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Special Studies in Biochemical Instrumental Methods

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Special Studies in Biochemical Instrumental Methods

Chemistry 491

by

Parvin Waymack

for

Dr. W.W. Everett

May 22, 1967

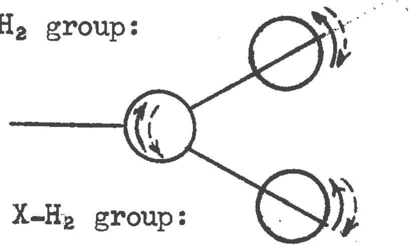
Two useful methods of biochemical analysis are infrared and ultraviolet spectroscopy. The occurrenceof absorption bands in the infrared region can be predicted for some of the simpler molecules. Theoretically, in order for a molecule to absorb radiation it must possess a vibrational or rotational frequency the same as that of the electromagnetic radiation, and a change in the magnitude and/or direction of the dipole moment must take place. The change in dipole moment may be caused by asymmetrical vibration or asymmetrical rotation of the molecule. A molecule has 3N-6 fundamental modes of vibration where N is the number of atoms in the molecule. The fundamental nomenclature of the vibrational modes is as follows:

Stretching of a X-H bond:

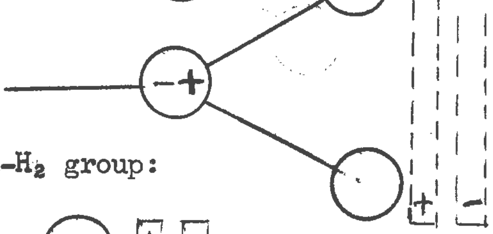
In-plane bend of an X-H bond:

Out of plane bend of an X-H bond:

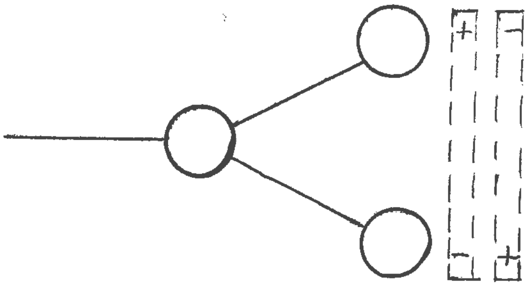
Rock-in-plane bend of an X-H_2 group:



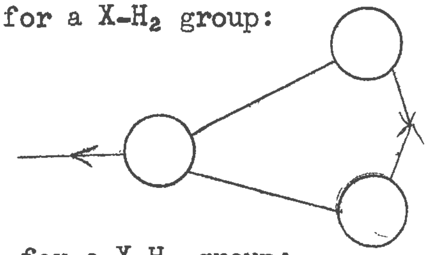
Wag-out of plane bend of an X-H_2 group:



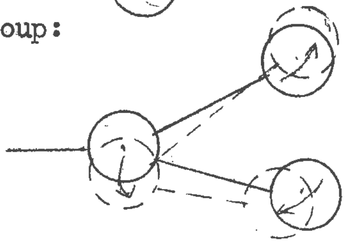
Twist- a vertical rotation of an X-H_2 group:



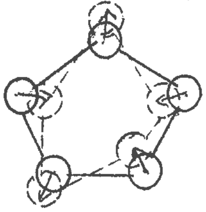
Bend- symmetrical for a X-H_2 group:



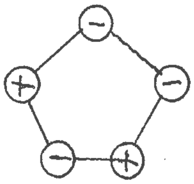
Bend- asymmetrical for a X-H_2 group:



Deformation in plane- all atoms move but in same plane:

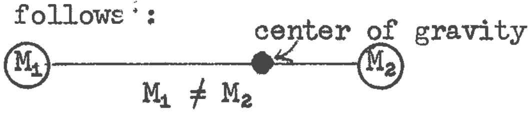


Deformation out of plane- all atoms move, some out of plane



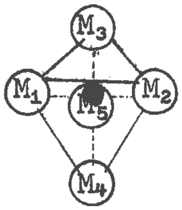
In addition to vibrating, a molecule may rotate about its center of gravity. The frequency of this rotation falls in the far infrared region. Some of the types are as follows:

A linear molecule.



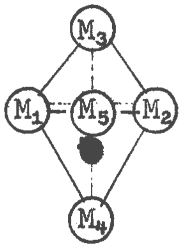
A spherical top molecule.

$M_1=M_2=M_3=M_4 \neq M_5$

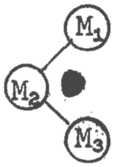


A symmetrical top molecule.

$M_1=M_2=M_3$



Asymmetrical top molecule



In many of the normal modes of vibrations the main participants in the vibration will be two atoms held together by a chemical bond. The frequency of the vibration is only slightly affected by the other atoms attached to the atoms concerned. Therefore these vibrational modes are characteristic of the groups in the molecule and are thus very useful in identifying a compound.

When molecules absorb in the ultraviolet region, valence or bonding electrons are raised to higher energy levels, with accompanying vibrational and rotational excitations. The close spacing of the vibrational-rotational levels in relation to electron levels cause each electron transition to result in a large number of slightly different possible energy levels. This along with solvent solute interaction tends to yield broad absorption bands in the ultraviolet. Molecules of rather loosely bound electron clouds afford absorption of energy in the ultraviolet. Therefore ultraviolet spectra are diagnostic of unsaturation in absorbing molecules, because with few exceptions only molecules with multiple bonds have excited states of sufficently low energy to give rise to absorption in the near ultraviolet. Organic analysis in the ultraviolet would be severely limited but for the fact that olefinic, acetylenic, and carboxyl functions will give strong absorption in the ultraviolet when conjugated with one another.

