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Microbiological entities in the atmosphere can be viewed as particles and studied for their particle properties, as packages of chemicals such as proteins, glucosides, DNA or RNA carriers, as potentially fluorescent or stainable entities or are as viable cells capable of reaction with an environment to produce detectable products. This report presents a discussion of each of the techniques found suitable to deal with some aspect of detection. In addition, a brief presentation is made of experimental probes of two unique approaches made within the time frame of the project. The final section presents the bibliography developed in the study. The reference material presented are those actually looked at and almost all were photocopied for convenient review. In each category they are arranged chronologically, the most recent first.

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Preface

This comprehensive literature search grew out of the U.S. Army need to identify evolving methods of microbiological agent detection. Some experimental work was carried out to test any novel or unique methods uncovered in the literature. The library facilities used were exclusively in the Washington, D.C. area. Three national exhibitions of current instrumentation were attended which provided valuable information relating to laboratory techniques. Consultations of a technical nature were conducted with:

Dr. Kadoba, University of Kentucky;
Professor Gallaher, Georgia Tech;
Dr. J. K. Taylor, National Bureau of Standards Laboratory;
Dr. R. Shaffer, National Bureau of Standards Laboratory;
Dr. J. Akins, National Bureau of Standards Laboratory;
Dr. Suneram, National Bureau of Standards Laboratory;
Professor G. Guilbault, University of New Orleans;
Professor Lockmuller, Duke University

The information provided by these experts proved to be valuable inputs to the effort.

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LITERATURE SEARCH FOR NEW PHYSICAL METHODS
OF BIOLOGICAL AEROSOL DETECTION

1. INTRODUCTION

The primary goal of the project was to do a comprehensive library study with a view to the identification of new rapid physical methods to detect microbiologicals introduced into the atmosphere. As it developed, the study included the use of all forms of radiant energy, all types of spectroscopies and a miscellaneous collection of techniques not classified into these broad groups, such as cell sorters or the use of stain techniques. In addition to the library work it was intended that some experimental work be carried out on any novel methods uncovered by the study.

This report describes the results of the work of Leonard Shapiro, who was assigned the primary responsibility for the library studies and for developing and performing some of the exploratory laboratory work which resulted.

All of the library work was done in the Washington, D.C. area using the following libraries:

Congressional Library	Washington, D.C.
Environmental Protection Agency Library	Washington, D.C.
Gelman Library of George Washington University	Washington, D.C.
Himmelfarb Medical Library	Washington, D.C.
National Medical Library	Bethesda, Maryland
Agriculture Department Library	Beltsville, Maryland
National Science and Space Agency Library	Greenbelt, Maryland
National Bureau of Standards Library	Gaithersburg, Maryland
Charles White Chemistry Library at Maryland University	College Park, Maryland
Math and Engineering Library at Maryland University	College Park, Maryland

The data-based search systems in these facilities were employed when available, and in one case a commissioned study was made for the project, using MEDLARS, at the National Medical Library. In the course of the study over 500 articles, or relevant portions of articles, were photocopied and these provide the basic material for the report.

During the course of the work a personal status report was given to the Technical Officer on seven occasions, and five detailed interim reports, as well as several smaller written reports. These were in addition to the required monthly reports.

During the course of the study three national exhibitions of current instrumentation were attended: The International Exhibition of Medical and Laboratory Instrumentation (AIMLI 82), the

American Chemical Society Exhibit, both in Washington, D.C., and the American Microbiological Society National Meeting in New Orleans, Louisiana.

In addition, consultations took place with a number of recognized authorities in several areas of interest. These included, among others, Dr. Kadaba of the University of Kentucky, and Professor Callagher of Georgia Tech., both regarding the subject of microwave analytical applications. Several visits were made to the National Bureau of Standards Laboratories. On the subject of piezoelectric devices a meeting took place with Dr. J. K. Taylor at the Bureau where his work with this technique was explained in detail. In subsequent visits to the Bureau Dr. R. Shaffer and Dr. J. Akins demonstrated their use of Fourier Transform IR instrumentation. Also, Dr. Suneram showed the microwave instrumentation used at the Bureau to measure the absorption of gases at low pressure.

The laboratory of Professor G. Guilbault, a leading authority on piezoelectric devices, was visited at the University of New Orleans where his instrumentation was demonstrated and some experiments requested by us were carried out.

Professor Lochmuller, an authority on photoacoustic measurements, at Duke University, was contacted and agreed to carry out experiments in this area which eventually provided us with over one hundred data curves useful to the project.

As the library work developed it became clear that microbiological detection was possible using any of a number of techniques and, therefore, each one of them had to be studied in detail to establish the advantages and disadvantages.

Microbiological entities in the atmosphere can be viewed as particles and studied for their particle properties, as packages of chemicals such as proteins, glucosides, DNA or RNA carriers, as potentially fluorescent or stainable entities or as viable cells capable of reaction with an environment to produce detectable products. This report presents a discussion of each of the techniques found suitable to deal with some aspect of detection. In addition a brief presentation is made of experimental probes of two unique approaches made within the time frame of the Project. The final section presents the bibliography developed in the study. The reference material presented are those actually looked at and almost all were photocopied for convenient review. In each category they are arranged chronologically, the most recent first.

In addition to reading material directly related to the techniques presented, it was found necessary to read background supplementary subject matter to more fully understand the techniques. The bibliography contains the supplementary material in a separate listing after the references relating directly to the techniques.

In the nature of the subject there is considerable interrelationship between several categories. As a single example, the subject of pyrolysis is applicable to gas and liquid chromatography as well as mass spectrophotometry and others. The placement of such references was therefore to some extent, arbitrary.

Table 1 lists the major areas of concentration for the literature search and idicates the bibliographic reference section appropriate to each area.

Table 2 lists supplementary areas of interest uncovered during the literature search and refers to the approprate area of the bibliography for reference material.

