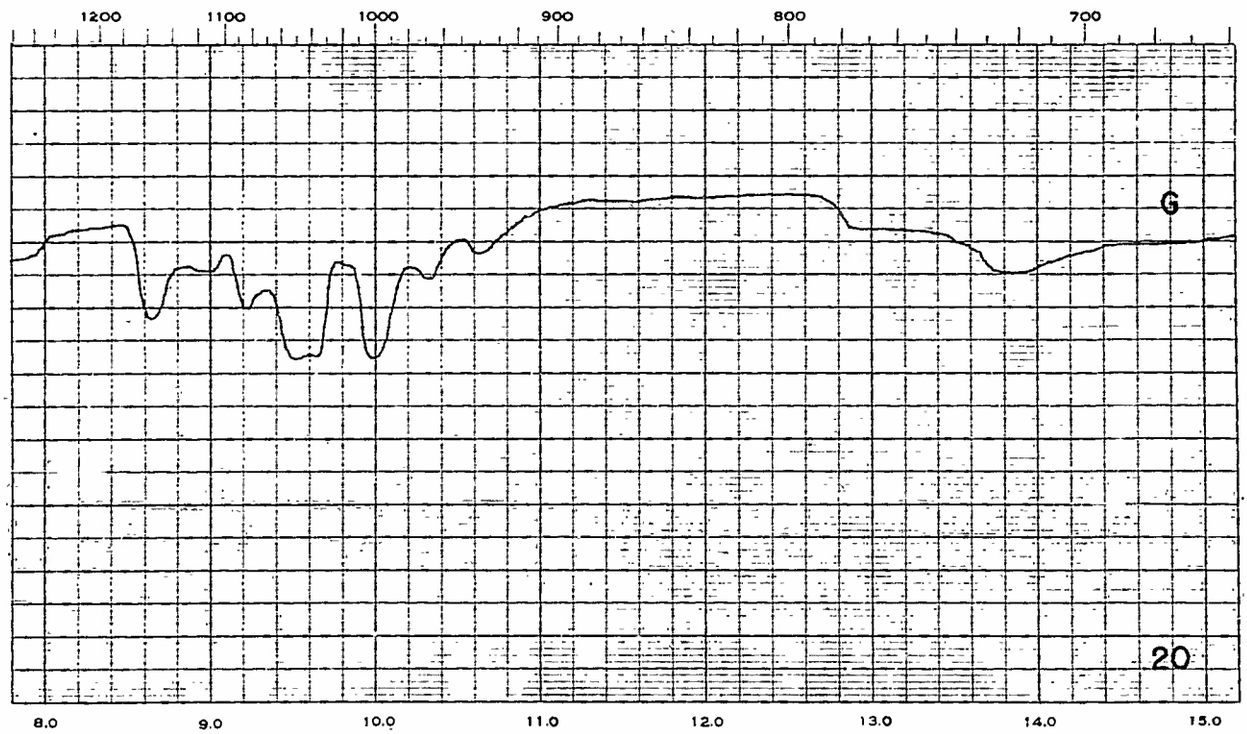




Wave Length in Microns

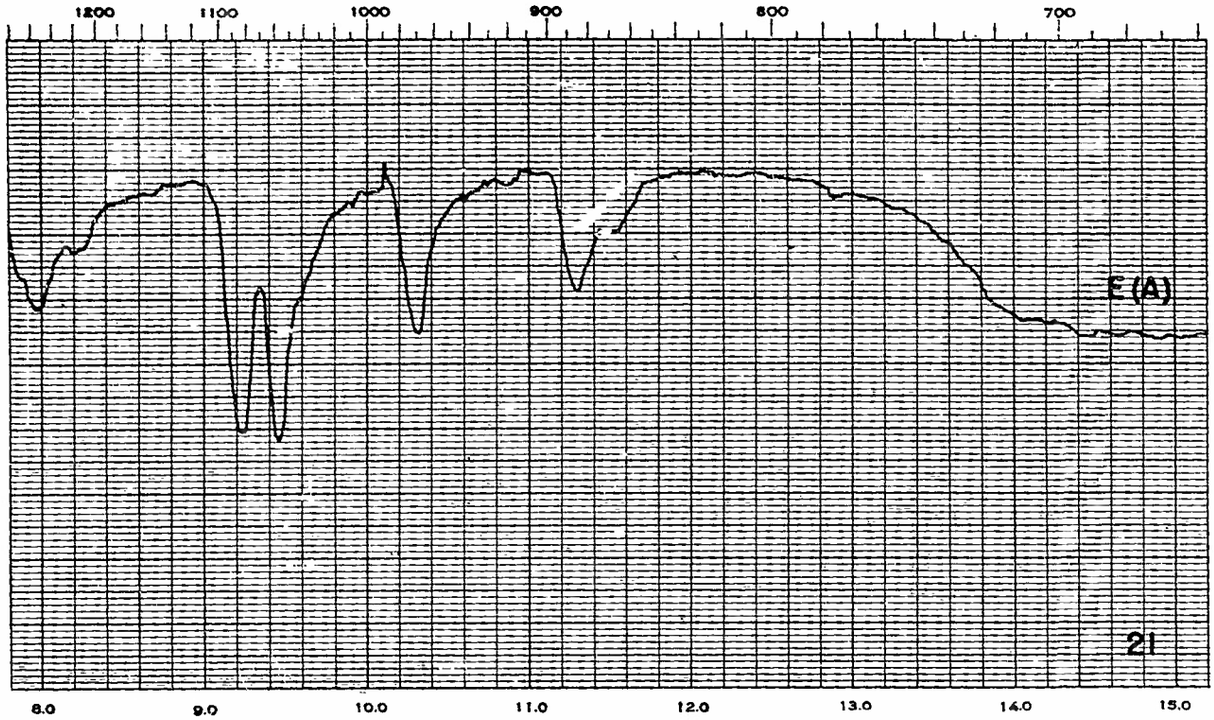
Wave Numbers in cm

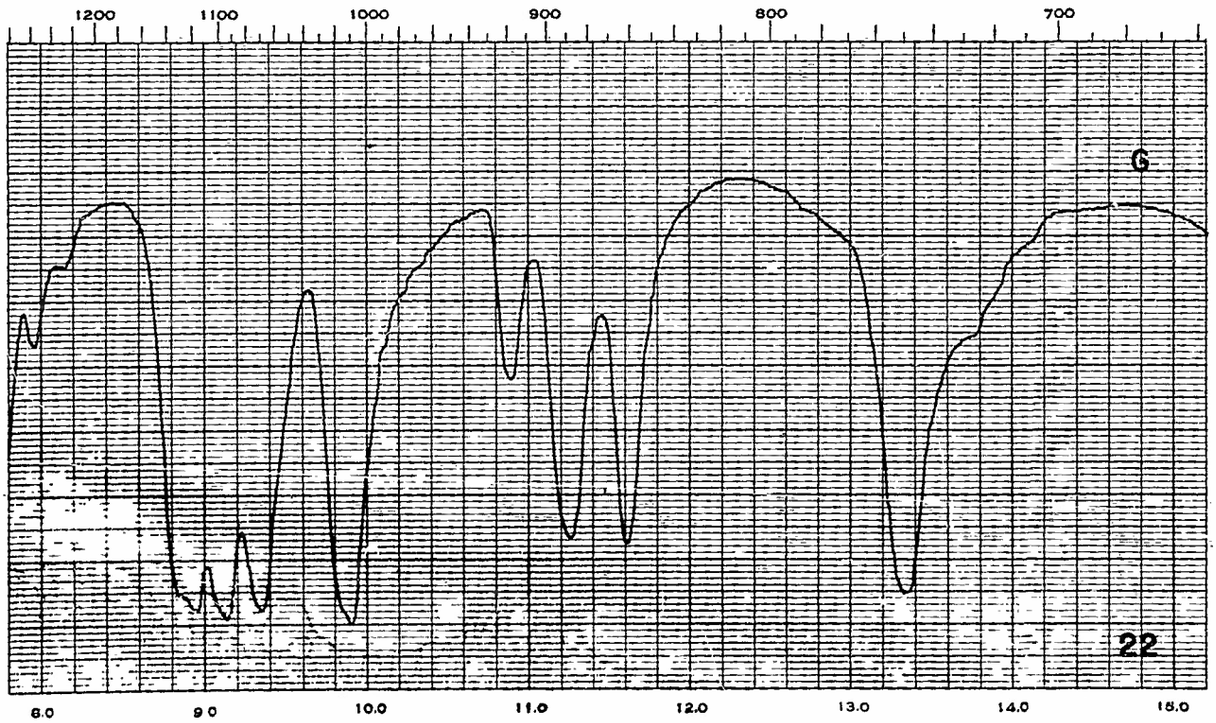




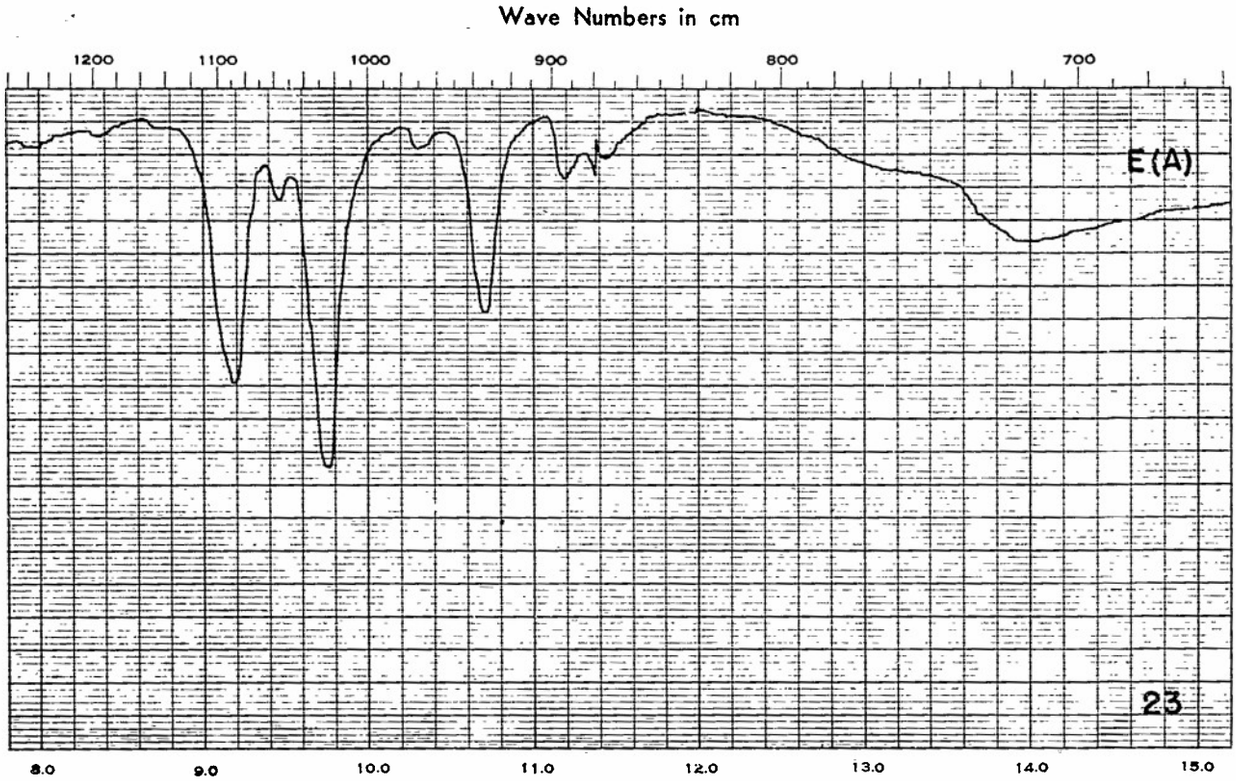
Wave Length in Microns

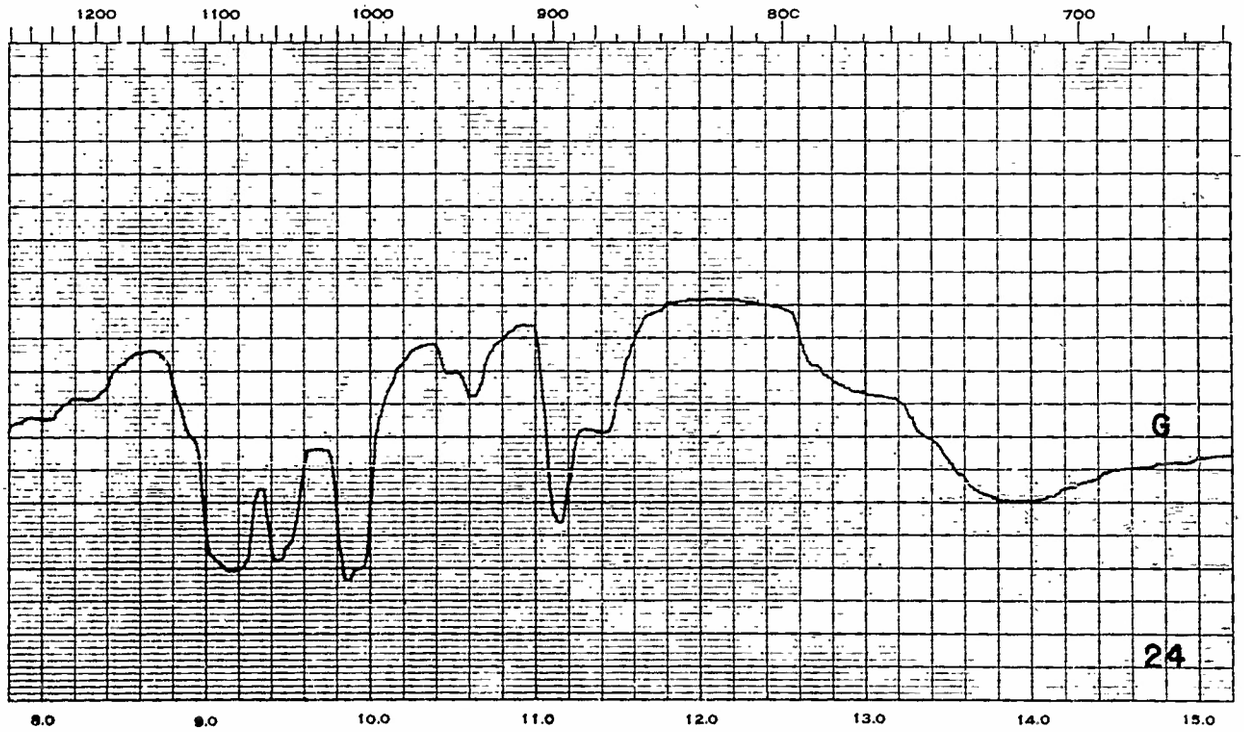
Wave Numbers in cm



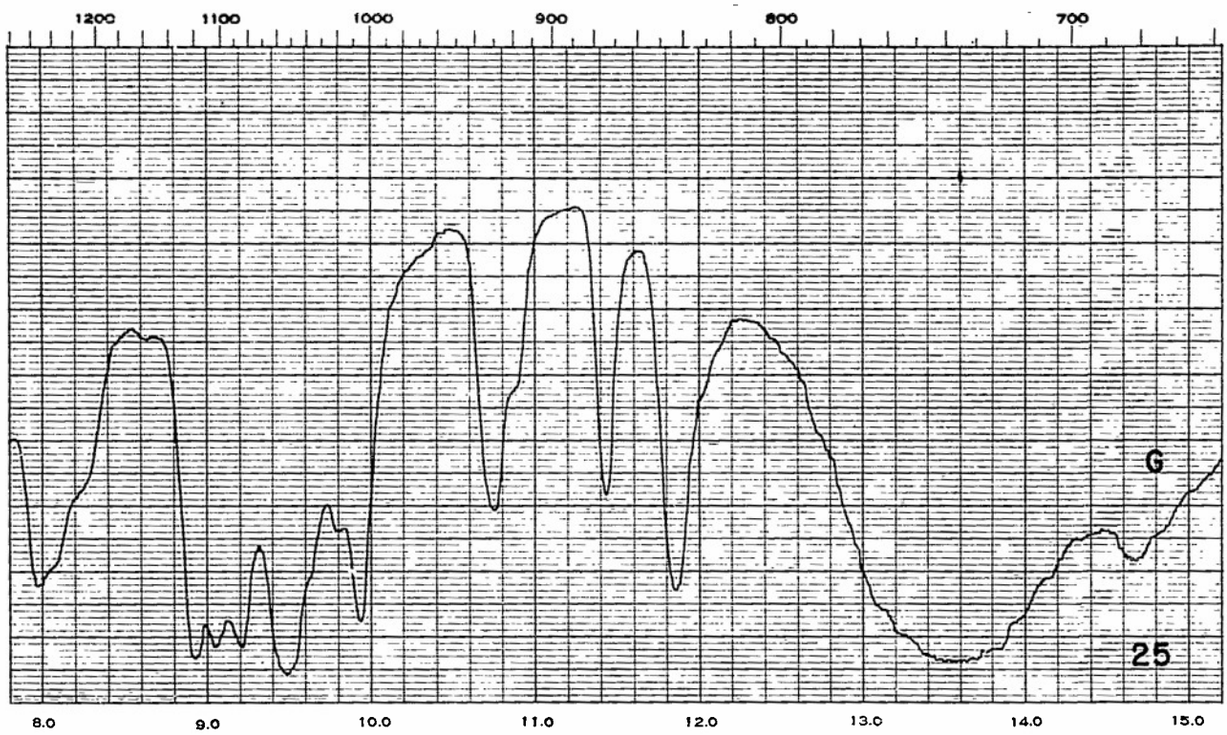


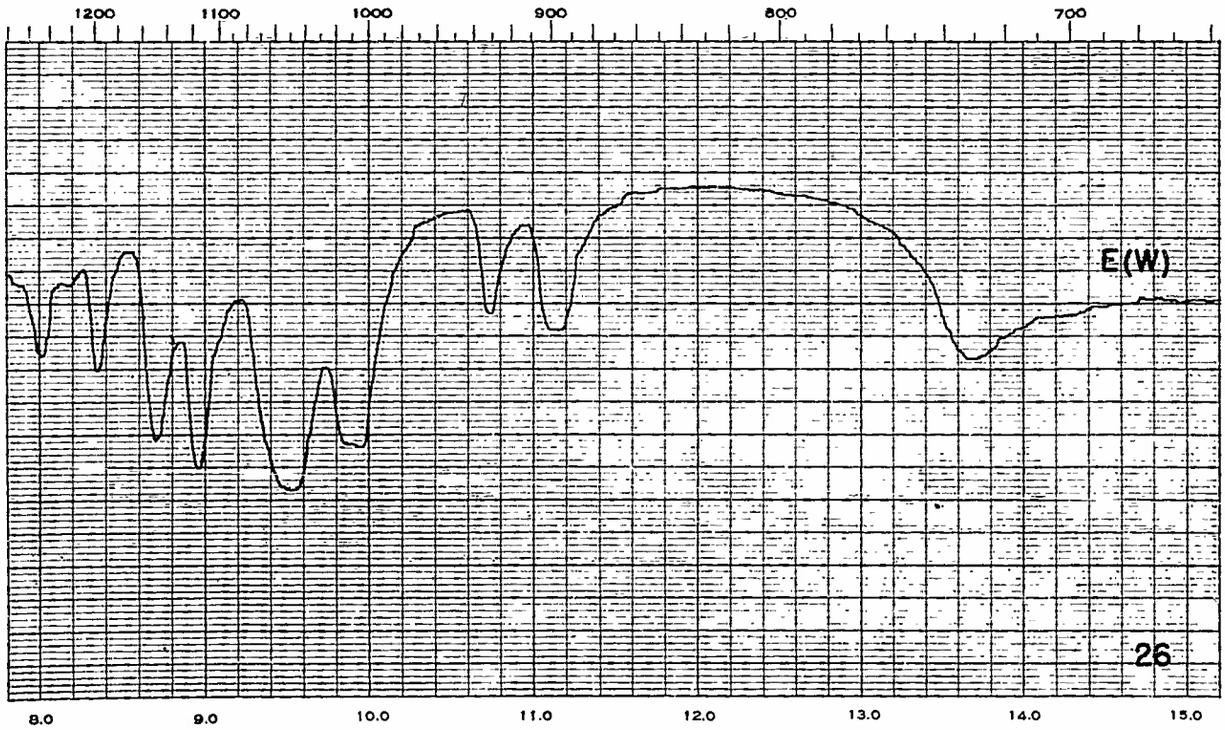
Wave Length in Microns


Wave Numbers in cm
1200
1100
1000
900
800
700
E(A)
23
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0


1200
1100
1000
900
800
700
G
24
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
Wave Length in Microns