EXPERIMENTAL

In order to learn more about the methods of obtaining infrared spectra, experiments were carried out using the basic methods of sample preparations. These included carbon tetrachloride solution, potassium bromide pellet and mineral oil mull.

Carbon tetrachloride is a popular solvent for infraed work because it is a comparitively inactive, non-polar compound which dissolves many organic compounds. More important is its lack of major absorption bands in the infrared region except one at 12.5 - 13.5 microns. Absorption in this region cannot be evaluated with carbon tetrachloride as a solvent because the solvent absorbs so much that the absorption of the sample in question cannot be detected in this region.

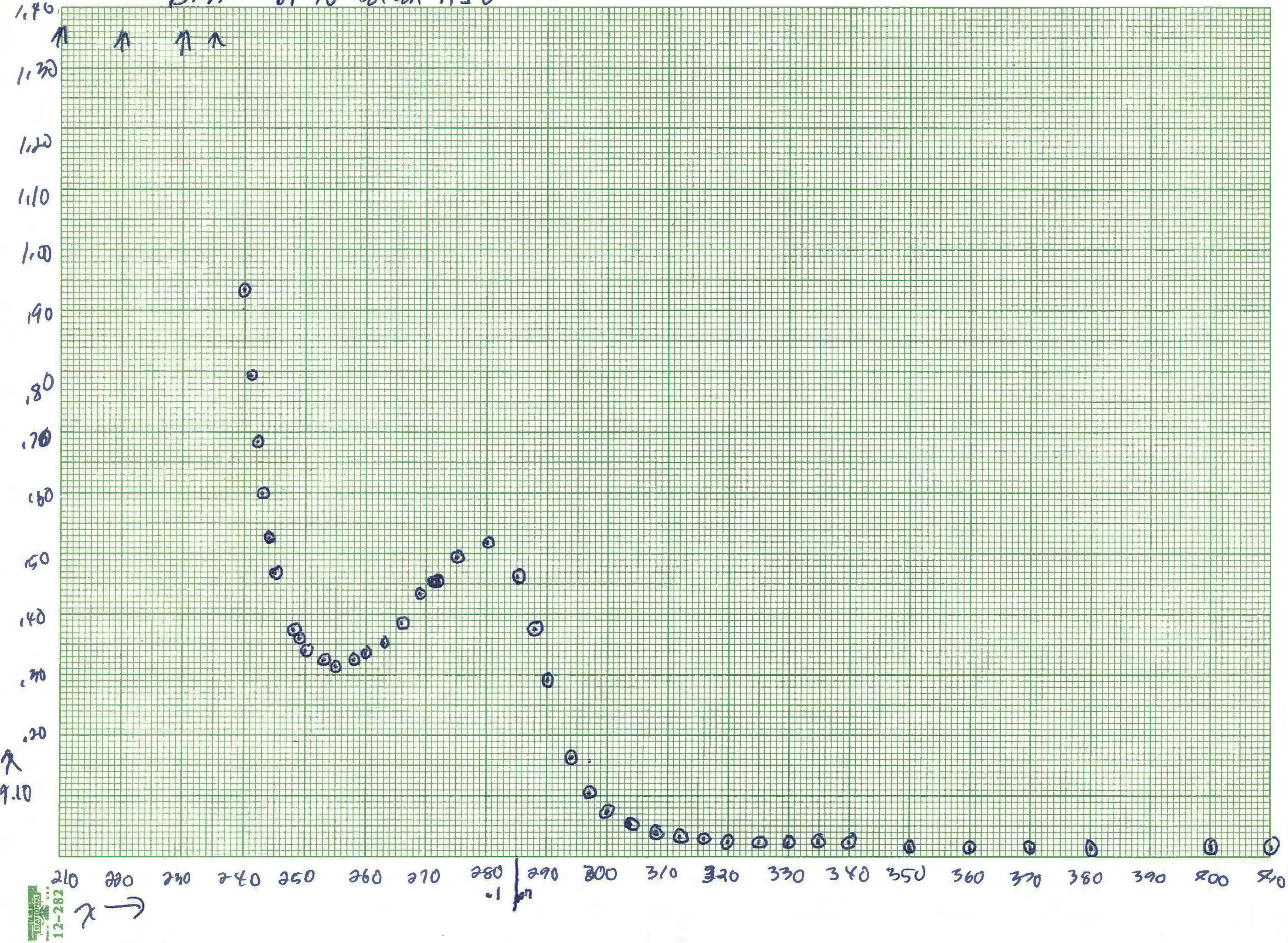
Excellent spectra were easily obtained by dissolving a small amount of sample in carbon tetrachloride. Using a double beam instrument with a carbon tetrachloride reference eliminated interfering absorption bands due to the atmospheric water and carbon dioxide, and the weaker absorption bands of carbon tetrachloride.