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2. MICROWAVE

This section describes the literature search covering microwaves made during the period 19 August through 30 September, [illegible]. Writers discussing the use of microwaves as an analytical tool are unanimous in their praise of the theoretical advantages of this over any other method of spectral analysis. Textbooks and monographs all begin by statements that are similar, by comparing its discriminating ability against infrared as being one to ten thousand times better. A great deal of theoretical work has gone into the electrical properties of circuit components such as microwave generators, wave guides, detectors, etc. as well as studies at the molecular level of energy transition, induced by microwave frequencies. From these much has been learned, especially about the structure of small molecules. The transitions which give rise to microwave emission and absorption are primarily rotational, involving whole molecules or vibrational, involving large structures within a molecule.

From the literature until the early 70's, the field appeared exceptionally promising, especially when two companies, Hewlett-Packard and Cambridge Scientific Instruments produced commercially available instruments and the associated documentation showing excellent analytical results for the composition of gases and vapors of many organic mixtures. Unfortunately, both companies found that they were not able to sell enough instruments to continue production due to lack of demand. Apparently, the state-of-the-art equipment was too expensive at that time relative to other analytical methods of obtaining similar results to generate broad interest. Nevertheless, development continued in microwave technology (primarily in relation to radar applications). Occasionally, there have appeared special purpose procedures using microwave absorption and up-dated devices and this sporadic trend will no doubt continue until microwave analysis will take a proper place in the analytical spectrum. But at present, in the 1980's, as the new organic texts appear, they contain chapters on IR spectra and NMR applications but no longer include even a reference to microwave spectra. Since 1970, one finds only occasional articles on applications of microwaves to analysis.

In 1979, an excellent monograph, part of a series called Chemical Analysis, was published (Varma and Hurbesh, 1979). It was devoted entirely to chemical analysis by microwave spectroscopy and describes the structure of several microwave instruments and some ingenious applications, all of them being done on gases at low pressure. The authors were very optimistic about the future of these methods, but at the same time they indicate an awareness of manipulative diffficulties that have been encountered.

Amoung the few applied papers in recent years three will be described briefly. In an article entitled Determination of Ammonia with a Stark Microwave Cavity Resonator (Hirose et al1982) ammonia in aqueous solutions was determined by measuring its absorption of 23.87 GHz at 4 torr pressure. The gas was generated

by adding strong alkali to the sample and the resultant vapor was carried by nitrogen diffusion through a teflon membrane into a Stark cell. This procedure was specific for ammonia and is in use in Japan to determine ammonia in industrial waters.

Another recent application was given in an article entitled Application of Microwave Spectroscopy for the Simultaneous Detection of Toxic constituents in Tobacco Smoke (Kadaba, 1979). In this work, 50 cc of tobacco smoke was transferred to a Stark cell covered with dry ice. Microwave frequencies 22-38 GHz were passed through and measured. The authors used known compounds to calibrate the output, as well as absorption lines published in the literature to establish the composition of the tobacco smoke.

They make the statement that water vapor absorption of radiation in the microwave range, unlike that with infrared, was no problem. They further claim that the instrumentation developed could be used to monitor the atmospheric air for the presence of toxic constituents. Personal communication with one of the authors indicated that the main problem encountered involved the wave guide, specifically absorption of the gas on the walls.

A third paper entitled Diagnosis of Air Pollution by Microwave Spectroscopy (Graff and Suffet, 1972), while not a description of an applied procedure, was actually an excellent summary of the state-of-the-art microwave spectroscopy up to mid 1971. Along with many suggestions and directions, such as concentration of the sample, etc., the authors warn of the difficulties to be expected. They state that pressure broadening of absorption lines of oxygen and water vapor were the main troubles. Perhaps these difficulties account for the lack of widespread application of this potentially useful technique.

2.1 Microwaves as Applied to Bacterial Detection

A large fraction of the published material on microwaves in the biological field deals with their effect on living tissue. Much of the early work was based on poorly understood principles (and the use of broad band radiation) and the effects were later shown to be the result of local heating of water in the tissues, as in microwave ovens. Later, work with narrow band radiation and lowe power showed that real effects, not attributable to heat, could occur at specific frequencies. A paper often quoted relative to effects on bacteria entitlled "Absorption of Microwaves by Microorganisms" (Webb and Booth, 1969) described the effects on E. Coli cells from 65-76 GHz radiation. The authors found that metabolism was stimulated or delayed at highly specific wavelengths. Furthermore, they seem to be the first to have run a microwave absorption curve on whole cells. They placed films of his culture on a thin mica window, dried them under 80% relative humidity and used a mica cover glass. They comment that the IR spectra of different microbes vary in small detail, too small to be of value in cell identification, but that millimeter waves may enable differences to be enlarged. They also showed absorption

curves for spectra of DNA and RNA between 64 and 75 GHz which are clearly very different and also differ from that of protein. The authors used whole cells of E. Coli, and did not use an evacuated system and obtained spectra which, while very broard, are clearly defined. A later article by Russian workers (Bainikov and Roshkov, 1980) refers to the possibility of identifying intact bacteria by a combination of dispersed light in the range 30-200 cm^{-1} and the spectra of microwave absorption in the range 65-96 GHz. They also present an absorption curve with a peak at 41.268 GHz for E. Coli.

It is obvious from the previously stated references that at least some bacteria, under the proper conditions, have shown absorption phenomena at very specific wavelengths. It was also well established that bacterial species differ in their chemistry to a significant degree.

It must be recognized that, unlike infrared measurements which detect chemical bond structures and therefore vibrations directly related to the chemistry of molecules, microwaves detect dipole rotations and some other vibrations more related to architecture and location of masses and charges than chemistry. Thus, for example, molecules with identical chemical composition but with different isotopes or different isomers of the same compound will give different absorption spectra at microwave frequencies. This may be an advantage because in addition to the different chemistries there are likely to be different molecular structures, even for the same chemistry among different species of bacteria. As all successful previous work has been done using microwaves placed through a waveguide with gases at very low pressure, it must be concluded that if bacteria are pyrolyzed to produce vapors and these are treated as gaseous mixtures at low pressure, the procedure to identify bacteria should be perfectly straightforward. The probability that useful results could be attained with specimens at atmospheric pressure are considered very low by microwave experts who were consulted. Spectral line broadening, as one goes above very low pressures, is considered to be too extreme to yield useful results.