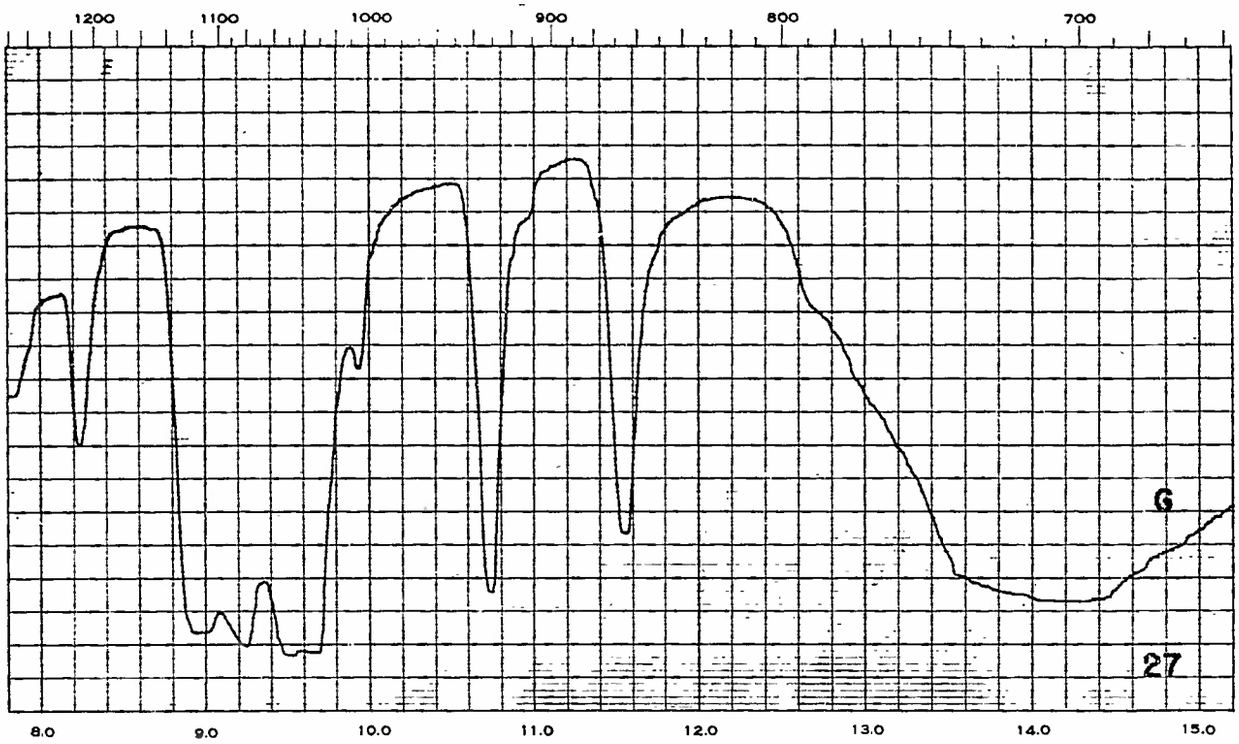
Wave Numbers in cm

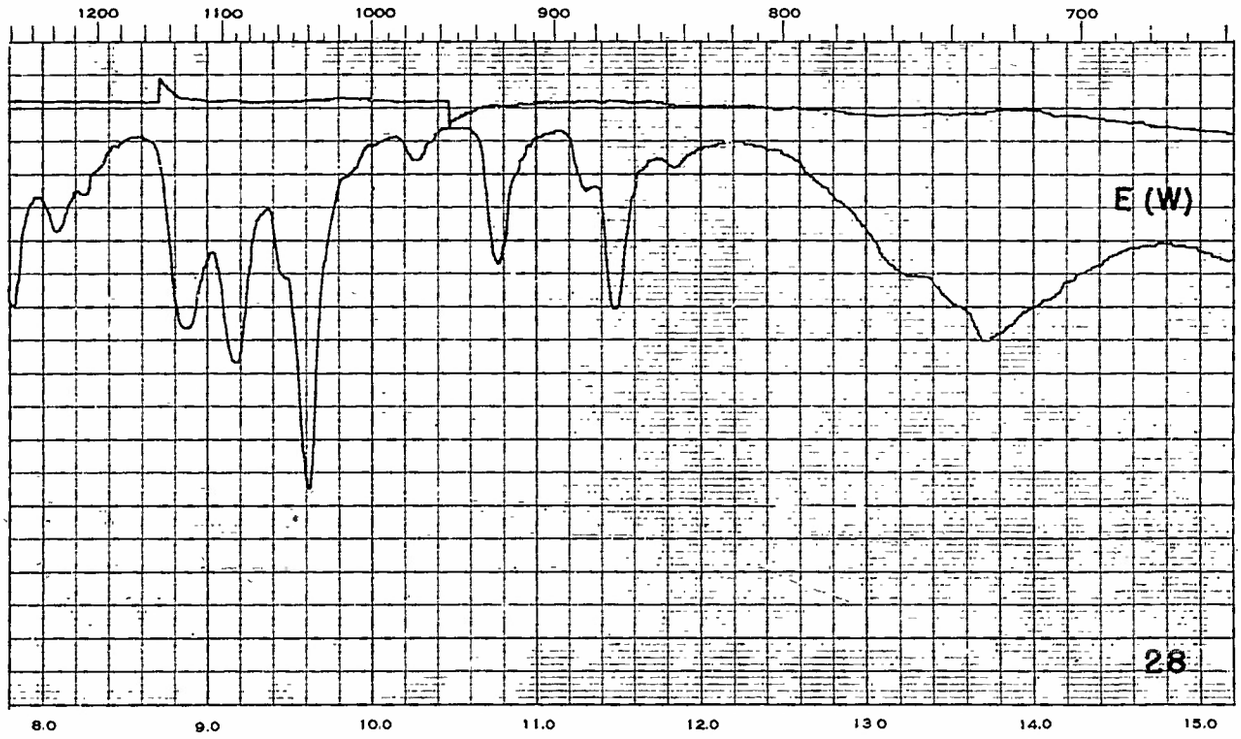




Wave Length in Microns

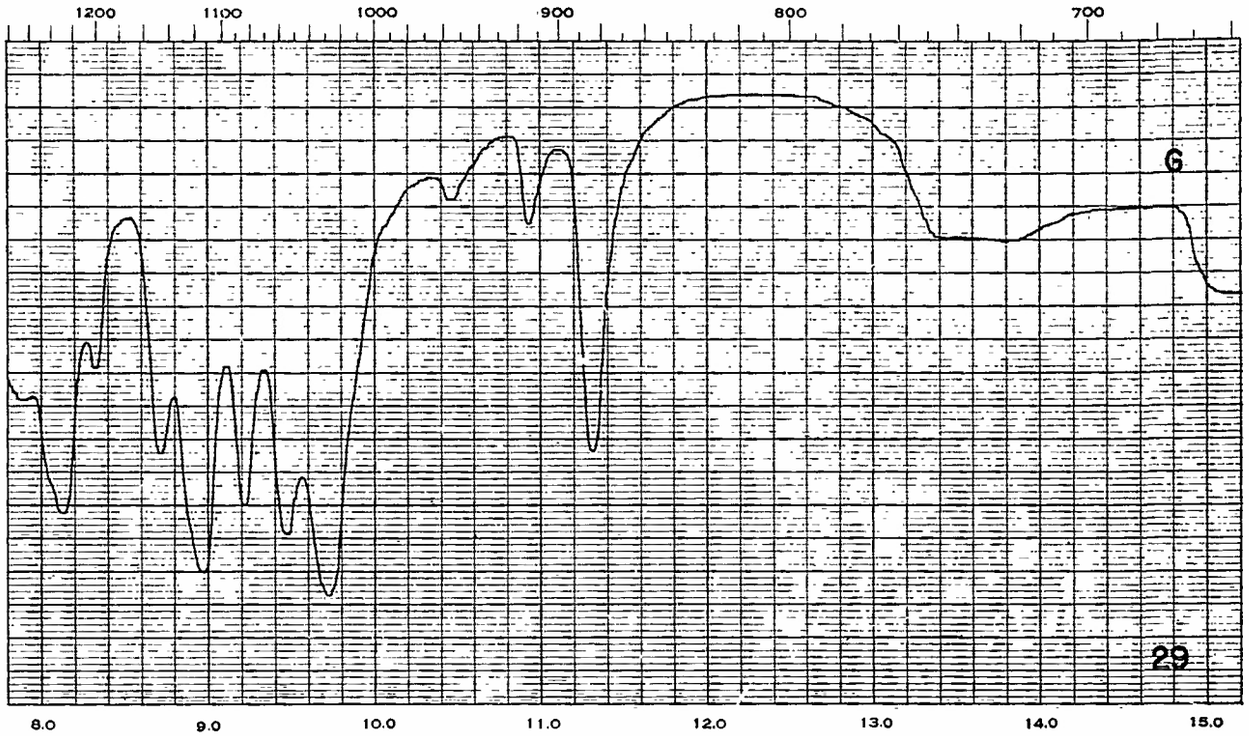
Wave Numbers in cm

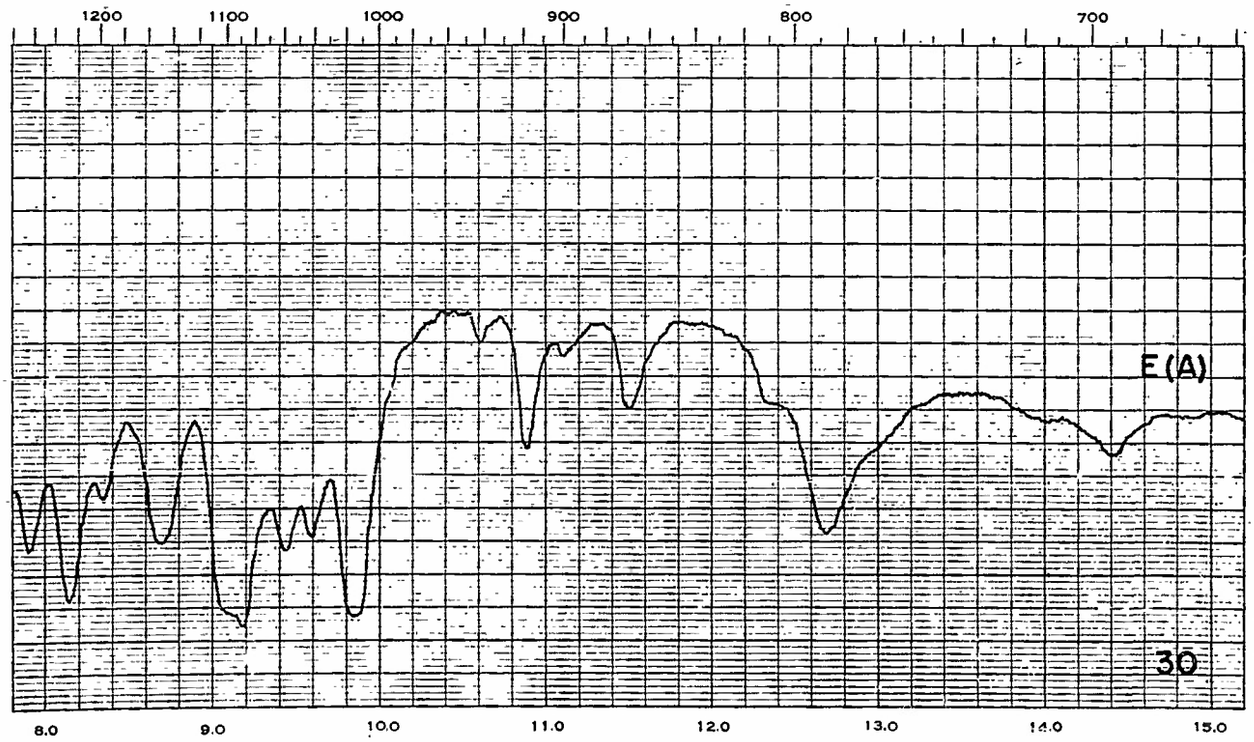




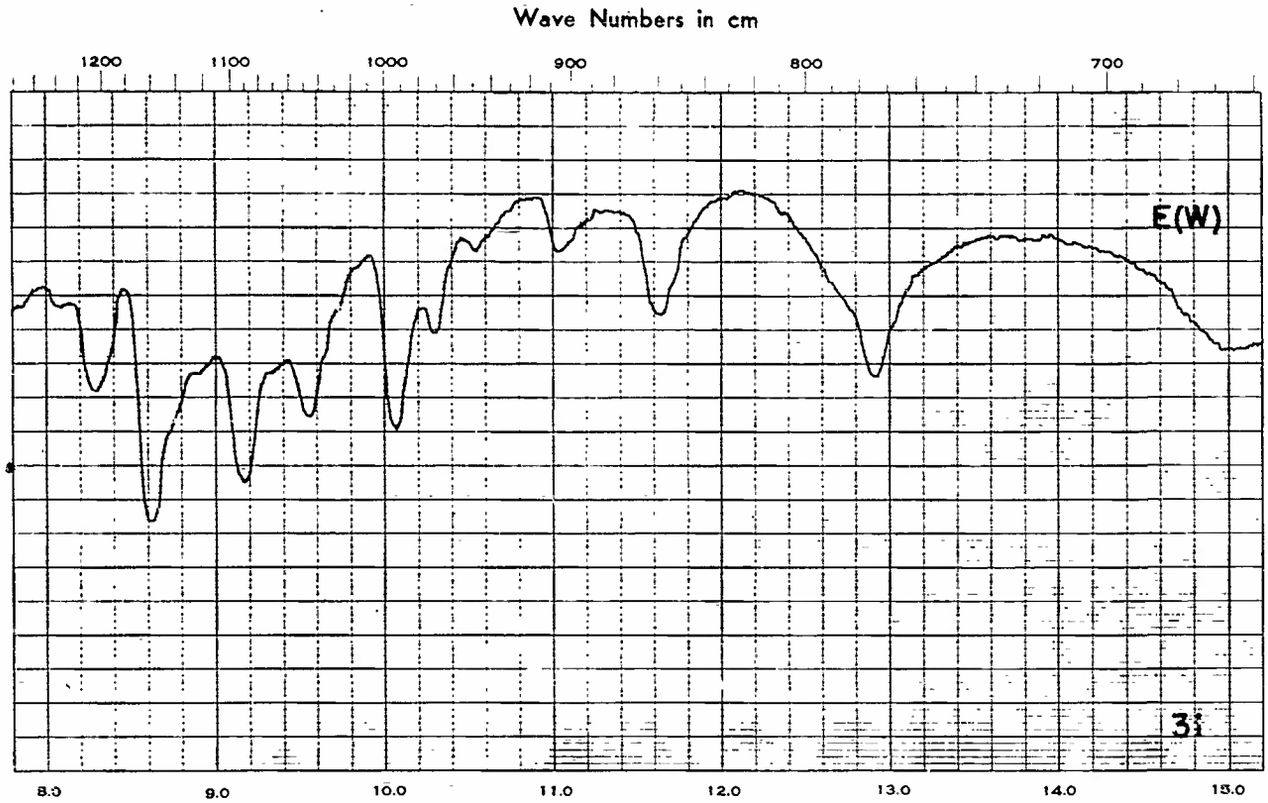
Wave Length in Microns

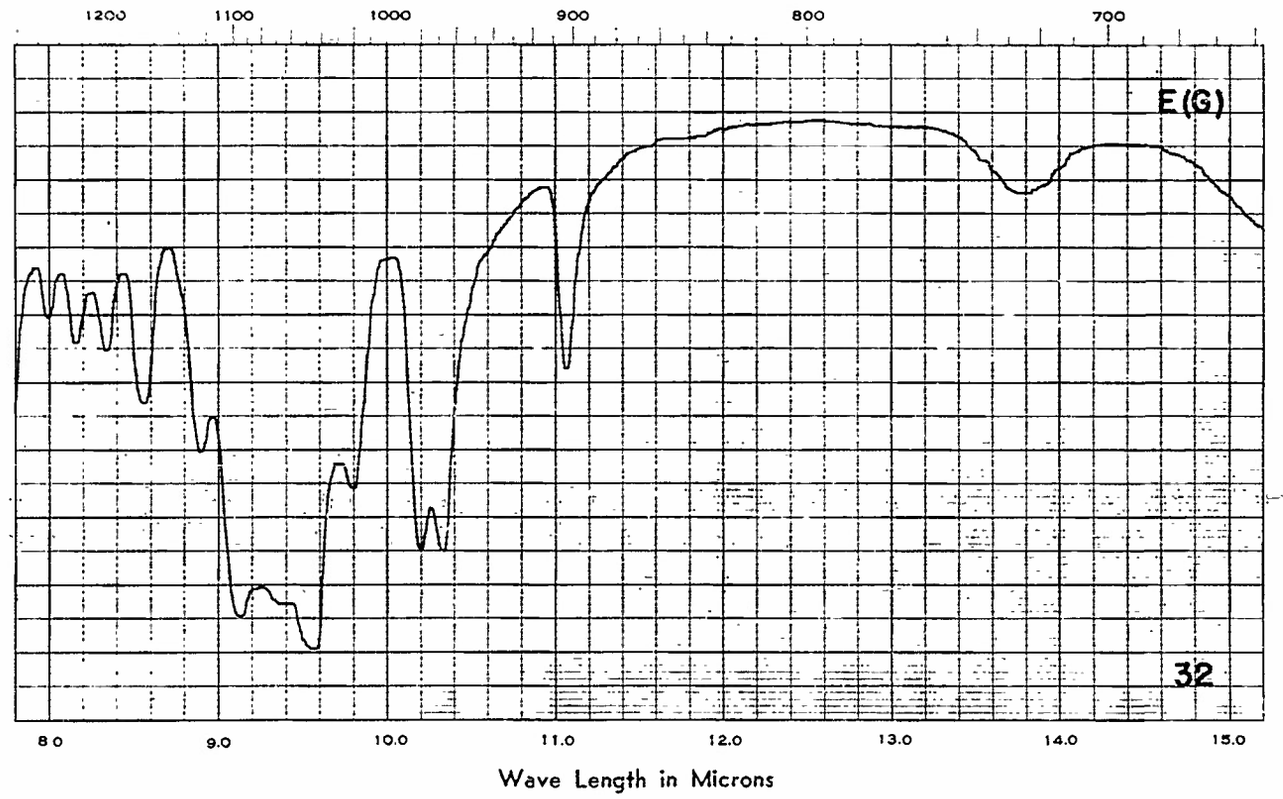
Wave Numbers in cm



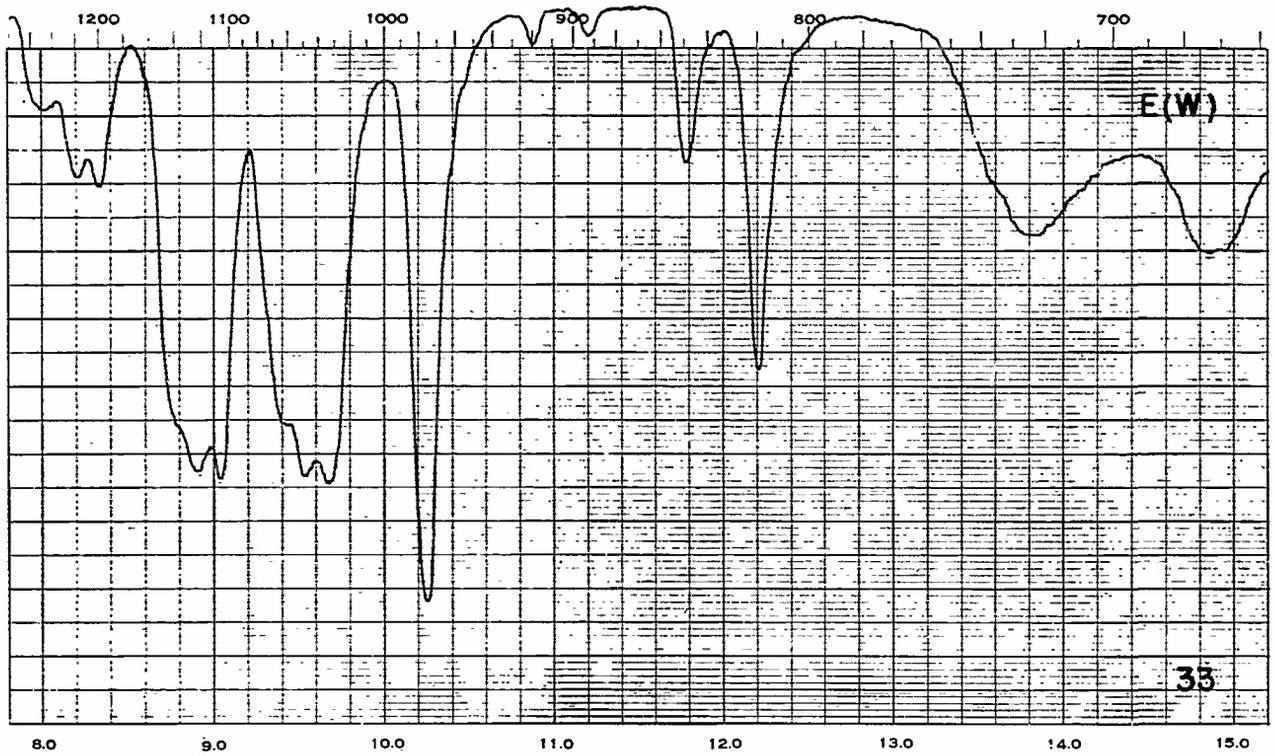


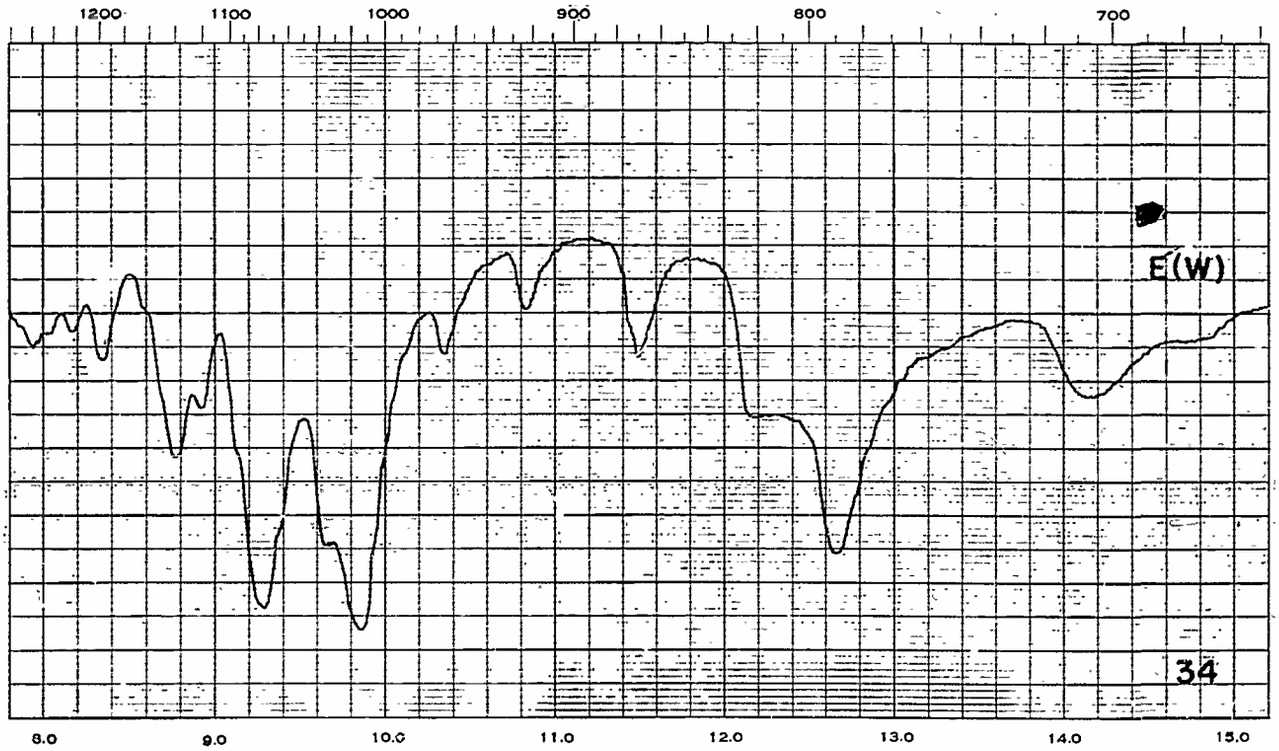
Wave Length in Microns


Wave Numbers in cm
1200
1100
1000
900
800
700
E(W)
31
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0