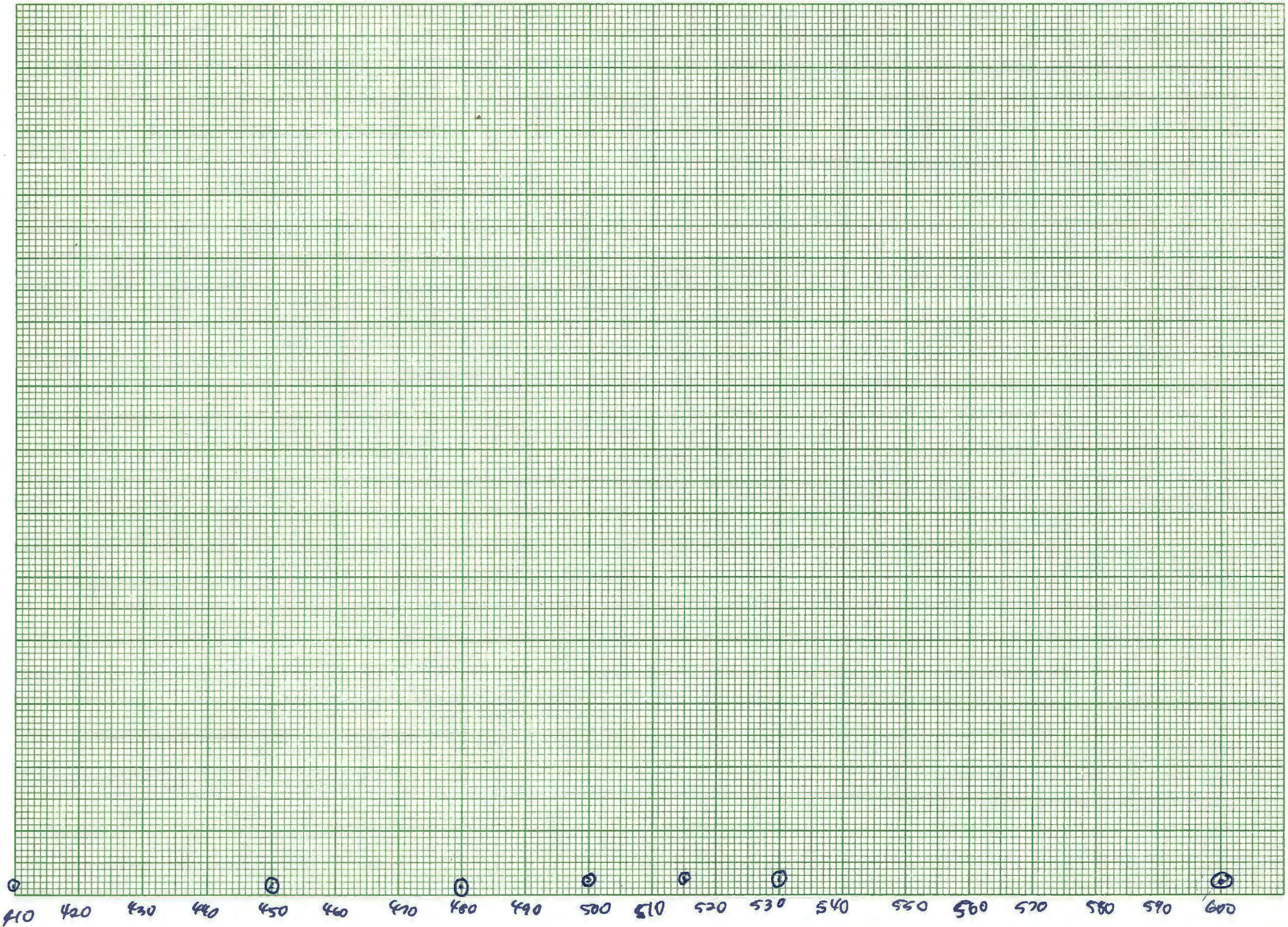
Some solid substances cannot be successfully analyzed in a liquid solvent because of interfering associations or solubility. A pellet of a mineral salt has been successfully used in these cases. Usually potassium bromide is used because it is inexpensive and useful over a wide range. The potassium bromide is oven dried and finely pulverized. A small amount of sample (approximately 5%) and potassium bromide are ground together until completely mixed. The pellets, reference, and sample were made with a homemade hydraulic press by trial and error methods. Each pellet was made mounted in filter paper cut to mount in the instrument sample holder. Satisfactory spectra were obtained by this method, although opacity of the reference and sample were encountered. A minute pressing time at maximum pressure was necessary for best results.

The third method employed was a mineral oil mull. It is widely used because of its simplicity for those adept at the mull technique. Mineral oil or Nujol has few major bands in it. The method consists of making a slurry of the sample with mineral oil which is ground to achieve a finely divided suspension of the solid in the mineral oil. A sample of benzoic acid in mineral oil was run versus air with the double beam instrument. All of the major absorption peaks of benzoic acid were detected except in the region where the mineral oil absorbed strongly. The method is one which depends heavily on the operator's technique.

To become acquainted with ultraviolet spectroscopy methods a point by point spectra was made. The sample was a .1% solution of Bovine Plasma Albumin. It exhibited an absorbance peak at 280 microns as reported by the literature.

BPA .1% sol in H_2O




410
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430
440
450
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470
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510
520
530
540
550
560
570
580
590
600