2.2 Conclusions

As a result of the literature survey of microwave applications as they might possibly relate to bacterial detection, certain inferences may be made. It is well established that gases at low pressures, a few torr or less, can give measurable and well-defined absorption spectra in the microwave region. It may be assumed that if bacteria are vaporized by pyrolysis and the vapor treated as gases at low pressure, well-defined spectra wou'd be obtained for them as well. It is improbable that at the low levels of bacterial matter in the open atmosphere microwave absorption could distinguish bacteria from background.

3. INFRARED

This report describes the results of a literature search covering infrared (IR). The study was made during the period 1 October through 10 December, 1982.

Since 1970, IR equipment saw a strong upsurge in popularity due to the introduction of Fourier Transform techniques and the availability of computers. At the same time microwave manufacturers discontinued the production of analytical instruments and that whole approach suffered from loss of interest. This report will elaborate on how IR has been used for the detection and identification of microorganisms and what the state of the art is at present.

3.1 Instrumentation

As previously discussed under Microwaves, the physical parameter being measured was predominantly related to molecular rotation and its energy levels. Infrared measurements arise from vibrations of structural groups within molecules and are directly related to chemical composition. An enormous amount of work has been undertaken successfully and is still done to establish molecular structure using infrared absorption measurements. For example, the presence of a ketone structure in a molecule or any other grouping invariably causes absorbance at fixed positions in the IR spectral region. When trying to identify the structure of any complex molecule, an IR curve is invariably made early in the study. Because of this high demand, instrument makers had responded with excellent and continually improving equipment.

Until 1970 the basis of IR spectroscopic instruments was the use of a prism of grating as with any other light spectroscopy. The light from a source was passed through a sample and then dispersed in this way. A narrow slit backed by a photedetector moved past the dispersed light and measured the spectra, wavelength by wavelength. A scan might require the better part of an hour.

In the early 70's this instrument was revolutionized by the introduction of the computer and Fourier Transform Infrared (FTIR). In this instrument the prism or grating is replaced with a Michelson interferometer. Light from a source, after passing through a sample as above, passes to a beam splitter which sends half the light 90 degrees to a mirror fixed in position, and transmits the other half of the light to a moveable mirror. The light reflected back from the moveable mirror meets and combines with the light reflected from the fixed mirror at the beam splitter and passes through the beam splitter to a detector.

The distance of the moveable mirror to the beam splitter at the start is a little greater than that of the fixed mirror to the beam splitter. The mirror is then moved at a fast rate towards the beam splitter while the light intensity is being measured. The

measured light intensity is the net result of interference between the two reflected beams. At the point where the two distances are exactly equal the scan measurement is started. The complete scan represents an interferogram in which peaks, troughs and intermediate positions have added and subtracted. The intensities throughout the scan have been digitized and recorded all within a second or less. The Fourier Transform in the form of pre-packaged software calculates the spectral curve from the digitized data.

This scan may be done repeatedly a hundred times or more to enhance the sensitivity and the results averaged so that even weak spectra can be detected. Accuracy is extremely high because the position of the moving mirror is continously tracked with the He-NE laser with extreme precision. Textbooks in the subject (Griffiths, 1978, Marshall 1982) describe the advantages of this instrument over the dispersive type as follows:

The Fellget advantage is that a broad beam of light is measured rather than the light through a small slit, that is, the throughput optics are better.

The Connes advantage is that the He-Ne laser controlled interferometer accurately positions the mirror.

3.2 Applications to Microorganisms

About the earliest published work on the use of IR to identify whole bacteria was done by Stevenson and Boluan (1952) working for the Chemical Corps, Biological Laboratories at Camp Detrick, Maryland. They smeared a few colonies taken from a culture onto a silver chloride plate and allowed the film to dry. Transmission curves were made on a Perkin-Elmer Model 21 Double-Beam Infrared spectrophotometer and an identification scheme, based on the relative heights of peaks on the curves, was developed. They pointed to the lack of a catalogue of spectra of organisms required for rapid identification.
After this excellent start a great deal of work followed, using the whole bacteria, the products formed in growth media and various parts of a bacteria, expecially the cell walls.

A comprehensive survey of this field up to 1971 is given in the book "Applications of Infrared Spectroscopy in Biochemistry, Biology and Medicine" (Parker, 1971). Although the book was written prior to the availability of FTIR, the author cites 58 references of work done between 1952 and 1971 on microorganisms. Included are a number of absorption curves for bacterial species as well as for fungi and viruses.

Many special interest groups have applied the IR approach to identification of bacteria related to their own interests from which much can be learned. Thus, Reynolds et al (Reynolds, 1967) was interested in a rapid identification of microorganisms in hydrocarbon fuel. They used cultures of aerobacter aerogenes, bacilluscereus, escherichia coli and pseudomonas aeruginosa as well

as three fungi. Like Stevenson they smeared cultures on slides and air-dried them. They used a Perkin-Elmer Model 137 double beam sodium chloride spectrophotometer and adjusted the transmittance to be 80% at 5.0 microns and 40% at 9.0 microns before making the curves. They point out that the chemical composition of bacteria are so close as to give similar appearing curves but the relative peak heights were sufficiently different. The ratio of peaks at 6.85, 7.15, 8.05 and 9.2 microns were used to differentiate organisms. It was also stated that the composition of the culture and method of treatment had to be uniform to obtain reproducible results. Apparently bacteria, to some extent, are like people in that they are what they eat.

In the late 1960's an intermediate stage is seen in the straight-forward measurement of IR on bacteria and their media. The technique of gas chromatography was developed and applied broadly. Chromatography separates different molecules by their differing abilities to migrate through a medium which retards them differently. Thus, complex molecular mixtures can be divided into their components. As bacteria can be considered complex mixtures of molecules it was natural to apply chromatography to their analysis. A number of papers appeared (Howard, 1966, Quinn, 1974, Jantzen, 1976, Mitruka, 1976), which describe the application of gas chromatography to bacteria, after either vaporizing, solubilizing or converting to derivatives of the whole bacteria or bacterial products produced in media or in people.

These methods did not identify the molecular species which where being separated but they were able to provide characteristic profiles which could be compared to known materials of similar composition and identification could be made in this way. They also could accomodate extremely small samples in the order of nanogram or even picogram size samples.

