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ABSTRACT

Title of thesis: Study of Acetone Extractable Organic Matter From Airborne Particulate Matter From An Urban Site

Wenhui Wu, Master of Science in Chemistry, 1989

Thesis directed by: Dr. Arthur Greenberg

Two seasonal periods (Winter 1988; Summer 1988) of daily Newark urban air samples were collected on glass fiber filters. Each filter was first extracted with dichloromethane and subsequently with acetone. Quarterly composites of each were then tested for mutagenicity. The results of the analysis of the less polar dichloromethane extracts were reported in the thesis of Junghen Lwo. A modified fractionation scheme involving acid-base partitioning and silica gel column chromatography has been used as the first step in the bioassay-directed search for significant levels of mutagenic compounds in extracts of inhalable (IP 10) ambient air particulates. The biologically "hot" fractions (fractions having highest specific mutagenicities) were separated and analyzed chemically and subfractionated to isolate and concentrate "hot" subfractions which were then chemically analyzed by GC/MS and FTIR.

The Ames assay of mutagenicity has been employed using the unactivated TA98 strain of Salmonella and enzyme-activated (TA98 + S9) assays. In addition, some assays were performed in this present study using TA98NR (TA98-nitroreductase deficient) and

TA98DNP (TA98-dinitropyrene reductase deficient). In essence, mutagenicity was used as the chromatographic detector to pinpoint the most active fractions and compounds which are responsible for mutagenicity (and possibly carcinogenicity) in the air, and then monitor them as well as assess their reactivity.

The comparison of winter and summer samples indicated that the mass and mutagenicity profiles were similar in the two periods. However, the most interesting result related to the acetone extract is that its weak acid fraction has the most bioactivity among all fractions and subfractions isolated from both acetone and dichloromethane extracts of the particulates.

STUDY OF ACETONE EXTRACTABLE ORGANIC MATTER FROM AIRBORNE PARTICULATE MATTER FROM AN URBAN SITE

by

Wenhui Wu

Thesis submitted to the faculty of the Graduate School of the New Jersey Institute of Technology in Partial fulfillment of the requirements for the degree of Master of Science in Chemistry

1989

APPROVAL SHEET

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CHAPTER ONE

INTRODUCTION

Urban air particles contain extractable organic matter which is both mutagenic (1) and carcinogenic (2,3). The ATEOS study of ACE extractable particles, which is the basis of this thesis, focused on the measurement of pollutants and biological activity thought to be associated with chronic diseases, notably cancer. Attempts have also made to identify the specific compounds related to pollution.

The organic fraction extracts from ambient particles have been shown to exhibit mutagenic activity in bioassays by several investigators (4). In the past decade, because of the relativly higher rate of lung cancer mortality in urban areas compared to rural areas and also because of the fact that concentrations of airborne particulate matter are generally higher in urban areas, investigators have focused increased attention on the carcinogenic and mutagenic activity of the organic material associated with airborne particles.

The EPA study estimates that roughly half of the cancer incidence assigned to air pollution is attritutable to products of incomplete combustion (PICs). PIC consists of organic compounds, including polycyclic aromatic hydrocarbons (PAH), as well as numerous PAH derivatives (nitro compounds, quinones, hydroxy and hydroxynitro compounds), PAH degradation products (aldehydes, carboxylic acids, and anhydrides), and heteroanalogs (notably containing nitrogen and sulfur, e.g. Aza and Thia-

Heterocyclic Hydrocarbons), as well as numerous as yet unknown classes. (5)

Previously, research in the area (6) had been "chemically-driven". That is to say, one would have advance knowledge of carcinogens known to be produced and found in the air and monitor them as well as assess their reactivity. Additionally, GC/MS and other techniques have been used to explore the presence of other known classes of carcinogens and mutagens in the air. However, detection of the specific compounds responsible for both mutagenic and carcinogenic activity is limited by the complexity of the extracts. Consequently, a new approach rests upon the fractionation of the extracts into less complex fractions to facilitate detection of the mutagenic compounds, using biological monitoring, mutagenicity for example, as a detector. Biologically "hot" fractions are separated and analyzed chemically or subfractionated to isolate "hot" subfractions which are then chemically analyzed. Thus, there are no preconceptions about which compounds or classes of compounds to examine. One attempts to concentrate all techniques, GC/MS, HPLC/MS, FTIR, HPLC, GC, etc. on the "hot" fractions or subfractions in order to identify compounds of interest. (7,8) This is an approach we have adopted in this study. The separation scheme adopted in this study was developed by Peterson (9) and was later employed by Nishioka, 1985 (8). The method of Nishioka et al (10) involves four levels of chromatography which were applied to the most active (polar neutral) fraction: 1) Extraction into fractions, 2) Silica column chromatography into subfractions, 3) HPLC of the

most active subfraction to provide "subsubfraction" which can then be separated to yield a "third-order subfraction". It was at this fourth stage that Nishioka et al identified hydroxynitropyrenes as important mutagenic components of airborne particulate matter.(8)

One significant modification in the Nishioka et al scheme has been introduced: washing the initial extract composites with pH 7.0 water to remove the strong acids (e.g. carboxylic acids, ROOH), strong bases (e.g. alkylamine, RNH_2) and possible highly polar neutrals from the extraction mixture. This is an attempt to simplify the fraction so that the identification of the compounds in the extract will be easied.

In order to quantify the contributions of various compound classes, and specific constituents to the mutagenicity of urban air particulate matter (< 1.7 um) samples were collected from the rooftop of the Clifford Street Boys Club in the Ironbound Section of Newark urban area. The air particles were sohxlet extracted with DCM (dichloromethane) and ACE (acetone) sequentially, and the extracts were fractionated to weak-base, weak-acid, strong-acid, strong-base and nonpolar neutral fractions. A further separation of the nonpolar neutral fraction was done using silica-gel open column chromatography to yield four fractions.

Those fractions were then tested for mutagenic activity by Dr. T. Atherholt at Coriell Institute for Medical Research. Dr. Atherholt employed the TA98 Salmonella strain (±) on the basis of the ATEOS experience. In addition, nitroreductase-deficient (TA98NR) and dinitropyrenereductase-deficient (TA98DNP) strains

have also been employed.

The analytical methods employed include Fourier Transform Infrared Spectroscopy (FTIR) (the work was being done under the advice of Dr. David Bugay of Squibb Co.). Samples were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS), by Drs. Robert Rosen and Tom Hartman of the Center for Advanced Food Technology (CAFT) at Cook College, Rutgers University.

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CHAPTER TWO

SAMPLING, EXTRACTION, FRACTIONATION

2-1. Principle of Method:

Extracts of individual samples of airborne particles are composited on the basis of equivalent air volumes to provide sufficient material for the Ames mutagenicity assay. The organic extractable matter in each composite should be approximately 10 mg. More EOM should be used if the composite is going to be fractionated. The minimum necessary EOM varies with solvent but is approximately 5 mg. Composites of blank filter extraction solvents are prepared so that the proportions of solvent extract are equal to those in the samples. (A preliminary calculation method is presented in Appendix I.)

2-2. Sampling

24-hour IP10 samples were collected on pre-fired 8" x 10" quartz filters (supplied by NJDEP) using a Anderson size-selective inlet (AD $\leq$ 10um) high volume sampler during sampling. The sampling filter was dried in a dessicator and weighed before and after sampling to calculate the accurate particle mass collected.

The sampler was located on the roof-top of the three-story Newark Ironbound Boys Club building at Clifford Street in Newark. The site is at the interface of an industrial and a residential neighborhood, and is also near the Newark international airport.

Three periods of high volume air samples were collected during this project. Since most analytical results (Ames assay and GC/MS) for fall 1988 were not completed, and since we did not have enough time to do FTIR analysis on the extract and fractions for this period, the mass data (which are the only analytical results available for the fall 1988 samples) of the extract, fractions and subfractions listed at the end of this chapter are only treated as a reference. In winter 1988, a total of 40 hi-vol 24-hour samples were collected using 4 samplers for 10 days; the collection period was: 1/6 - 1/9, 1/11 - 1/15, 1/17 - 1/20/88. In summer 1988, a total of 60 hi-vol samples were collected during the period of: 7/27 - 7/31, 8/2 - 8/6, 8/8 - 8/12, 8/14 - 8/19/88. In fall 1988, a total of 40 hi-vol 24-hour samples were collected during the period of: 11/11 - 15/11, 19/11 - 23/11.

A total air volumes of about 70,000, 100,000 and 70,000 m^3 were collected winter, summer and fall respectively. The total weight of air particles collected on filters was 3.30150 g for winter 1988 (W88), 4.92785 g for summer 1988 (S88), and 4.03350 g for fall 1988 (F88). The samples were then extracted using dichloromethane (DCM) and acetone (ACE).

2-3. Blank Filter Testing

Checks of blank samples for mutagenicity and GC/MS backgrounds were done prior to the sample filter extraction. It was based on 2000 ml DCM (10 * 200 extracts which were concentrated to 10 ml) and similarly, a 10ml ACE blank composite of 10 filters. The procedure used was as follows: ten pre- fired

hi-vol quartz filters (supplied by NJDEP) were each soxhlet extracted sequentially with 200 ml dichloromethane (DCM), then with 200 ml acetone (ACE) for a 24 hour period. (The DCM and ACE solvents used here were the same as the sample filters extraction mentioned below.) Ten individual extracts were concentrated using Kuderna-Danish apparatus and then combined. These extracts were EOM determined and 5 ml of each extract sent to Dr. Thomas Atherholt at the Coriell Institute for Medical Research for Ames assay; and 4 ml of each extract was sent to Drs. Robert Rosen and Thomas Hartman at the Rutgers University CAFT for GC/MS.

The Ames assay results indicated that the blanks were essentially free of mutagens for both W88 and S88 blank filters testing.

The GC/MS results were not as satisfactory, indicating significant levels of esters and other plasticizers. The source of the problem was at first considered to be solvent as well as the use of polyethlene bottles containing methanol for glassware cleaning at the period of chemical treatment of the W88 sample. But even though the subsequent work of S88 involved use of Fisher "Optia" dichloromethane, J.T. Baker HPLC methanol and no further use of plastic dispenser, the same plasticizer problem remained. Based upon this, Burdick-Jackson Distilled-in-Glass quality solvent was used for the Fall, 1988 part of the study. Since we are still waiting for the GC/MS results of Fall 1988, we are not certain how good the Burdick-Jackson Distill-in-Glass solvent is at present.

2-4. Extraction

In order to determine the total masses of airborne particulates collected, each filter was first extracted with 200 ml DCM, followed by soxhlet extraction with 200 ml ACE sequentially for 20 hours at a rate of 10 minutes a cycle. The individual extracts were then concentrated using Kuderna-Danish apparatus and combined.

A total of 8,000 ml DCM and ACE was used in the W88 extraction period separately, 12,000 ml DCM and ACE for S88 separately, 8,000 ml DCM and ACE for F88 separately.

The extracted solvents were totally concentrated to 10 ml for both DCM and ACE fraction at W88. In S88 and F88 both fractions were reduced to 25 ml.

A 150 ul aliquot of each extract was used for a residue mass measurement and a 1 or 2 ml aliquot was also removed and prepared for bioassay. The volume remaining for further fractionation was 7.85 ml (10.0 - 0.15 - 2.0 = 7.85 ml) for W88, and 23.85 ml (25.0 - 0.15 - 1.0 = 23.85 ml) for S88 and F88. Moreover, all glassware was cleaned by a special procedure: (1) cleaned with strong base detergent; (2) washed with 5% HNO_3 solution; (3) rinsed with distilled water; (4) rinsed with methanol (three times); (5) dried in the oven.

2-5. Fractionation of Extract into Compound Classes

Each seasonal extract composite was further fractionated first by liquid-liquid partition into to weak base, weak acid,

strong base, strong acid and neutral fractions. The neutral fraction was further separated by open column chromatography.

The modified fractionation scheme employed here is based on the scheme of Peterson et al (1), which involved acid-base partitioning to separate the extract initially into organic acid, organic base, and neutral component fractions. We feel that this scheme is more useful than a similar one used by the Rome research group (2). However, one significant modification was made in this scheme because we are interested in attempting to separate classes of acids and bases at fractionation (extraction) level. A schematic representation of the entire fractionation procedure is shown in Figure 1. For purposes of discussion, the fractions are referred to numerically as B1, A2, B3, A4, P5, N6 etc, which represents weak base, weak acid, strong base, strong acid, polar neutral and less polar neutral.

In the fractionation scheme, the extract is first washed with water to get polar and less polar fractions. Since the acetone is completely miscible with water, solvent exchange must be done before the fractionation precedure. For W88, S88 and F88 samples the ACE extracts were solvent exchanged to hexane (HEX), blowing off the initial acetone solvent under nitrogen and adding hexane several times, with a final volume of the hexane of 25 ml. Because of the difference in polarity between ACE and HEX, at the of time solvent exchange a significant precipitate of insoluble materials formed. These insolubles dissolved when washed with base (sodium hydroxyide) in the fractionating precedure and, thus, they are probably organic acids.

The reason for extraction with pH 7.0 water, which is a

modification of the Nishioka et al scheme (3,4), is to attempt to separate strong acids (e.g. carboxylic acids, RCOOH), strong base (e. g. alkylamines, RNH2) and possible highly polar neutrals and inorganic salts, from the remaining substances.

At the same time, an aliquot of 150 ul was removed for the EOM (Extractable Organic Matter) determination and these fractions were also submitted for Ames bioassay. If the fraction gave a significant Ames response, chemical analysis was then carried out.

In the separation of Fall 1988 extracts, the base-acid partition separation scheme was employed, the only difference between this procedure and that of acid-base partition, which we employed in winter and summer 1989, is that the hexane solvent, after being extracted with pH 7.0 water, was extracted with 5% NaOH instead of with 5% H_2SO_4 first. This is an attempt to see the effect of the extraction sequence to the recoveries.

As noted earlier by Nishioka (4), the neutral component of the dichloromethane extract is the most active fraction, so it is further separated by column chromatography using 5% H_2O-deactivated silica-gel (70-150 mesh, Woehlm Pharma) eluted with solvents of increasing polarity. The silica gel columns (2.5 cm i.d. * 15cm long) are packed with 20 g of silica in a hexane slurry and the gel retained with a glass frit. Columns are prepared for each organic solution to be partitioned. An additional column is also prepared to check the accuracy of the silica gel deactivation before partitioning the neutral organic solution. This is done by measuring the volume of hexane required

to elute 500 ug of anthracene. The migration of the anthracene is monitored by a 366-nm UV lamp, and the volume of hexane is measured during the migration. When the silica gel is deactivated by 5 percent water, anthracene starts to elute from the column after the addition of 140 $\pm$ 10 ml of hexane. For 3 percent and 7 percent deactivation, the volumes of hexane required are 270 $\pm$ 12 ml and 115 $\pm$ 8, respectively.

Upon assurance that the silica gel is 5 percent H_2O deactivated, the neutral organic compounds are further fractionated. Four elution solvents are used. They are applied to the column and the eluent is collected in the following sequence: 100 ml hexane, 100 ml hexane/ benzene (1:1), 100 ml methylene chloride, and finally , 200 ml methanol. The collected fractions, which are then called subfractions, are named: N6-S1, N6-S2, N6-S3, N6-S4. Aliphatics, aromatics, moderately polar and high polar components are separated in this procedure based upon the polarity of the extraction solvent. These isolated fractions are evaporatively concentrated to 10 ml for W88, S88 and F88. EOM, Ames assay and GC/MS are carried out on them.

From the winter 88 and summer 88 mutagenic results (see chapter 3 for more detail) we find that unlike the results of Nishioka et al from the DCM extraction fractions (which indicated that the neutral fraction has the highest mutagenic activity), in the ACE extract fraction the weak acid fraction has the highest mutagenic activity among all the isolated fractions and subfractions. In order to isolate the weak acid fraction, in Fall 88 the same silica gel open column chromatography subfractionation method was employed in the F88-ACE-A2 fraction.

2-6. EOM Results and Discussion:

The method of Nishioka et al (5) involves four levels of separation (including three chromatography levels) which were applied to the most active (polar neutral) fraction: 1) Extraction into fractions, 2) silica column chromatography into subfractions, 3) HPLC of the most active subfraction to provide "subsubfractions" which are termed second-order subfractions, 4) HPLC of the "subsubfraction" to produce third-order subfractions. It was at this fourth stage that Nishioka et al (5) identified hydroxynitropyrenes and hydroxynitrofluoranthenes as important mutagenic compounds in airborne particulate matter. However, we tried to adopt the first three stages of the Nishioka et al procedure for the most active fraction. We completed level 1, fractionation and fractioned the neutral fractions of the ACE composite using silica gel open column chromatography to form subfraction (level 2), but unfortunately the subsubfractionation was not possible for ACE extracts because of the lack of time and the low mass and mutagenicity activity of the subfractions (see chapter 3 for more data information).

In Table 2.1, summary of the data for the fractions and subfractions is presented. Table 2.2 shows filter extract mass balance results for the Winter 88, Summer 88 and Fall 88 periods. Tables 2.3, 2.4 and 2.5 show the mass balance results for the first two levels of separation for Winter, Summer and Fall 88 acetone composite extracts. The recoveries of the organic mass through the acid-base partitioning are 21.6%, 39.5% and 16.2% for

winter and summer respectively. While it is obvious that the recovery of mass in the fractions of ACE has improved considerably in the summer 88 period. Between the first and second separations, the total recoveries for ACE are significantly lower than DCM (6) in part, due to the fact that a significant portion of the ACE extract is comprised of inorganic and probably hydrogen-bonding substances.

The mass balances of the subfraction for W88-ACE, S88-ACE and F88-ACE are listed in tables 2.6, 2.7 and 2.8. The mass recoveries are 33.9%, 58.9% and 102% for winter, summer and fall neutral fraction by silica gel column chromatography with only four elution solvents - hexane, hexane and / benzene, methylene chloride and methanol. Because we did not use the acidic methanol to remove the last neutral fraction, some dark-colored organic material remained at the top of the silica gel column after the fractionation stage. This indicated that the extract contained extremely polar compounds which did not migrate through the silica gel bed. According to the Nishioka study (3), presumably this unrecovered material would be classified as extremely polar neutral compounds and might have accounted for as much as 20% of the original extract mass. In addition, the Nishioka group also used acidic methanol [2% 2N HCl in Methanol (v/v)] to pull out the extremely polar neutral compounds from the silica gel column. However, the mutagenic activity both with and without activation of the acidic methanol fraction was quite low and less than any other mutagenic fraction. Therefore, this subfraction was ignored. In Table 2.9 the result of silica gel column chromatography separation of ACE-A2 fraction is presented as

further refernce.

In Figure 2.2, the masses of W88-ACE and S88-ACE are tracked (corrected for total recovery) throughout the fractionation procedure. The similarity of the winter and summer profiles was somewhat surprising.