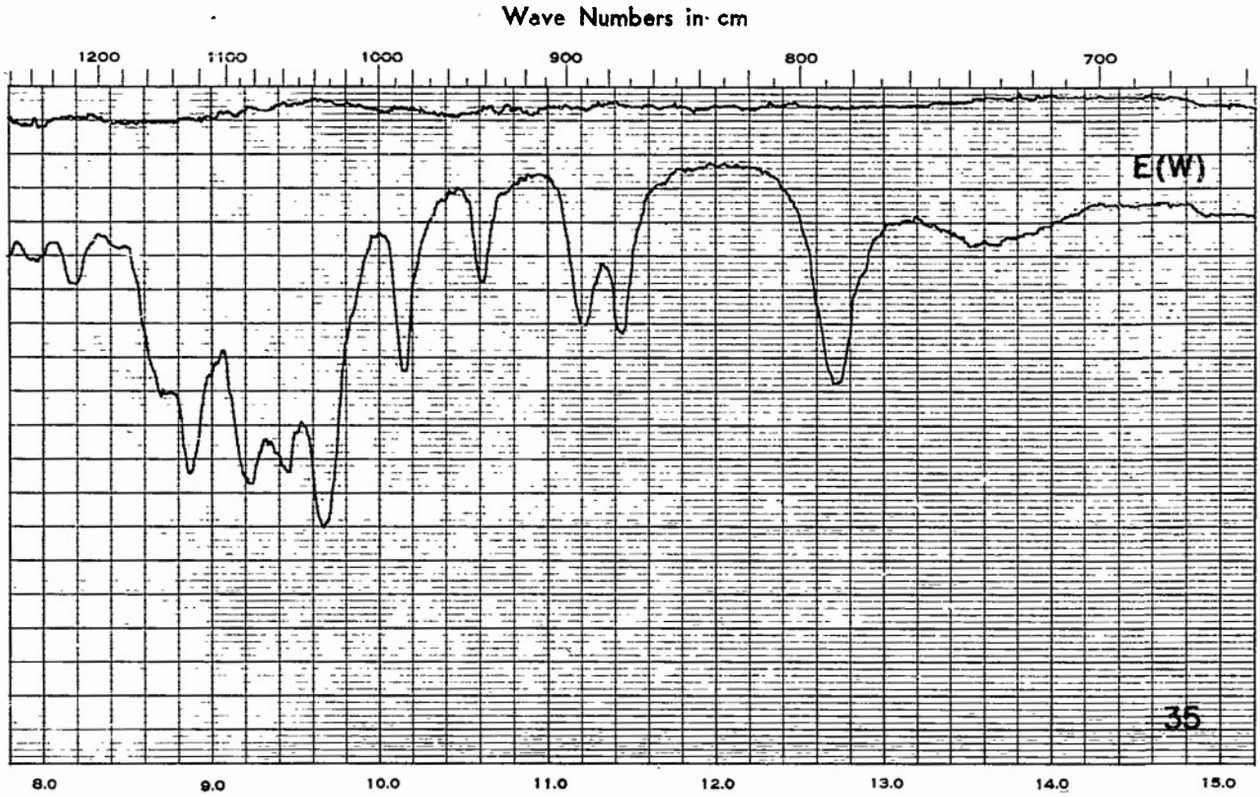

1200
1100
1000
900
800
700
E(G)
32
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
Wave Length in Microns

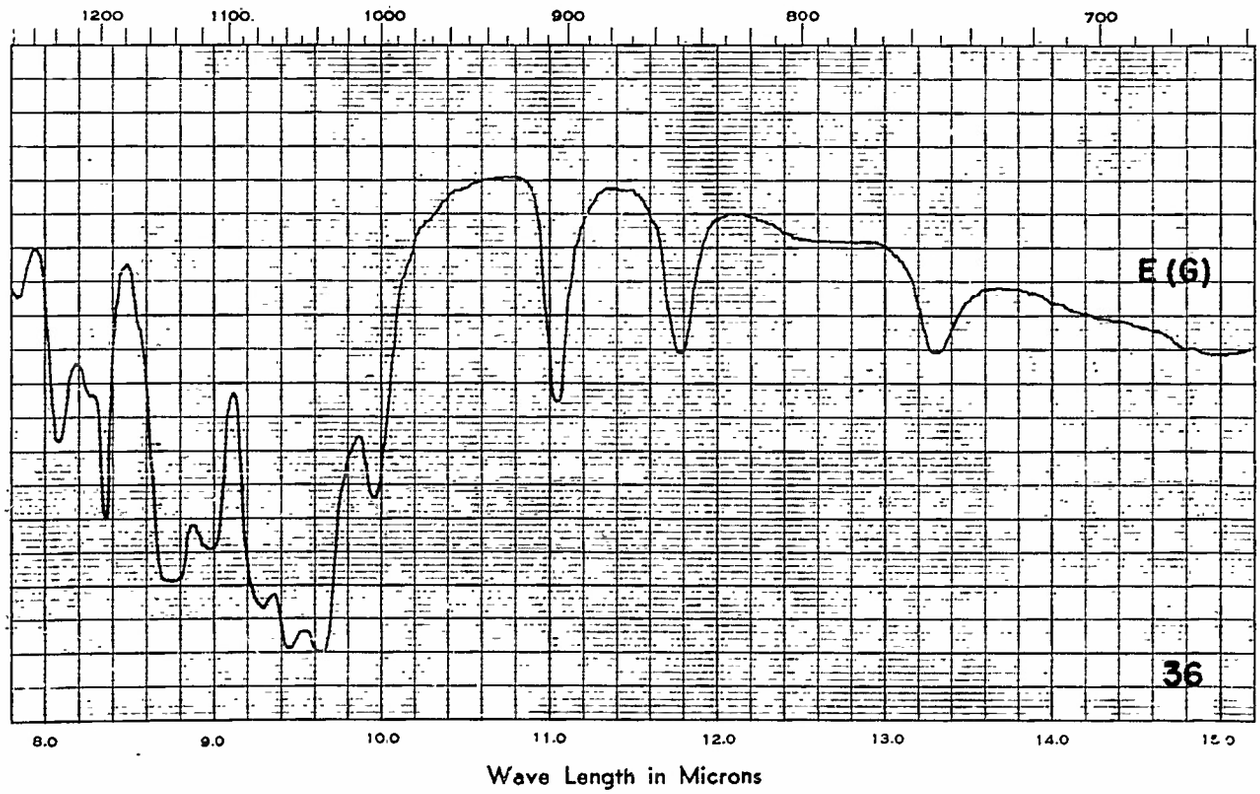
Wave Numbers in cm



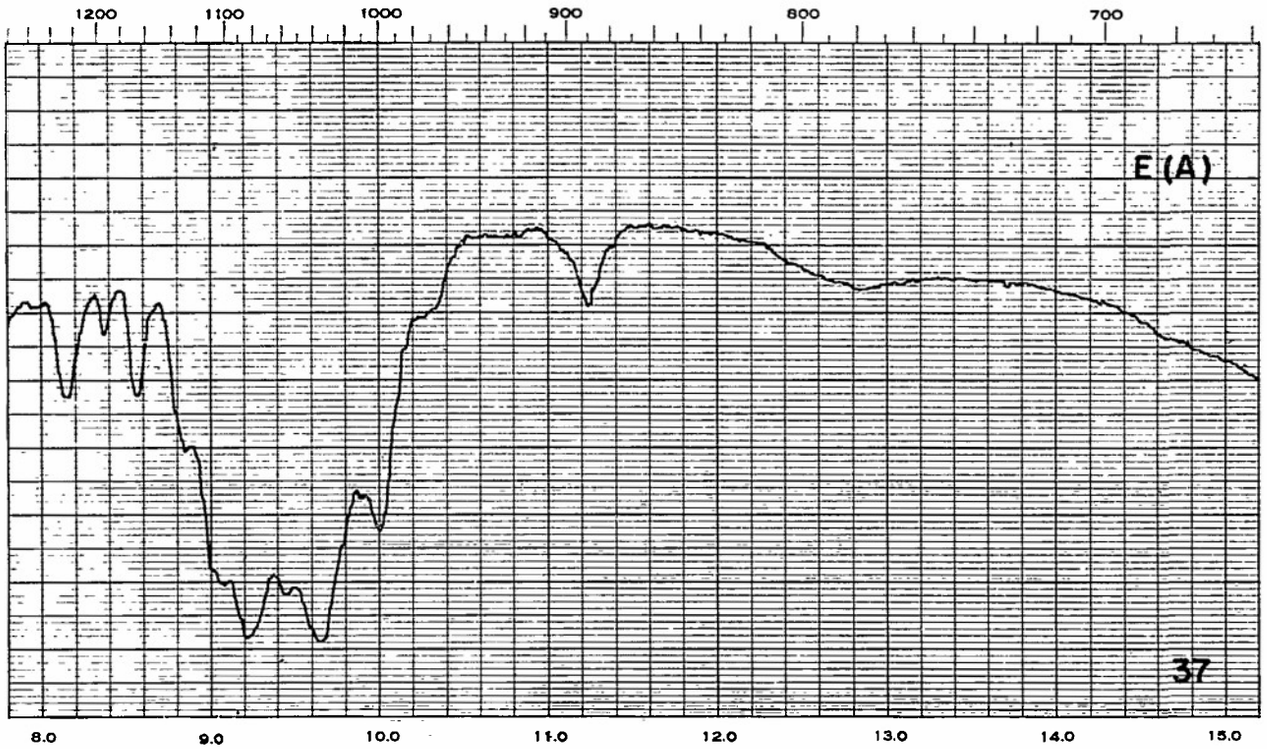


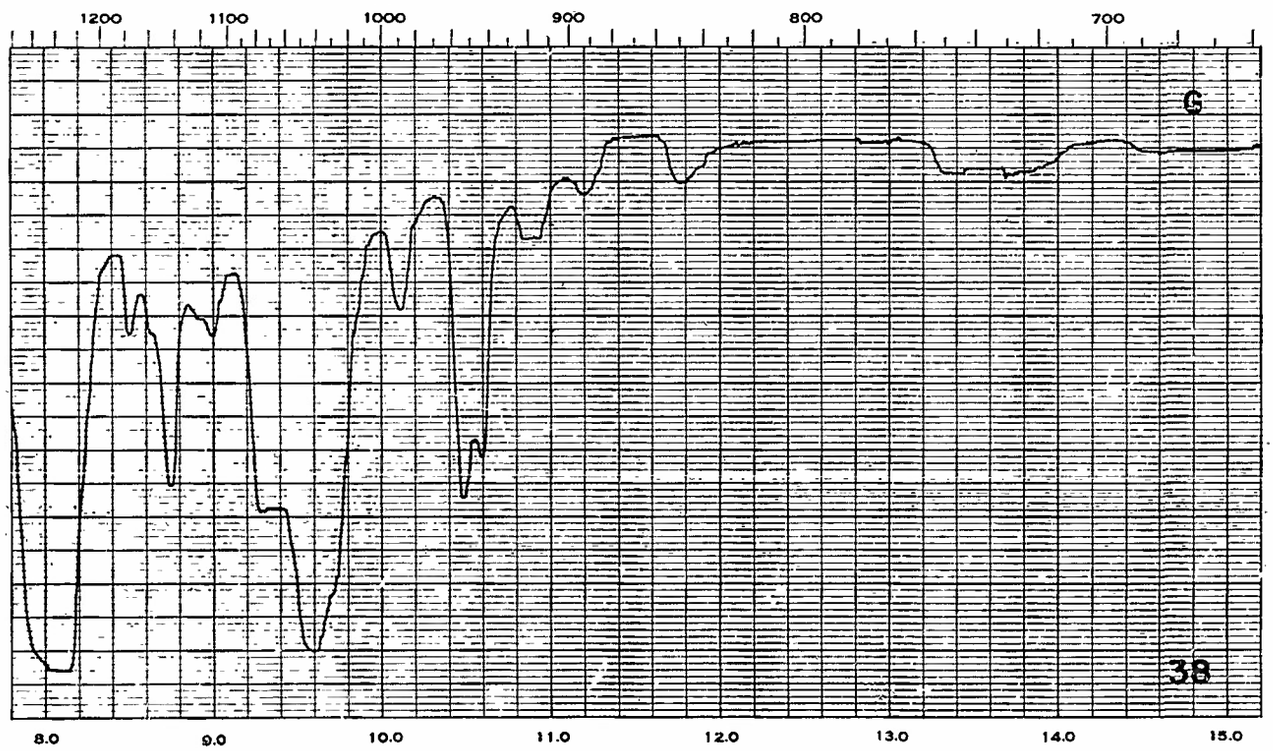
Wave Length in Microns


Wave Numbers in cm
1200
1100
1000
900
800
700
E(W)
35
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0


1200
1100
1000
900
800
700
E (G)
36
8.0
9.0
10.0
11.0
12.0
13.0
14.0
Wave Length in Microns