The area of analysis called analytical pyrolysis makes heavy use of gas chromatography and among the many papers in this field there are a number devoted to bacteria. Menger (1972) wrote on the subject of computer matching of pyrolysis of pathogenic microorganisms. A symposium on analytical pyrolysis (Jones, 1977), held in Amsterdam in 1976, heard a number of papers on this application. Generally, 90-100 micrograms dry weight of bacteria were used and "fingerprints" were used rather than attempting to identify the molecular composition. A considerable amount of information on cell chemistry as well as on the volatile metabolites of microoganisms of bacteria and fungi, which include in part ethylene, acetylene, propylene, ethane, propane, aldehydes, alcohols, ketones, esters, methyl mercaptan, dimethyl sulfide, etc. is provided in a recent book by Aaronson (1981).

The next stage, which may be considered the current one takes the output of the chromatographic column, as each molecular entity comes from the column and submits it to an identification technique, most commonly IR or mass spectrometry.

Using such techniques and especially if capillary columns are used for the gas chromatographic samples, quantities as low as nanogram and even picogram amounts can be run. Considering that 1 bacteria can weight about 1 picogram (10^{-12} g) and that, as Anas (1978) has reported, a cubic meter of air normally contains 2000-4000 bacteria, or up to 4 organisms per liter, it is, at least theoretically, possible to have sufficient material from 1 cubic meter to detect and identify bacteria if one could collect them. Gaudy (1980), in addition to giving useful information on the chemical composition of cells points out that bacteria are 50 to 75% water and the actual dry weight which is less than half of the speciment is of course the basis of a correct estimate. However, the intended application is for an enhanced concentration in the environment so that 1 cubic meter should yield a sufficient sample if the gas chromatograph/IR technique (GC/IR) were used.

A great deal of work along these lines has been done successfully for clinically applications by Mitruka (1976) and described in an excellent book entitled "Methods of Detection and Identification of Bacteria". In all cases bacteria are first grown as colonies after which the GC/IR techniques rapidly identify the microorganisms. Samples are usually in microgram amounts, a thousand times more than is reasonably expected to be acquired directly from air samples. Nevertheless, the micro capillary GC/IR mentioned above, although not reported to have been applied to bacteria, are likely to give results similar to those obtained by Mitruka but without culturing.

The interfacing of FTIR with the computer leads to ingenious treatments which enable handling the complex IR spectra to obtain a variety of desired information. Powell (1978) describes computer identification of IR spectra by correlation-based file searching. Lam (1982) describes the technique of cross-correlation to enhance the signal/noise ratio. The ordinary treatment of the IR interferogram to obtain the frequency spectra is by auto-correlation, by means of which the power intensity at any given amount is multiplied by its intensity at a slightly later time. A repetition of this calculation yields data needed to calculate the spectra. However, if a single desired compound such as carbon monoxide is needed to obtain an interferogram, and this is stored in the computer, if any unknown is then scanned and its interferogram is correlated with that of the carbon monoxide (cross-correlation), a very quick and accurate measure of carbon monoxide in the sample is obtained. This has been widely used in IR instruments in high-flying airplanes to study individual components in the upper atmosphere. Perhaps spectra of bacteria can be recorded and stored for cross-correlation purposes in future IR detection devices.

An area in which IR spectra has been widely use is in studies of air pollution. Ferraro (1978) describes an FT-IR study in detail of air pollution in the Los Angeles area, using a kilometer path length (22.5 meter tube with folded optics). This study yielded information on the gaseous constituents of the

atmosphere and needed the long path length to increase sensitivity to the ppm level. Microorganisms were not considered in this study.

The Los Angeles smog seems to be a popular place to study with IR techniques. In 1982 Hanst (Hanst, 1982) reported detection in the parts per billion range on 12 or more atmospheric constituents including hydrocarbons. He reported on the rise and fall of these constituents over a 2-day period using a Digilab FT IR instrument which allowed 52 reflections of the beam within the cell to an operating length of 1170 meters. A fan was run for 120 minutes to exchange the air and 3 minutes was allowed for settling. The scan took 7 minutes. No one seems to have thought of it but perhaps if a second portion of air had been brought in across a very hot section they might have been able to detect other pyrolyzed products of microorganisms by subtracting the results of the first scan from the second.

3.3 Variations of the Basic IR Techniques

A number of unique techniques involving IR have been reported in the literature some if which have been applied to bacterial detection. Raman spectra including the IR region have been used especially with Laser sources, and this procedure has been particularly sensitive when used at certain frequencies to give so-called Resonance Raman which has been used to detect and identify bacteria in water (Howard, 1980). Raman spectra will be discussed later.

If the speciment can be spread across a surface either as a layer of cells or as a liquid by virtue of air passed through a solvent, IR can be used in a number of ways. Internal reflection spectroscopy, also called attenuated total reflection (ATR) has been widely used for obtaining IR spectra of solid surfaces. Small liquid samples can be run using special cells for this purpose which are commercially available. (Barnes Analytical, 1983, harrick Scientific, 1983, Update Instrument Inc., 1983). Another technique to measure IR absorption of material on a surface, as well as the UV and visible light is the use of photoacoustic spectroscopy (Low, 1980).

Photoacoustics will be covered later, but at this point it will be mentioned that a commercially available attachment suitable for any spectrophotometer has been reported (EG and G Instruments, 1983).

A novel approach to the handling of micro samples for the purpose of IR absorption measurements was described by Anderson (1975). To accommodate 50 nanogram samples he made a KBr pellet and used watch jewels as sample apertures to mask the sample. He used a Digilab Model FTS-14 spectrophotometer and 100 one second scans stored in a minicomputer so that a composite could be made after noise reduction. Much of the work of preparing the sample required a low power microscope and considerable care but excellent curves were shown on less than 100 nanogram samples.

3.4 Conclusions

Bacteria can be detected and identified using IR measurements if they are cultured to produce sufficient sized samples. High speed detection would require direct determination without the slow stage of growth. Analysis of very small samples of other substances has been done at an order of magnitude comparable to the number of organisms found in normal dirty city atmospheres in 1 cubic meter volume. At this time no work has been reported on a direct determination of microorganisms without culturing.