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Table 2.1: List of Winter, Summer & Fall Fractions and Subfraction

Name	Identity	
W88-ACE-B1	Winter 1988 ACE Extract:	weak bases in DCM
W88-ACE-A2		weak acids in DCM
W88-ACE-B3		strong bases in DCM
W88-ACE-A4		strong acids in DCM
W88-ACE-N6		nonpolar-moderate polar neutral in hexane
W88-ACE-N6-S1	W88-ACE-N6 Subfractions:	hexane eluant
W88-ACE-N6-S2		1:1 hexane-benzene eluant
W88-ACE-N6-S3		dichloromethane eluant
W88-ACE-N6-S4		methanol eluant
S88-ACE-B1	Summer 1988 ACE Extract:	weak bases in DCM
S88-ACE-A2		weak acids in DCM
S88-ACE-B3		weak bases in DCM
S88-ACE-A4		weak acids in DCM
S88-ACE-N6		nonpolar-moderate polar neutral in hexane
S88-ACE-N6-S1	S88-ACE-N6 Subfraction:	hexane eluant
S88-ACE-N6-S2		1:1 hexane/benzene eluant
S88-ACE-N6-S3		dichloromethane eluant
S88-ACE-N6-S4		methanol eluant

Table 2.1 (continue)

Name	Identity	
F88-ACE-B1	Fall 1988 ACE Extract:	weak bases in DCM
F88-ACE-A2		weak acids in DCM
F88-ACE-B3		strong bases in DCM
F88-ACE-A4		strong acids in DCM
F88-ACE-N6		nonpolar-moderate polar neutral in hexane
F88-ACE-N6-S1	F88-ACE-N6 Subfractions:	hexane eluant
F88-ACE-N6-S2		1:1 hexane-benzene eluant
F88-ACE-N6-S3		dichloromethane eluant
F88-ACE-N6-S4		methanol eluant
F88-ACE-A2-S1	F88-ACE-A2 Subfractions:	hexane eluant
F88-ACE-A2-S2		1:1 hexane-benzene eluant
F88-ACE-A2-S3		dichloromethane eluant
F88-ACE-A2-S4		methanol

Table 2.2 Extraction Mass Balance for Winter, Summer and Fall 1988 Samples:

	W88[a]		S88[b]		F88[c]	
	Mass/ml	Tot(mg)	Mass/ml	Tot(mg)	Mass/ml	Tot(mg)
DCM Extract	5.94	594	21.3	532	17.1	428
ACE Extract	5.66	566	20.9	523	16.1	403
Tot. Ext. Mass		1160		1055		831
Tot. Sample Mass		3302		4928		2338
Mass Recovery		35%		21%		31%

a. The mass was collected from 40 hi-vol sample filters, the extract was concentrated to 10 ml finally.

b. The mass was collected from 60 hi-vol sample filters, the extract was concentrated to 25 ml finally.

c. The mass was collected from 40 hi-vol sample filters, the extract was concentrated to 25 ml finally.

Table 2.3 Mass Balance of Fractions from Acetone Extract of Winter 1988 (40 Filter, original total mass is 566mg in 10.0 ml)

FRACTION	NAME	SENT to CIMR	MASS/ml[a]	TOTAL MASS (mg)	CORR TOTAL[b] MASS (mg)
#B1	Weak Bases	1.0 ml	0.214	1.070	1.36
#A2	Weak Acids	1.0 ml	3.337	16.687	21.25
#B3	Strong Bases(1/2)	1.0 ml	1.487	7.435	18.94
#A4	Strong Acids(1/2)	1.0 ml	2.994	14.970	38.14
#P5	Hi-Polar Neutral	ca 8.30 aq.	----	------	-------
#N6[c]	Non-Polar Neutral	1.0 ml	3.358	33.580	42.78
				TOTAL MASS	122.5
				RECOVERY	21.6%

a. All solutions adjusted to a total volume of 5 ml except N6 to a volume of 10 ml

b. Fractions B3 and A4 are only half of the original. Therefore total masses must be multiplied by 2 prior to doing the correction; then all masses are divided by 0.785 to account for the fact that from a total of 10 ml of the original W88-ACE extract, 2 ml was used for Ames assay and 0.15 ml was used for EOM determination.

c. Mass/ml of W88-ACE-N6 was measured by CIMR

Table 2.4 Mass Balance of Fractions from Acetone Extract of Summer 1988 (60 filters composite, original total mass is 523 mg in 25 ml.)

FRACTION	NAME	SENT TO CIMR	MASS/ML[a]	TOTAL MASS (mg)	CORR TOTAL[b] MASS (mg)
B1	Weak Bases	12.0 ml	0.408	8.160	8.59
A2	Weak Acids	7.0 ml	1.248	24.960	26.27
B3	Strong Bases (1/2)	5.0 ml	0.506	10.120	21.31
A4	Strong Acids (1/2)	5.5 ml	1.489	29.780	62.69
P5[c]	Hi-Polar Neutral	-------	------	--------	-------
N6	Nonpolar Neutral	2.0 ml	4.168	83.360	87.75
				TOTAL MASS	206.61 mg
				RECOVERY	39.5%

a. All solutions ajusted to a total volume of 20.0 ml

b. For fraction B3 and A4, total mass is multiplied by 2 (see the explanation in table 2.2 footnote b). All masses divided by 0.950 to account for the fact that from a total of 25.0 ml of the original S88-ACE extract, 1.0 ml was used for Ames assay and 0.15 ml was used for EOM determination.

c. Due to the extremely low mutagenic activity of this fraction (see the mutagenic testing result presented in chapter 3), P5 was discarded.

Table 2.5 Mass Balance of Fraction from Acetone Extract of Fall 1988 (40 filter, orginal total mass is 403 mg in 25 ml) Samples

Fraction	Name	Sent To CIMR	Mass/ml[a]	Total Mass (mg)	CORR Total[b] Mass (mg)
B1	Weak Bases	11.8	0.1015	1.5225	1.60
A2	Weak Acids	2.0	1.2540	18.810	19.72
B3	Strong Bases(1/2)	13.85	0.2578	3.8663	8.11
A4	Strong Acids(1/2)	11.85	0.7000	10.500	22.01
N6	Nonpolar Neutral	2.0	0.8892	13.338	13.98
				Total Mass	65.42
				Recovery	16.25%

a. All solutions adjusted to a total volume of 20.0 ml.

b. See footnote "b" in Table 2.4.

Table 2.6 Mass Balance of Subfractions from W88-ACE-N6 Open Column Chromatography Isolation (29.72mg N6 fraction was employed in this procedure)

FRACTION[a]	DESCRIPTION	MASS/ML	MASS TOTAL	CORR TOTAL[b] MASS (mg)
N6-S1	Aliphatics	0.202	2.02	2.28
N6-S2	Aromatics	0.186	1.86	2.10
N6-S3	Moderately Polar	0.150	1.50	1.69
N6-S4	High Polar	0.470	4.70	5.31
			Total Mass	11.38
			Recovery[c]	33.9%

a. All the subfractions were concentrated to 10 ml. The four subfractions were subsequently collected in Hexane for N6-S1; Hexane/Benzene (1:1) for N6-S2; DCM for N6-S3; and MeOH for N6-S4.

b. Since only 8.85 ml N6 fraction was used in the fractionation (0.15 ml was used for EOM determination, 1 ml was sent to CIMR). To get the original total mass of each subfraction, their total masses were divided by 0.885 which was then called "correct total mass".

c. The original mass of W88-ACE-N6 is 33.58 mg.

Table 2.7 Mass Balance of Subfractions from S88-ACE-N6 Open Column Chromatography Isolation (74.4 mg N6 fraction was employed in this procedure)

FRACTION[a]	DESCRIPTION	MASS/ML	MASS TOTAL	CORR TOTAL[b] MASS (mg)
N6-S1	Aliphatics	1.285	12.85	14.40
N6-S2	Aromatics	0.131	1.31	1.47
N6-S3	Moderately Polar	0.33	3.3	3.80
N6-S4	Highly Polar	2.637	26.37	49.12
			TOTAL MASS	49.12
			RECOVERY[c]	58.9 %

a. The subfractions for S88-ACE were contained in the same solvents as described in Table 2.6 footnote a. All the subfractions were concentrated to 10 ml as well.

b. Since only 17.85ml N6 fraction was used in the fractionation procedure (0.15 ml was used for EOM determination, 2 ml was sent to CIMR). TO get the original total mass in each fraction, their total masses were divided by 0.8925.

c. The original mass of S88-ACE-N6 is 83.4 mg.

Table 2.8 Mass Balance of Subfractions from F88-ACE-N6 Open Column Chromatography Isolation (11.29 mg N6 fraction was employed in this procedure)

FRACTION[a]	DESCRIPTION	MASS/ML	MASS TOTAL	CORR TOTAL[b] MASS (mg)
N6-S1	Aliphatics	0.05275	0.5275	0.6230
N6-S2	Aromatics	0.06750	0.6750	0.7648
N6-S3	Moderately Polar	0.09775	0.9775	1.1545
N6-S4	High Polar	0.9370	9.370	11.067
			Total Mass	13.609
			Recovery[c]	102%

a. The subfractions for F88-ACE-N6 were contained in the same solvents as described in Table 2.6 footnote "a". All the subfractions were concentrated to 10 ml.

b. Since only 12.7 ml N6 fraction was used in the fractionation procedure (0.30 ml was used for EOM determination, 2 ml was sent to CIMR). To get the original total mass in each fraction, their total masses were divided by 0.8467.

c. The original mass of F88-ACE-N6 is 13.338 mg.

Table 2.9 Mass Balance of Subfractions from F88-ACE-A2 Open Column Chromatography Isolation (13.6059 mg A2 fraction was emplyed in this procedure)

FRACTION[a]	ELUANT SOLUANT	MASS/ML	MASS TOTAL	CORR TOTAL[b] MASS (mg)
A2-S1	Hexane	0.08250	0.8250	1.1406
A2-S2	1:1 Hexane/ Benzene	0.04375	0.4375	0.60484
A2-S3	DCM	0.04525	0.4525	0.62558
A2-S4	MeOH	1.4760	14.760	20.406
			Total Mass	22.78
			Recovery[c]	121%

a. All the subfractions listed in this Table were concentrated to 10 ml.

b. Only 10.75 ml A2 fraction was used in the fractionation procedure (0.15 ml for EOM, 2 ml for Ames assay; 1 ml for GC/MS analysis; 1 ml left behind). So, to get the original mass contained in the original 15 ml A2 fraction, the total mass of each subfraction had to be divided by 0.7.

c. The total mass of F88-ACE-A2 in the original 15 ml fraction is 18.81 mg.

Figure 2.1: FRACTIONATION PROCEDURE FOR ACE EXTRACT

(VOLUME PRESENT IN THIS FIGURE IS GAINED FROM WINTER 88 CAMPAIGN, THE SAME PROCEDURE IS EMPLOYED FOR SUMMER 88)

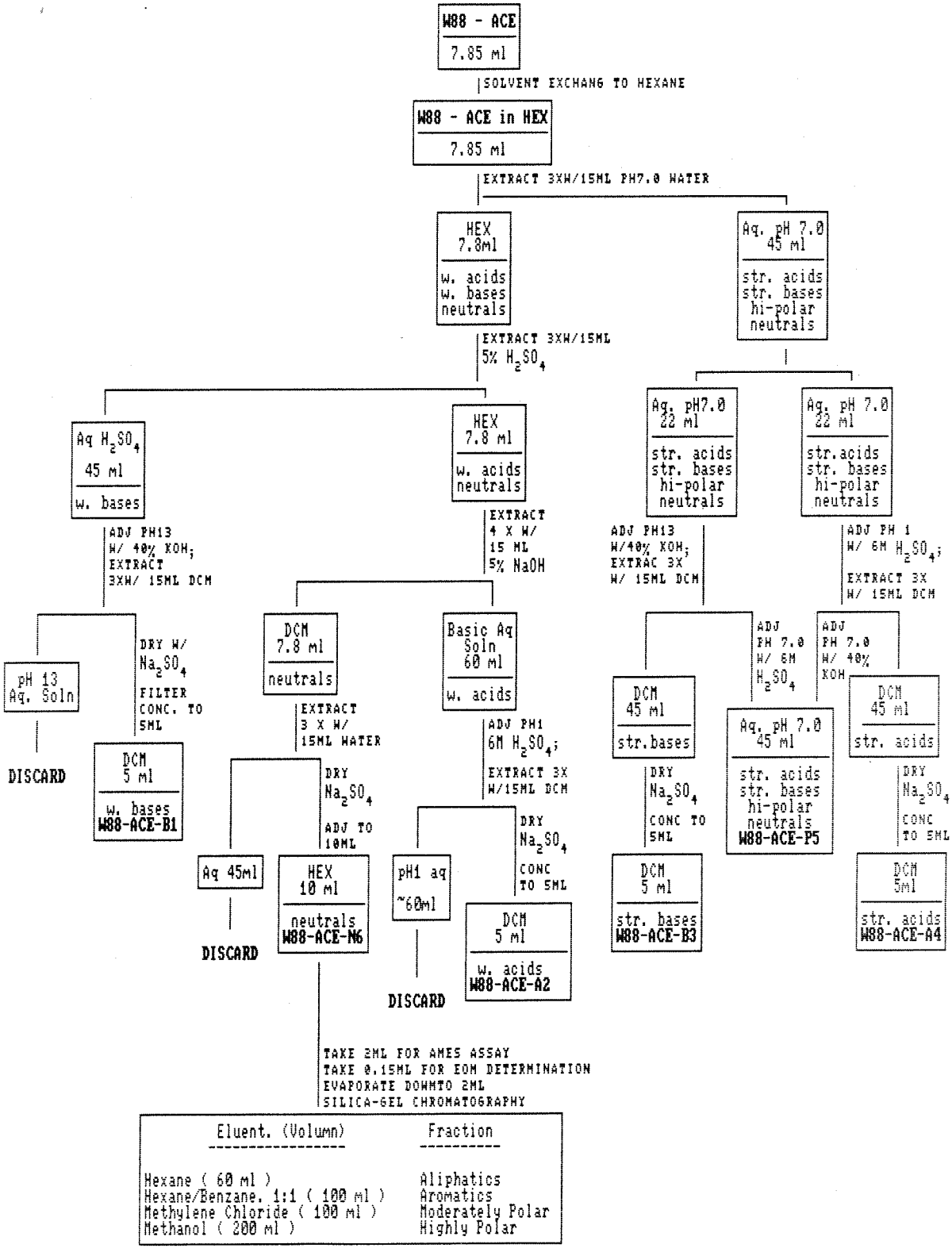
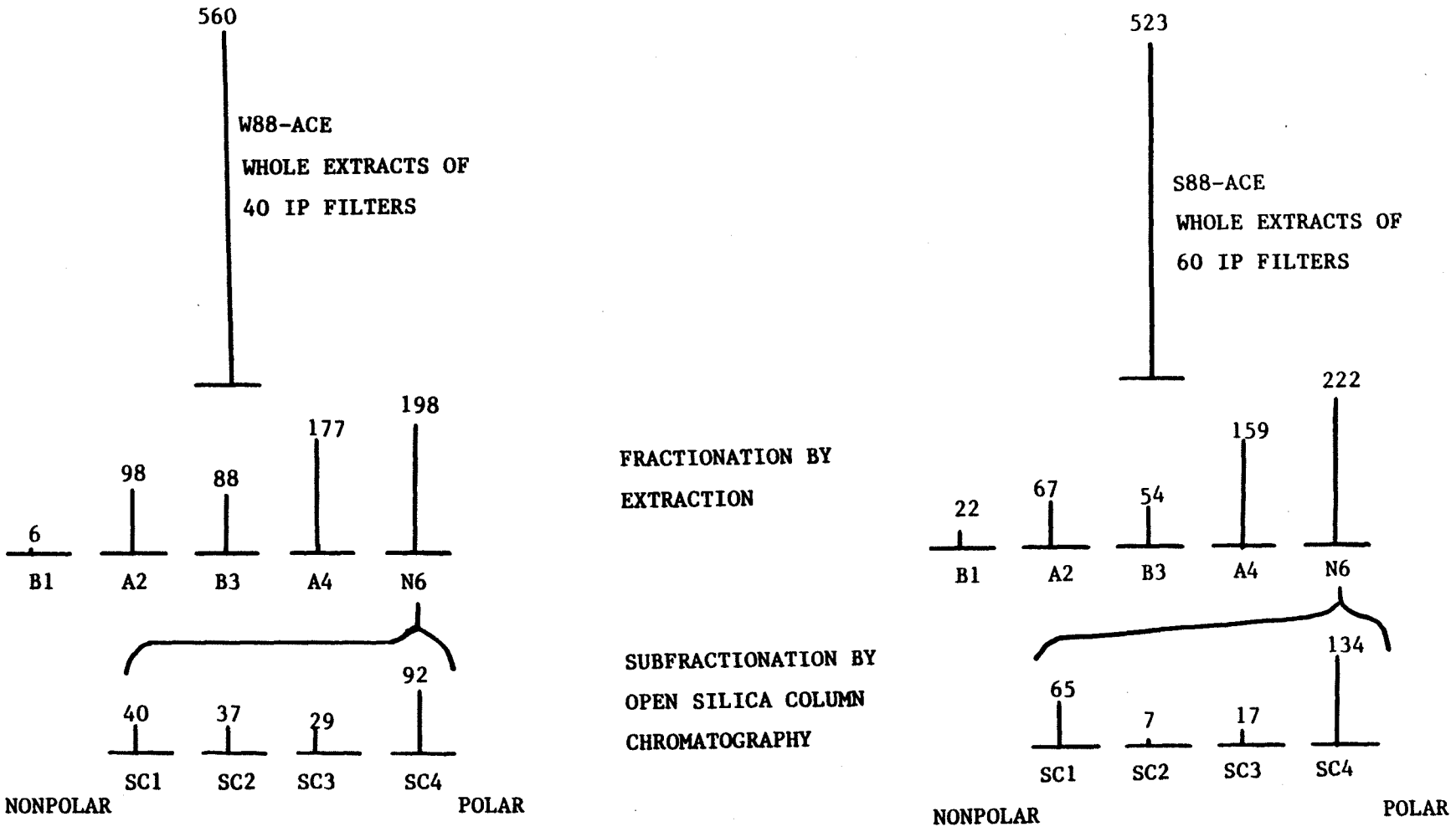


Figure 2.2 Tracking of mass distributions (mg) for total W88-ACE and S88-ACE and selected fractions, subfractions and subsubfractions (All masses have been normalized to equal 100 % recovery at each stage- assumptions of equal and total recoveries not actually justified)



APPENDIX FOR CHAPTER TWO:

I. Preliminary Calculations

1. Average total volume/10 ml extract (whole filter) per 24 hour daily sample for composite period.
2. Average total mg/10 ml extract (whole filter) per 24 hour daily sample for composite period.
3. Using average mass for these samples, estimate how many ml of each extract will be needed to obtain the total required mass for mutagenicity testing (typically 5 mg or 20 mg / fractionated extract). Whole extract Ames testing requires a minimum of approximately 1 mg/test.
4. Using the Apple computer program "Composite", calculate the mg/aliquot, and air volume/aliquot.
5. Record total ml composite, total cubic meters of air, and total mg composite (should be approximately 10 mg) on the composite data sheet.

The total of column fraction of filter blank = the total of column ml composite/10. If there are 30.9 ml in the composite, it will take 3.09 filter blanks.

II. Procedure for Obtaining Air Sample Volumes

1. Air volumes are obtained from pressure transducer chart readings (flow rate) and sample times (read from digital time meter on sampler.

2. Volume = (meter cubed/min) * (hrs./sample) * (60 min/hr.).
3. Air volumes represent meter cubed/whole filter which equals meters cubed/10 ml extract.
4. Record meters cubed/10 ml extract on composite sample data sheet.

III. Weighing Procedure (EOM Determination):

Samples must be weighed on a Cahn 26 microbalance to calculate total mg.

1. Zero and calibrate balance using 50 mg weight.
2. Zero balance on a 5 mg range.
3. For the tare, use an aluminum pan with a small piece cut off.
4. Empty aluminum sample pans have been soaked in cyclohexane for 30 minutes, dried 10 -12 hours at 100°C, and stored in a dessicator until used.
5. Slide warmer should be set to about 40 °C (or slightly above boiling point of solvents).
6. Duplicate weighings of empty pans should agree to within 0.0005 mg.
7. Average the pan weights.
8. Pipette 50 ul and 100 ul sample into 2 preweighed aluminum pans and place on slide warmer to dry (pipettes are Wiretrol 1001)
9. Weigh samples when dry as previously described using counterbalance tare pan. Complete weighings within 20 minutes. Higher slide warmer weighings or longer drying

periods may result in loss of some volatile organic materials.