absorption expected at 2900, 1450 & 1375 cm-1

SPECTRUM NO.
DATE
SAMPLE Mineral oil
single beam

SOURCE
STRUCTURE

PATH ___ mm ___
SOLVENT
CONCENTRATION
PHASE
COMMENTS

ANALYST P. Waymack

Beckman®

INFRARED
SPECTROPHOTOMETER

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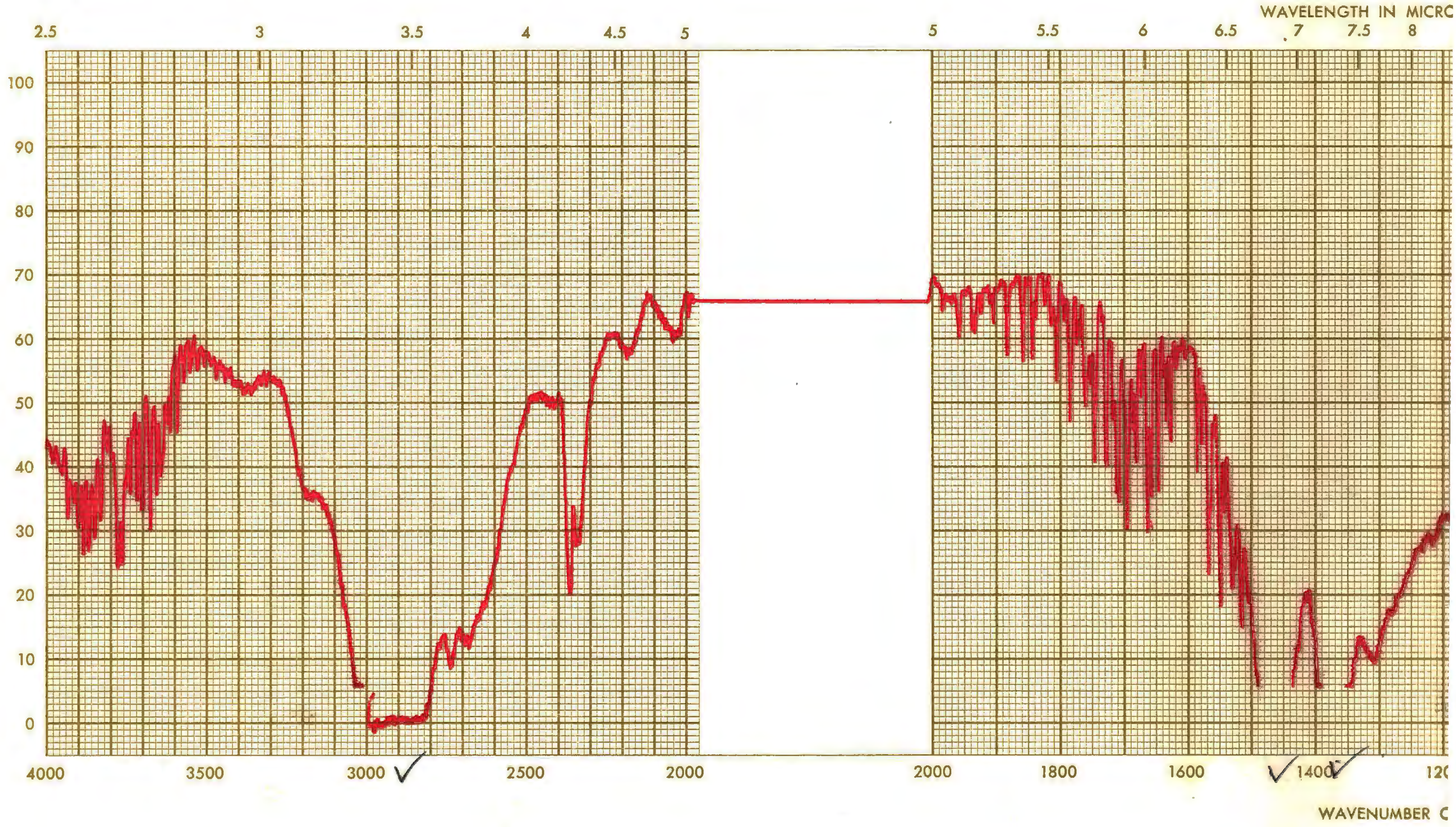

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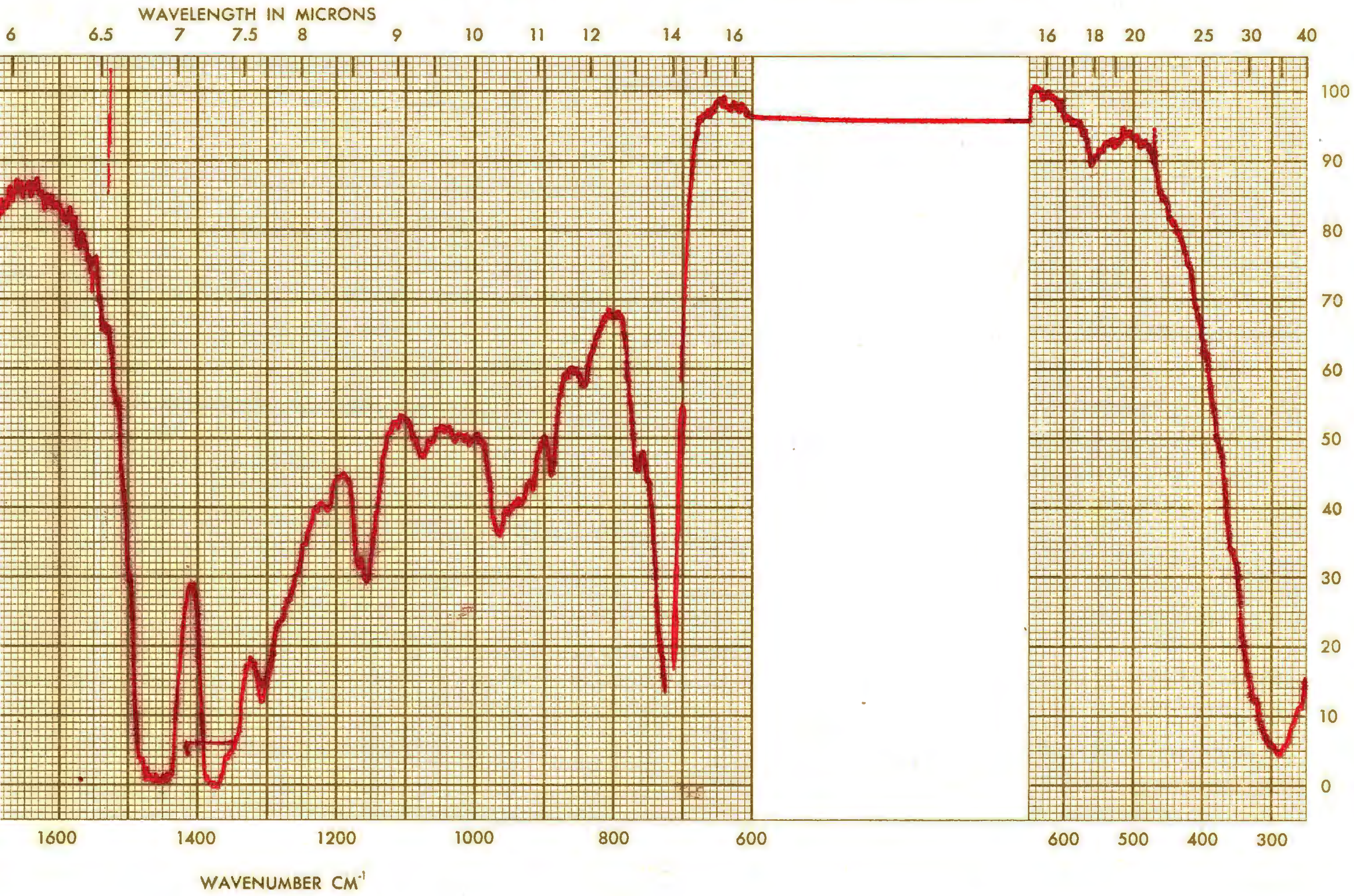