Nearly all manufacturers of laboratory equipment make FT-IR instruments. These are generally compact and relatively easy to operate. In a recent advertisement one of them claims that it requires only 0.2 seconds to do a scan. There remains only the problem of how to gather all the organisms in 1000 liters of air into a suitable form for the task.

4. PHOSPHORESCENCE

In the report on microwaves we dealt with relatively long wavelengths measured in the millimeter range and therefore of low energy. The rotation of the molecule, or large sectiosn capable of rotation, were effected at these energies and the absorption spectra was measured. The report on infrared radiation dealt with wavelengths about 1000 times shorter, in the micron range. Here the molecular phenomena was vibration of atoms bonded to each other within the molecules. Each type of bond between atoms vibrates with a characteristic frequency so that the spectral absorption of the infrared energy was measured to identify what structures were present in the investigated molecule.

In phosphorescence we are dealing with electronic transitions which involve shorter wavelengths of higher energy and we measure the light emitted by the stimulated electrons as they fall back to a ground level. Phosphorescent emission generally is observed across the whole visible range and slightly beyond on either side.

Although phosphorescence has been known for a long time and used in various ways such as in marking microbial organisms with phosphorescent stains it was only proposed as a quantitative technique in 1944 (Lewis, 1944), and first applied as a quantitative technique by Kiers (Kiers, 1957). To avoid interferences from fluorescence and the quenching effects of molecular collisions the methods employed liquid nitrogen to cool specimens to about 77 degrees K. Such cooling was required to immobilize molecular motion giving conditions where strong phosphorescence could be observed and measured. In the late 60's and early 70's much work was being done with paper chromatography and it was found that some of the spots produced on paper showed good phosphorescence without cooling. In 1974 (Wellons, 1974) first proposed that room temperature phosphorescence (RTP) could be used successfully as a quantitative technique. It was in fact shown that samples one tenth the amounts

previously required could be detected and determined quantitatively on paper. Since then many applications have been published using paper, silica and other substrates. RTF is much simpler to work with and to a large extent has now replaced low temperature phosphorescence (LTP) as the prevalent method. Many discussions of RTP are found in the literature (Chen, 1976, Winefordner, 1972, Vo Dinh, 1977, Yen Bower, 1980, Parker, 1980). In 1980 room temperature phosphorescence was extended to solutions by the use of micellar compounds (Cline-Love, 1980, 1981, Skrilec, 1980). It was found that detergents such as sodium lauryl sulfate in the presence of heavy atoms could isolate the phosphor in micelles which increased intersystem crossover to greatly enhance phosphorescence while reducing fluorescence. Sensitivity was claimed to be comparable to low temperature and paper phosphorescence.

4.1 Theory

Certain molecules, when irradiated with visible or ultraviolet radiation emit a visible radiation of longer wavelength for either a brief or long time when the original radiation is discontinued. This is called luminescence. If this luminescence exists for only a short time (10^{-7}-10^{-10} seconds) it is called fluorescence. Longer periods (10^{-3}-10) seconds of light output are called phosphorescence. The mechanism by which they occur follows:

Electrons entering into the above phenomena are only those in the outer orbitals, which are those involved in chemical reactions and form bonds between atoms. Electrons in a plane which includes the 2 atomic centers joined by a bond are called ^{o}electrons and are held strongly in position. Electrons in orbitals outside this plane are called πelectrons and are less strongly held. In large molecules with multiple bonded atoms some electrons (η electrons), are even more loosely held and move over less restricted distances. The η and π electrons give rise to luminescence.

When a molecule is relaxed, at rest, all of the energy components are considered to be in their ground state. Vibrations are going on as though the bonds were springs and the atomic cneters were heavy masses with electric and magnetic charges delimiting and defining the ranges of vibration. When electromagnetic energy of the right frequency passes close, within 10^{-15} seconds a π or η electron can absorb enough energy to jump from its lowest evel (T_0) to a higher state of excitation. This new level is really a band of sublevel but it quickly loses energy by heat loss and goes to its lowest level of excitation (T_1) within this band. At this point it should be pointed out that prior to its energy jump the electron was sharing an orbital with another electron and by the Pauli exclusion principle the two electrons were spinning in opposite directions. This is required to conserve momentum for the whole atom so that the two together had a net spin of 0. When the jump to a higher level occurred the spins were still opposite to each other but now each is alone in its orbital.

The electron at T_1 can be subjected to three possible actions. It can, due to collisions, lose enough energy and thus produce a little heat to arrive back at T_0, with no radiation. It can drop back down giving off its surplus energy in the form of radiation called fluorescence, which is of longer wavelength than the original stimulating radiation because some energy was lost in the sublevels of T. If this path is taken it will be done in 10^{-7}-10^{-10} seconds. Third, it can lose a small amount of energy, reverse its spin and be at an excited level S_1. This electron is now called T^*. This one can loiter around between 10^{-3}-10 seconds before flipping down to T_0 giving its energy up as radiation (phosphorescence). It might, due to collisions, acquire a little energy as it goes down and shift back to T_1 after which it might then fall down to T_0 giving delayed fluorescence.

While the electron was spinning in its original form, balanced by another spinning in the opposite direction, the atom was in a so-called singlet state as the net spin momentum was zero. When the spin direction was flipped over in the above S_0 state there were now 2 electrons spinning in the same direction and the atom is said to be in the triplet state. This is the so-called intersystem cross-over. Phosphorescence always arises from the triplet state and is always of longer wavelength than fluorescence which arises from the singlet state.

Which of the above phenomena occur, and to what extent, are governed by a variety of factors. Molecular structures, such as those found in aromatic molecules in which electrons are orbiting loosely over a wide structure with multiple bonding cause enhancement. In media where collisions can occur easily, radiationless transfers of energy diminishment occur. Oxygen and moisture have a tendency to reduce triplet electrons and thus quench phosphorescence (McAleese, 1980).

An interesting method of inducing phosphorescence, but without the use of radiant energy is chemiluminescence or bioluminescence. Certain chemical reactions such as the firefly glow have been used to measure bacteria. All living cells contain ATP, a single bacteria having about 2.5×10^{-10} ng, so that 1000 have about 2.5×10^{-10} ng. Marino (Marino, 1981) has provided a data acquisition system that will detect 10^{-7} ng of ATP by its reaction with the firefly-produced luciferase. A luminol/hydrogen peroxide reaction catalyzed by bacterial porphyrins has also been used, as have other bioluminescent methods (Owens, 1973, Picciolo, 1968, Vo Dinh, 1978).