10. Average the sample weights when weighings agree to within 0.0005 mg (double 50 ul weight to average with 100 ul weight.).
11. Since 100 ul represents 1/100 of extract, multiply average value by 100 to calculate total mg in 10.0 ml.
12. Record on composite sample data sheet.

CHAPTER THREE

MUTAGENICITY ASSAYS

3-1. Introduction

The primary interest of the present study is the biological effect (carcinogenicity) of airborne particulates on humans. In recent years the Ames Assay of mutagenicity (1) has been employed as a screen in testing enviromental samples. Since it is accepted that cancer can be initiated by an alteration in DNA, mutagenicity appears to be a reasonable first-order surrogate. Furthermore, 83% of the known animal and human carcinogens have been detected as mutagens using the Ames Assay (2). In the present study, Dr. T. Atherholt of the Coriell Institute for Medical Research has employed the TA-98 strain of Salmonella for the assay since this has been shown to be highly sensitive to airborne mutagens (3). The assay has involved unactivated (TA 98-S9) and enzyme-activated (TA98+S9) assays. The significance of the former is that some substances are known to be direct mutagens capable of reacting with DNA without metabolism ("activated") before attacking DNA. In addition, some assays have been performed in the present study using TA98NR (TA98- nitroductase deficient) and TA98DNP (TA98- dinitropyrene reductase deficient). If there are significant reductions in mutagenicity using these microorganisms, then the active compounds are presumed to be mononitrated or dinitrated respectively.

The purpose of the Ames Assays of extracts and fractions of extracts is to point out the most mutagenic fractions of the extracts. In essence, we are using mutagenicity as our chromatographic detector and, thus, employing biology to "drive" our chemical strategy. This approach has been used to deduce that most of the mutagenic activity of airborne particulates is associated with polar nitrated compounds,(4, 5) a finding consistent with the results of the ATEOS study.(3)

3-2. Methodology

The methodology for the Ames assay has been discussed elsewhere.(3) As described in chapter 2, the ACE extract was fractionated and isolated into five fractions and four subfractions by using a modification of the Nishioka-Peterson scheme and silica gel column chromagraphy. Then the whole extracts, each fraction and subfraction for winter and summer samples were bioassayed using the TA98 strain with and without enzyme metabolic activation (S9), as well as by some other assays.

3-2-1. W,S88-ACE extract and fraction Ames assay mutagenic testing

Processing:

1). The initial volume was measured.

2). The EOM was determined using a 50 ul and 100 ul aliquot from each extract (30 min, 40 C on slide warmer).

3). All extracts were solvent-exchanged to acetone (two 5 ml aliquots of acetone used during solvent exchange process) and brought to a predetermined final acetone

volume.

4). The final EOM/ml data for each extract were also calculated.

3-2-2. Toxicity Test

1). All ACE fractions were submitted to an abbreviated toxicity test using TA98 only (-S9) to determine the proper dose levels to use in the Ames assay.

2). An abbreviated form of the toxicity test was performed (TA100 was not used) to conserve sample for the Ames assay.

3). All revertant/plate data are from one plate only (not duplicate). From these data, appropriate dose levels for each sample were chosen for Ames assay.

3-3. Mutagenicity Assay Result

The calculated distribution of mutagenic activities coupled with EOM (extractable organic matter) results for the first two levels of fractions in winter and summer are shown in Tables 3.1 and 3.3; the mass and mutagenic activity distribution between fraction and subfractions are listed in Table 3.2 and Table 3.4. For easy estimation and comparison of the mutagenicity results between fractions and between subfractions, the mutagenicity data for fractions and subfractions of winter and summer are graphically displayed in figure 3.1. In S88-ACE, after its fractionation, the remaining 2ml of initial S88-ACE extract was lost and that is why no data are presented for it and why it does

not appear in figure 3.1.

The total mutagenicity in both winter and summer, was fairly evenly distributed in the weak acids, weak bases and neutral fractions. Because of the result, that the amount of mutagenic activity in the summer DCM extract (6) is considerably smaller than for the winter extract, we are reasonably confident that the same conclusions hold true for the ACE extracts.

The relative percentage distribution of the mutagencity in fractions is listed in Table 3.5. The percentage distribution of subfractions is shown in Table 3.2 and Table 3.4. From the tables, we can more easily understand that the ordering of mutagenic activities for unactivated TA98 strains are slightly different between winter and summer samples. Specifically, the orderings are Winter: weak acids > strong acids > neutrals > strong bases > weak bases and Summer: strong acids > neutrals > weak acids > strong bases > weak bases, respectively. However, the mutagenic recovery per ug for S88-ACE-A2 is still the highest. Its total mutagenicity is lower than that of the strong acids and neutrals, because the weak acids fraction contains much less mass than the other two fractions. Additionally, the orderings for activated TA98 strains are Winter: neutrals > weak acids > strong acids > strongs bases > weak base and Summer: neutrals > weak acids > strong bases > strong acids = weak acids, but the weak acids fractions still are very important.

In appendix of this chapter, the most recent details of mutagenicity results from Dr. Altherholt on Summer 88 (including DCM extract portion) is attached for reference.

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Table 3.1: Mass and AMES Assay Results* for W88-ACE Extract and Fractions

(The mass balance and Ames assay results for the ACE extract are listed here. The mass recoveries are worse than that of the DCM extract, while the mutagenicity recoveries are better. This may reflect the presence of large amounts of water-soluble, nonmutagenic inorganic salts in ACE).

	Whole DCM	B1 Weak Base	A2 Weak Acid	B3 Strong Base	A4 Strong Acid	P5 Hi-Polar Neutral	N6 Nonpolar -Polar Neutral	Total (1-6)	% Rec.
10.0ml Mass(ug)	566,000	1,360	21,250	18,940	38,140	----	42,780	122,500	21.6%
98-S9 (per ug)	0.51	NEG	2.69	(0.28)	1.30	----	0.95	----	----
98-S9 (Total)	288,660	0	57,163	5,303	49,582	(2,772)	40,641	152,689	53.9%
98+S9 (per ug)	0.26	NEG	0.95	(0.23)	0.34	----	0.55	----	----
98+S9 (Total)	147,160	0	20,188	4,356	12,968	(1,628)	23,529	61,041	42.6%
98NR-S9 (per ug)	0.21	NEG	0.70	----	(0.36)	----	0.36	----	----
98NR-S9 (Total)	188,860	0	14,875	0	13,730	----	15,401	44,006	37.0%
98DNP-S9 (per ug)	(0.04)	NEG	NEG	----	NEG	----	0.34	----	----
98DNP-S9 (Total)	22,640	0	0	0	0	----	14,545	14,545	64.2%

*. Data in parentheses have poor correlation coefficients and are uncertain.

Table 3.2: Mass and Ames Assay Results for W88-ACE-N6 Subfractions

Subfraction	Eluant	Final Vol.	mg/ml	Total ug*	Strain	Rev/ug**	Total Rev.***	% Activity
W88-ACE-N6-S1	Hexane	10 ml	0.202	2,020	TA98-S9 DNP-S9	NEG NEG	NEG NEG	0% 0%
W88-ACE-N6-S2	Hex:Benz (1:1)	10 ml	0.186	1,860	TA98-S9 DNP-S9	0.56 (0.17)	1,040 320	45% 29%
W88-ACE-N6-S3	DCM	10 ml	0.150	1,500	TA98-S9 DNP-S9	0.48 0.26	720 390	31% 36%
W88-ACE-N6-S4	MeOH	10 ml	0.470	4,700	TA98-S9 DNP-S9	0.12 0.08	560 380	24% 35%

*. The amount of solution chromatographed was 7.85 ml of original 10.00 ml; thus percent mass recovery and percent mutagenicity recovery was based on 0.785 of fraction 6 Values.

**. Total Mass Recovered = 10,080 ug (% Rec: 30% based on 42,780 ug in 10 ml; 33,582 ug in 7.85 ml).

***. Total Rev (% Recov from W88-ACE-N6): TA98-S9: 2,320 (7%) DNP-S9: 1,090 (10%).

Table 3.3: Mass and Ames Assay Results[A] for S88-ACE Extract and Fractions

	Whole ACE[B]	B1 W. BASE	A2 W. ACID	B3 ST. BASE	A4 ST. ACID	N6 NEUTRAL	TOTAL	%REC
20.0 ml Mass (ug)	523,030	8,590	26,270	21,310	62,690	87,750	206,600	39.5
98-S9 (rev/ug)	-----	NEG	2.06*	0.47	1.37	0.75	-----	---
98-S9 (Total rec)	-----	0	54,116*	10,016	85,885	65,813	215,829	---
98+S9 (rev/ug)	-----	NEG	(0.32)	(0.19)	NEG	0.16	-----	---
98+S9 (Total rec)	-----	0	8,406	(4,049)	0	14,040	26,495	---
98NR-S9(rev/ug)	-----	NEG	0.62	NEG	NEG	0.33	-----	---
98NR-S9 (Total rec)	-----	0	16,287	0	0	28,958	45,245	---
98DNP-S9 (rev/ug)	-----	NEG	NEG	NEG	NEG	NEG	-----	---
98DNP-S9 (Total rec)	-----	0	0	0	0	0	0	---

A. The initial Ames Assay data are summarized in Table AI-4 (APPENDIX). Data in parentheses have poor correlation coefficients and are uncertain. TA98-S9 data marked with an asterisk (*) are also less certain.

B. The 1.0 ml sample of S88-ACE for Ames assay was lost.

Table 3.4: Mass and Ames Assay Results for S88-ACE-N6 Subfractions

Subfraction	Eluant	Final Vol.	mg/ml	Total ug*	Strain	Rev/ug**	Total Rev.***	% Activity
S88-ACE-N6-S1	Hexane	10 ml	1.285	12,850	98-S9 98DNP-S9	0.18 NEG	2313 0	8% 0%
S88-ACE-N6-S2	Hex:Benz (1:1)	10 ml	0.131	1,310	98-S9 98DNP-S9	0.83 NEG	10,873 0	36% 0%
S88-ACE-N6-S3	DCM	10 ml	0.33	3,300	98-S9 98DNP-S9	2.01 NEG	6633 0	22% 0%
S88-ACE-N6-S4	MeOH	10 ml	2.637	26,370	98-S9 98DNP-S9	0.40 (0.09)	10,548 (2373)	35% 100%

*. The amount of solution chromatographed was 17.85ml of original 20.00 ml; thus percent mass recovery and percent mutagenicity recovery was based on 0.8925 fraction 6 Values.

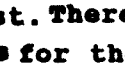
**. Total Mass Recovered = 49,120 ug (% Rec: 59% based on 83,400 ug in 10 ml; 74,400 ug in 7.85 ml).

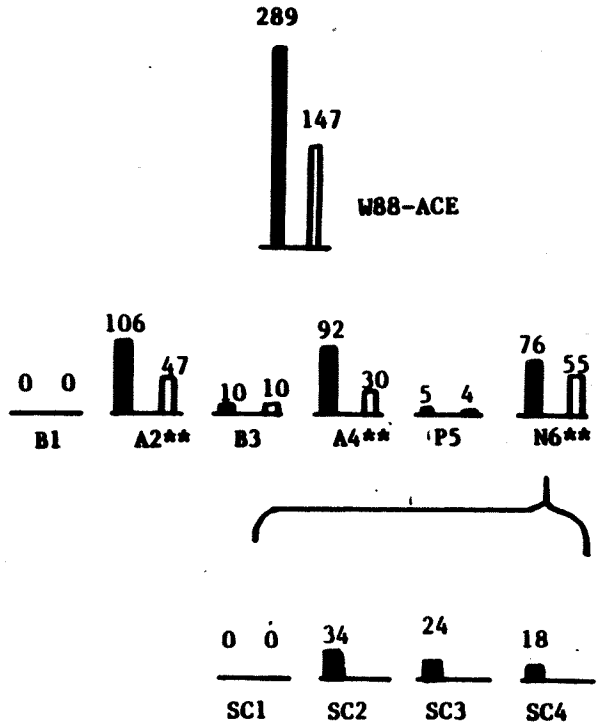
***. Total Rev (% Recov from S88-ACE-N6): 98-S9: 30,367 (42%)

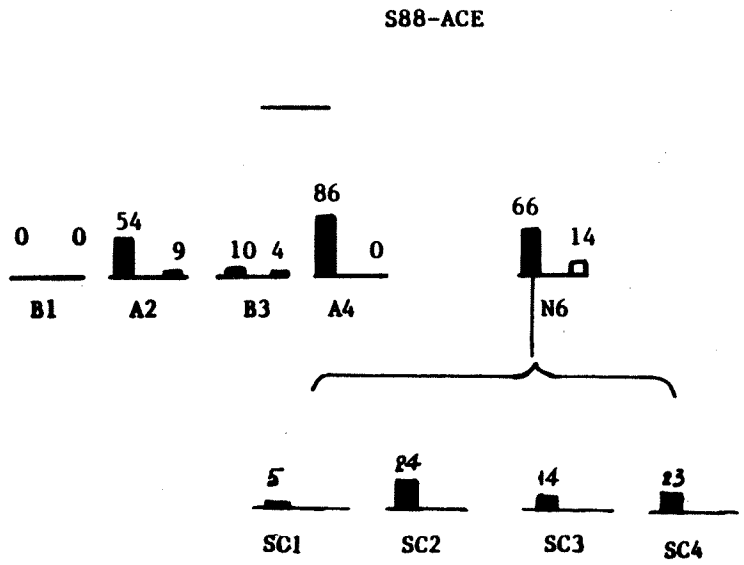
Table 3.5: Percentage Distribution of Mass and Mutagencity in Winter and Summer Fractions

Fraction	Mass(%)	TA98-S9(%)	TA98+S9(%)
W88-ACE-B1	3%	0	0
W88-ACE-A2	17%	37%	33%
W88-ACE-B3	16%	4%	7%
W88-ACE-A4	31%	33%	21%
W88-ACE-N6	35%	27%	39%
S88-ACE-B1	4%	0	0
S88-ACE-A2	13%	25%	32%
S88-ACE-B3	10%	5%	15%
S88-ACE-A4	30%	40%	0
S88-ACE-N6	42%	30%	53%

Figure 3.1: Total mutagenicities (Total rev. in thousands) for W88-ACE (composites of 40 Hi-Vol IP filters), and S88-ACE (composites of 60 Hi-Vol IP filters) at Newark, NJ site. The W88-ACE fractions were corrected to assume unit recoveries (not actually justified). The S88-ACE sample for Ames assay was lost. Therefore, its mutagenicity could not be reported and the values for the fraction cannot be normalized or directly compared with the W88-ACE samples.







APPENDIX FOR CHAPTER THREE, PART I:

Ambient Air HPLC Fractionation Project: Summer 1988 Subfractions

Repeat Mutagenicity Assay

Due to unexpected high levels of cytotoxicity in three of the four S88-ACE-N6 subfractions, the results of the initial mutagenicity assay were not satisfactory. Therefore, repeat analysis was performed on the small amount of sample which remained following the initial assay. All samples were tested with TA98-S9. There was enough sample remaining in subfractions S3 and S4 to test with TA98/1,8DNP$_6$ also.

Processing

From an initial extract volume of 1.50 ml, approximately 0.52 ml of each extract remained following the initial Ames assay. The samples were further processed as follows:

1. Each sample was solvent-exchanged two more times (2 X 5 ml acetone aliquots) and the final volume was brought to 1.0 ml.

2. The EOM was determined using a 25 µl and a 50 µl aliquot from each sample. The following data were obtained:

Sample	EOM[a] mg/ml
S88-ACE-N6-	
-S1	0.952
-S2	0.239
-S3	0.509
-S4	2.132

[a] total volume = 1.0 ml

3. A toxicity test was performed using TA98-S9 to find a suitable dose range for the repeat assay.

4. With the remaining 0.67 ml of each sample, appropriate dilutions were prepared and a repeat Ames assay was conducted.

Results

Toxicity Test

1. Results of the toxicity test are shown on the following pages.

2. Assuming initial observed toxicity levels per µg EOM were unchanged in these samples following the additional solvent-exchange procedure, samples S2 and S3 should have displayed some evidence of toxicity at the highest dose tested. They did not. Therefore, the toxicity observed in the initial assay may have been due to toxic solvents present in the extracts following an incomplete solvent exchange procedure. This is possible, but by no means certain because most of the dose levels tested in this toxicity test were below those which were toxic in the initial Ames assay.

HPLC Air Fractionation Project
Ames Assay Results
(initial assay)

A. S88 Samples

1. DCM extract and fractions

   a. Mutagenic activity (per ug) was fairly evenly distributed over all fractions except N6 which was lower than the othters.
   b. There were indirect-acting mutagens in the initial extract and in all fractions. Such mutagens were detected primarily by TA100 but by TA98 as well in some cases (although the -S9 and +S9 confidence intervals overlap in all cases except N6 with TA98).
   c. The data from the nitroreductase-deficient strains are of lower quality, especially for TA98/DNP. Therefore, conclusions are drawn here with some caution. Nitro group-containing mutagens appear to be present in A2 and possibly B1 and dinitropyrene-type mutagens may be responsible for at least some activity in B1, A2, B3 and perhaps A4 as well.

2. ACE fractions

   a. TA98 was generally unresponsive to all fractions (a low level of activity was observed in N6). TA100 responded to mutagens in all fractions except B1. The order of activity (per ug) was A4 ≈ A2 > N6 > B3.
   b. TA100 did not detect indirect-acting mutagens in any fraction (except possibly B1). The data from TA98 were too poor to draw any conclusions in this respect.
   c. It is not immediately apparent why activity was seen with TA98/NR but not TA98 in fractions A2 and A4. Again, the data was not terribly good to begin with especially in the ACE as compared to the DCM samples.

B. Comparisons to the W88 extracts and fractions

The mutagenic activity per ug in the S88 samples was lower than in the W88 samples in most, but not all cases. It is not known how the respective samples compare in terms of revertants per cubic meter of air sampled and the absence of the S88 acetone extract may prevent such analyses.

1. DCM Extract

As in the ATEOS project, a low level of indirect-acting mutagenic activity was observed in the summer extract which was not seen in the winter extract. It may be that higher levels of direct-acting mutagens mask the presence of indirect-acting mutagens in the winter samples. Nitro group-containing mutagens comprised a smaller percentage of the total activity in the summer extract compared to the winter extract.

2. DCM fractions

a. The B1 and N6 fractions contained S9-metabolized mutagens in both the summer and winter smaples. The other fractions (except A4) had such mutagens as well in the summer, but not the winter (see TA100 data).

b. The pattern of responses of the nitroreductase-deficient strains was very similar for both summer and winter samples.

c. The TA98 specific activity in the N6 fraction was lower than in the other fractions in both summer and winter. In the winter samples, the B1 specific activity was low also. This was not true in the summer sample.

3. ACE fractions

a. There were little or no S9-metabolized mutagens in these fractions in either season. Again some of the S88 data is of low quality. Therefore, definitive conclusions in this regard are not possible.

b. TA100 specific activity profiles (high to low) for the various fractions were similar for both seasons. Because the S88 TA98 data was inconclusive, no TA98 seasonal comparisons can be made.

c. As in the winter samples, TA100-responsive mutagens were particularly evident in fractions A2-A4.