SPECTRUM NO. ______

DATE ______

SAMPLE mineral oil vs air

SOURCE ______
STRUCTURE

PATH ______ mm ______

SOLVENT ______

CONCENTRATION ______

PHASE ______

COMMENTS ______

ANALYST P Waymack

Beckman®

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SPECTROPHOTOMETER

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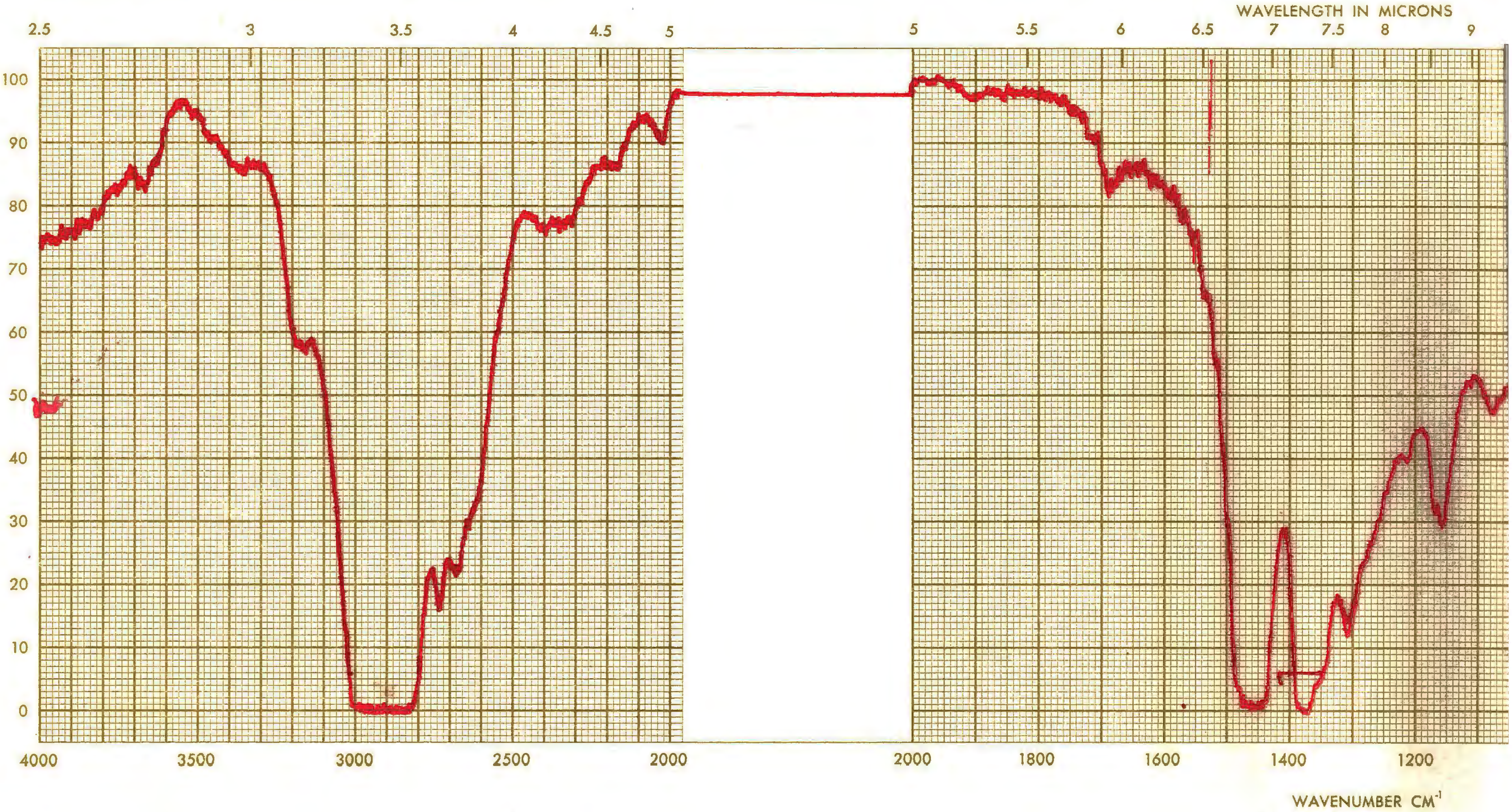