4.2 Factors Which Affect Phosphorescence

Phosphorescenece can be diminished by any condition that causes electrons in the triplet state to lose energy by collision or by thermal means. These cause a drop of excited electrons to a lower orbit without radiation or to enable electrons to pick up a small amount of energy to cross over to the singlet state. Lowering the temperature to a point where liquids solidify prevents these

events and therefore permits efficient phosphorescence. The presence of moisture and gaseous oxygen increases collisions, particularly oxygen which itself has a high triplet state which degrades the triplet electrons and must therefore be absent as much as possible (McAleese, 1980).

The fact that samples dried on paper or silica surfaces can give good phosphor scence at room temperature is often explained by the reduced mobility of molecules on a surface or by some surface forces.

An interesting finding was made that heavy atoms when present will greatly increase phosphorescence on paper. As a result it is now usual to add sodium iodide to a sample before dehydrating it (Vo Dinh, 1976). This increase was theorized to be caused by the more labile electrons present in metals.

Room temperature phosphorescence can also be accomplished in aqueous solutions successfully in the presence of micelles (Cline-Love, 1980, 1981 Skrilec, 1980). If compounds with polar end nonpolar ends, such as the detergent sodium lauryl sulfonate are present in sufficient cncentration molecules capable of phosphorscence are apparently protected from collisions by the nature of the detergent surrounding these molecules. The addition of heavy atoms here too has a large enhancing effect. In addition, fluorescence which normally gives a troublesome background is vastly reduced. This makes possible simpler measurements, as total emission, only, need be measured. The use of micelles discovered only in 1980, has not yet been fully exploited but would appear to have cnsiderable potential.

The use of dye lasers, and especially pulse dye lasers, has allowed considerable control over the time of reading phosphorescence relative to its stimulation (Fisher, 1972, Bergquist, 1973, Kasatiya, 1974). Because molecules vary in the wavelengths that will stimulate them, their emission spectra and the time of maximum emission, all of these factors can be utilized to differentiate among phosphorescent species. By generating a series of emission spectra versus excitation spectra for different time periods, three dimensional maps or fingerprints can be made to differentiate similar molecules.

An interesting approach using some of the above properties was described by Vo Dinh (Vo Dinh, 1978, Vo Dinh and Gammage, 1978), using synchronous luminescence spectrometry. In this method he varies the exciting wavelength as he measured the emitted wavelength intensity such that the difference between the two of them is constant, i.e. $\Delta\lambda$ is fixed. He applied this to phosphorescent mixtures and showed that as each significant emission wavelength is approached it is greatly enhanced, whereas background is diminished. He claimed that this procedure increased selectivity and decreased measurement time. It was interesting to note that results were different for different sizes of and useful information could be obtained by both narrow and broad differences.

4.3 Instrumentation

Instrumentation varies with the type of application and with the usage intended. Research instruments have different requirements than instruments designed for a specific application. For example, research oriented instruments, many of which are commercially available, are able to provide a broad range of wavelengths as radiant energy, but an instrument intended for a specific application need only a specific wavelength. Likewise, the spectra of the emitted light intended for research must be measured more tightly than is required for many specific applications.

Furthermore, low temperature phosphorimetry, intended for molecules in a solution, which in the past has been the most common, required a means of cooling the sample in liquid nitrogen. Room temperature phosphorimetry does away with this and is usually done on a paper or silica carrier after drying.

In any case, in spite of variations with type of application all must have four components: a source of illumination including some means of choosing the desired wavelength, a means of carrying a sample, a filter or other means of selecting the radiant wavelengths, and a detector to measure the output energy.

The instrumentation used for low temperature phosphorimetry, which for many years was the only widely used procedure has been well described by Schulman (1977). He discusses each of the four components in some detail which need not be reviewed here, except to say that commercial instruments for fluorimetry, which are widely available are easily adapted to phosphorimetry.

In general, phosphorescence has been measured using instrumentation designed for fluorescence with the added feature that a time delay between a few milliseconds to several seconds must be built into the system and subject to control. This delay between the time of irradiation and the measurement of systems uses a can with 2 slits 180 degrees apart. The can sits over the sample and rotates. The sample is irradiated from one side as one slit passes the sample and is read on the other side as the can rotates. The speed of rotation controls the time lag between irradiation and reading. An alternate to this uses a shaft with two multi-bladed fans with one fan on each side of the smaple. The placement of the blades is such that as the shaft rotates, illumination coming from one direction is either blocked or allowed to strike the sample. The blades of the fan on the far side of the sample allow phosphorescent emission to enter a detector or not depending on the speed of rotation and placement of the blades. A third system, used for measuring the phosphorescence of spots on a tape roll in an automated analyzer uses a mirror on a rotating shaft set at 45 degrees to the spot. A source of illumination is focused on the mirror and is reflected onto the sample. As the mirror turns away from the illumination it reflects the phosphorescent light onto a detector. Another appr ach using pulsed sources with gated

detectors was found to have advantages over mechanical techniques. Pulsed energy can be delivered with more peak energies at shorter intervals. This produces more intense output and allows better selectivity-advantage for short life-time phosphors (0.5-50 milliseconds).

Room temperature phosphorescence (RTP) has mostly been applied to measurements of phosphorescence of a surface or a substance on a surface. A recent book by Hurtubise (1981) provides a fine discussion of devices for this purpose. Essentially, thelse devices have the same four basic components but involve lasers as the excitation source to a greater degree. As RTP lends itself easily to automation, especially in the form of paper tape as a sample carrier, ingenious ways of reflecting light onto and off spots on paper with suitable time-delays have been devised (Vo Dinh, 1977, Yen Bower, 1979).

The area called chemiluminescence or bioluminescence uses a chemical reaction rather than incident radiation to generate a light emission. In this case a source of illumination is not needed. Simple and ocmpact equipment to use this approach to bacterial detection after culturing on media is much in favor by exo-terrestrial scientists working for NASA. Light measurements are made after injection of suitable chemicals onto the cultured speciment (Mitz, 1969, Strange, 1972). Because such methods can be easily automated they are excellent candidates for further development.