Ambient Air HPLC Fractionation Project: Summer 1988 (S88) Sample Extracts and Fractions

A. DCM Extract and Initial Fractions

Processing

1. Pertinent processing data can be found in Table AI-1

2. The initial volume was measured.

3. The amount of extractable organic matter (EOM) was determined using a 50 ul and 100 ul aliquot from each sample (30 min, 40°C on slide warmer).

4. All extracts were solvent-exchanged to acetone (two ~5 ml aliquots acetone used during solvent exchange process) in small Kuderna-Danish glassware according to standard procedures and bought to a predetermined final acetone volume (see Table AI-1).

5. The final, calculated EOM/ml data for each extract is also shown in Table AI-1.

B. ACE Extract and Initial Fractions

Processing

1. Pertinent processing data can be found in Table AI-2.

2. The initial volume was measured.

3. The EOM was determined using a 50 µl and 100 µl aliquot from each extract (30 min, 40°C on slide warmer).

4. All extracts were solvent-exchanged to acetone (two 5 ml aliquots of acetone used during solvent exchange process) and brought to a predetermined final acetone volume (see Table AI-2.

5. The final EOM/ml data for each extract was also calculated (Table AI-2).

C. Toxicity Test

1. The DCM extract and all DCM and ACE fractions except fractions DCM-B1 and B3 were submitted to an abbreviated toxicity test using TA98 only (-S9) to determine the proper dose levels to use in the Ames assay.

2. An abbreviated form of the toxicity test was performed (TA100 was not used) to conserve sample for the Ames assay.

3. The results are shown in Table AI-3 All revertant/plate data are from one plate only (not duplicate). From these data, appropriate dose levels for each sample were chosen for the Ames assay.

D. Mutagenicity Assay

1. The dose-response slope data is provided in Table AI-4. Hard copy of all of the raw data was sent to Roy Meyer, NJ DEP under separate cover.

2. The results are summarized on the following pages and in a cover letter to Dr. Greenberg (enclosed)

Table **AI-1**

Processing Data for S88 DCM

Extract and Initial Fractions

	NJIT Data		CIMR Data					
Material	Vol. to CIMR (ml)	Total EOM (mg)	Vol. (ml)	EOM (mg/ml)	Total EOM (mg)	Vol. after EOM deter. (ml)	Assay[a] Vol. (ml)	EOM/
DCM ext.	3	21.279	2.85	6.156	17.543	2.70	5.00	3
DCM-Fr. B1	5	5.096	5.00	0.825	4.125	4.85	2.80	1
DCM-Fr. A2	5	11.663	4.85	2.732	13.250	4.70	3.25	3
DCM-Fr. B3	5	3.970	4.82	0.714	3.441	4.67	3.00	1
DCM-Fr. A4	5	8.744	5.09	1.670	8.500	4.94	3.25	2
DCM-Fr. N6	1	14.453	0.94	13.094	12.308	0.79	3.25	3

[a]following solvent-exchange to acetone.

Table **AI-2**

Processing Data for S88 Acetone

Extract and Initial Fractions

	NJIT Data		CIMR Data					
Material	Vol. to CIMR (ml)	Total EOM (mg)	Vol. (ml)	EOM (mg/ml)	Total EOM (mg)	Vol. after EOM deter. (ml)	Assay[a] Vol. (ml)	Ca EOM/m
ACE ext.	3	20.920	2.87	0.000	0.000	*	*	
ACE-Fr. B1	6	4.896	5.60	0.750	4.228	5.45	3.25	1.
ACE-Fr. A2	3.5	8.763	3.39	2.244	7.607	3.24	3.25[b]	2.
ACE-Fr. B3	5	5.060	4.80	1.033	4.958	4.65	3.25	1.
ACE-Fr. A4	5.5	8.190	5.49	1.675	9.193	5.34	3.25[c]	2.
ACE-Fr. N6	2	8.336	1.92	3.948	7.579	1.77	3.25	2.

[a] following solvent-exchange to acetone (all samples solvent-exchanged)
[b] Ames assay: volume = 5.50 ml; EOM = 1.220 mg/ml.
[c] Ames assay: volume = 6.00 ml; EOM = 1.376 mg/ml.
*Sample extract clear. Probably received sample blank by mistake.

Table **AI-3**

Ambient Air HPLC Project
Summer 1988 (S88) Initial Extracts and Extract Fractions

Toxicity Test — Assay Date: 11/14/88

Material Tested[a]	Dilution	E.O.M.[b] (ug)	Revs/plate[c]	Toxicity
Acetone[d]	-	-	28	
DCM Ext.	1/128	2.6	39	
	1/64	5	33	
	1/32	10	45	
	1/16	21	50	
	1/8	42	74	
	1/4	83	104	
	1/2	166	160	
	und.	332	265	No Tox.
DCM Fr. B1	not done (insufficient sample)			
DCM Fr. A2	1/128	3	30	
	1/64	6	27	
	1/32	12	38	
	1/16	25	39	
	1/8	49	57	
	1/4	99	85	
	1/2	198	104	
	und.	395	159	No Tox.
DCM Fr. B3	not done (insufficient sample)			
DCM Fr. A4	1/128	2	31	
	1/64	4	18	
	1/32	8	29	
	1/16	16	40	
	1/8	32	29	
	1/4	63	42	
	1/2	127	73	
	und.	254	87	No Tox.
DCM Fr. N6	1/128	2.5	33	
	1/64	5	21	
	1/32	10	30	
	1/16	20	37	
	1/8	40	27	
	1/4	80	38	
	1/2	159	49	
	und.	318	61	No Tox.

Table AI-3 (Con't)

Material Tested[a]	Dilution	E.O.M.[b] (ug)	Revs/plate[c]	Toxicity
ACE Ext.	not done (received wrong sample)			
ACE Fr. B1	1/128	1	16	
	1/64	2	19	
	1/32	4	28	
	1/16	8	21	
	1/8	16	20	
	1/4	32	25	
	1/2	63	14	
	und.	127	40	No Tox.
ACE Fr. A2	1/128	2	39	
	1/64	4	28	
	1/32	7	36	
	1/16	14	28	
	1/8	28	49	
	1/4	56	66	VST?
	1/2	112	42	ST
	und.	224	18	ST/T
ACE Fr. B3	1/128	1	20	
	1/64	2	25	
	1/32	5	19	
	1/16	9	29	
	1/8	18	37	
	1/4	37	28	
	1/2	74	48	
	und.	148	52	No Tox.
ACE Fr. A4	1/128	2	21	
	1/64	4	45	
	1/32	9	31	
	1/16	17	33	
	1/8	34	43	
	1/4	69	51	VST
	1/2	138	40	ST
	und.	275	25	ST/T

Table **AI-4**

Mutagenicity Data
HPLC Air Fractionation Project
S88 Extracts and Fractions

Fraction	Tester Strain +/-S9	Rev./ug. +/- 95% C.I.[a]	Corr. Coeff.[b]	Calc. Dose/ Dose[c]
S88-DCM Extract	98-S9	0.31 ± 0.08	0.86	5/5
	98+S9	0.48 ± 0.10	0.91	5/5
	98NR-S9	0.34 ± 0.14	0.77	4/5
	98DNP-S9	0.22 ± 0.10	0.79	3/5
	100-S9	0.83 ± 0.10	0.97	5/5
	100+S9	1.39 ± 0.10	0.99	5/5
S88-DCM-B1	98-S9	0.46 ± 0.23	0.84	2/3*
	98+S9	0.68 ± 0.07	0.99	3/3
	98NR-S9	[0.22 ± 0.14]	0.66	3/3*
	98DNP-S9	[0.15 ± 0.17]	0.51	2/3*
	100-S9	0.61 ± 0.21	0.92	2/3
	100+S9	1.51 ± 0.43	0.94	2/3
S88-DCM-A2	98-S9	0.55 ± 0.18	0.93	3/4
	98+S9	0.55 ± 0.06	0.98	4/4
	98NR-S9	[0.13 ± 0.08]	0.59	4/4
	98DNP-S9	[0.12 ± 0.08]	0.75	2/4
	100-S9	0.52 ± 0.10	0.94	4/4
	100+S9	0.98 ± 0.05	1.00	4/4
S88-DCM-B3	98-S9	[0.45 ± 0.33]	0.71	2/3*
	98+S9	0.58 ± 0.20	0.87	3/3
	98NR-S9	0.46 ± 0.04	0.99	2/3
	98DNP-S9	[0.16 ± 0.20]	0.44	2/3
	100-S9	0.48 ± 0.17	0.86	3/3
	100+S9	1.17 ± 0.14	0.98	3/3
S88-DCM-A4	98-S9	[0.27 ± 0.16]	0.78	2/3*
	98+S9	0.27 ± 0.05	0.97	2/3
	98NR-S9	[0.28 ± 0.18]	0.76	2/3*
	98DNP-S9	[0.06 ± 0.07]	0.45	2/3*
	100-S9	0.48 ± 0.20	0.89	2/3
	100+S9	0.53 ± 0.14	0.93	3/3
S88-DCM-N6	98-S9	0.08 ± 0.04	0.72	3/3
	98+S9	0.29 ± 0.13	0.88	2/3
	98NR-S9	[0.13 ± 0.11]	0.65	2/3
	98DNP-S9	[0.03 ± 0.02]	0.64	3/3
	100-S9	0.25 ± 0.11	0.87	2/3
	100+S9	1.03 ± 0.12	0.99	2/3

Table AI-4 Con't (see note "a" in Table 3.3)

Fraction	Tester Strain +/-S9	Rev./ug. +/- 95% C.I.[a]	Corr. Coeff.[b]	Calc. Dose/ Dose[c]
S88-ACE-extract	(no sample available for testing)			
S88-ACE-B1	98-S9	neg ?		*
	98+S9	neg ?		*
	98NR-S9	neg ?		*
	98DNP-S9	neg		
	100-S9	neg		
	100+S9	[0.24 ± 0.10]	0.82	3/3
S88-ACE-A2	98-S9	neg ?		*
	98+S9	0.35 ± 0.13	0.77	5/5
	98NR-S9	0.62 ± 0.25	0.83	3/5*
	98DNP-S9	neg		
	100-S9	1.82 ± 0.49	0.89	4/5
	100+S9	neg		*
S88-ACE-B3	98-S9	neg ?		*
	98+S9	[0.19 ± 0.14]	0.60	3/3
	98NR-S9	neg ?		*
	98DNP-S9	neg ?		*
	100-S9	0.42 ± 0.15	0.91	2/3
	100+S9	0.37 ± 0.18	0.86	2/3
S88-ACE-A4	98-S9	neg ?		*
	98+S9	[0.48 ± 0.34]	0.72	2/4
	98NR-S9	1.04 ± 0.42	0.89	2/4
	98DNP-S9	neg ?		*
	100-S9	2.06 ± 0.42	0.93	4/4
	100+S9	0.95 ± 0.23	0.93	3/4
S88-ACE-N6	98-S9	[0.40 ± 0.28]	0.74	2/4
	98+S9	0.16 ± 0.06	0.79	4/4
	98NR-S9	[0.21 ± 0.11]	0.73	3/4
	98DNP-S9	neg ?		*
	100-S9	0.77 ± 0.06	0.99	4/4
	100+S9	0.48 ± 0.10	0.93	4/4

[a] = number of revertant colonies per ug ± 95% confidence intervals.
[b] = Corr. coeff. = correlation coefficient.
[c] = calc. dose/dose = # doses used for linear regression analysis/# doses tested.
[ ] = mutagenic activity equivocal.
* = Dose response not linear.

Table AI-4 Con't)

Material Tested[a]	Dilution	E.O.M.[b] (ug)	Revs/plate[c]	Toxicity
ACE Fr. N6	1/128	2	31	
	1/64	3	28	
	1/32	7	29	
	1/16	13	36	
	1/8	27	33	
	1/4	54	60	
	1/2	107	57	
	und.	215	66	VST?

---

a = Volumes tested = 0.1 ml unless otherwise indicated. One plate per dose unless indicated.
b = E.O.M. = extractable organic matter.
c = Incubation time = 48 hours. TA98-S9.
d = Mean of 3 plates.
und. = undiluted extract

Toxicity Code
VST = Very slight toxicity. Only slight decrease in confluency of background lawn.
ST = Slight toxicity. Background lawn shows definite signs of toxicity.
ST/T= Subtoxic. Background lawn reduced to a few survivors; revertant colonies still distinguishable from survivor colonies.
T = Toxic. Essentially all cell growth inhibited.

Ambient Air HPLC Fractionation Project: Summer 1988 (S88) Sample Extracts and Fractions

Repeat Mutagenicity Assay Using TA98 and Nitroreductase-deficient (NR^-) Tester Strains

For reasons previously outlined, the mutagenicity assay data for the S88 samples was sub-optimal. Therefore, repeat testing was done on the small amount of each sample that remained following the initial assay. All samples were tested with TA98 without metabolic activation (-S9). In a few cases, there was sufficient sample remaining for analysis with metabolic activation as well or with one or more of the NR^- tester strains. As many strains and/or conditions were used as sample quantity would allow.

Processing

Each sample was further processed for repeat testing as indicated.

	Initial Ames Assay			
Sample	Vol. used (ml)	Vol. remaining (ml)	EOM (mg/ml)	Further Processing
DCM Ext.	2.78	≈ 1.99	3.324	5 dilutions prepared (0.8 ml e
DCM B1	2.40	0.36	1.429	Vol. increased to 0.514 ml; assayed at 4 different volumes
DCM A2	2.60	0.40	3.951	4 dilutions prepared (0.31 ml each)
DCM B3	2.50	0.52	1.111	Vol. increased to 0.578 ml; 4 dilutions prepared (0.25 ml each)
DCM A4	2.40	0.47	2.538	Vol. increased to 0.596 ml; assayed at 4 different volumes
DCM N6	2.40	≈ 0.60	3.183	4 dilutions prepared (0.31 ml each)
ACE B1	2.40	≈ 0.60	1.267	4 dilutions prepared (0.3 ml)
ACE A2	2.90	2.00	1.029	5 dilutions prepared (1.0 ml)
ACE B3	2.40	≈ 0.60	1.478	4 dilutions prepared (0.3 ml)
ACE A4	2.00	≈ 3.80	1.429	5 dilutions prepared (1.0 ml)
ACE N6	2.60	0.34	2.150	Vol. increased to 0.812 ml; assayed at 4 different volumes

Mutagenicity Assay

1. The dose-response slope data is provided in Table **AI-5.** Hard copy of the raw data was sent to Roy Meyer, NJ DEP under separate cover.

2. The repeat assay data were much improved over the initial data set (see respective slope and r^2 values).

3. The repeat assay data points out one of the hazards of comparing -S9 and +S9 slope data when testing complex mixtures. Namely, differential toxicity in the absence verses the presence of S9 may make it appear that indirect-acting mutagens are present when in fact none are present (see below).

4. DCM extract and fraction data:

   DCM Extract

   1. The TA98/DNP$^-$ strain data were similar for both assays.

   2. The TA98-S9 slope was 2.5 times greater in the repeat assay.

   3. Although the TA98+S9 slope was also greater, there was now no evidence for the presence of indirect-acting mutagens. Previous evidence was therefore artifactual due to greater toxicity in the -S9 assay vs the +S9 assay.

   DCM Fractions

   1. There was enough sample to test each fraction with TA98-S9 only.

   2. The B1 slope was 1.8-2.6 times greater in the repeat assay (depending on the number of doses used in regression analysis in the second assay). Indirect-acting mutagens may be present, but it is now much less certain (see DCM extract data).

   3. The comparative potencies (rev/ug) are such that B1 is now the most potent of all the fractions with A2, B3, and A4 having comparable but lesser potencies. As in the initial assay, N6 was the least potent.

   4. B1 was among the two lowest potency (rev/ug) fractions in the W88 samples but was the most potent in the S88 samples.

   5. The potencies of the DCM extract and all fractions except B1 were greater in the W88 samples than in the S88 samples.

   6. Nitrogroup containing mutagens now appeared to comprise roughly the same percentage of the total activity in the S88 DCM extract as it did in the W88 DCM extract. This was not the case in the initial S88 data set.

ACE Fractions

1. Fraction B1 remained negative with TA98-S9, but all of the other fractions now displayed mutagenic activity. B1 was also negative in the W88 samples.

2. A2: depending on the number of dose levels used for regression analysis, this fraction contained between 0.60 to 3.2 rev/ug with the most likely range falling between 1.2 and 2.1 rev/ug. There were no indirect-acting mutagens present. The TA98/DNP data was again negative. It is not certain why the 98/NR data was negative in the repeat assay and positive in the initial assay except that the dose-response was not linear in the initial assay and was also influenced by toxicity. Therefore, most or perhaps all of the activity here is due to nitro-group mutagens.

3. B3: Tested with TA98-S9 only. It is not unlikely but not certain that this fraction contains indirect-acting mutagens.

4. A4: The sample contains nitro group direct-acting mutagens only. It is not certain why the 98/NR data was positive in the initial assay and negative in this assay. Toxicity resulted in only two doses being used to calculate the slope in the initial assay and therefore the initial assay data may not be too reliable.

5. N6: about half the activity in this fraction is due to nitro group mutagens and confirms this finding in the initial assay. There are undoubtedly no indirect-acting mutagens in this or any other ACE fraction.

6. Fraction potencies (rev/ug) in the S88 ACE samples were not too different than in the W88 sample and therefore interfraction comparisions are similar for both seasons.

7. As in the W88 fractions, some TA100-responsive mutagen(s) may be present in fraction A4.

Table AI-5

Repeat Mutagenicity Assay Data

HPLC Air Fractionation Project

S88 Extracts and Fractions

Fraction	Tester Strain +/-S9	Rev./ug. +/- 95% C.I.[a]	Corr. Coeff.[b]	Calc. Dose/ Dose[c]
S88-DCM Extract	98-S9	0.77 ± 0.08	0.98	5/5
	98+S9	0.79 ± 0.16	0.92	5/5
	98NR-S9			
	98DNP-S9	0.13 ± 0.05	0.73	5/5
S88-DCM-B1	98-S9	1.19 ± 0.52	0.88	2/4
		(0.84 ± 0.28)	(0.87)	(3/4)
S88-DCM-A2	98-S9	0.64 ± 0.10	0.97	3/4
S88-DCM-B3	98-S9	0.52 ± 0.12	0.92	4/4
S88-DCM-A4	98-S9	0.54 ± 0.11	0.95	3/4
S88-DCM-N6	98-S9	0.29 ± 0.12	0.87	3/4
S88-ACE-extract	(no sample available for testing)			
S88-ACE-B1	98-S9	neg.		
S88-ACE-A2	98-S9	2.06 ± 1.16	0.72	3/5
		(1.16 ± 0.60)	(0.68)	(4/5)
	98+S9	[0.32 ± 0.23]	0.46	5/5
	98NR-S9	neg.		
	98DNP-S9	neg.		
S88-ACE-B3	98-S9	0.47 ± 0.18	0.90	2/4
S88-ACE-A4	98-S9	1.37 ± 0.40	0.87	4/5
	98+S9	neg.		
	98NR-S9	neg.		
	98DNP-S9	neg.		
S88-ACE-N6	98-S9	0.75 ± 0.32	0.82	3/4
	98+S9			
	98NR-S9	0.33 ± 0.18	0.66	4/4
	98DNP-S9			

[a] = number of revertant colonies per ug ± 95% confidence intervals.
[b] = Corr. coeff. = correlation coefficient.
[c] = calc. dose/dose = # doses used for linear regression analysis/# doses tested.
[ ] = mutagenic activity equivocal.