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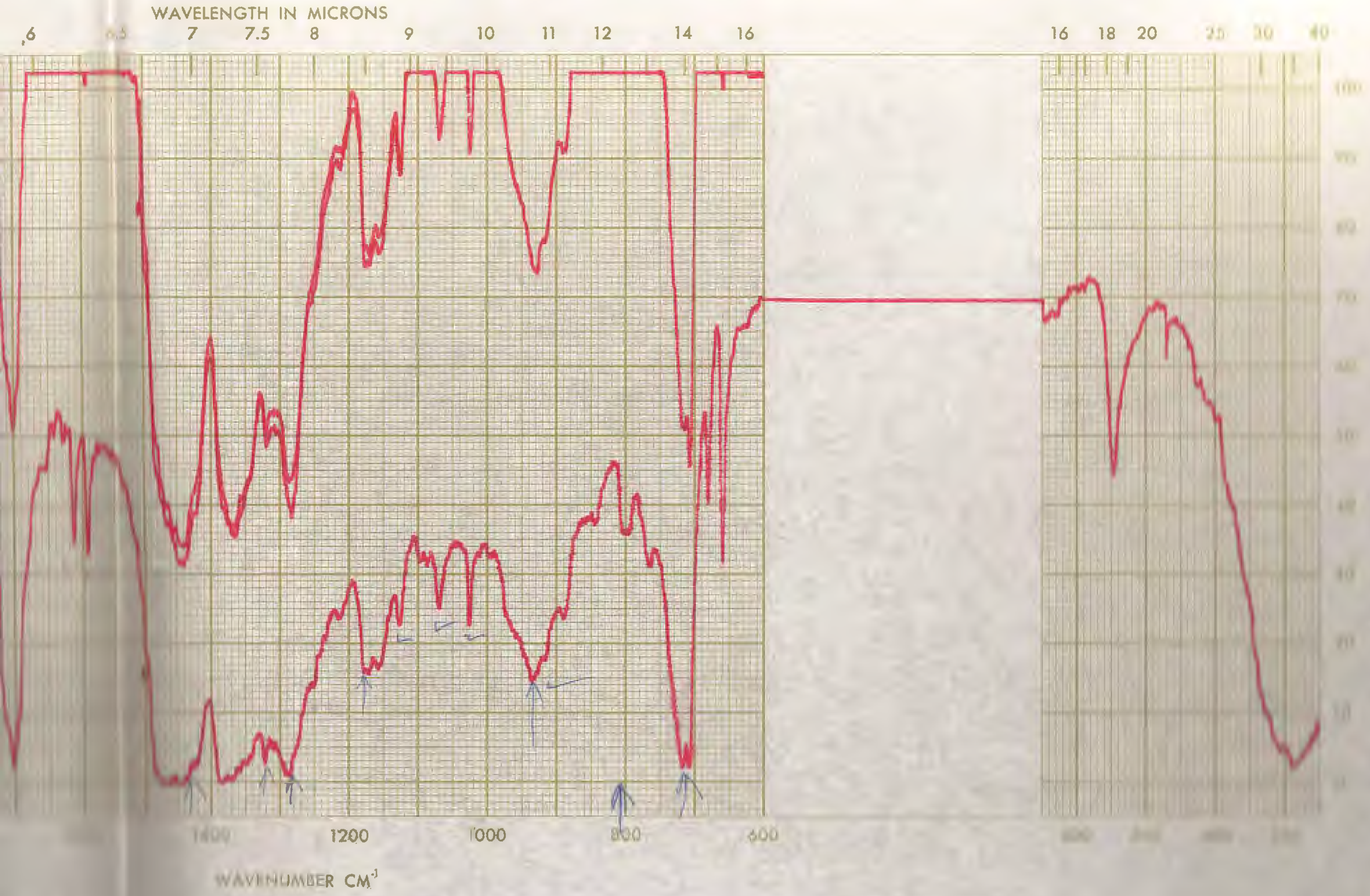

SPECTRUM NO

DATE

SAMPLE Benzoic acid, mulled in [illegible]