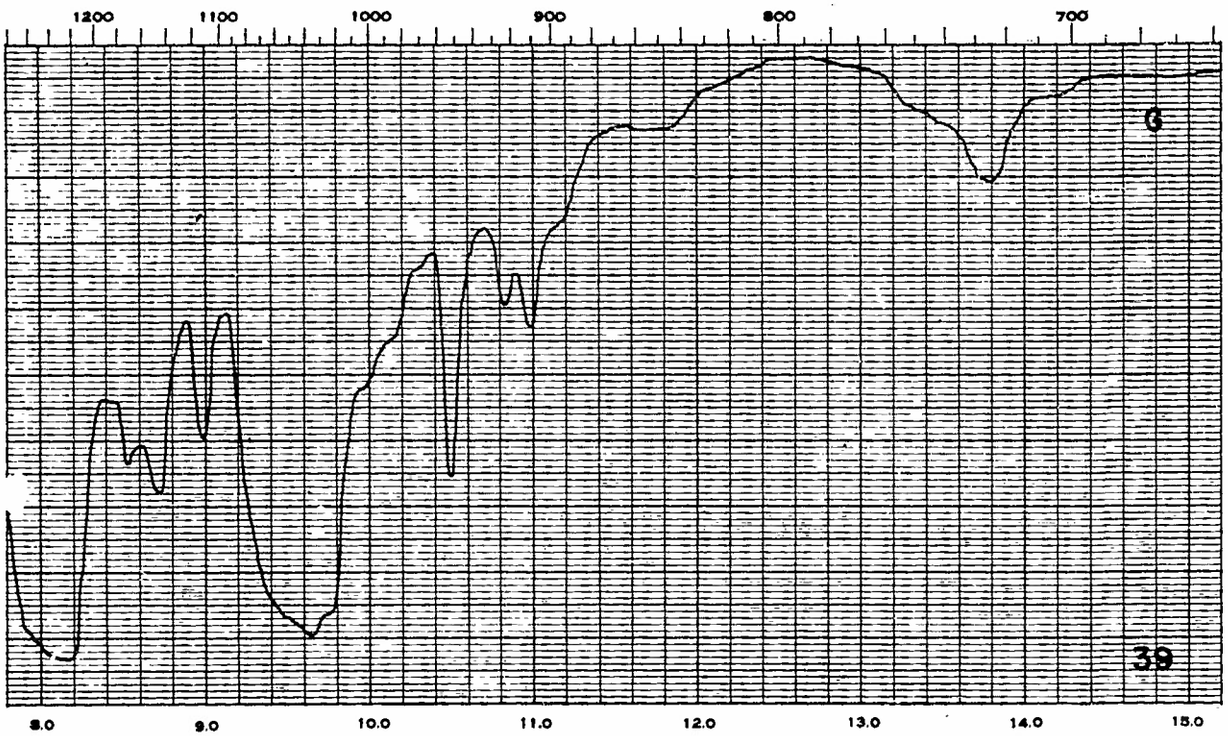
Wave Numbers in cm

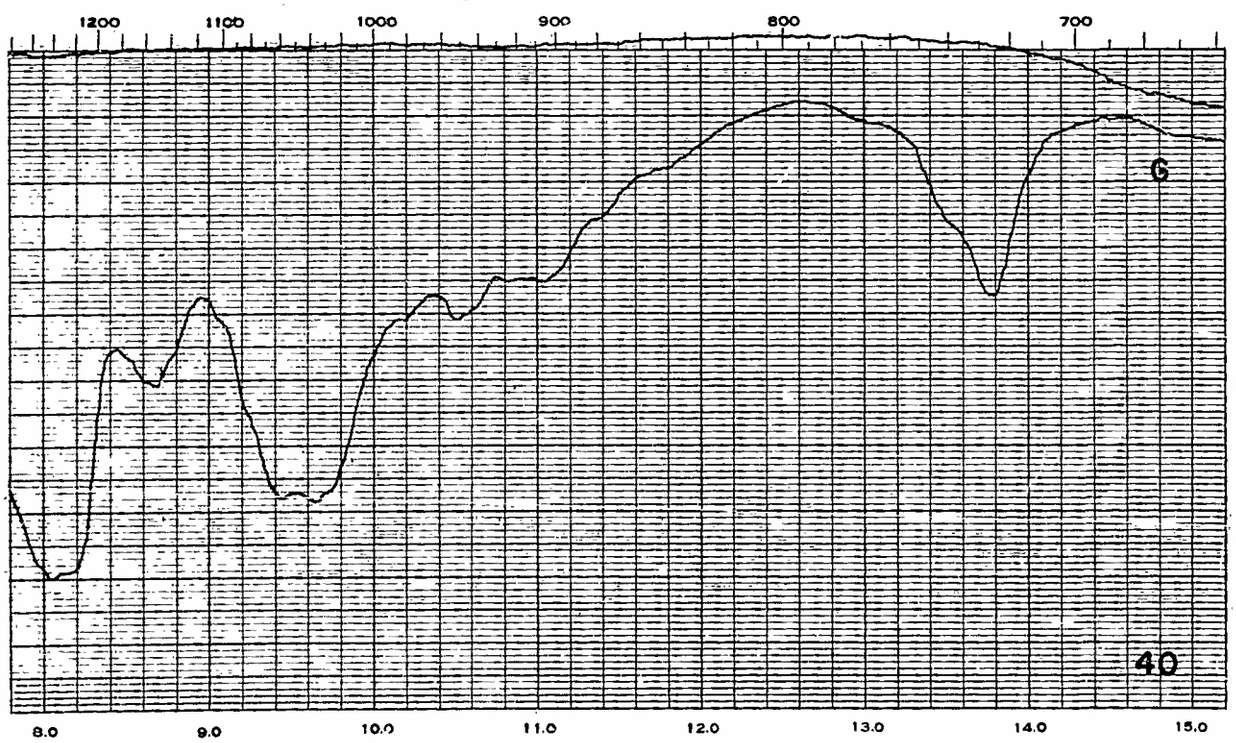




Wave Length in Microns

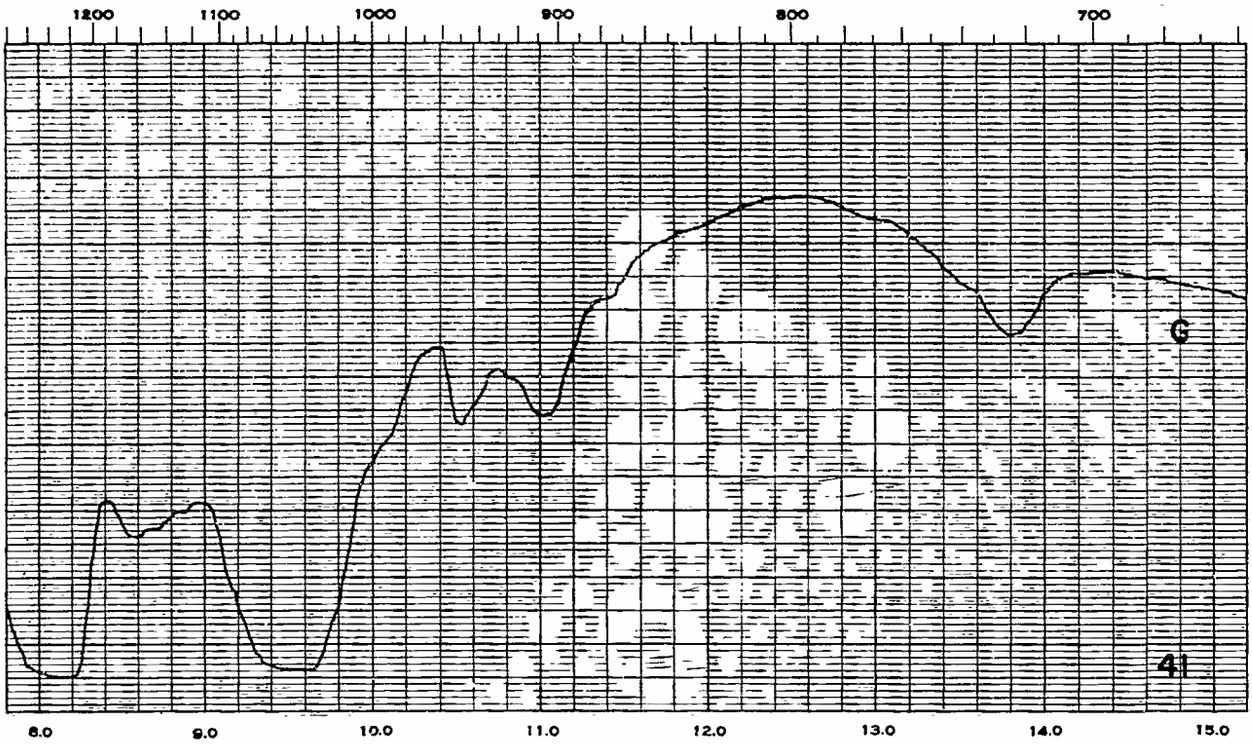
Wave Numbers in cm

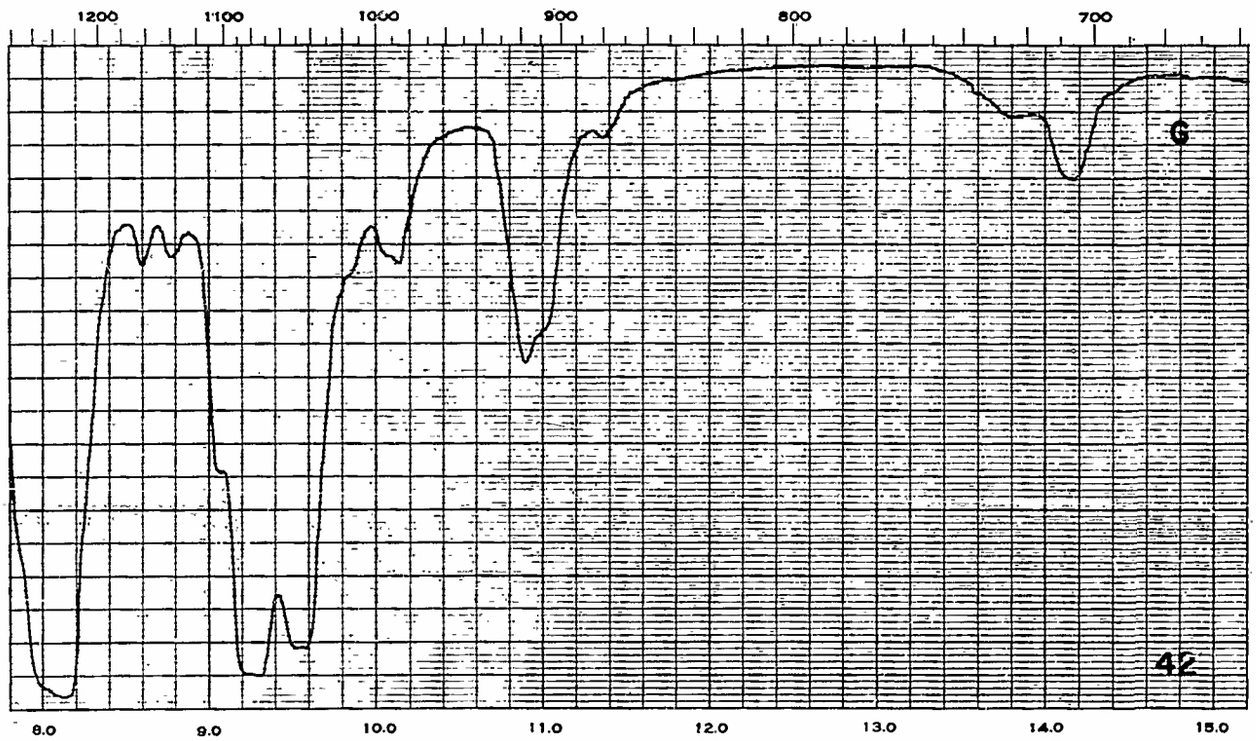




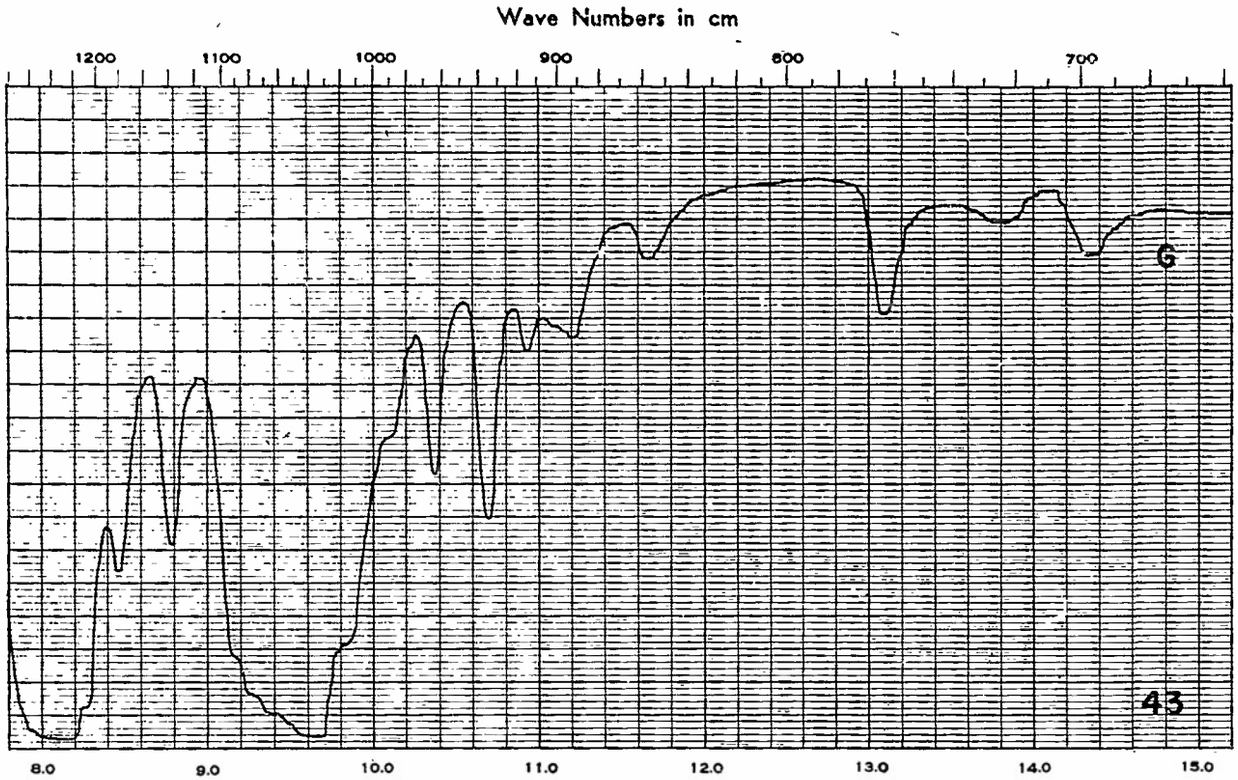
Wave Length in Microns

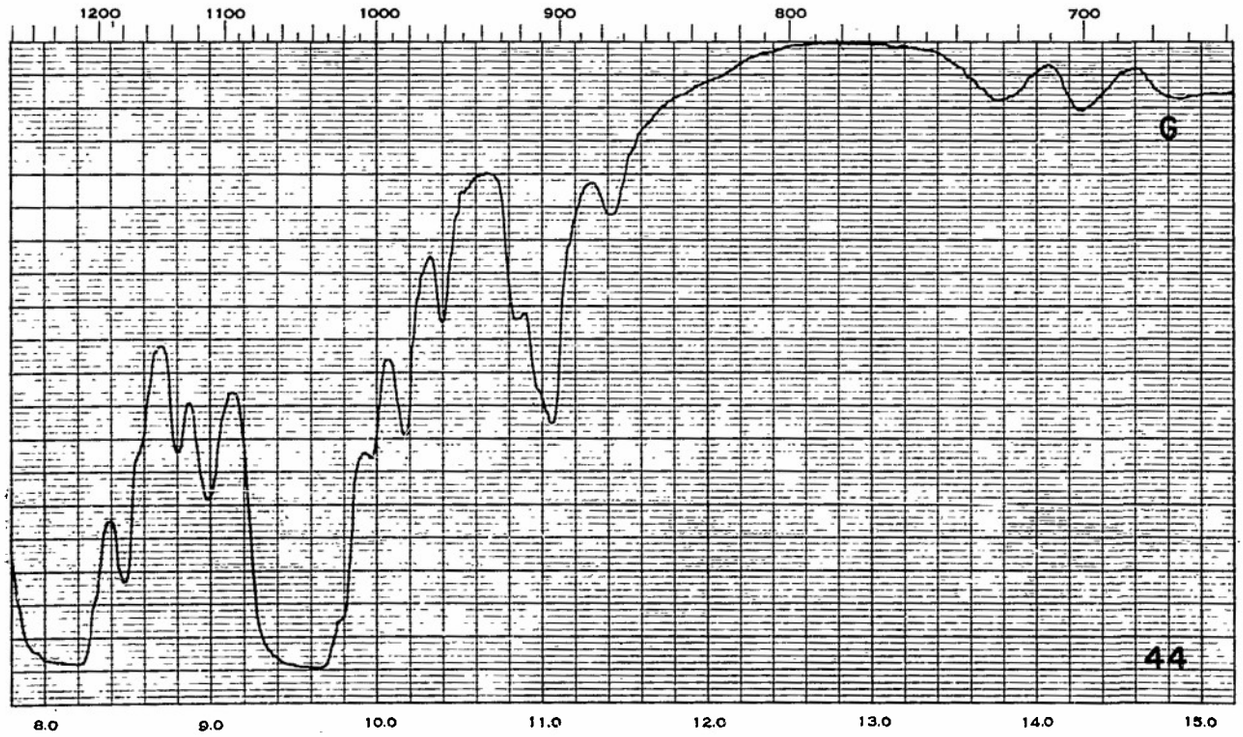
Wave Numbers in cm



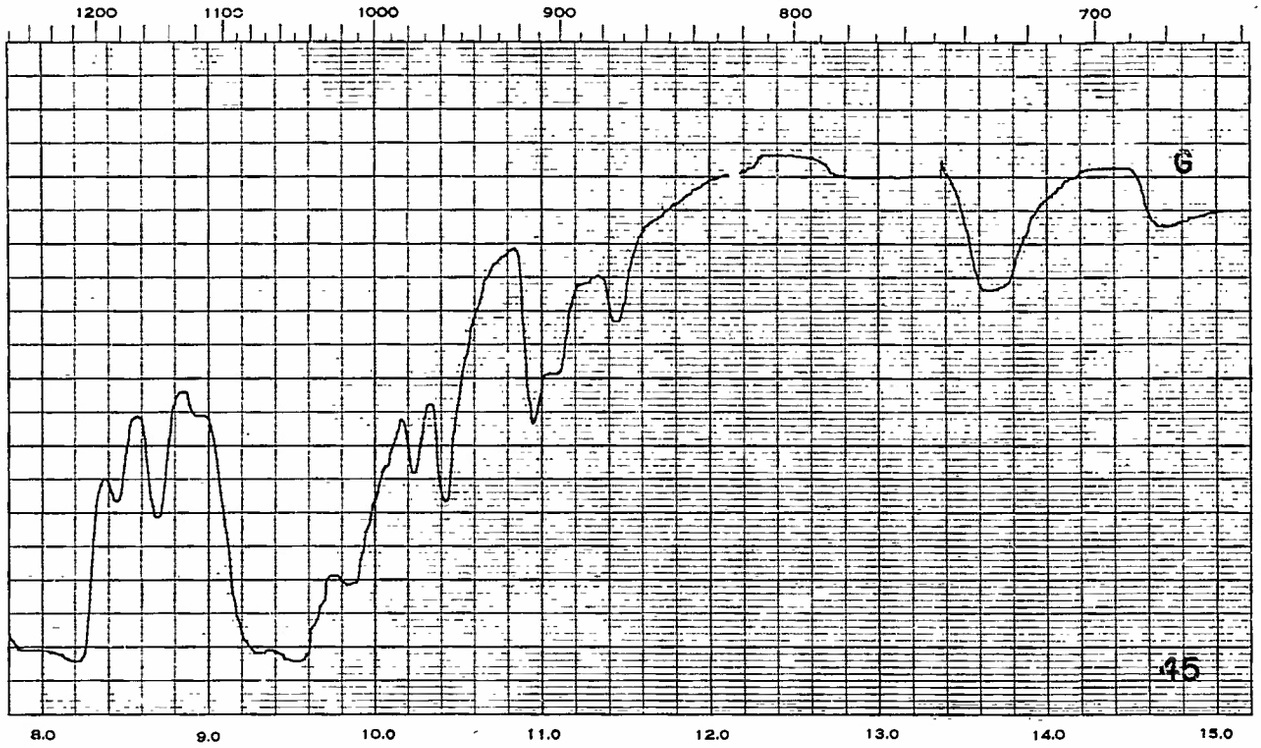


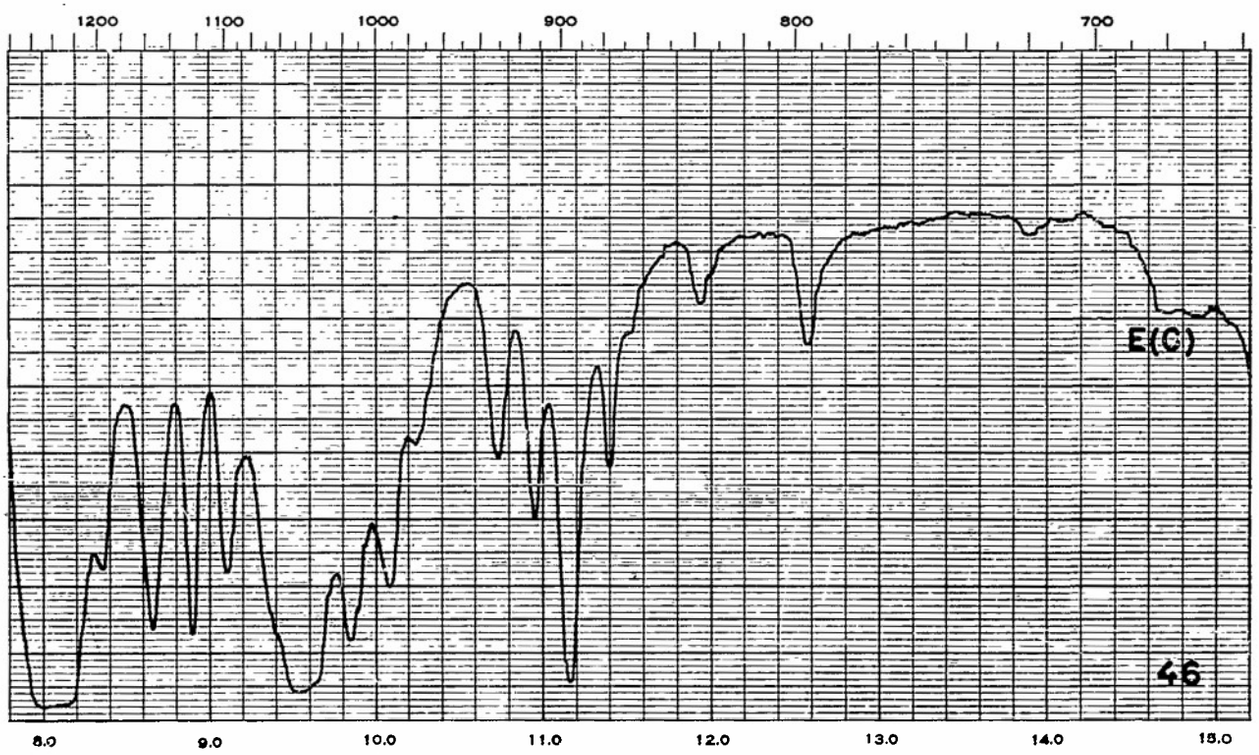
Wave Length in Microns


Wave Numbers in cm
1200
1100
1000
900
800
700
G
43
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0


1200
1100
1000
900
800
700
G
44
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
Wave Length in Microns