APPENDIX FOR CHAPTER THREE, PART II:

Ames assay of S88 Subfraction

1. The results are shown on the following pages and are summarized as follows:

Fraction	Tester Strain +/-S9	Rev./ug +/- 95% C.I.	Corr. Coeff.	Calc. Dose/ Dose
S88-ACE-N6-				
-S1 (Hex)	98-S9	0.25 ± 0.07	0.92	3/3
-S2 (Hex/benz)	98-S9	1.86 ± 0.80	0.88	2/3
-S3 (DCM)	98-S9	5.56 ± 0.68	0.98	3/4
	98DNP-S9	[0.72 ± 0.52]	0.52	4/4
-S4 (Meth)	98-S9	0.72 ± 0.21	0.91	4/4
	98DNP-S9	[0.13 ± 0.10]	0.58	4/4

[ ] = mutagenic activity equivocal

2. Intersample mutagenic potency comparisons of the four samples support the results of the initial assay from a qualitative standpoint.

3. Sample S4 was not toxic in both assays. Its activity was 1.8 (TA98) or 1.4 (TA98/DNP) times higher in the repeat assay compared to the initial assay.

4. Subfraction S3 was the most mutagenic per µg of EOM followed by S2, S4 and S1.

5. It appears as if most of the mutagenic activity in fractions S3 and S4 is due to the presence of nitro group-containing mutagens. Although there was insufficient material to test S1 and S2 with TA98/DNP in the repeat assay, results from the initial assay indicated that probably much of the activity in these fractions was due to nitro group-containing mutagens as well.

Table AII-1

Processing Data for Subfractions of

	Subfraction	Vol. (ml)	EOM/ml (mg)	Total EOM (mg)	Vol. after EOM deter. (mg)	Extract Data for Ames Vol. (ml)	Extract Data for Ames Calc. EOM, (mg)
				DCM - N6			
S1	Hex	4.94	2.146	10.599	4.79	2.0	5.138
S2	Hex/Benz	6.54	0.248	1.619	6.39	2.0	0.791
S3	DCM	6.51	0.768	4.996	6.36	2.0	2.441
S4	Methanol	3.15	4.178	13.159	3.00	2.0	6.266
				ACE - N6			
S1	Hex	4.91	1.351	6.633	4.76	1.5	4.287
S2	Hex/Benz	9.47	0.159	1.506	9.32	1.5	0.988
S3	DCM	5.60	0.286	1.599	5.45	1.5	1.037
S4	Methanol	3.62	2.017	7.302	3.47	1.5	4.669

Table **AII-2**

Mutagenicity of the S88: DCM-N6 and ACE-N6

Subfractions

Fraction	Tester Strain +/- S9	Rev./ug +/- 95% C.I.[a]	Corr. Coeff[b]	Cal. dose/ Dose[c]	
DCM-N6 Subfractions					
S1 Hexane	98-S9	0.06 ± 0.03	0.66	4/4	
	98+S9	0.12 ± 0.02	0.96	4/4	
	98DNP-S9	neg.	--	4/4	
S2 Hexane/Benzene	98-S9	1.76 ± 0.28	0.96	4/4	
	98+S9	2.51 ± 0.19	0.99	4/4	
	98DNP-S9	0.57 ± 0.21	0.80	4/4	
S3 DCM	98-S9	1.68 ± 0.26	0.96	4/4	
	98+S9	2.40 ± 0.46	0.94	4/4	
	98DNP-S9	0.30 ± 0.07	0.91	4/4	
S4 Methanol	98-S9	0.28 ± 0.06	0.94	4/5	
	98+S9	0.27 ± 0.03	0.96	5/5	
	98DNP-S9	[0.03 ± 0.02]	0.56	5/5	
ACE-N6 Subfractions					
S1 Hexane	98-S9	0.18 ± 0.05	0.94	2/4	Highest t three dos cytotoxic
	98DNP-S9	neg.		2/4	
S2 Hex/Benz	98-S9	0.83 ± 0.45	0.82	2/4	
	98DNP-S9	neg.		1/4	
S3 DCM	98-S9	2.01 ± 0.97	0.85	2/4	
	98DNP-S9	neg.		1/4	
S4 Methanol	98-S9	0.40 ± 0.07	0.95	4/4	
	98DNP-S9	0.09 ± 0.02	0.93	4/4	

[a] = number of revertant colonies per ug of EOM ± 95% confidence interval.
[b] = correlation coefficient.
[c] = number of doses used for linear regression analysis/number of doses tested.
[ ] = mutagenic activity equivocal.

VIII. HPLC Air Fractionation Project: S88-DCM-N6 and S88-ACE-N6 Subfractions

Processing

1. DCM fraction N6 or ACE fraction N6 were layered onto the top of a silica gel column.

2. Four solvents were passed through the column successfully and collected separately to obtain four subfractions for each sample as follows:

Subfraction	Eluting Solvent
1	Hexane
2	Hexane/benzene (1/1)
3	DCM
4	Methanol

3. Pertinent processing data for these subfractions can be found in Table AII-1.

4. The initial volume was measured.

5. The EOM was determined using a 50 ul and 100 ul aliquot from each extract (30 min, 40°C on a slide warmer).

6. All extracts were solvent-exchanged to acetone (three 5 ml aliquots used during the solvent-exchange process) and brought to a predetermined volume. (see Table AII-1).

7. The final EOM/ml data for each extract was also calculated.

Analysis

1. Each extract was assayed at 4 or more dose levels.

2. The DCM-N6 subfractions were assayed with strains TA98 (+ and -S9), and TA98 1,8DNP$_6$.

3. The ACE-N6 subfractions were assayed with strains TA98 and TA98/1,8DNP$_6$ (-S9 only).

4. A summary of the dose-response data is provided in Table AII-2

Conclusions

1. The major conclusions drawn from this portion of the subfractionation project are provided in an 1/30/89 letter to Dr. Greenberg (enclosed).

CHAPTER FOUR

FOURIER TRANSFORM INFRARED (FTIR) SPECTRA ANALYSIS OF ACETONE EXTRACTABLE AIRBORNE PARTICULATES

4-1. Introduction.

We have attempted to gain insight into the nature of funtional groups and classes of organic compounds in our airborne particulate extracts by employing Fourier Transform Infrared (FTIR) spectroscopy on the 15 individual fractions (B1, A2, B3, A4) and subfractions (N6-S1, N6-S2, N6-S3, N6-S4) of W88-ACE and S88-ACE extracts.

4-2. Experimental

The FTIR technique has been discussed elsewhere(1). For the present study, samples dissolved in different solvents were evaporated to dryness on 13 * 2 mm KBr windows and scanned 32 times on a Nicolet 740 FTIR spectrophotometer equipped with a sensitive MCT detector. The spectra were obtained under the supervision of Dr. David Bugay of Squibb Corp., New Brunswick.

Fourier Transform Infrared (FTIR) Spectroscopy is a potentially powerful technique for analyzing classes of pollutants on ambient particulates. In the present study fractions and subfractions of the ACE extract of W88 and S88 composite sample IP 03 were obtained using liquid-liquid partition and silica-gel open column chromatography. In the following, each FTIR spectra of the fraction and subfraction will

be discussed in turn. All the samples were layered on a 13 * 2 mm KBr window and the solvent evaporated.

The spectra for the fractions and subfractions of W88 and S88 are presented at the end of this chapter from Figure 4.1 to Figure 4.15. No S88-ACE-N6-S2 was analyzed by FTIR because of the limited sample.

4-3. FTIR Spectrum Analysis Results

4-3-1. Weak Base Fraction (B1)

A quick glance at the transmittance scales of Figure 4.1 and Figure 4.2 indicated that is little difference between them. However, both of them have very strong bands at 2957 cm^{-1}, 2917 cm^{-1}, 2849 cm^{-1}, 1472 cm^{-1}, 1376 cm^{-1}, and 1261 cm^{-1} which could correspond to alkanes. In the winter fraction, there is a hint of unsaturated and/or aromatic hydrocarbons as shown by the small shoulder around 3030 cm^{-1} and the small peak around 1600 cm^{-1}; moreover, the carbonyl absorption around 1713-1728 cm^{-1} is likely to be phthalates or other saturated or unsaturated ketones. Actually, phthalates have carbonyl absorption around 1720 cm^{-1}. For the winter weak base fraction (W88-ACE-B1), the band at 1123 cm^{-1} could correspond, along with the 3100 cm^{-1} band, to an alcohol or to an ester. This is a significant difference between winter and summer weak bases fractions, since the summer fraction lacks this. But in the summer fraction, an interesting double band appeared at 1095 cm^{-1} and 1018 cm^{-1} which could corrrespond to the unconjugated straight chain anhydrides. The bands near 952-909 cm^{-1} and 1260 cm^{-1} in this sample correspond to cyclic

anhydride C-CO-O-CO-C stretch. This might be the second significant difference between the winter and summer weak base fractions because the winter fraction lacks this feature.

4-3-2. Weak Acid Fractions (A2)

The winter (Figure 4.3) and summer (Figure 4.4) weak acid fractions have very similar IR spectra: both have carbonyl bands at 1712 cm^{-1} and a weak aromatic band above 3000 cm^{-1} as well as aromatic C-C stretch around 1600 cm^{-1}. In addition, the O-H absorption peak around 3280 cm^{-1} might be due to some fatty acid contribution, although moisture is possible. It is interesting that very weak asymmetric stretching (1515-1550 cm^{-1}) and symmetric stretching (1345-1385 cm^{-1}) bands for the nitro group are found in these two fractions. In this regard, it is worthwhile remembering the earlier-cited observations of a number of research groups that most of the mutagenic activity is associated with nitrated compounds. This is, perhaps, the reason why these weak acid fractions were the most mutagenic at the first level of separation. The carbonyl absorption at 1712 cm^{-1} is probably due to phthalates, because the mass spectrometric results indicated that samples were contaminated with phthalates.

4-3-3. Strong Base Fraction (B3)

The IR spectra of the strong base fractions are shown in Figure 4.5 and Figure 4.6. There is a significant level of alkanes which appear to compose most of its mass. Thus, there is less mutagenicity in these two samples. It appears, as in the weak base fractions, that levels of nitro group cannot be

obviously found in these fractions and Ames assay results showed no TA98NR-S9 and TA98DNP-S9 response in W88-ACE-B3 and S88-ACE-B3 fractions. There might be a carbonyl band (1712-1728 cm^{-1}) which is likely to be associated with an ester. A weak aromatic band at around 1600 cm^{-1} was also found in the spectra of summmer strong bases fraction, but not in that of winter fraction.

4-3-4. Strong Acid Fraction (A4)

In Figure 4.7 and Figure 4.8, the spectra of strong acid fractions displayed intense and wide carbonyl bands at 1720 cm^{-1} which could correspond to carboxylic C=O stretch, 1715 cm^{-1}, and the band around 1280 cm^{-1} might represent the C-O, dimer, stretch in carboxylic acid. The aromatic absorption band above 3000 cm^{-1} was seen in the winter fraction only. Also in the winter fraction, there might be N-H existing, as indicated by the two absorption bands at 3500 cm^{-1} and 3100 cm^{-1}. Furthermore, the band around 1550 cm^{-1} and the band at 1370 cm^{-1} could correspond to the nitro group.

4-3-5. First Neutral Subfraction (S1)

These two subfractions (W88-ACE-N6-S1 and S88-ACE-N6-S1) eluting from an open silica gel column with hexane are the non-polar and second most massive fractions at the second level of separation. Their FTIR spectra are shown in Figures 4.9 and 4.10. In the winter fraction, only the alkane bands at 2918 cm^{-1} and 2850 cm^{-1} and the band above 3000 cm^{-1} which correspond to aromatics could be seen. The FTIR spectra of the summer fraction

shows the alkane bands at 2963 cm^{-1}, 2924 cm^{-1}, 2851 cm^{-1} corresponding to C-H stretch of alkanes, as well as 1461 cm^{-1}, 1340 cm^{-1} corresponding to C-H out-of-plane bendings; two bands at 1090-1020 cm^{-1} may correspond to unconjugated straight chain anhydrides, and a cyclic anhydride C-CO-O-CO-C stretch near 900 cm^{-1} as well as a 1261 cm^{-1} band are found in this sample.

4-3-6. Second Neutral Subfraction (S2)

These hexane/benzene (1:1 v/v) eluant neutral subfractions should correspond to polycyclic aromatic hydrocarbons (PAH) compound, although most parent PAH compounds are found in this fraction of the DCM extracts. The spectrum is shown in Figure 4.11 (FTIR analysis was not done on S88-ACE-N6-S2 fraction because of the limited mass of the sample). The aromatic C-H stretching band at 3050 cm^{-1} is evident and the most characteristic absorption of polycyclic aromatics resulting from C-H out-of plane bending in the 900-675 cm^{-1} region is also found in Figure 4.11. Thus, possibly, some parent PAH compounds remain in this fraction.

4-3-7. Third Neutral Subfraction (S3)

The aromatic C-H stretching bands are still observable at 3050 cm^{-1} in Figures 4.13 and 4.14. Weak C-C ring stretch occurs at 1580, 1487 and 1466 cm^{-1}. Unexpectedly, it seems to us that the W88-ACE-N6-S3 spectra do not clearly show (the S88-ACE-N6-S3 gives a few clear hint) asymmetrical and symmetrical stretching of the nitro group in the regions of 1550-1515 cm^{-1} and 1385-1345 cm^{-1}. However, a C-N stretching vibration of nitro aromatic

compounds appear near 860 cm^{-1}. Asymmetrical stretching in the NO_2 group of organic nitrates results in strong absorption in the 1660-1625 cm^{-1} region and symmetrical vibration absorbs strongly near 1300-1255 cm^{-1}. In addition, in the spectra of W88-ACE-N6-S3, the carbonyl band at 1729 cm^{-1} could correspond to benzoates because conjugation of an aryl group or other unsaturated linkage with the carbonyl group causes this C=O stretch to be at lower than normal frequency (e.g. benzoates absorb at ca. 1724 cm^{-1}); but in S88-ACE-N6-S3, the carbonyl band appears at 1743 cm^{-1}, along with two strong bands at 1076 cm^{-1} and 1095 cm^{-1} corresponding to an ester. The band around 1635 cm^{-1} could well be due to PAH-quinones since extended quinones are known to absorb in the 1655-1635 cm^{-1} region.

4-3-8. Fourth Neutral Subfraction (S4)

In the W88-ACE-N6-S4 spectra shown in Figure 4.14, we can only clearly observe three hydrocarbon bands at around 3000 cm^{-1}. There might be some aromatic hydrocarbons, because of the band at above 3000 cm^{-1}. In the spectrum of S88-ACE-N6-S4 (Figure 4.15), except those bands at 2963 cm^{-1}, 2916 cm^{-1}, 2848 cm^{-1} which are for hydrocarbon, the two bands at 1094 cm^{-1} and 1021 cm^{-1} are due to the unconjugated straight chain anhydrides; we can also find the cyclic anhydride C-CO-O-CO-C stretch near 952-909 cm^{-1} and 1261 cm^{-1} in this sample.

REFERENCES

1. P.R. Griffiths and J.A. de Haseth, Fourier Transform Infrared Spectrometry, Wiley, New York, 1986.
2. K. Nakanishi, Infrared Absorption Spectroscopy Practical, Holden-Day, Inc., San Francisco, 1962.

Figure 4.1

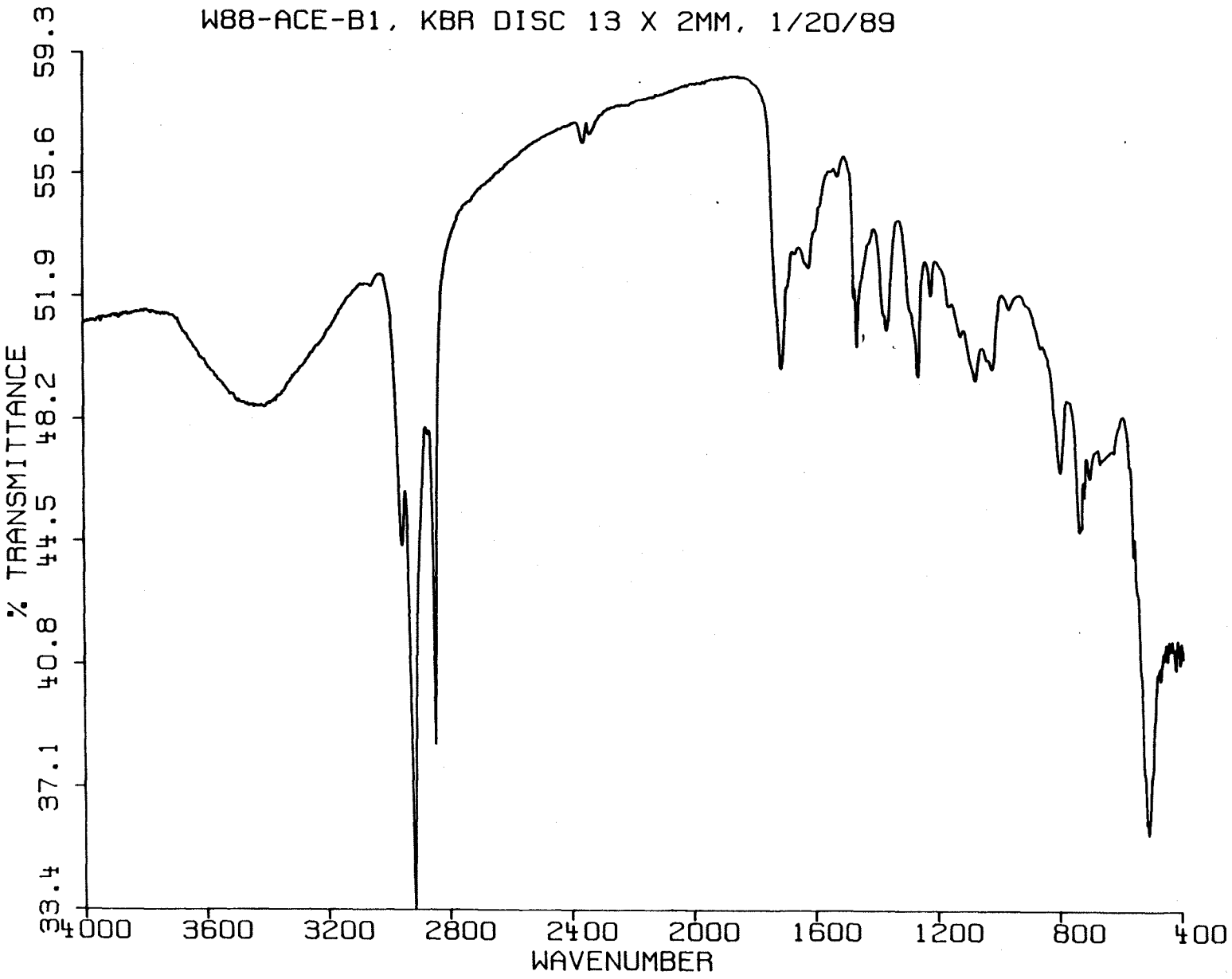

W88-ACE-B1, KBR DISC 13 X 2MM, 1/20/89
% TRANSMITTANCE
59.3
55.6
51.9
48.2
44.5
40.8
37.1
33.4
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER

Figure 4.2

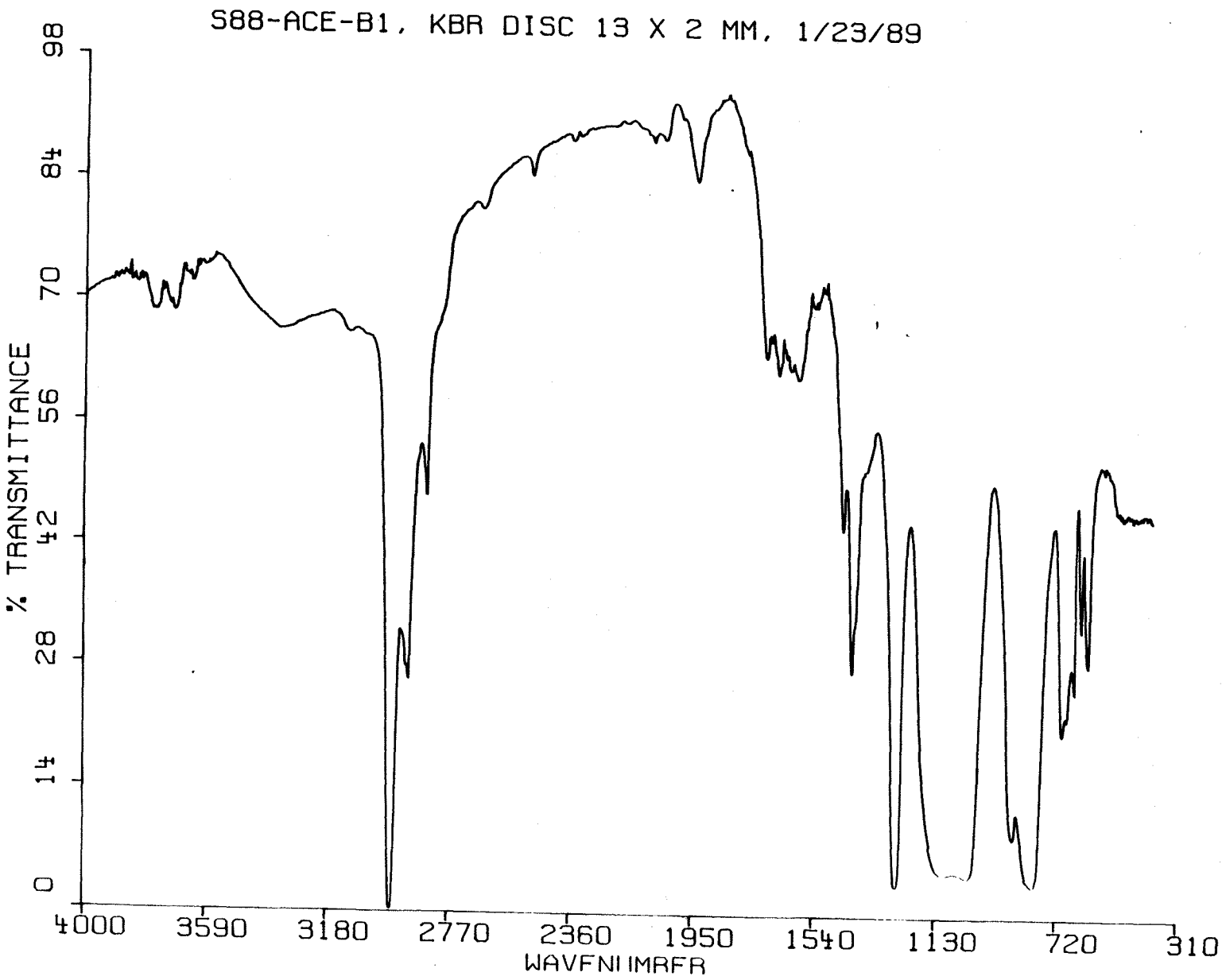

S88-ACE-B1, KBR DISC 13 X 2 MM, 1/23/89
% TRANSMITTANCE
98
84
70
56
42
28
14
0
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4.3

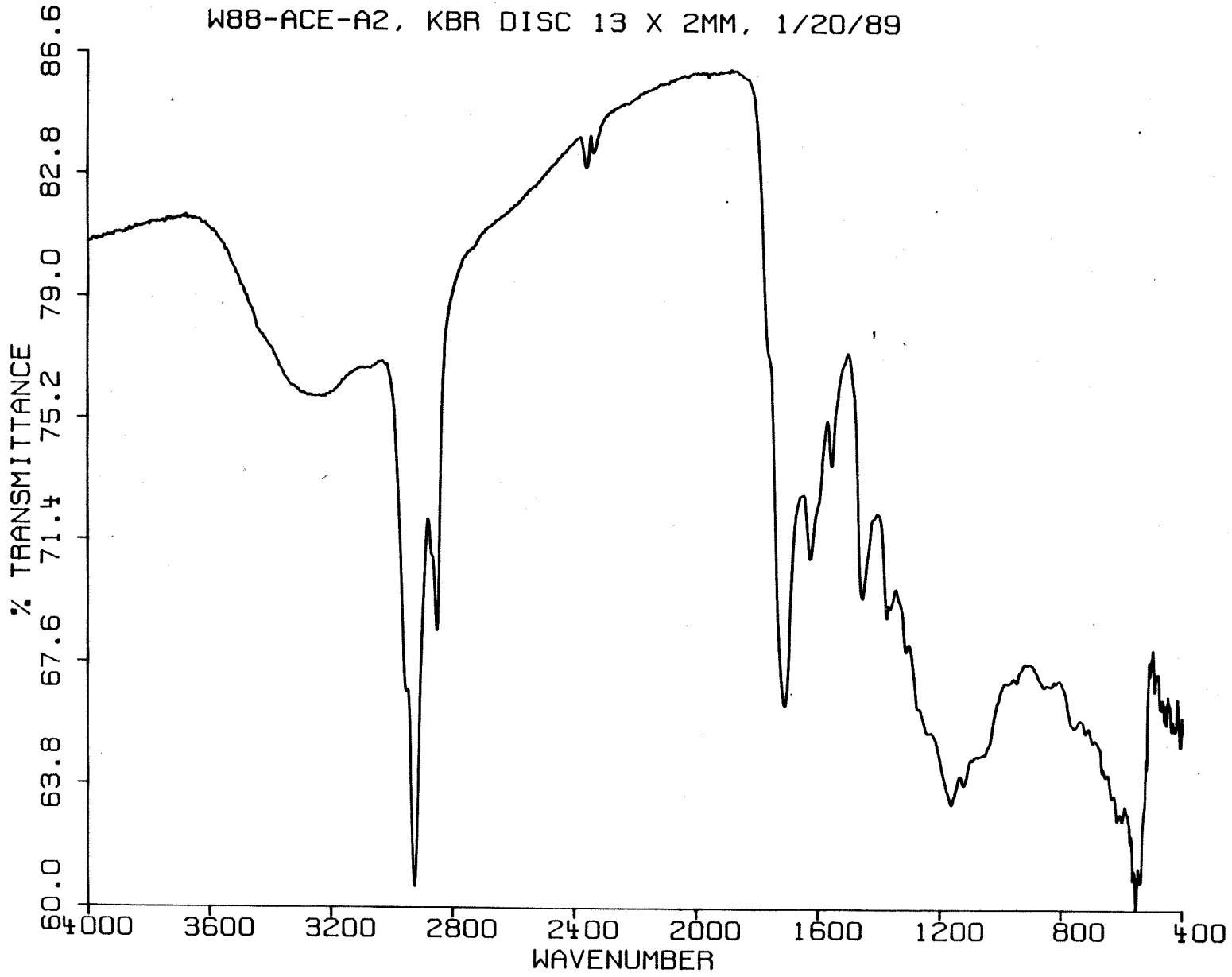

W88-ACE-A2, KBR DISC 13 X 2MM, 1/20/89
% TRANSMITTANCE
86.6
82.8
79.0
75.2
71.4
67.6
63.8
60.0
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER

Figure 4.4

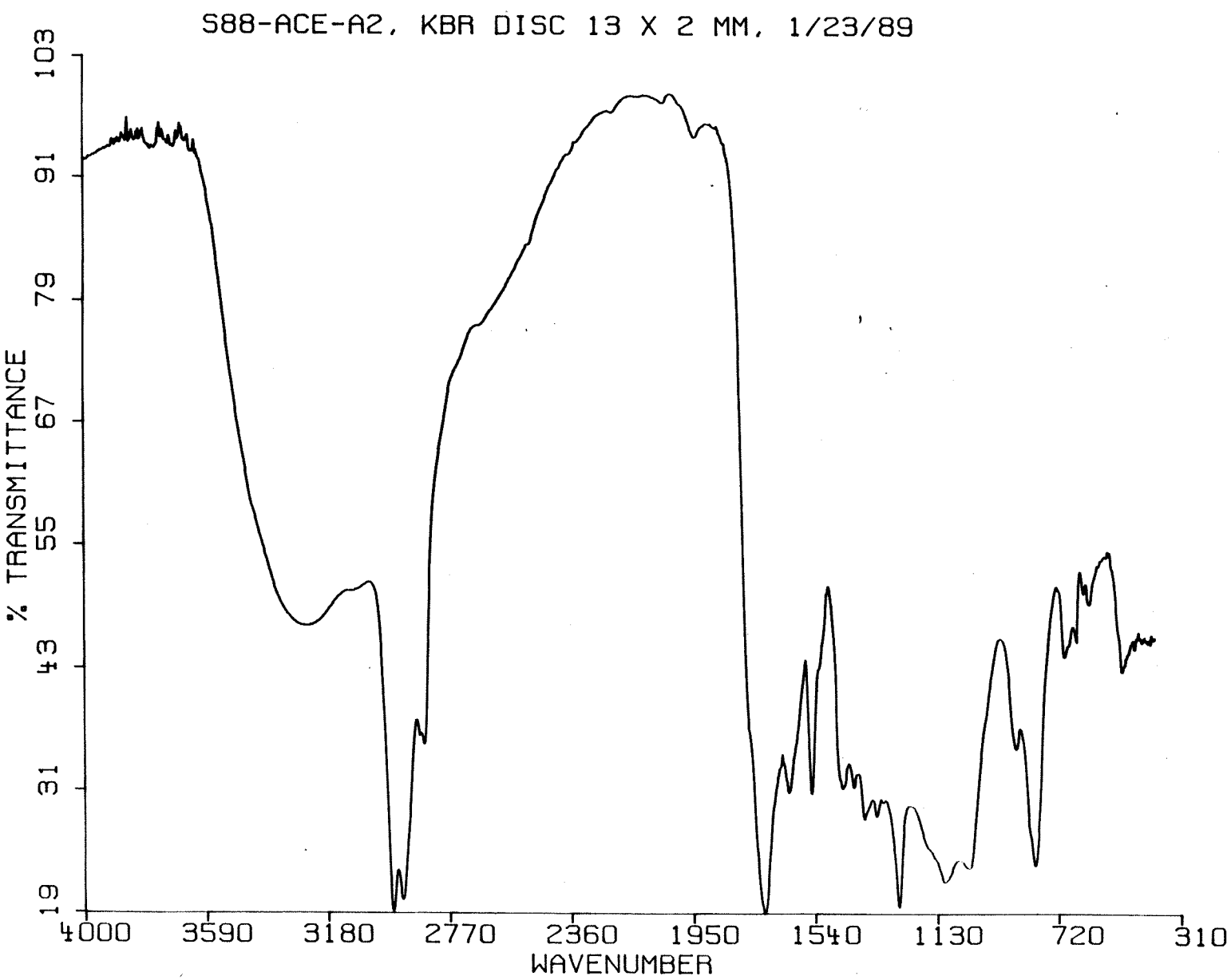

S88-ACE-A2, KBR DISC 13 X 2 MM, 1/23/89
% TRANSMITTANCE
103
91
79
67
55
43
31
19
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4.5

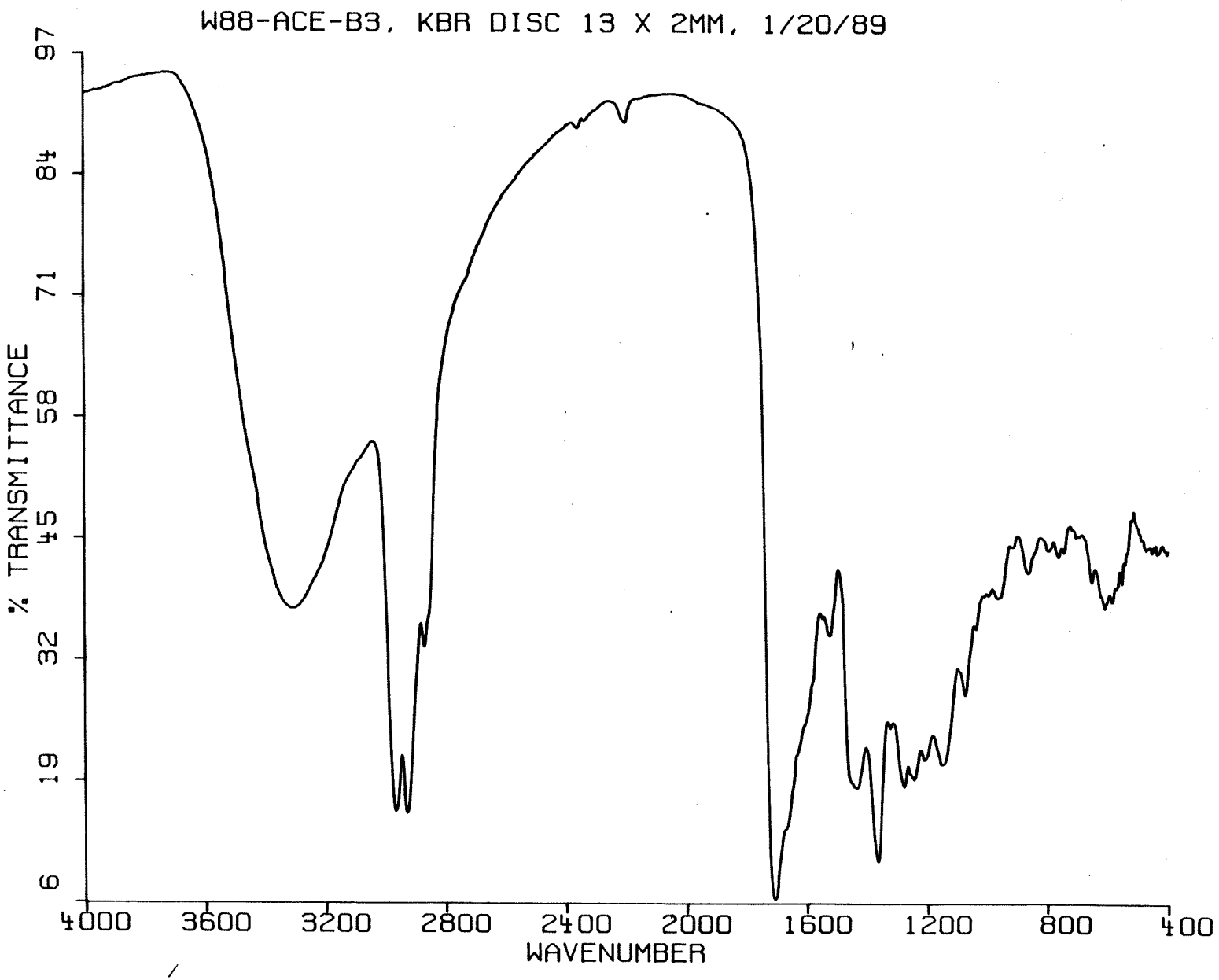

W88-ACE-B3, KBR DISC 13 X 2MM, 1/20/89
% TRANSMITTANCE
97
84
71
58
45
32
19
6
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER

Figure 4.6

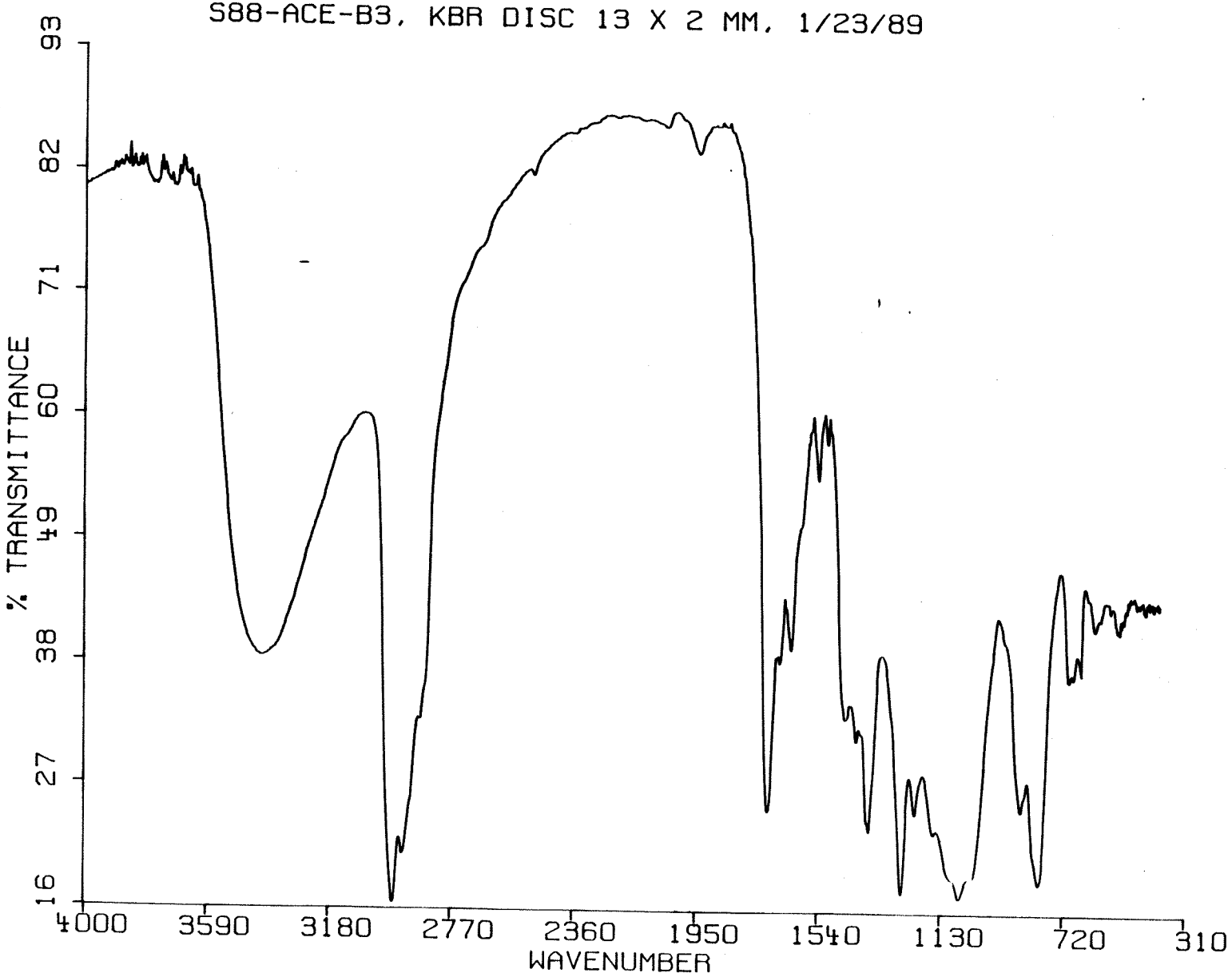

S88-ACE-B3, KBR DISC 13 X 2 MM, 1/23/89
% TRANSMITTANCE
93
82
71
60
49
38
27
16
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4.7