A great deal of work has been done with fluorescence in the study of biological molecules. Tagging of molecular groups with compounds known to fluorescence strongly is done by reaction with protein and the surplus washed away. The protein can then be detected and measured by the attached fluophor. In recent years immunofluorescence techniques have been applied to bacteria and viruses in which the fluorescent attachment is made to a micoorganism by the antibody for the microorganism. This requires highly specific antibodies to detect highly specific organisms and is a powerful identification technique. The emitted light is detected visually under a microscope or by photomultipliers.

Considerable work has been devoted to tryptophane and tyrosine in terms of their phosphorescence. It was found that excitation at 280 nm produces phosphorescence in both while excitation at 300 nm excites tryptophane only. The absorption spectra also varies somewhate in different proteins. Direct application of this information to a study of bacterial protein has not been found in the literature although the variation in the relative amounts of these compounds has been used to differentiate proteins.

4.4 Applications

The literature lists hundreds of compounds giving excitation and emission wavelength, their lifetimes and even their

limits of detection, many of them of biological interest (Becker, 1969. Rhys, 1981). Detection at nanogram levels is common using phosphorescence, and represents one of the few methods for detection at these levels. The majority of applications have been in the field of drugs including some of the most popular ones such as phenobarbital, cocaine, chlorpromazine and salicyclic acid in wine and blood. Air and water pollutants (Sawicki, 1969), hydroxyl substituted aromatics (Dalterio, 1982) and products from petroleum have also been given much attention. Proteins have been differentiated to some extent by the difference in relative amounts of tryptophane and tyrosine (Konev, 1967) based on their different phosphorescent spectra.

As pointed out by Shelley (1980) in discussing clinical applications, very little work has been done by clinicians although some work has used fluorescence to identify microbiological species. Shelley described measurements on compounds diffused into media by growing pseudomonas and plotting excitation vs. emission wavelengths which gave 3 dimensional plots that clearly were different for members of this group.

Unforunately no similar work on phosphorescence has been found in the literature. This may be accounted for at least in part by the fact that until recently the considerable background of broad fluorescence required extensive preliminary purification to obtain good specimens. Recent developments such as micelle techniques mentioned earlier might eliminate or reduce this problem.

4.5 Conclusions

Because phosphorescence is one of the most sensitive means of detecting specific molecules it would appear to be unusually desireable for microbial detection.

Developments of the past decade such as room temperature phosphorescence, heavy atom effects, micelle approaches and synchronous spectrometry have opened up possibilities which have not yet been explored.

The detection and identification of microorganisms has been attempted to a limited extent by associated techniques such as fluorescence, bioluminescence or immunofluorescence. They should now be examined by phosphorescence.

5. RAMAN

In the discussions on microwave and infrared measurements the method of observation was by absorption. Electromagnetic radiation passed through an array of molecules, stimulated to higher levels of excitation, thereafter losing some energy. The differnce between energy going in and coming out of this molecular array is usually small compared to the two values being differenced. Accuracy, or discrimination, based on amplication of this difference requires stable instruments such as are available at least for

infrared measurements. Using these methods advantage is gained by increased in input intensities, as the absorption is a fixed fraction of the input and the difference remains the same.

In phosphorescence and Raman we deal with emitted radiation and the signal of interest increases with an increase in the intensity of the exciting illumination. As a result, the interest in these phenomena has risen with the improved availability and decreased price of lasers.

To some extent Raman provides information chemically similar to that given by infrared, that is, the bond vibrations stimulated by infrared are also stimulated and measured by Raman but additional vibrations are also obtained by Raman. Furthermore, samples in water, or of high water content are difficult to measure using infrared but are no problem with Raman.

5.1 Background

Grasselli (Grasselli, 1981) has given a brief summary of the development of Raman spectroscopy through 1979. C.V. Raman was awarded the Nobel Prize in 1930 for his discovery of this effect in 1928 (Raman, 1928). Almost simultaneously Landsberg and Manderstam made a similar discovery in the Soviet Union. Within 10 years, 1753 papers had been published on the Raman effect and its chemical applications.

During the next twenty years excellent infrared equipment became commercially available and absorbed the interest of spectroscopists looking for vibrational information.

The advantage of Resonance Raman was first suggested by Brandmuller (1959) but as Morris (1979) points out, sources f sufficient intensity were lacking and the method was rarely used. In the late 60's when argon ion and krypton lasers became available, almost immediately Raman spectroscopy became a valuable tool for biochemical systems. Today commercially available dye lasers can now be used to cover almost the entire range in which conventional electronic absorption spectra can be obtained.

Starting in 1976 and to the present a new area called Coherent Anti-Stokes Raman Scattering (CARS) developed rapidly, in which two lasers at different wavelengths are employed. This approach has given rise to a series of variations and acronyms including SRS (Stimulated Raman Scattering), CSRS (Coherent Stokes Raman Spectroscopy), SRG (Stimulated Raman Gain), IRS (Inverse Raman Scattering, PARS (Photoacoustic Raman Spectroscopy) and Rikes (Raman Induced Kerr Effect Spectroscopy). These are all included in the general term Coherent Raman Spectroscopy because the scattered Raman radiation emerges from the sample as a coherent beam where the photons are all in phase. A summary of these methods appears in Analytical Chemistry (Borman, 1982). A number of recent books have described these methods (Barrett, 1981, Wright, 1981, Carriera, 1981). In 1978-1979 it was noted that about 3,600 pages were

published using Raman techniques compared with 8,200 using infrared spectroscopy.

5.2 Theory

When electromagnetic radiation approaches a molecule many things happen. As the wavelength of a typical illumination is 4000 A and a molecule is several angstroms, the whole molecule is stimulated at once. As discussed in previous sections some electrons are placed in more energetic orbitals, the geometry of the molecule may be shifted as a result, and rotations and vibrations are all energized to some degree.