VS air

SOURCE

STRUCTURE

PATH

SOLVENT

CONCENTRATION

PHASE

COMMENTS

ANALYST

Beckman

INFRARED
SPECTROPHOTOMETER

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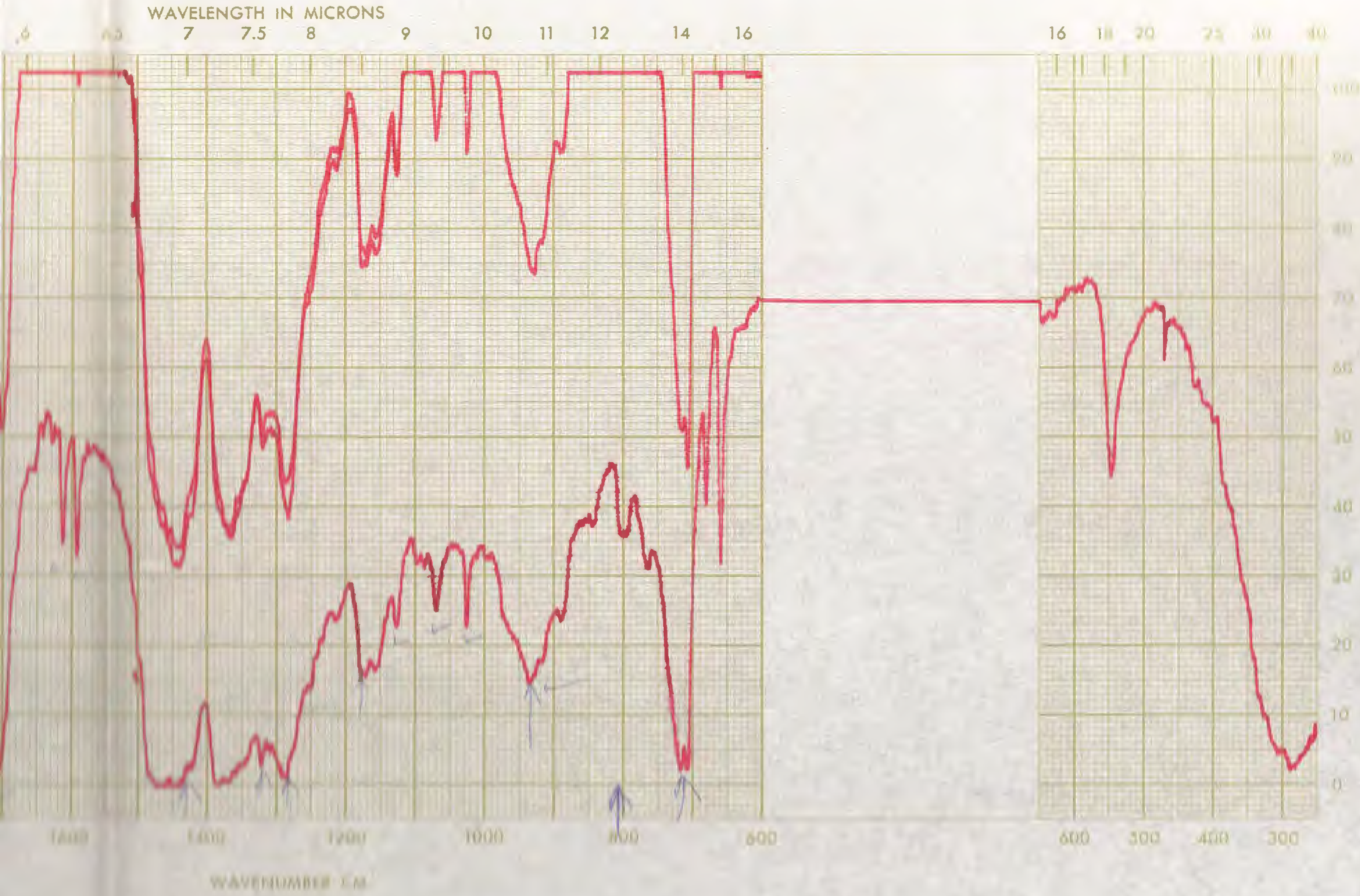

SPECTRUM NO.

DATE

SAMPLE Benzoic Acid, mulled in mineral vs air

SOURCE STRUCTURE

PATH

SOLVENT

CONCENTRATION

PHASE

COMMENTS

ANALYST P Waymack

Beckman®

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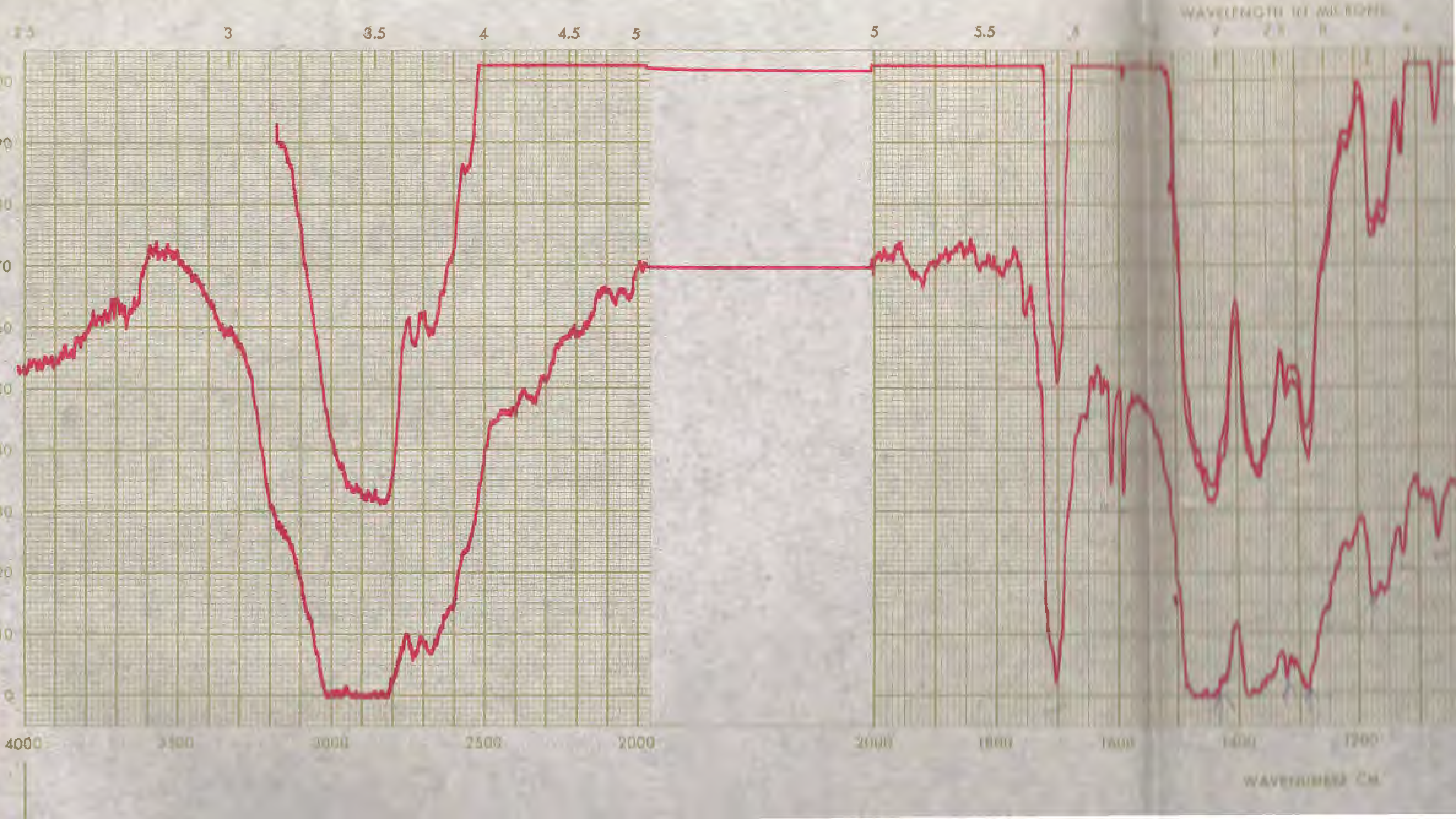
WAVELENGTH IN MICRONS
2.5
3
3.5
4
4.5
5
5
5.5
6
7
7.5
8
100
90
80
70
60
50
40
30
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10
0
4000
3500
3000
2500
2000
2000
1800
1600
1400
1200
WAVENUMBER CM