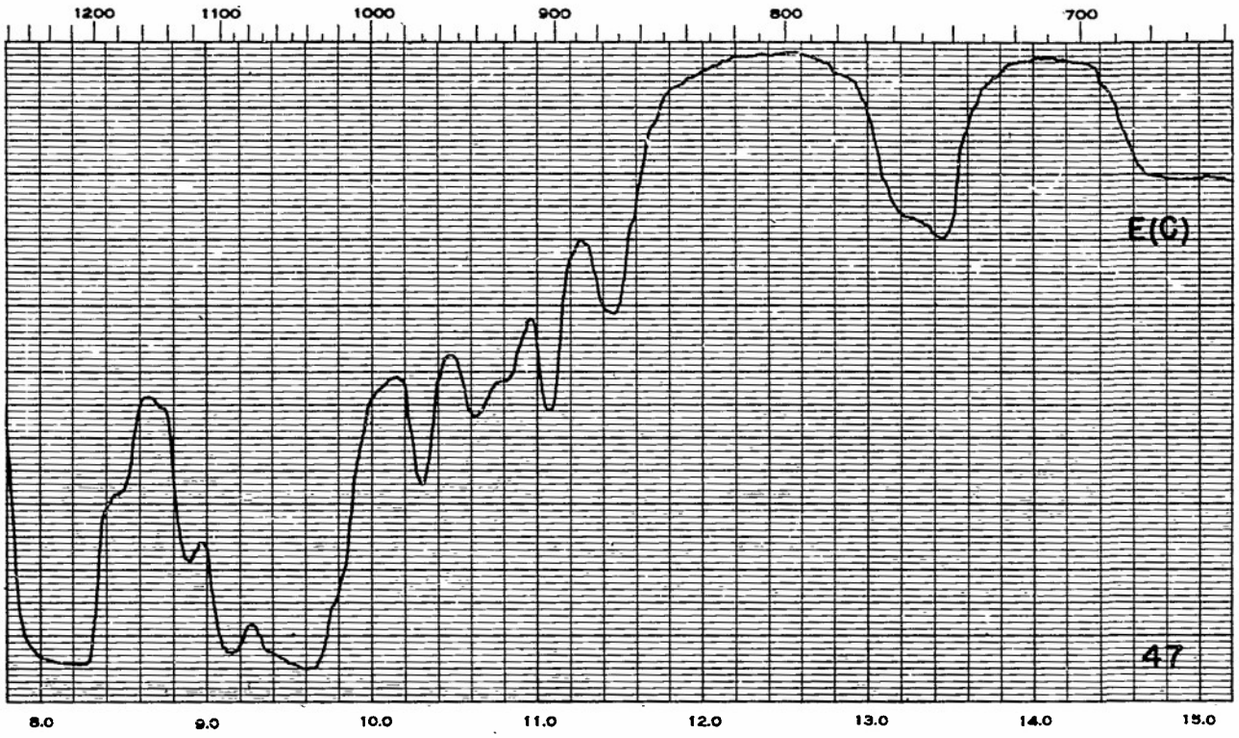
Wave Numbers in cm

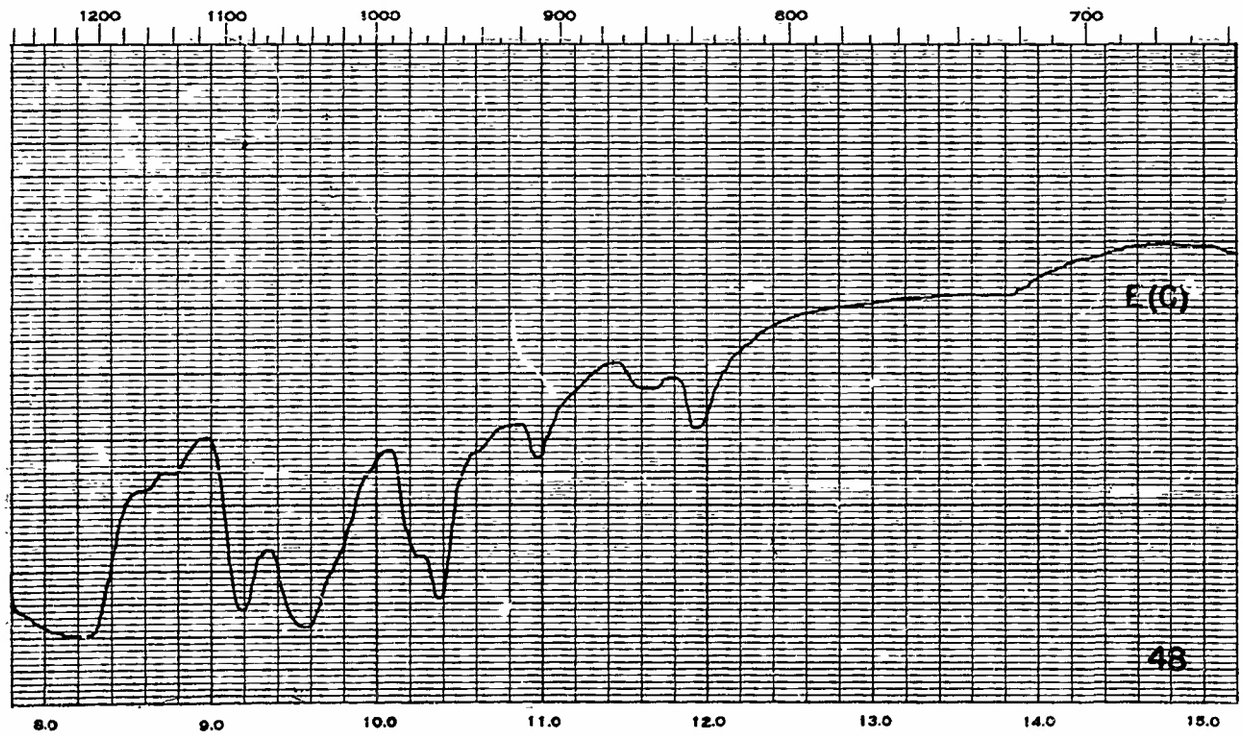




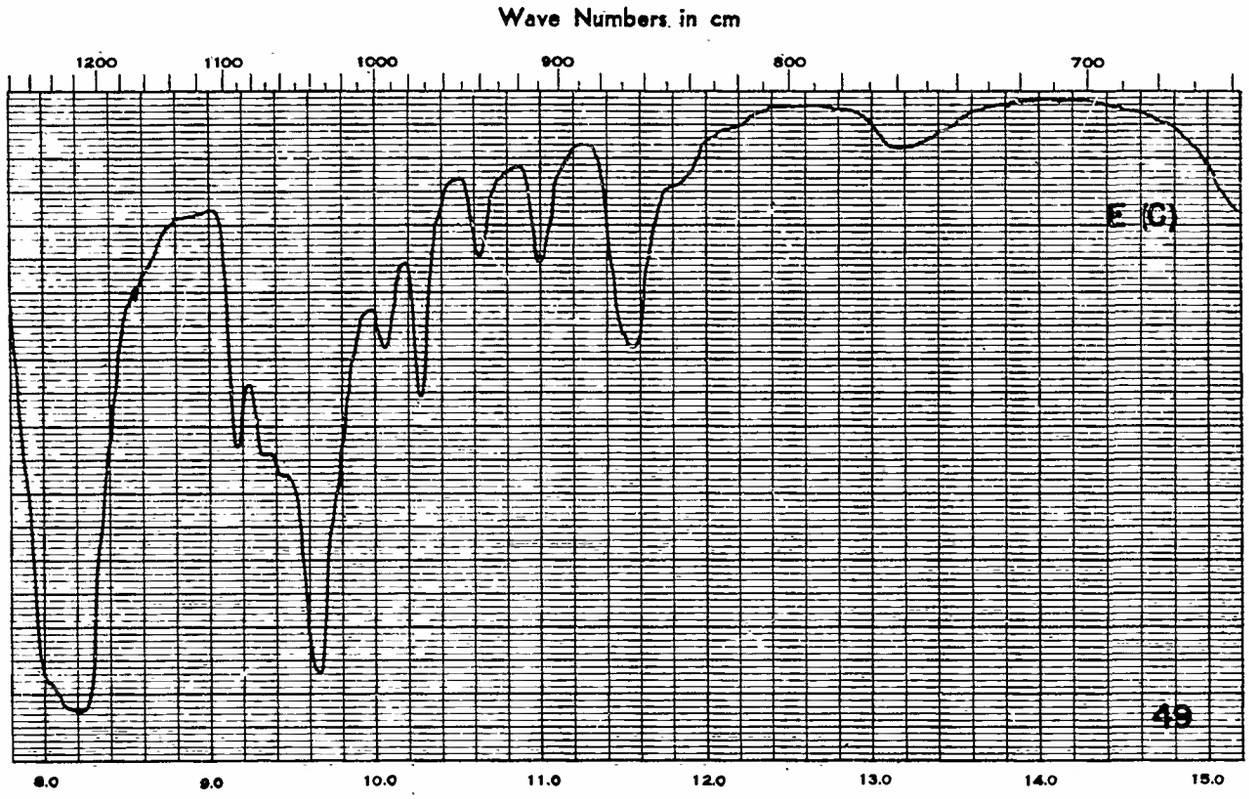
Wave Length in Microns

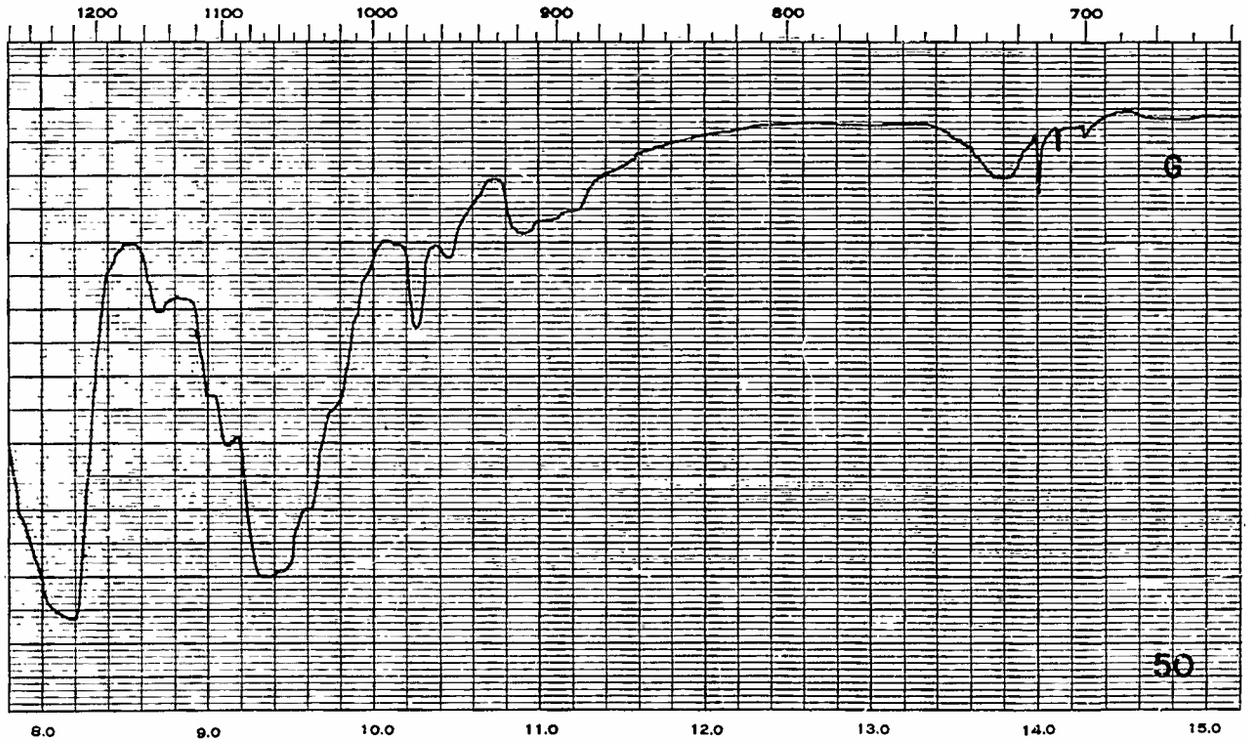
Wave Numbers in cm



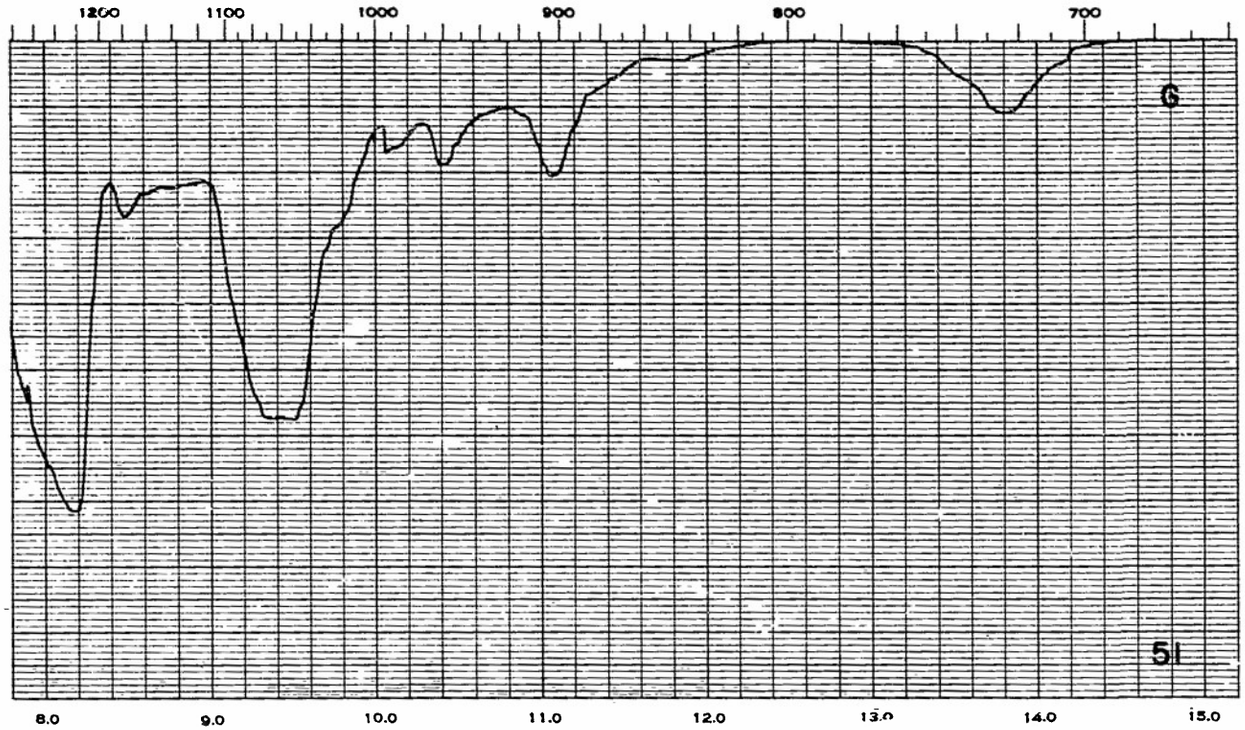


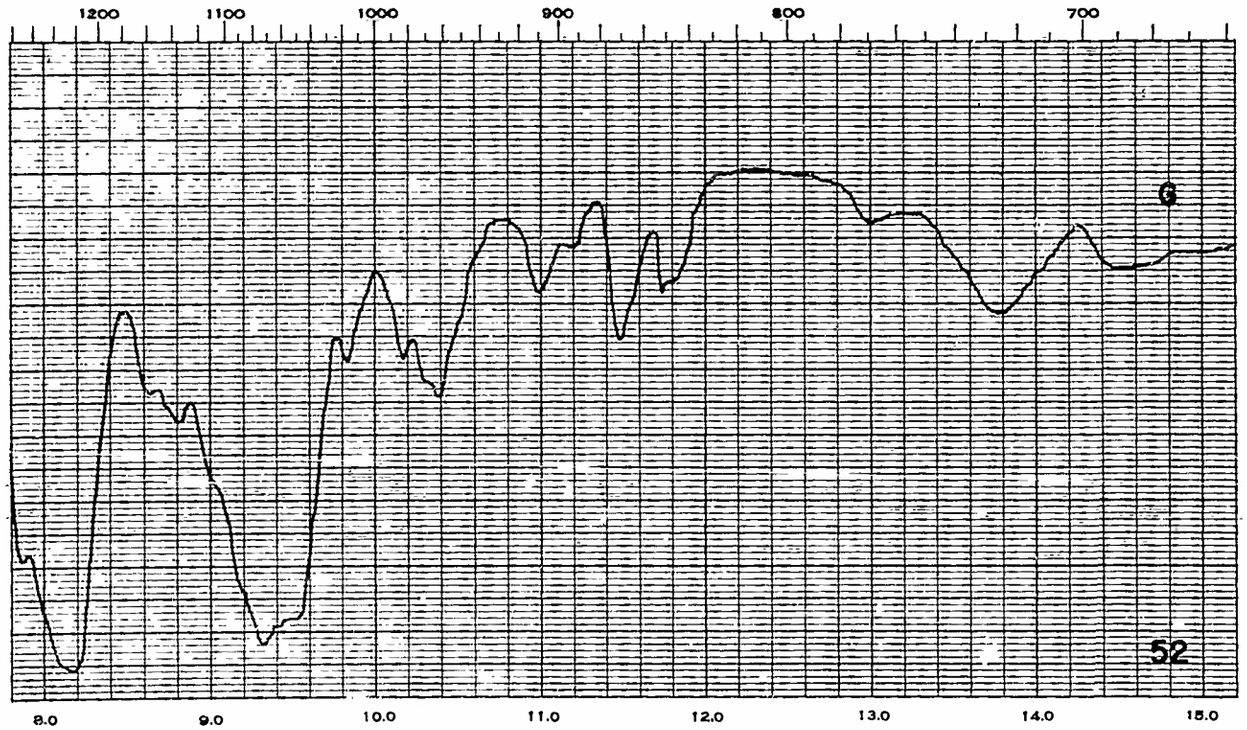
Wave Length in Microns


Wave Numbers in cm
1200
1100
1000
900
800
700
E (C)
49
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0


1200
1100
1000
900
800
700
G
50
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
Wave Length in Microns