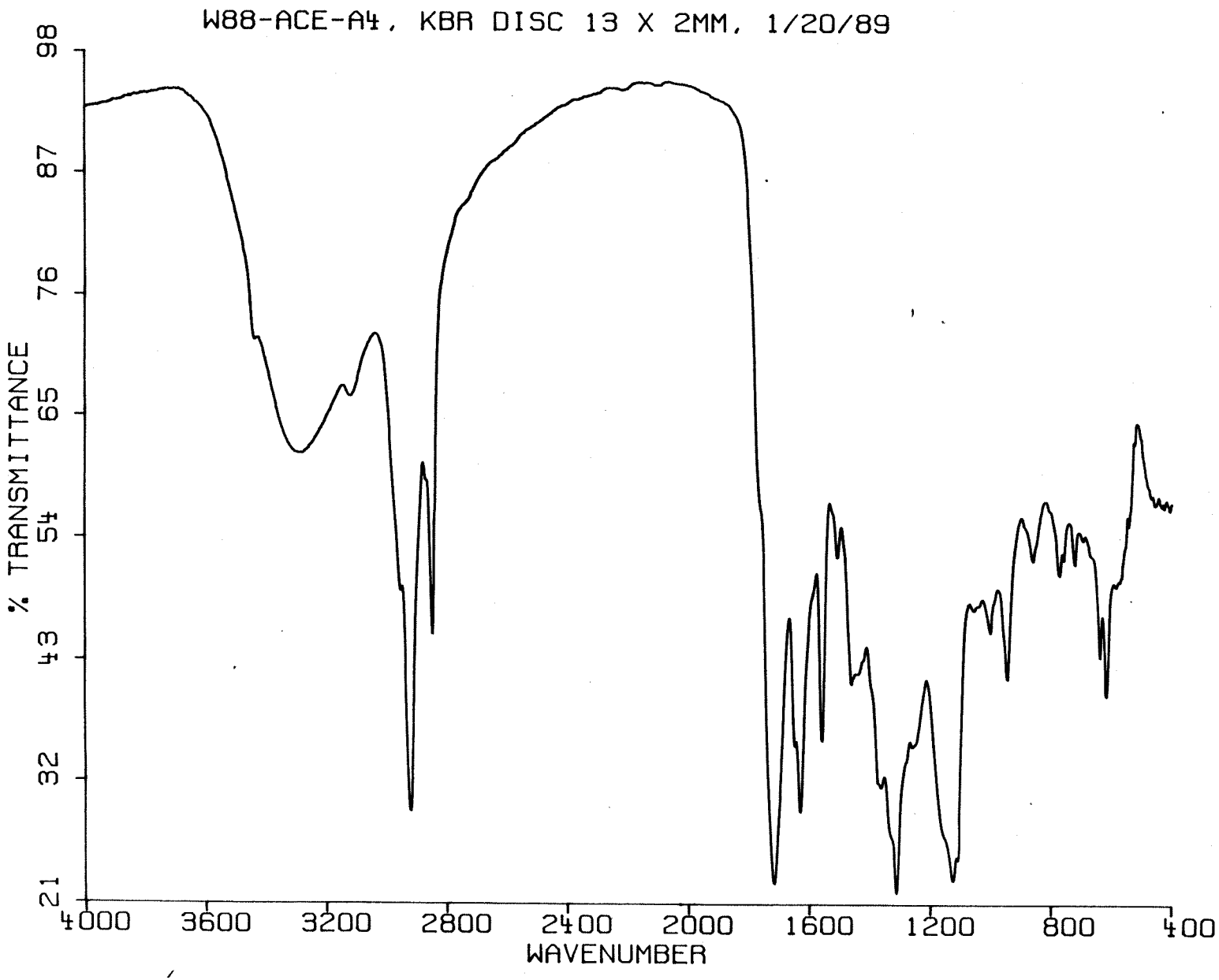

W88-ACE-A4, KBR DISC 13 X 2MM, 1/20/89
% TRANSMITTANCE
98
87
76
65
54
43
32
21
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER

Figure 4.8

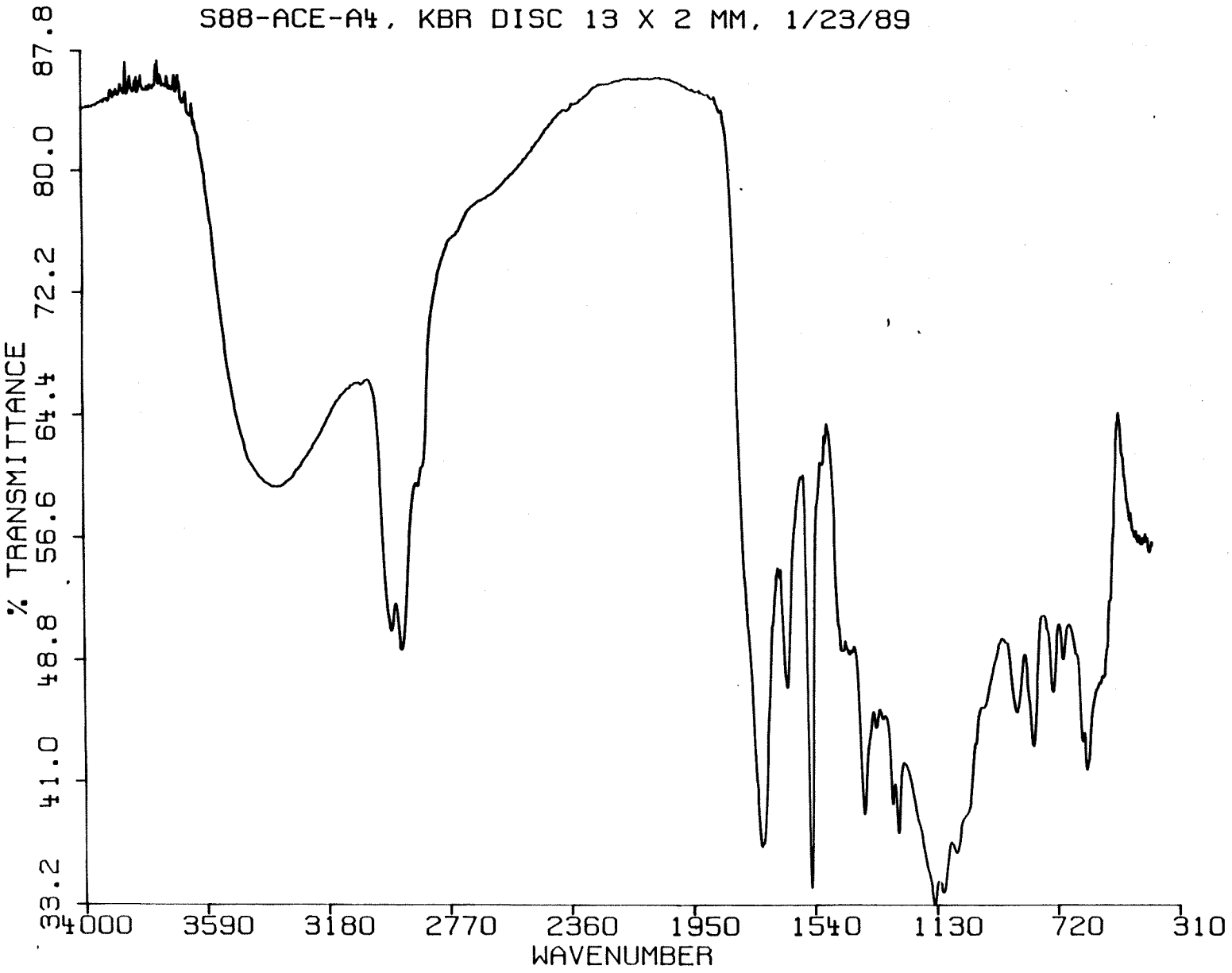

S88-ACE-A4, KBR DISC 13 X 2 MM, 1/23/89
% TRANSMITTANCE
87.8
80.0
72.2
64.4
56.6
48.8
41.0
33.2
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4.9

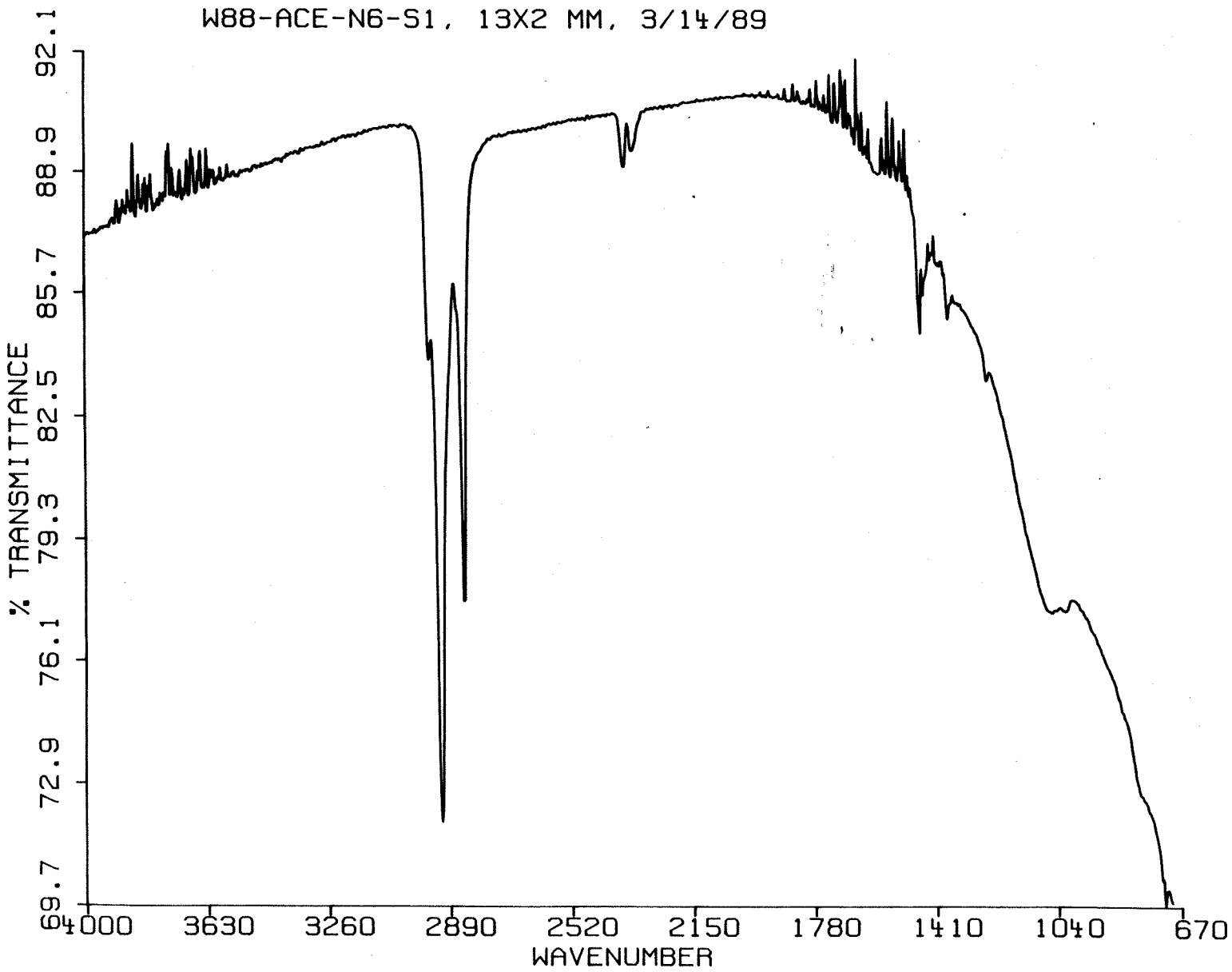

W88-ACE-N6-S1, 13X2 MM, 3/14/89
% TRANSMITTANCE
92.1
88.9
85.7
82.5
79.3
76.1
72.9
69.7
4000
3630
3260
2890
2520
2150
1780
1410
1040
670
WAVENUMBER

Figure 4.10

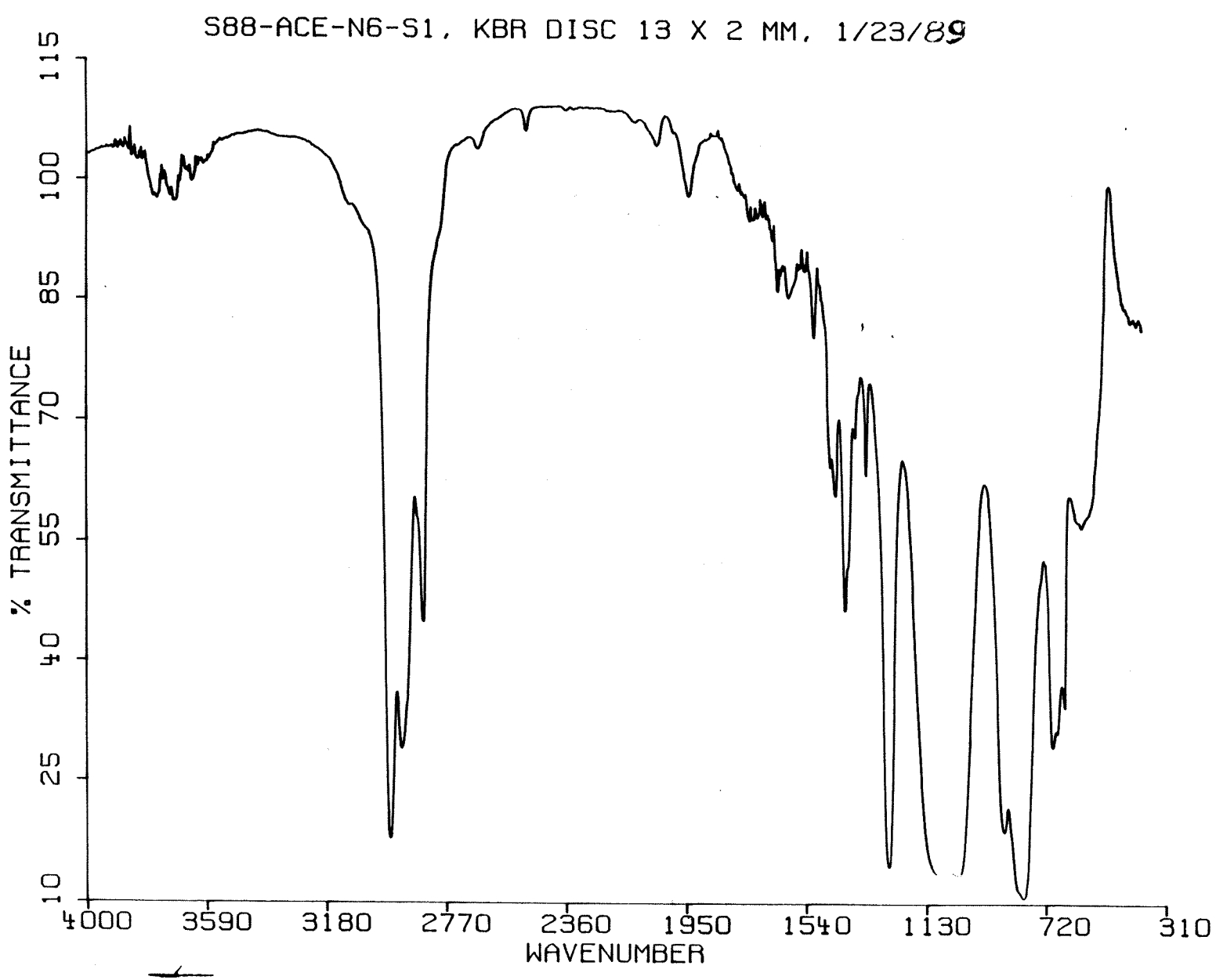

S88-ACE-N6-S1, KBR DISC 13 X 2 MM, 1/23/89
% TRANSMITTANCE
115
100
85
70
55
40
25
10
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4.11

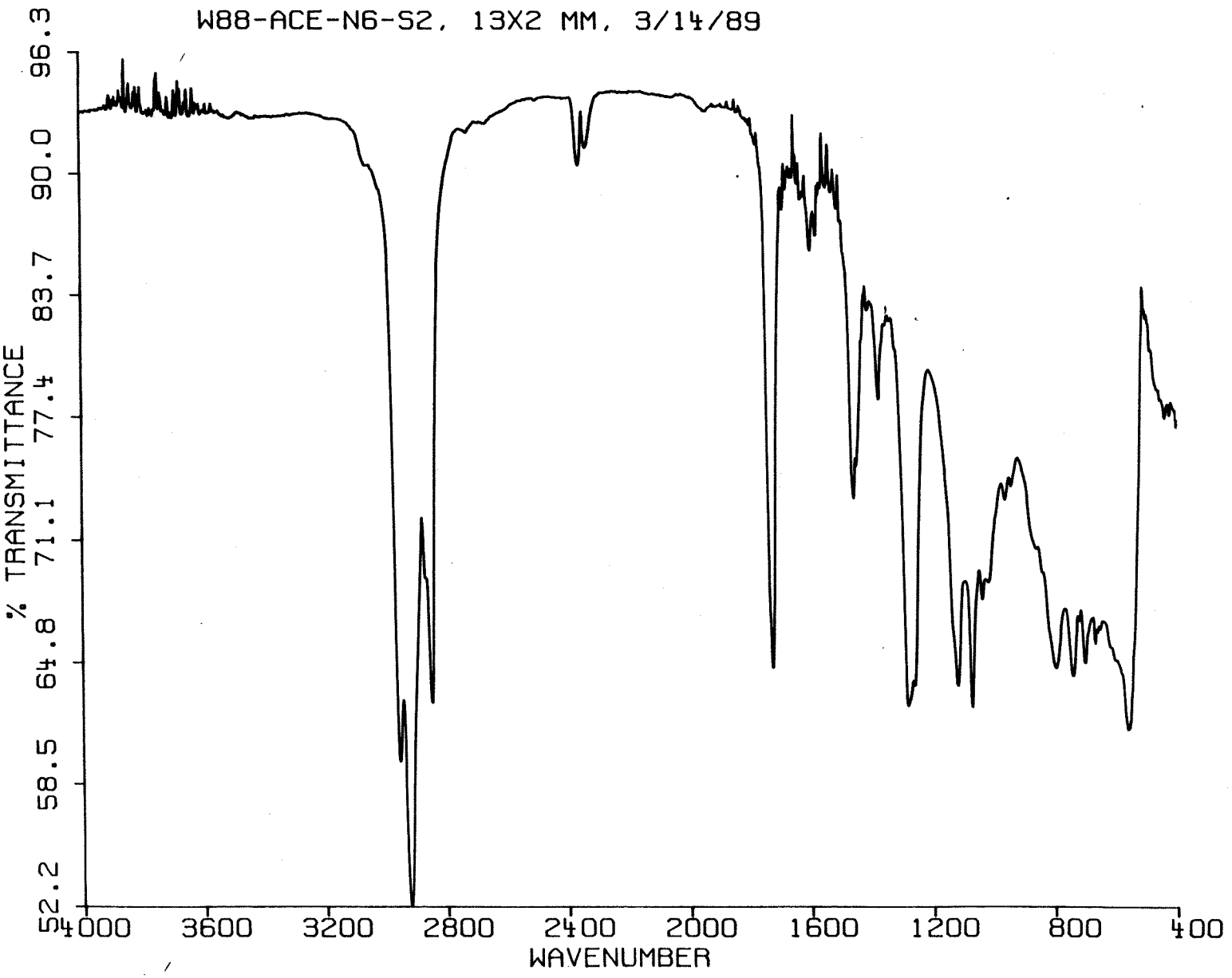

W88-ACE-N6-S2, 13X2 MM, 3/14/89
% TRANSMITTANCE
96.3
90.0
83.7
77.4
71.1
64.8
58.5
52.2
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER

Figure 4.12

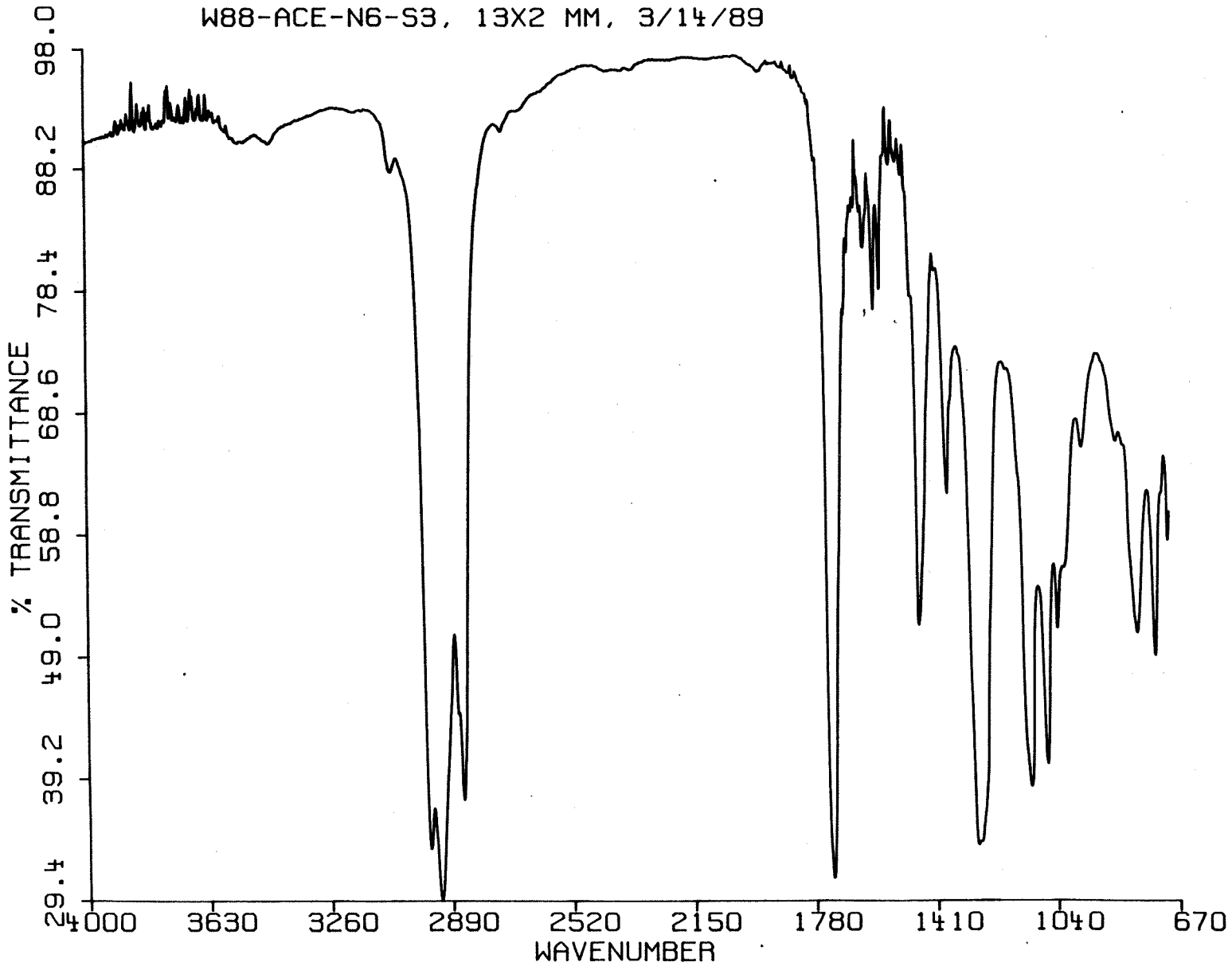

W88-ACE-N6-S3, 13X2 MM, 3/14/89
% TRANSMITTANCE
98.0
88.2
78.4
68.6
58.8
49.0
39.2
29.4
4000
3630
3260
2890
2520
2150
1780
1410
1040
670
WAVENUMBER

Figure 4.13

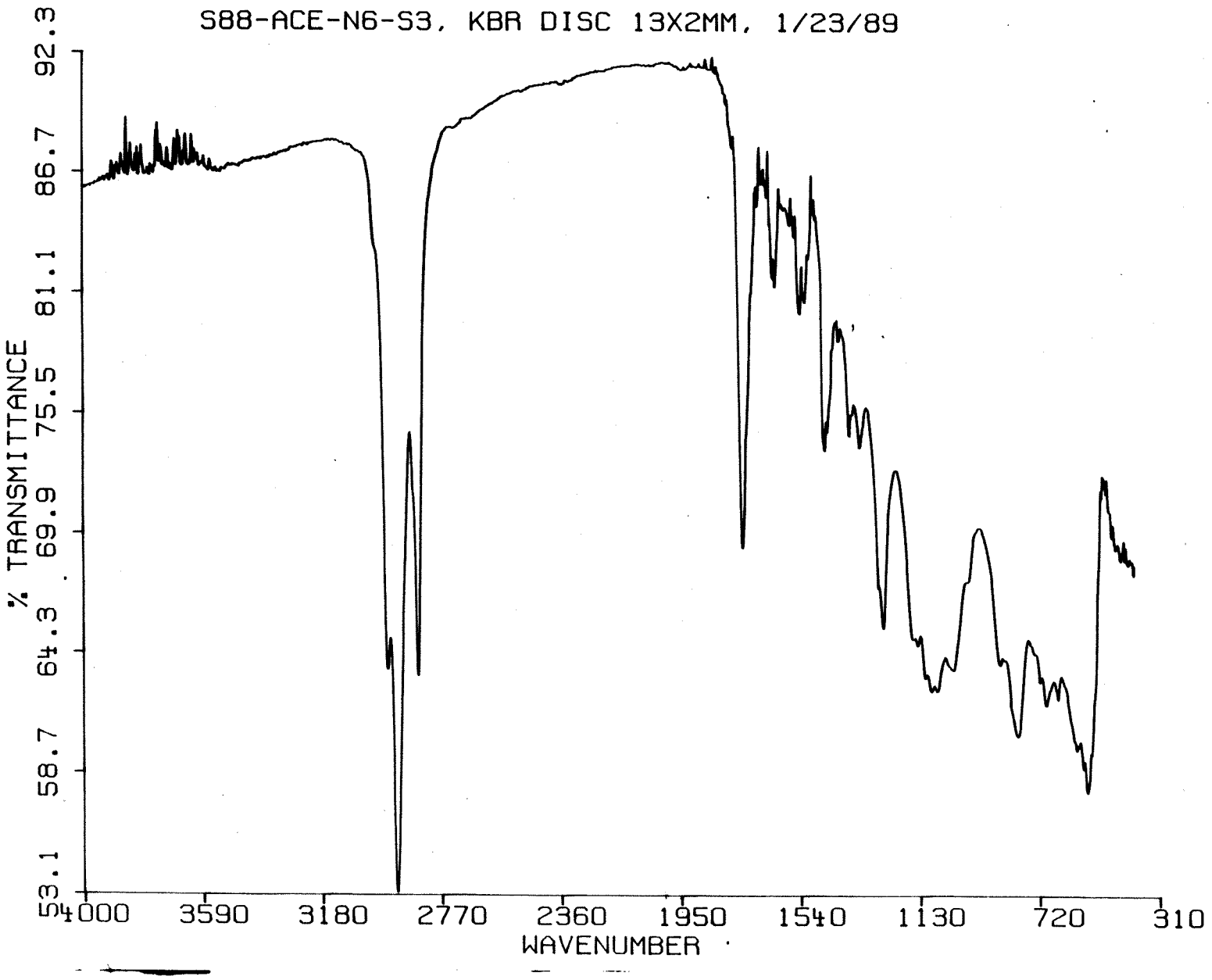

S88-ACE-N6-S3, KBR DISC 13X2MM, 1/23/89
% TRANSMITTANCE
92.3
86.7
81.1
75.5
69.9
64.3
58.7
53.1
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

Figure 4.14

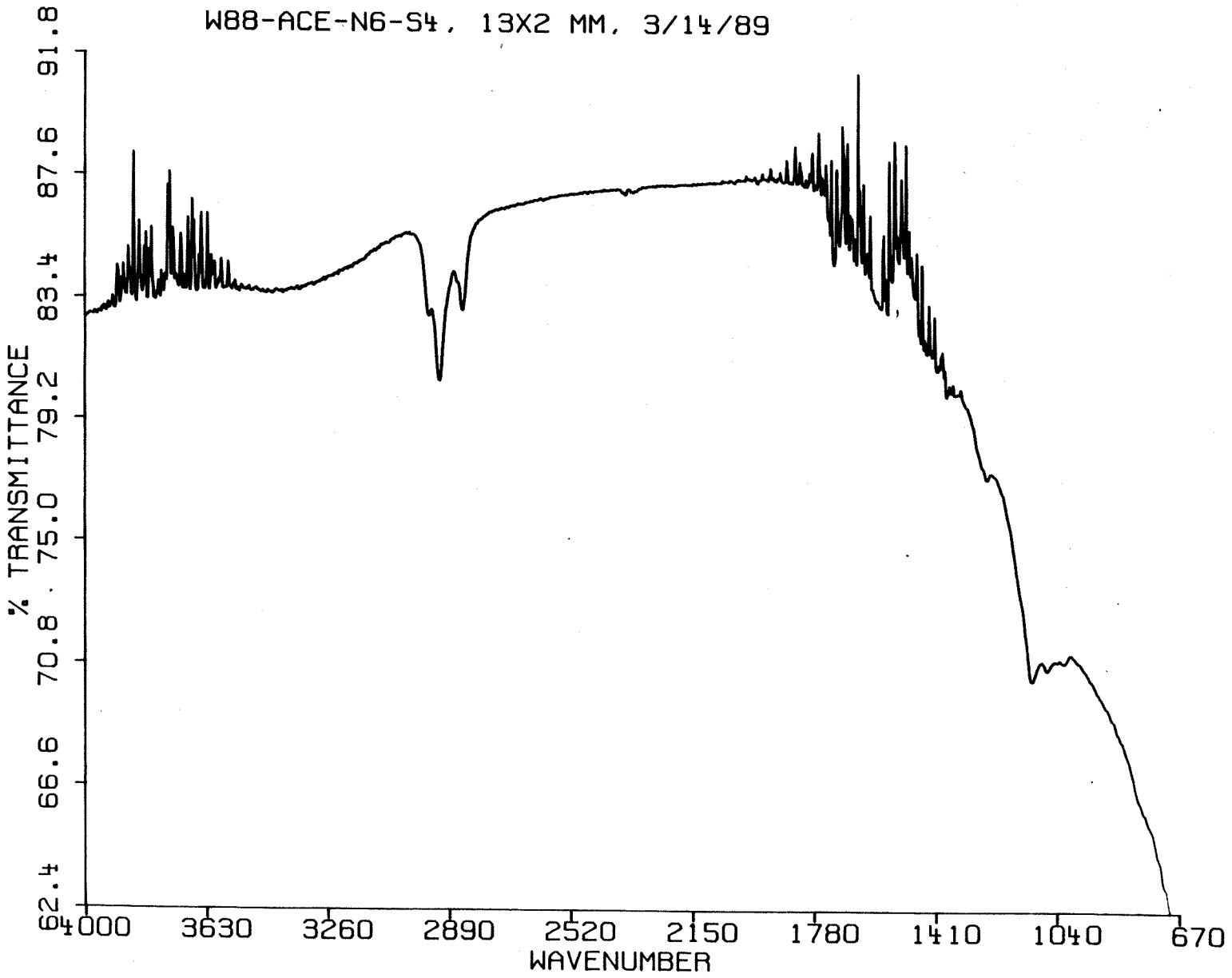
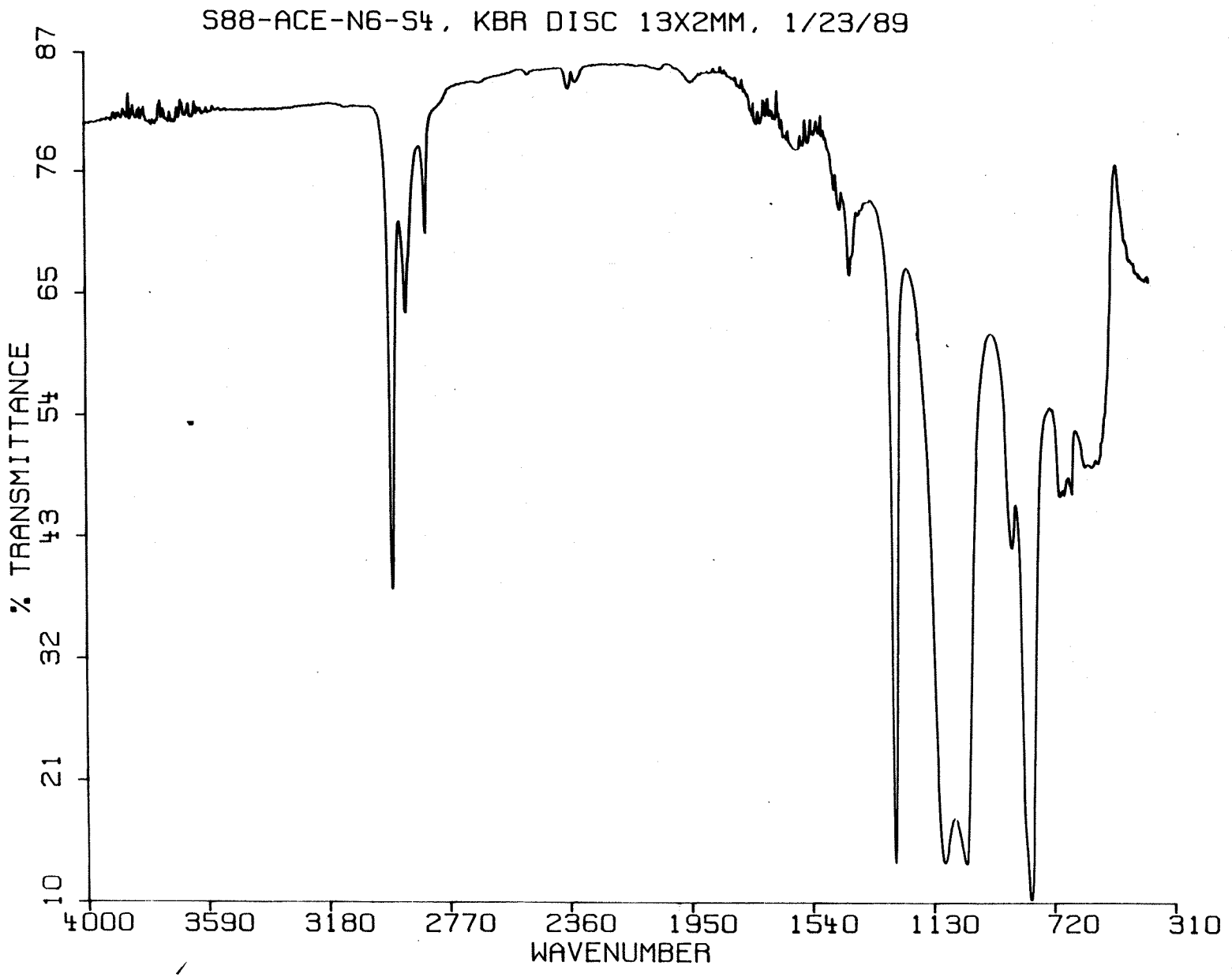

W88-ACE-N6-S4, 13X2 MM, 3/14/89
% TRANSMITTANCE
91.8
87.6
83.4
79.2
75.0
70.8
66.6
62.4
4000
3630
3260
2890
2520
2150
1780
1410
1040
670
WAVENUMBER

Figure 4.15


S88-ACE-N6-S4, KBR DISC 13X2MM, 1/23/89
% TRANSMITTANCE
87
76
65
54
43
32
21
10
4000
3590
3180
2770
2360
1950
1540
1130
720
310
WAVENUMBER

CHAPTER FIVE

GAS CHROMATOGRAPHY / MASS SPECTROMETRIC RESULTS

5-1. Introduction

A major research goal of this project was to obtain data on classes of organic compounds, presently unknown, which contribute to mutagenicity of airborne particulates. Limited success was attained in this endeavor.

As mentioned in chapter 3, a large portion of the mutagenic activity of the ACE extracts is associated with the weak acid fraction. Thus, particularly close attention is paid to the GC/MS results for this fraction. All major mutagenic fractions and subfractions of ACE extracts were analyzed by the GC/MS technique at the Center for Advanced Food Technology, Cook College of Rutgers University where samples were run by Drs. Robert Rosen and Thomas Hartman.

5-2. Experimental

All analysis were conducted using a Varian 3400 gas chromatograph directly interfaced to a Finnigan Mat model 8230 mass spectrometer. Data were acquired and processed using the SS-300 data system. Chromatography was performed using on-column injection techniques. Samples were injected on a 15 m * 0.32 mm i.d. DB-5 capillary column containing a 0.25 micron film thickness. The injector temperature was 260^{0}C. Various column programs were used. The GC-MS interface lines were maintained at 320^{0}C. The mass spectrometer was scanned at a rate of 1 second

per decade from mass 35 to 550 and mass spectra were produced using standard electron ionization (70 eV).

Since the presence of plasticizers obscured GC/MS results, some of the acidic fractions (e.g. weak acids fractions) were taken using acid/base extraction to further fractionate into strong acid (e.g. carboxylic acids) and weak acids (e.g. phenols). The original sample was evaporated under nitrogen, then a solution of $NaHCO_3$ was added to reach a pH of 8.4. The sample was partitioned with dichloromethane. The DCM extract was collected and designated weak acid fraction. The aqueous portion was acidified with HCl to a pH<2.0 and again partitioned with dichloromethane. This sample was collected and designated strong acid fraction. Samples were then concentrated and analyzed as was done with previous samples. Other fractions of samples were given similar treatment.

5-3 Results and Discussion

GC/MS results for selected fractions are listed in Tables 5.1-5.3. There are still some data awaiting analysis.
It is clear that the fractionation into acids and bases was not a neat one and many water-soluble compounds are present that are neither acid nor base. Furthermore, the presence of a lot of phthalates contaminated our samples and obscured GC/MS results. This is probably due to the ubiquitous nature of these plastics additives and perhaps due to plastics processing activity in the area. Even so, some interesting compounds were expected to be found from this GC/MS analysis.

Table 5.1: Analysis of W88-ACE-A2 ("weak acids"), following an extraction technique employed at CAFT to separate weak acids (e.g. phenols) from strong acids (e.g. carboxylic acids). This group is the weak acid (phenol) group.

ASSIGNMENT	MW
Methylpentenone	98
Dimethylpentanol	116
Pentanedione	114
Unknown	124
Dihydrodimethylfuranone	114
Dimethylpentenone	112
Hydroxybenzaldehyde	122
Dimethylundecane	184
Dimethylheptadienone	158
Heptanal	112
BHT	220
BHT Analog	180
Propanoic acid, 2 methyl-	286
1-(1,1 dimethylethyl)-2-methyl propanediol	
BHT Analog	234
Tetradecanoic acid	228
Hexadecanoic acid	256
Octadecanamide	284
Hexadecanamide	255
Octadecenamide	281
Unknown Adipate	

Note: Sample too contaminated by phthalates for further analysis.

Table 5.2: Analysis of W88-ACE-A2 ("weak acids") using CAFT extraction technique as in Table 5.1. This is the strong acids (carboxylic acids) extract.

ASSIGNMENT	MW
Dimethylpentanol	116
Dihydrodimethyl furanone	114
Methylbutanedioic acid	132
Unknown aliphatic acid	
Dimethylbutenoic acid	114
Hexanoic acid	116
Dimethylpentanoic, ethenyl ester	156
Ethylhexanoic acid	144
Octanoic acid	144
Unknown chlorinated acid	148
Hexadienal	96
Nonanoic acid	158
Decanoic acid	172
BHT Analog	180
Dodecanoic acid	200
Phthalates	