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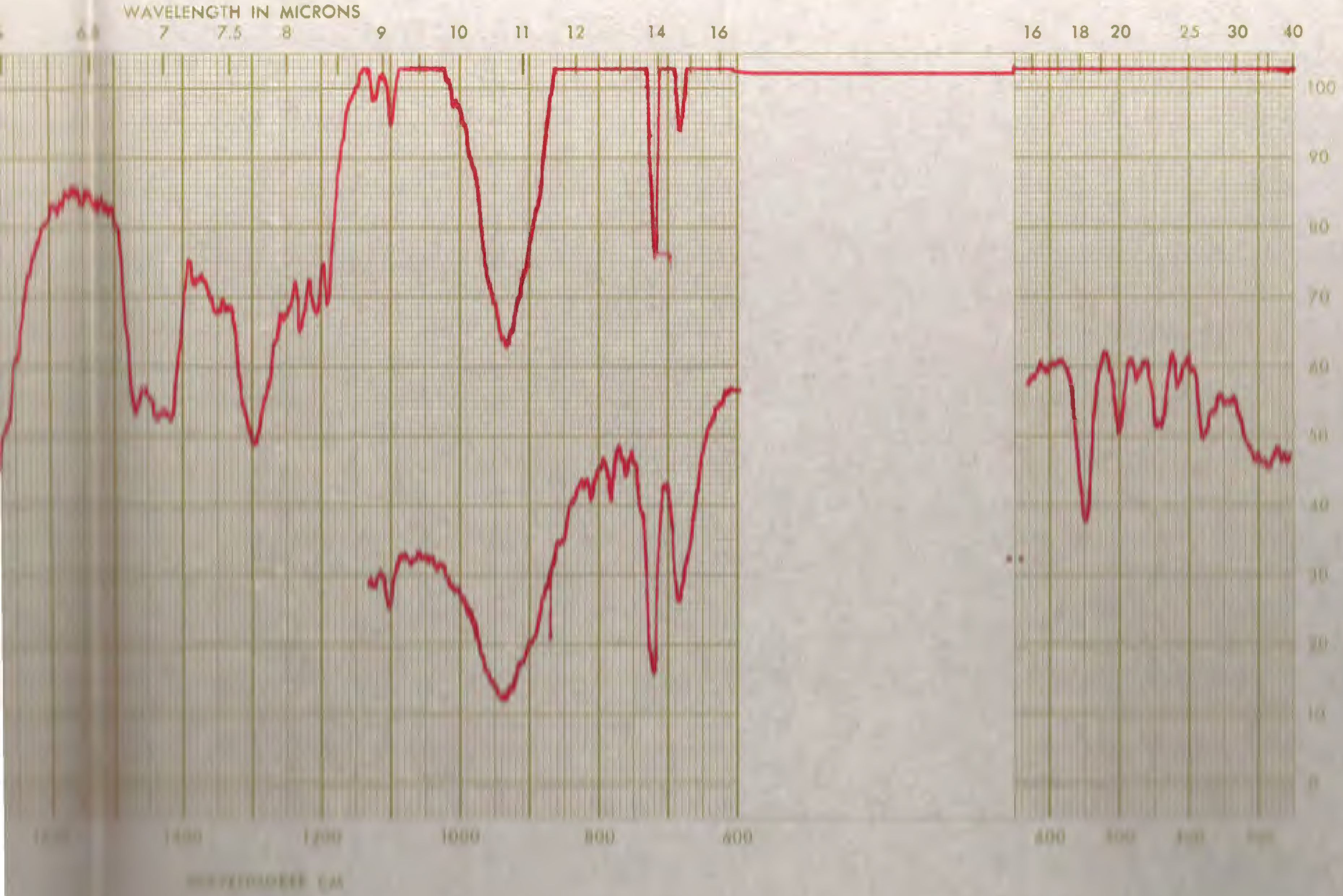

SPECTRUM NO.

DATE

SAMPLE Stearic acid

in KBr pellet vs KBr

SOURCE STRUCTURE

PATH mm

SOLVENT

CONCENTRATION

PHASE

COMMENTS Zottler 50

ANALYST P. Waymack

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