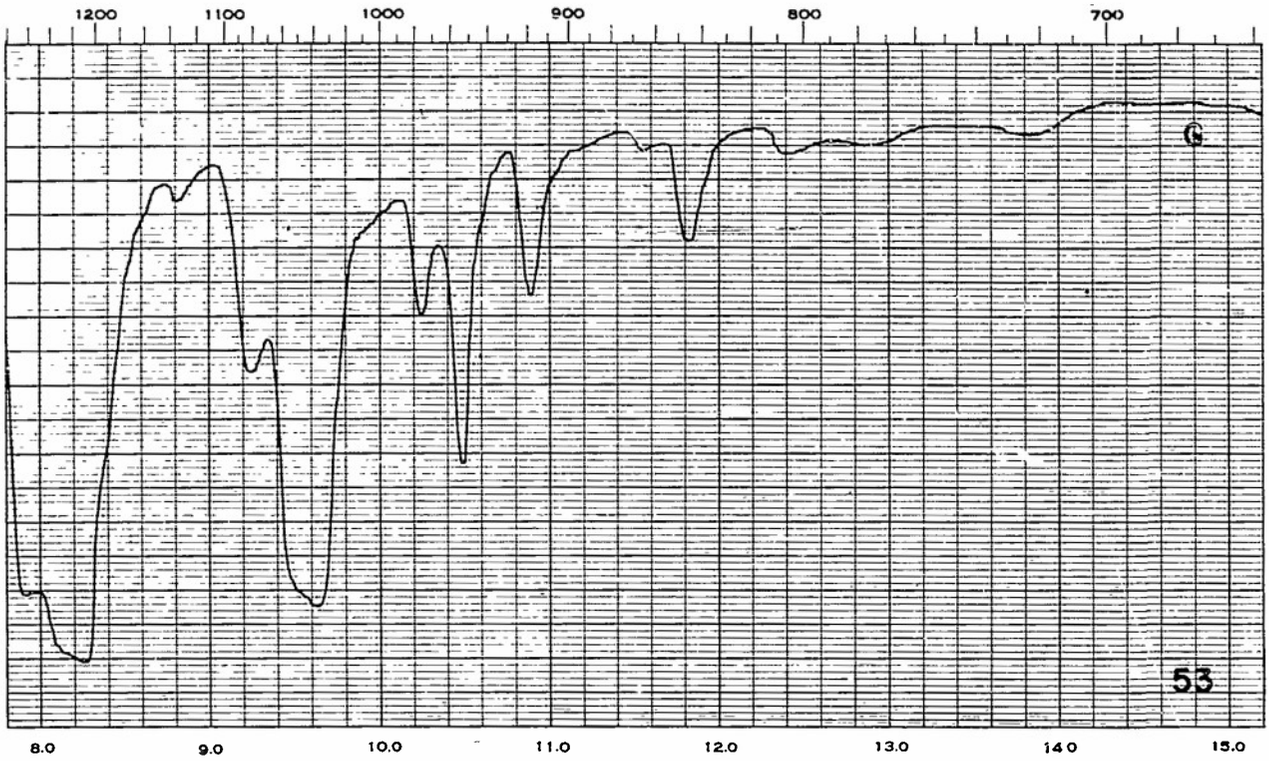
Wave Numbers in cm

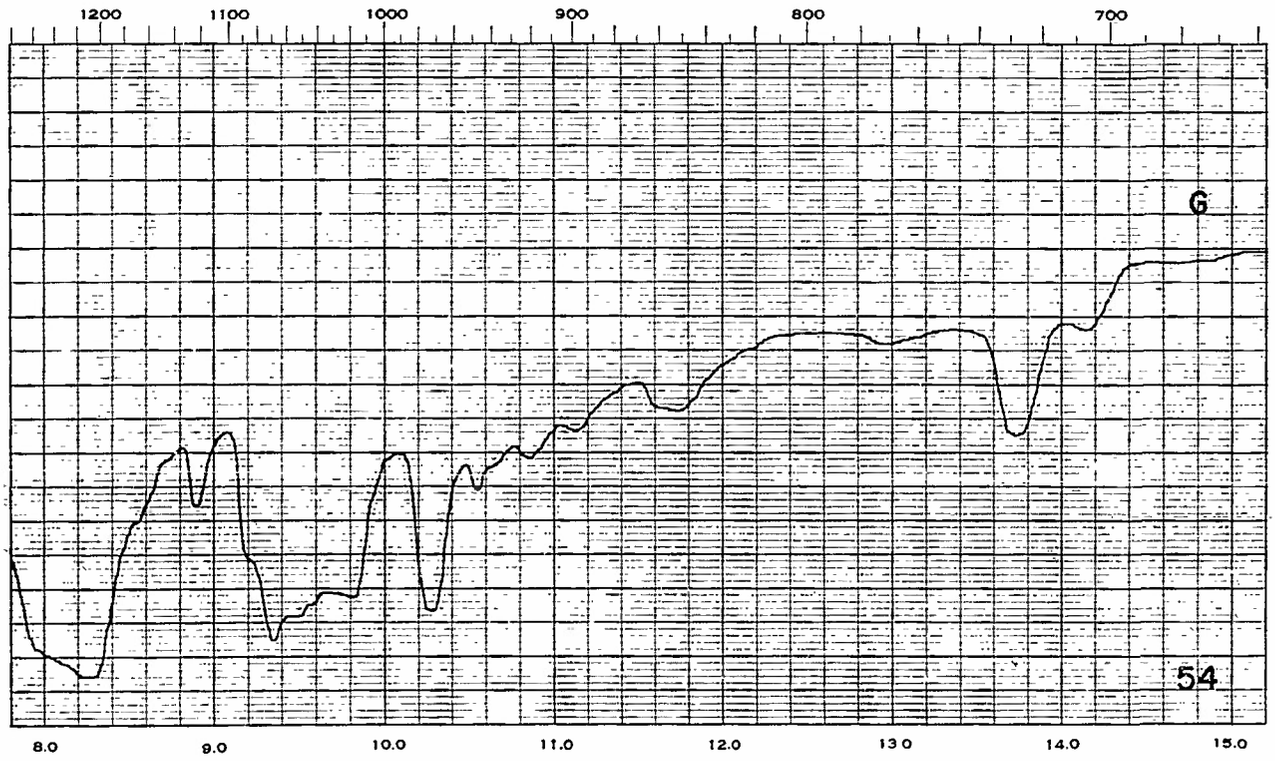




Wave Length in Microns

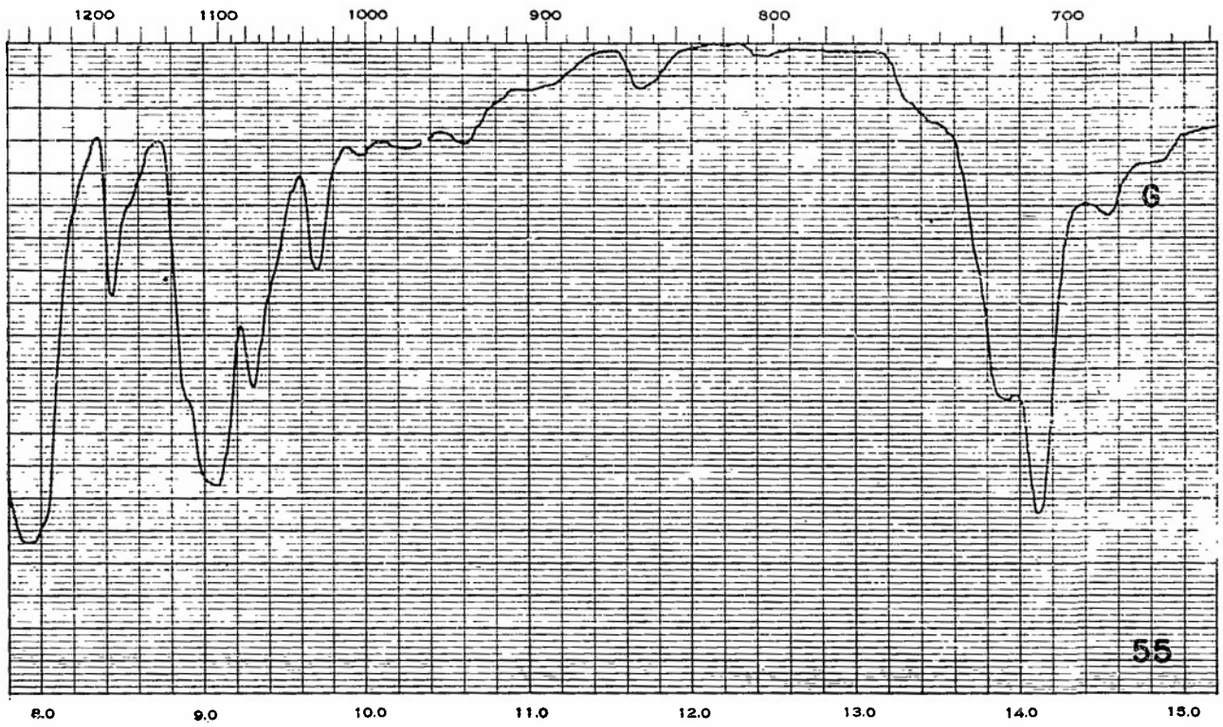
Wave Numbers in cm

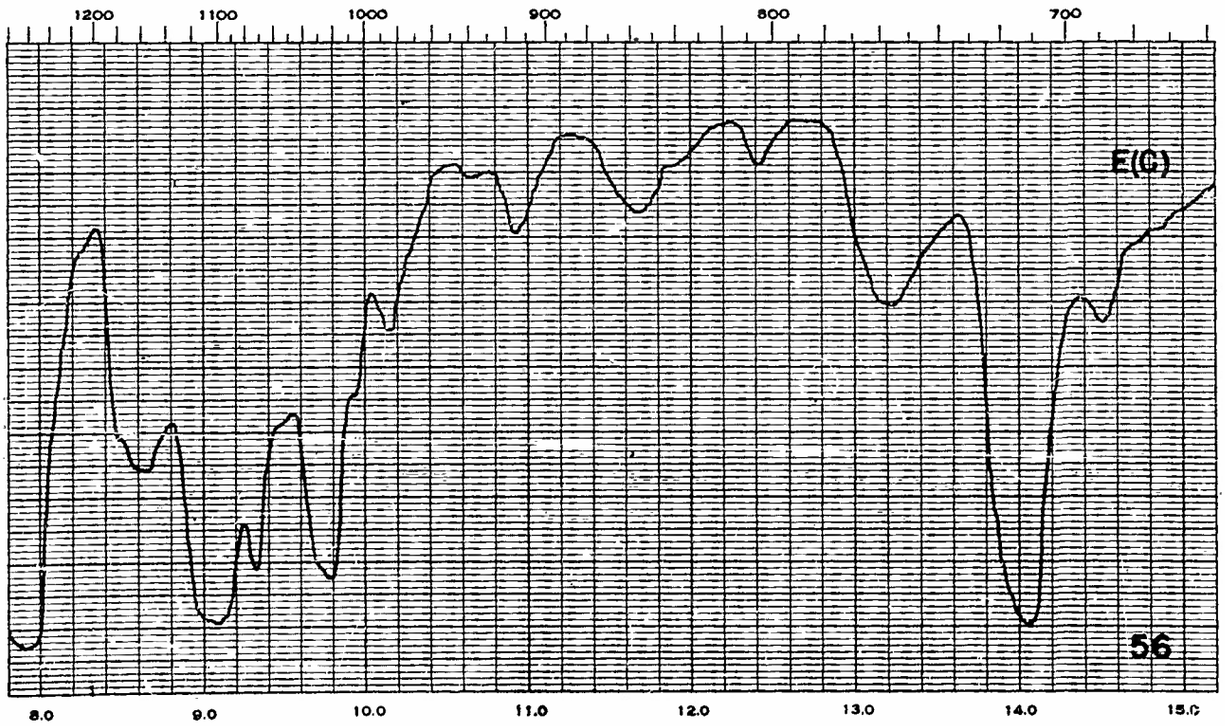




Wave Length in Microns

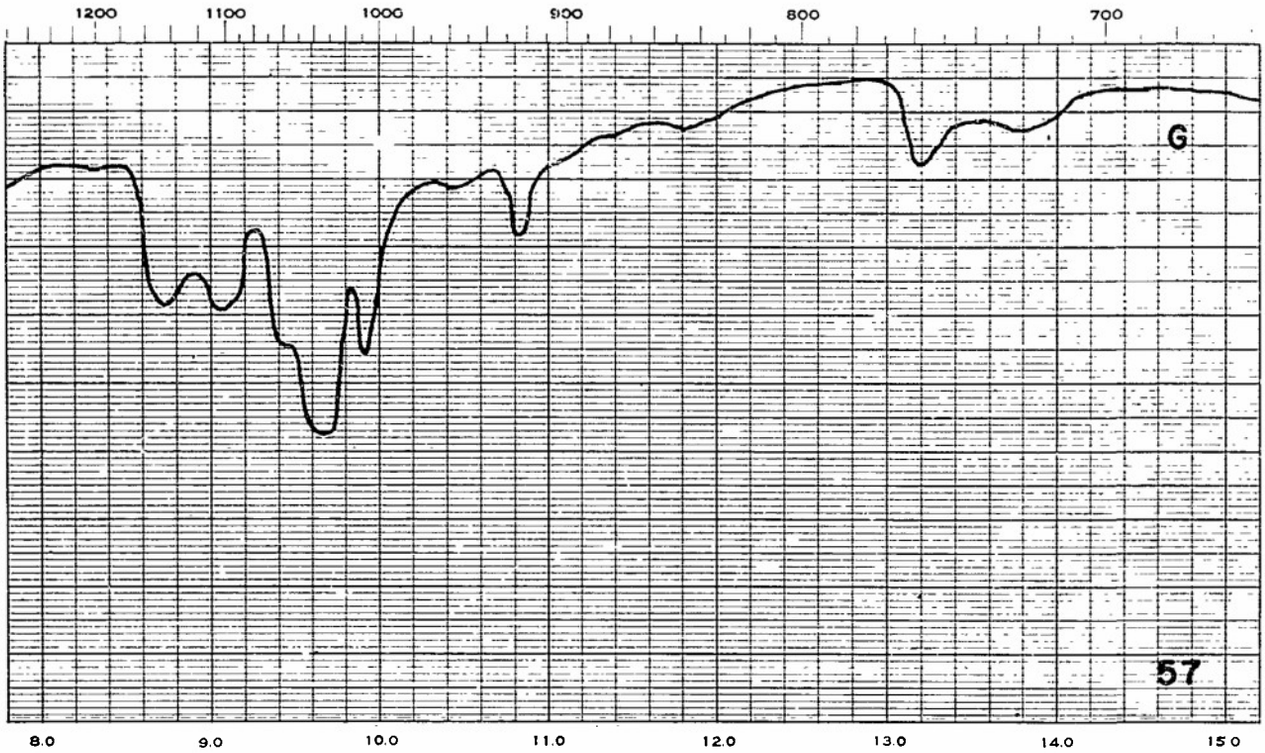
Wave Numbers in cm

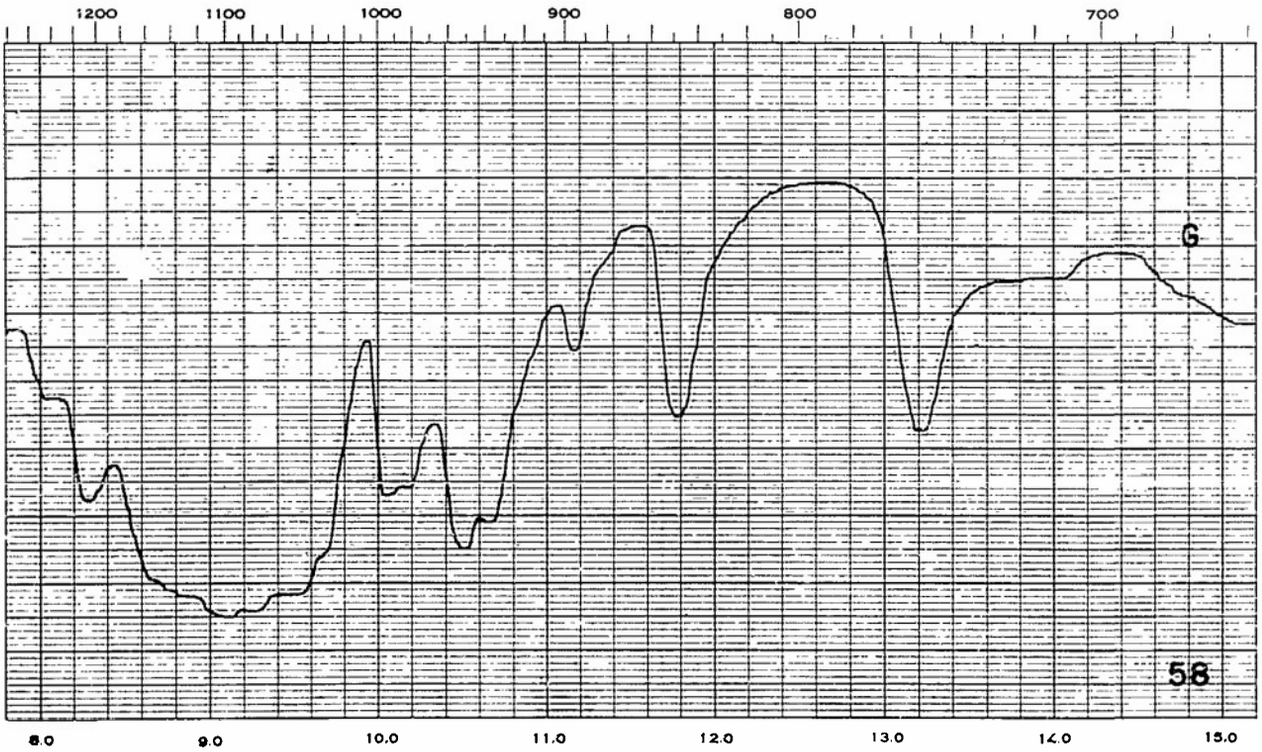




Wave Length in Microns

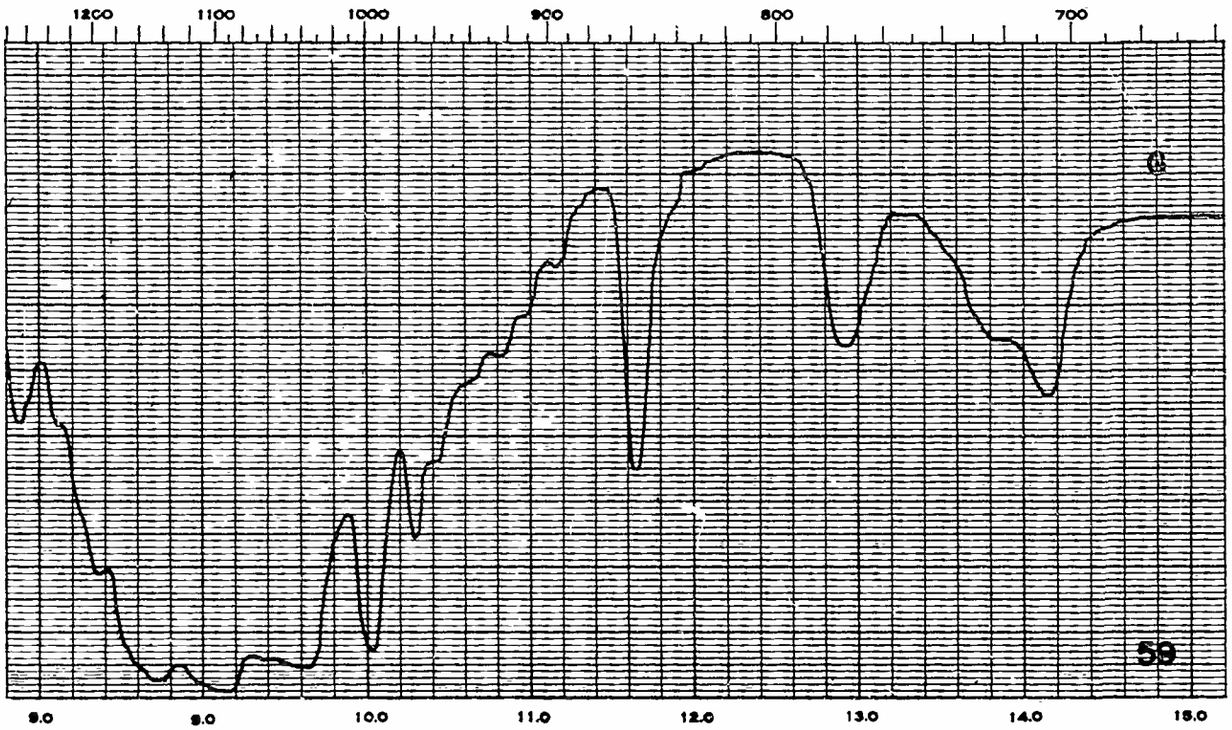
Wave Numbers in cm

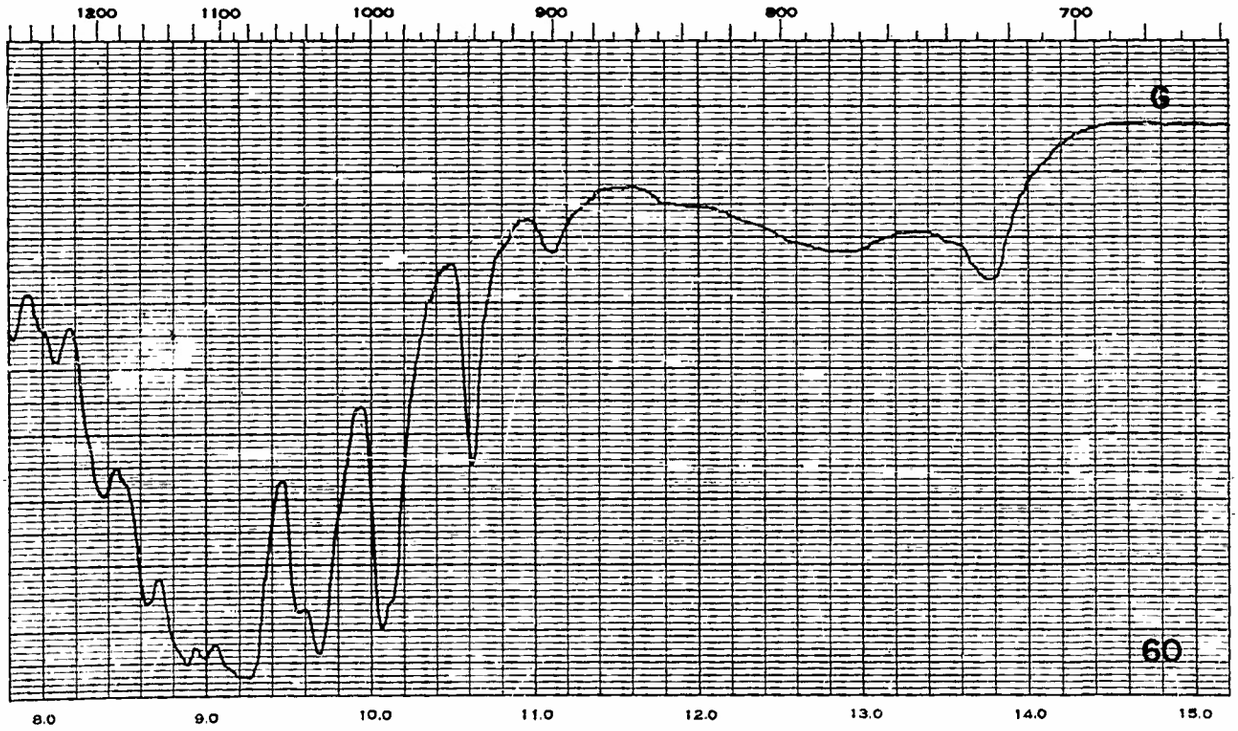




Wave Length in Microns

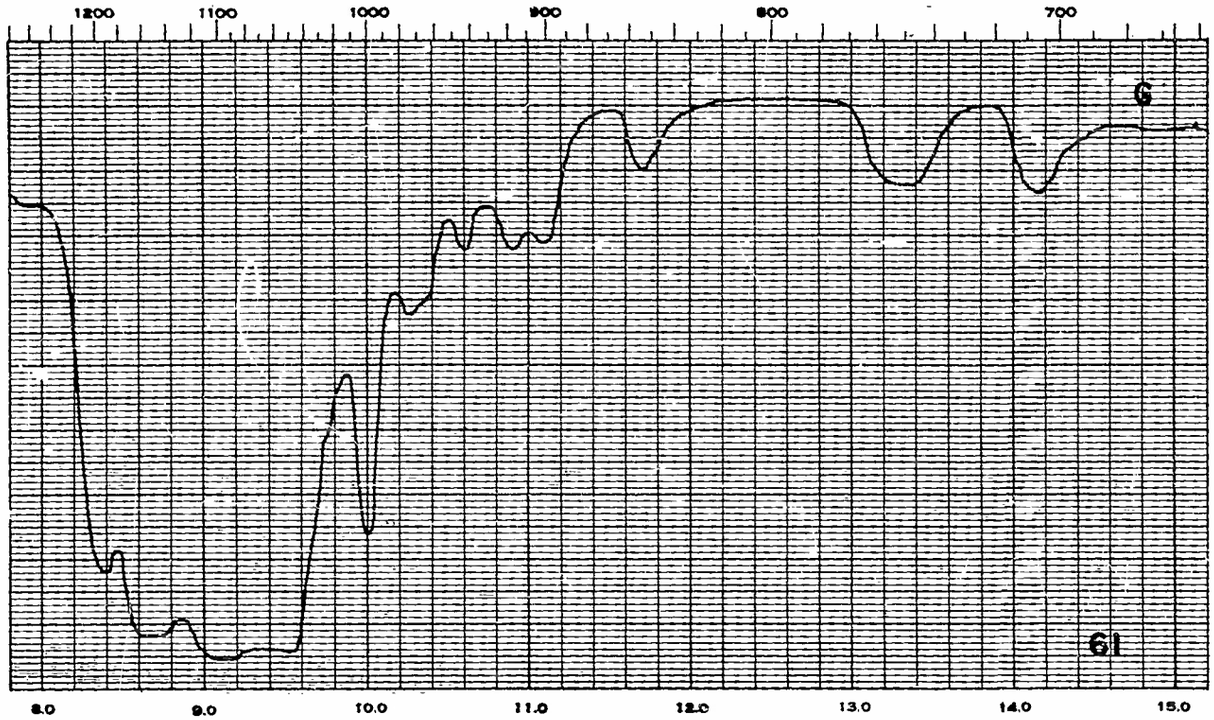
Wave Numbers in cm

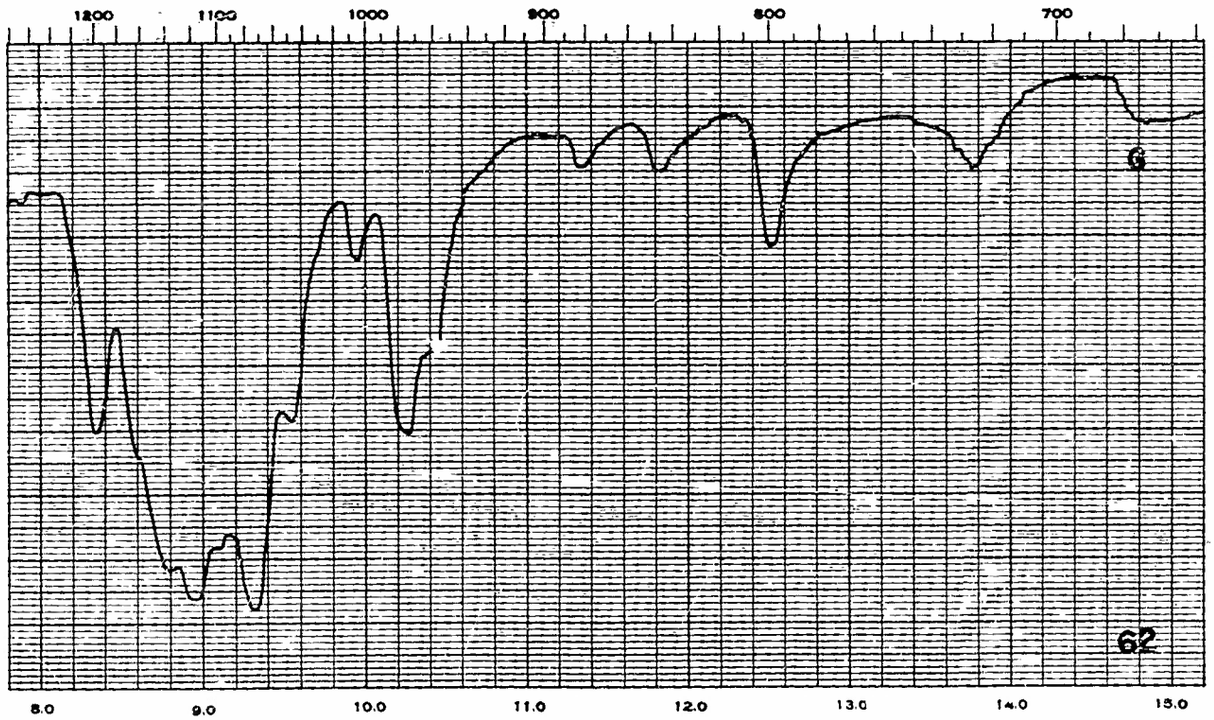




Wave Length in Microns

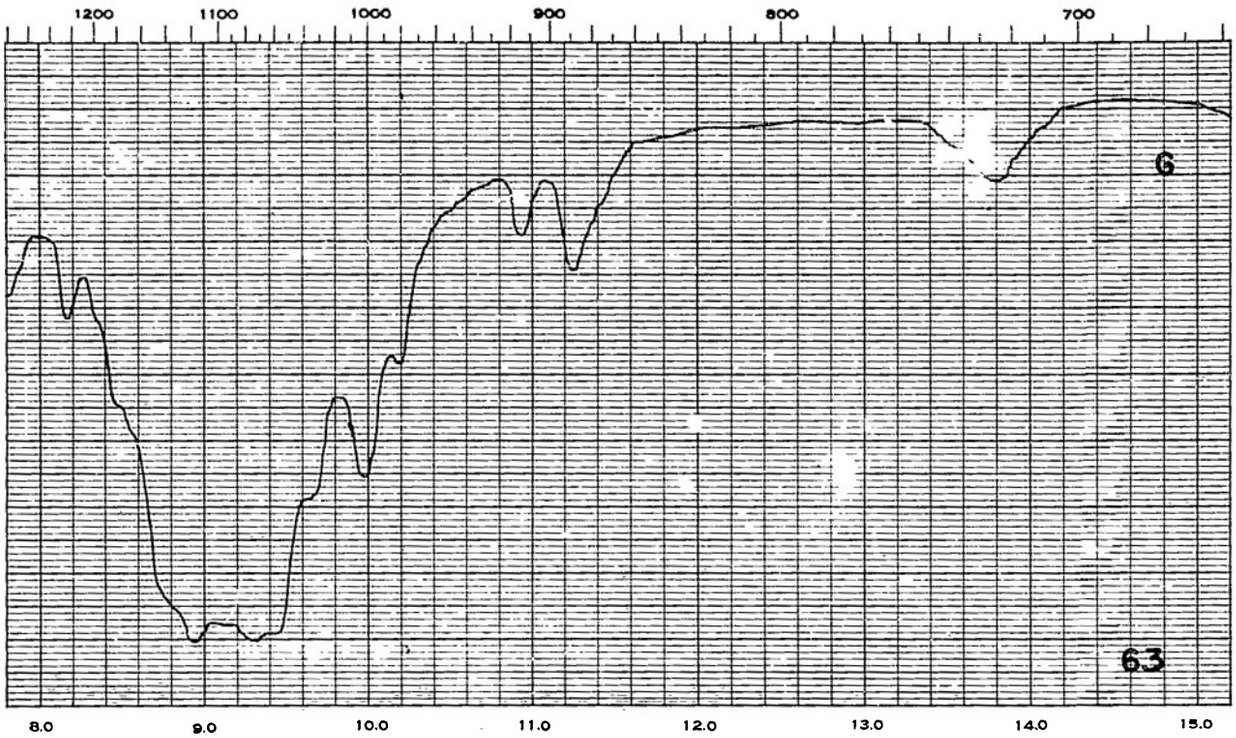
Wave Numbers in cm

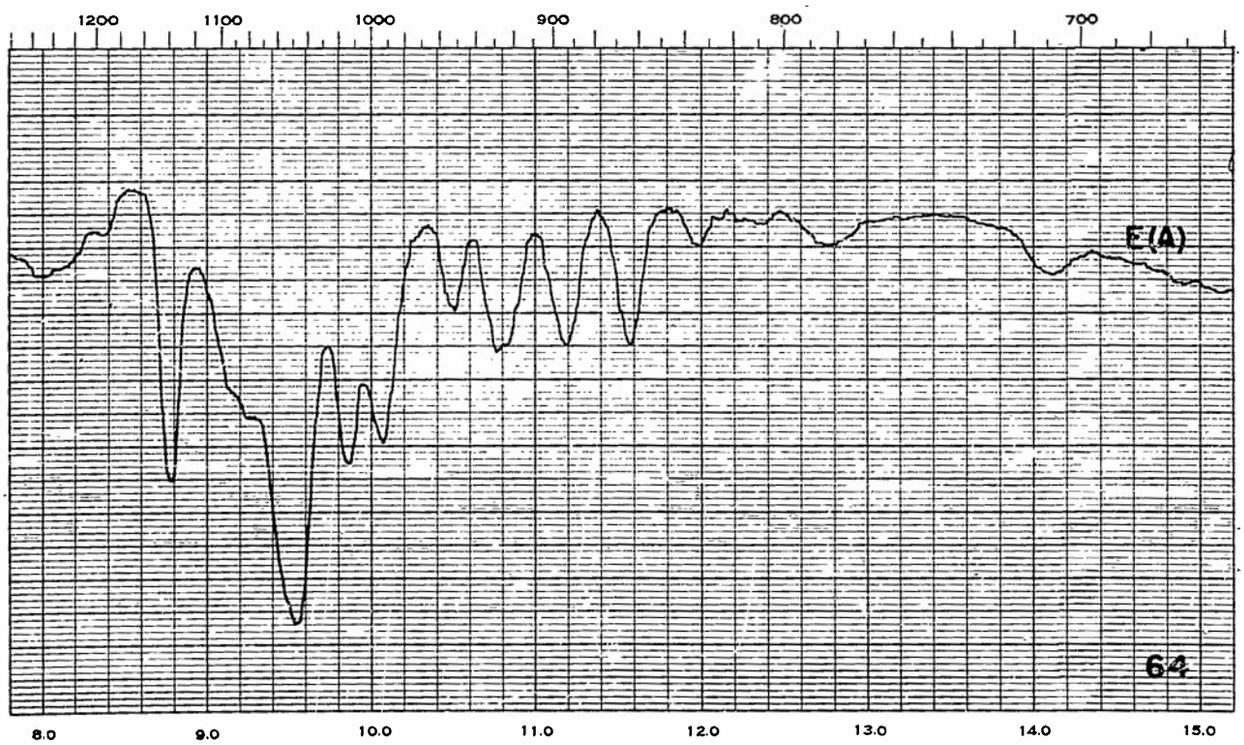




Wave Length in Microns

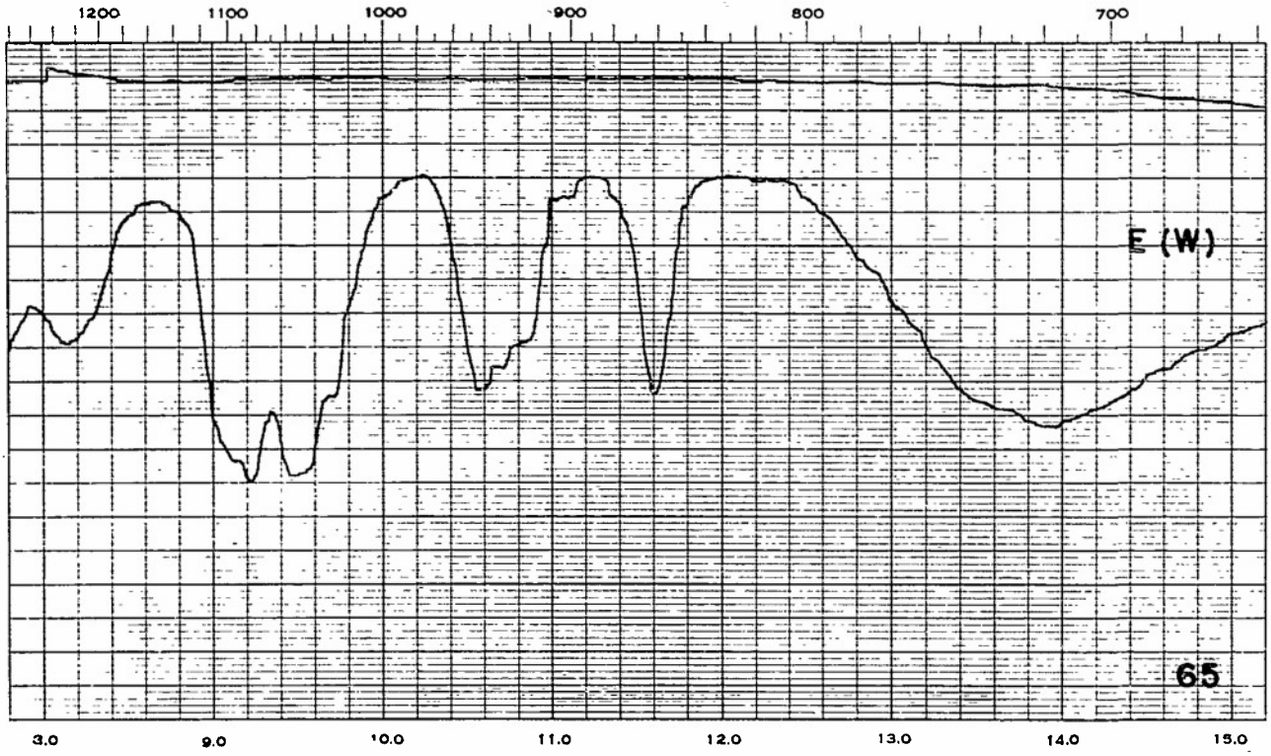
Wave Numbers in cm

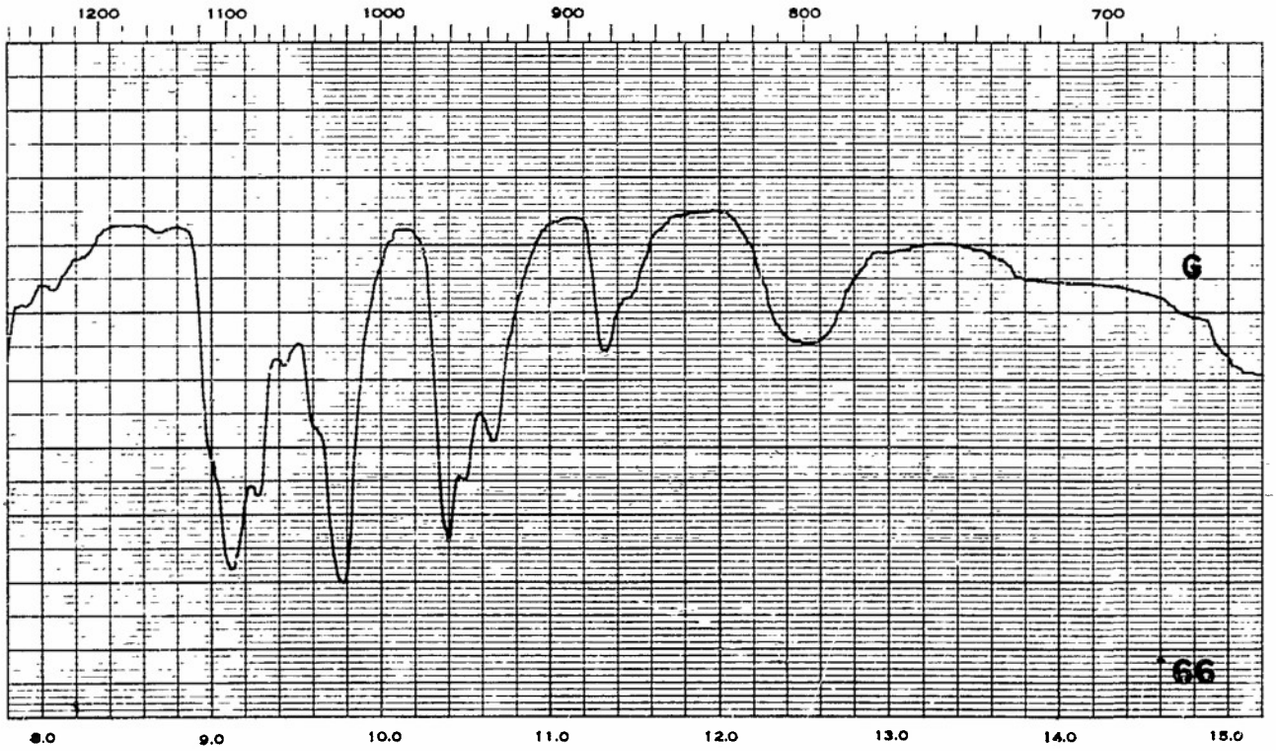




Wave Length in Microns

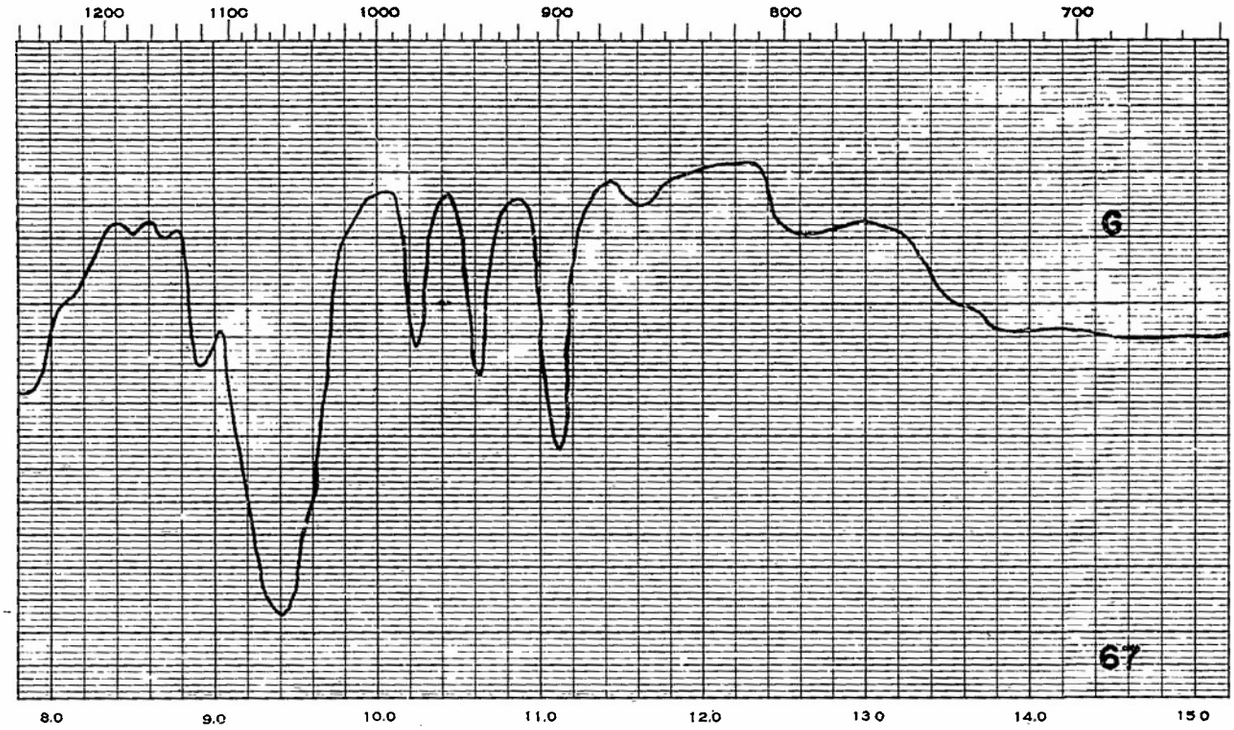
Wave Numbers in cm

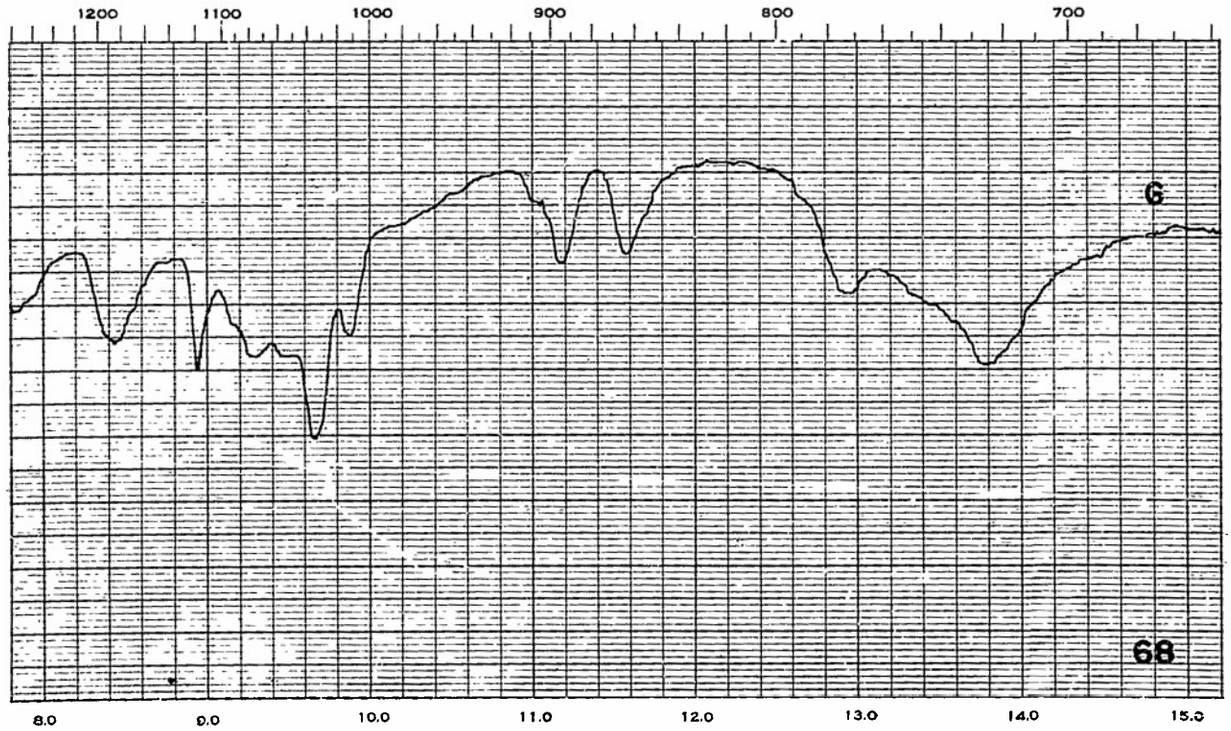




Wave Length in Microns

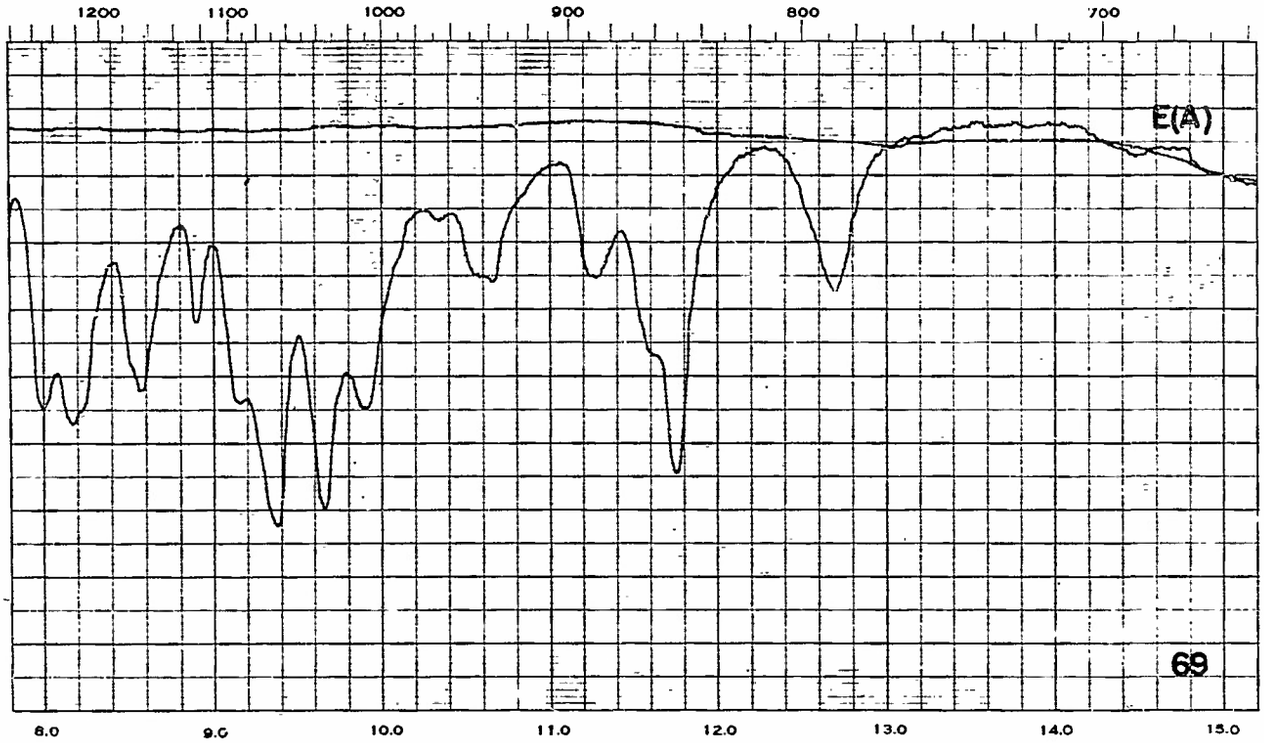
Wave Numbers in cm

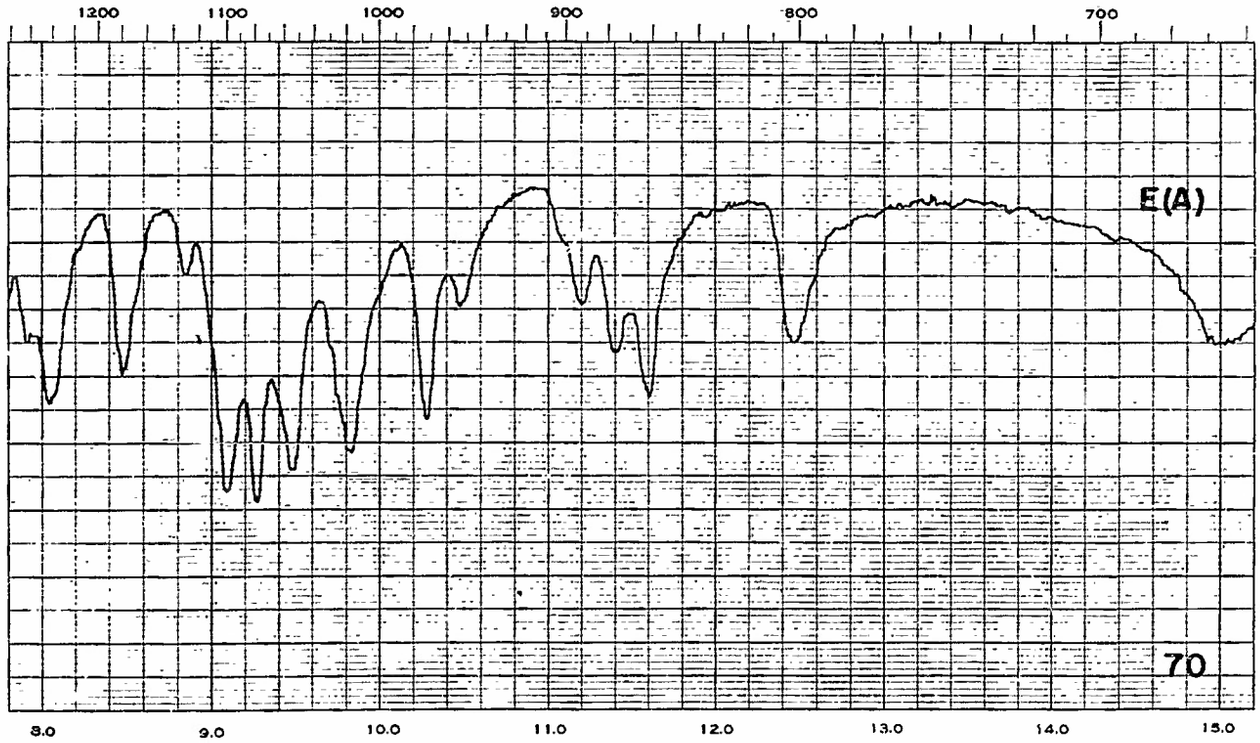




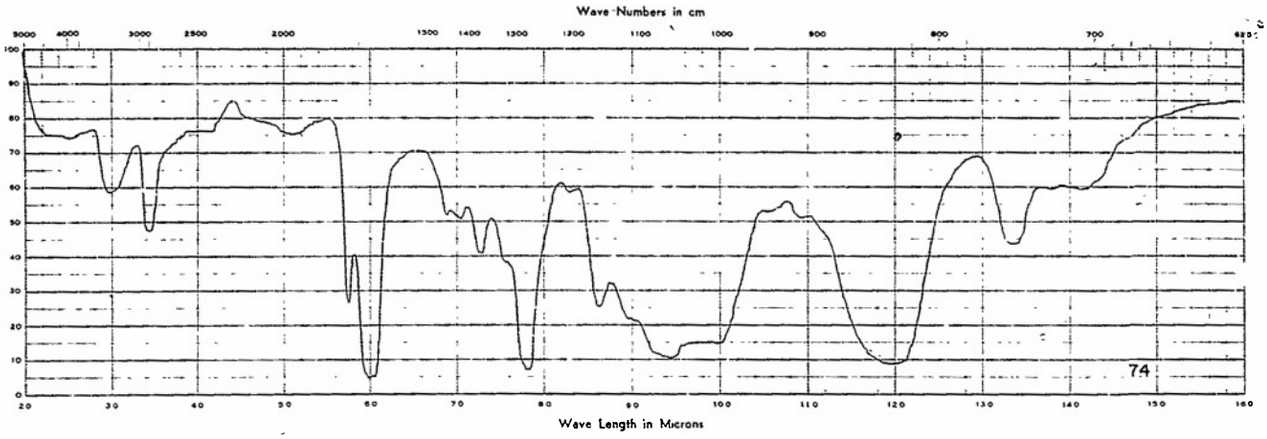
Wave Length in Microns

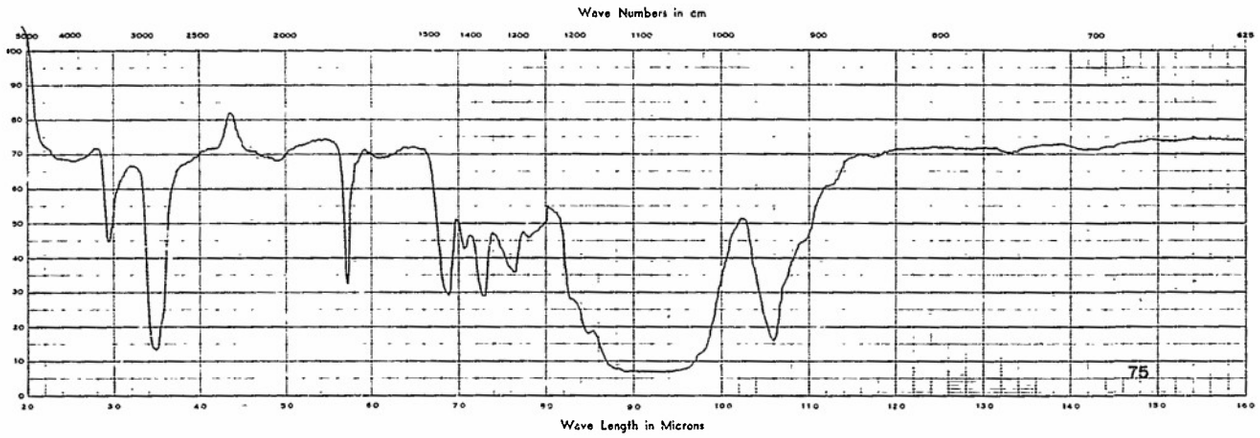
Wave Numbers in cm

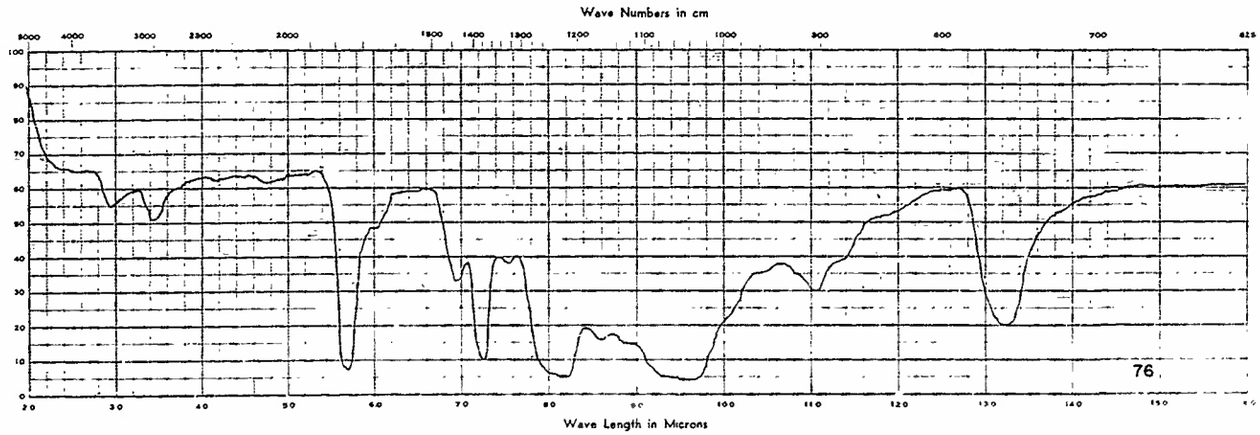


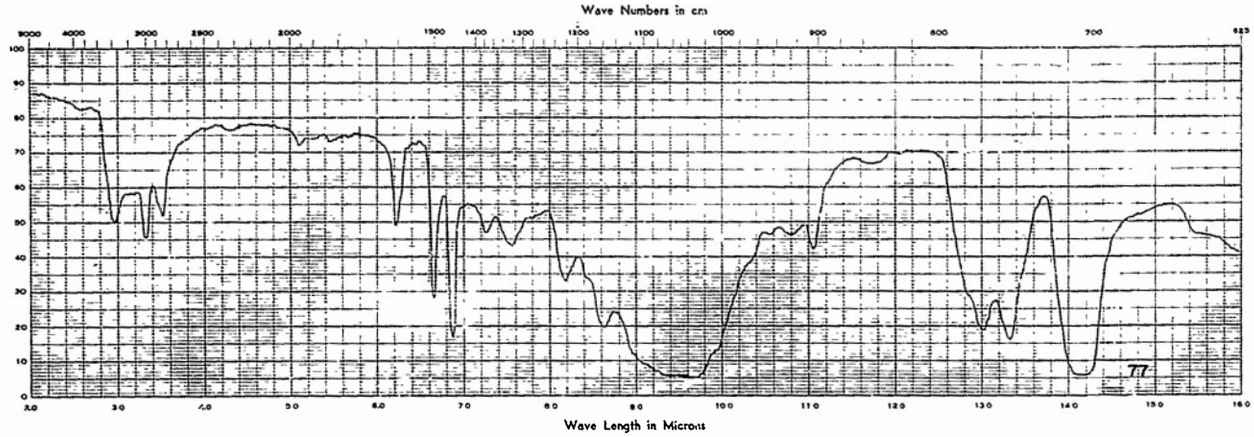


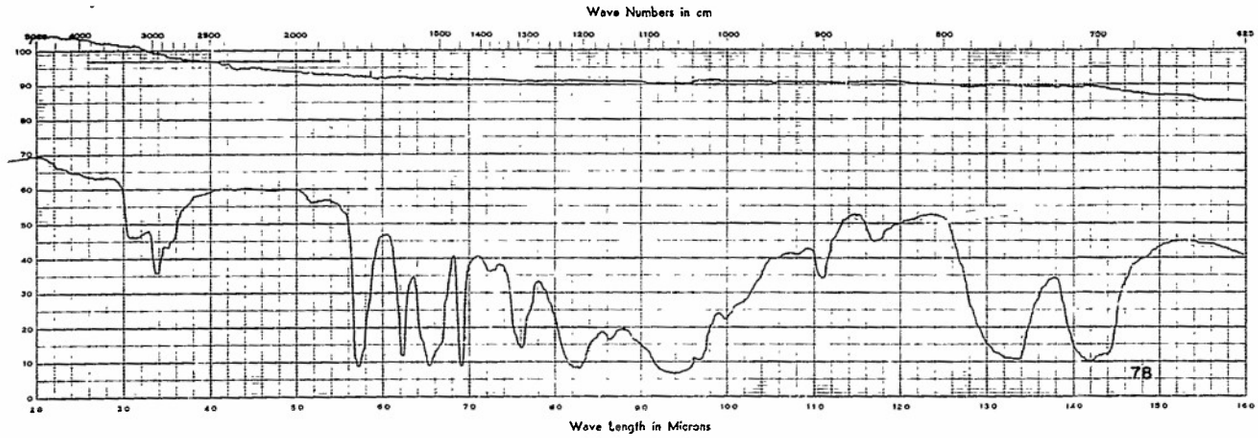
Wave Length in Microns

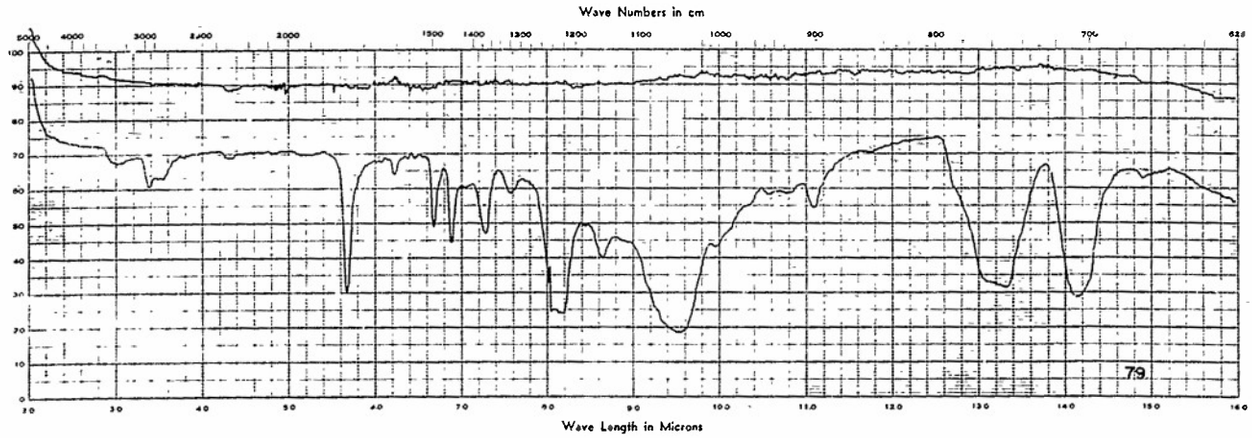

Wave Numbers in cm
Wave Length in Microns
74


Wave Numbers in cm
Wave Length in Microns
75


Wave Numbers in cm
Wave Length in Microns
76


Wave Numbers in cm
Wave Length in Microns
77


Wave Numbers in cm
Wave Length in Microns
78


Wave Numbers in cm
Wave Length in Microns
79

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