If the molecules are in an enclosure as in a solution or gas, free of particles, and light of a given wavelength enters from one side and emerges from the other, absorption is cause by vibrations stimulated at that wavelength. Most of the light will pass through. About one thousandth will be scattered in all directions by the molecules present. This scattered light arises from the same phenomena which gives rise to absorption but with some interesting and measurable variations. In all cases inter-atomic bonds vibrate at ground levels or higher energy levels, and electrons also orbit at ground levels or higher energy levels, and electrons also orbit at ground levels or higher energy levels. However, the manner of fall-back to ground level can vary in different combinations thus re-emitting their surplus energy with different results.

In the section ofn phosphorescence we have described the results of electronic energy levels. In this section we are concerned with vibration levels.

The incident light can be scattered in three ways. The most likely path occurs when it stimulates a vibration from ground level (the most prevalent state) to an excited level which immediately falls back to its ground level emitting a photon of the same wavelength. This is described as elastic scattering and is known as Rayleigh scatter. the next two phenomena change the wavelength and are called inelastic scattering. These occur to about one thousandth of the Rayleigh scatter.

Consider the three energy levels: the ground level V_0, the first excited V_1, and a higher level of excitation V_2. If the exciting radiation raises a vibrator from its ground level to V_2 and it then falls down to V_1 it will emit with less energy than the original radiation. If the incoming frequency was f_0, the output is $f_0 - f_1$ and is called Stokes Raman scatter. However, the exciting radiation may encounter a vibrator already at level V_1 and raise it to V_2. If it drops back all the way to V_0, the output has acquired the extra energy it had in V_1 and comes out as $f_0 + f_1$ and is called Anti-Stokes scatter. In either case the output is a shifted wavelength from the input wavelength.

Other scatter effects of less importance to us here include Brillouin scatter (Brillouin, 1922) predicted in 1922 and first observed by Gross in 1930 as a very slight Doppler shift usually not separated from the incident radiation and Mie scatter which occurs from dust and is at the same wavelength as the Rayleigh scatter and often confused with it.

Raman shift is generally described in terms of wave number (the reciprocal of wavelength) and the shift from the exciting wavelength is the number used. As Anti-Stokes involves the population already in an excited state is starts with a much smaller population than Stokes, generally 1% of it, and therefore the Stokes wavelengths, which are the more probable ones, are usually used.

Gases, liquids and solids give different Raman spectra because of their differences in freedom of movement and vibration. The energy transitions which occur in some cases are the same as found by infrared and microwave absorption but in other cases are found only in Raman.

It should not be forgotten that among all of the transitions which are occurring, phosphorescence and fluorescence are frequently present. Sometimes to an overwhelming extent. Although Raman spectra originate earlier than these, measurements may still overlap and various precautions must be taken to eliminate interferences.

5.3 Resonance Raman

A recent paper by Van Haverveke (1981) on the use of resonance Raman to detect pollutants in water discusses the first applications of theis technique. he points out that Bernstein in 1972 noted that when the frequency of the exciting light falls within an absorption band, close to its maximum, the Raman signal can be enhanced up to a million times while other molecules exhibit only the ordinary Raman spectrum. The first investigations on the applications of this effect were described in 1978 by Van Haverbeke (Van Haverbeke, 1978). He found detection limits 1,000 times mroe sensitive than with conventional Raman of industrial dyes at the 30-50ppb level.

As will be pointed out later most optics used for Raman are glass, as the wavelengths generally used are in the visible range. It is possible to conduct Resonance Raman in the UV but the suitable optics are required.

Resonance Raman has now been widely used and its applications and advantages will be discussed elsewhere.

5.4 Coherent Raman

A considerable number of papers have been written describing so-called Coherent Raman Spectroscopy (Melveger, 1977, Morris, 1979, Borman, 1982, Gratzer, 1978). Under the description

several techniques have been developed. Coherent Anti-Stokes Raman Spectroscopy (CARS) has received the most attention because of its applicability to chemical analysis. In the procedure two lasers, one of which is varied, are used to illuminate the sample. The difference frequency which is produced is relatively weak but as it is varied, whenever the difference corresponds to a Raman frequency the Anti-Stokes emission is vastly increased. If Stokes emission is measured, it becomes CSRS.

Many variations using multiple lasers and other optical properties such as polarization have generated an alphabetical zoo. CARE, RIKES, OHD-RIKES, SRS and PARS are a few that have been noted. These are described in a 1981 monograph by Eisley (Eisley, 1981).

5.5 Advantages of Raman for Microbiological Applications

The advantages of using Raman for microbiological applications are:

1) Raman spectra can be obtained even for dilute samples in water because water has a weak Raman spectra. Infrared measurements are severly hampered by the presence of water.
2) Liquids, solutions, gases, films, surfaces and solids are al readily studied with Raman.
3) Extremely small amounts of material are needed. The active volume, in the nanoloter range is governed by the size of a focused laser beam.
4) The equipment geometry is very flexible due to the use of laser beams.
5) Symetrical bonds such as C-C and S-S can give intense Raman bands. These give very weak bands or no spectra in the infrared region.
6) The entire vibrational spectrum from 10 to 4,000 cm^{-1} is covered in a single scan with conventional glass optics. Infrared spectroscopy requires more restricting equipment and some changes to cover this scope.
7) Complete spectra can be recorded in less than 1 second.

5.6 Disadvantages

The disadvantages of using Raman for microbiological applications are:

1) The intense illumination can cause chemical changes.
2) Fluorescence can be troublesome. There are means to cope with this problem.
3) Normal Raman spectroscopy is not extremely sensitive and usually requires a clear solution.

5.7 Special Advantages With Resonance Raman

The use of a Resonance Raman provides the following special advantages:

1) Much more sensitive than normal Raman.
2) One can be selective and "pick out" a single desired chromophore in a complex biological mixture. Biological chromophores in the 250-750 nm range have been studied by Resonance Raman.
3) The spectra are relatively simple.

5.8 Coherent Raman--Advantages

Coherent Raman is now in a high state of change and is likely to develop further shortly. However, the following advantages can already be seen:

1) Conversion efficiences are 5 times greater than with normal Raman.
2) CARS is generated in a laser-like beam and therefore collection is excellent.
3) A monochromater is not needed, the spectral line width being determined by the laser line width.
4) Laser induced fluorescence does not interfere.
5) Low incident power is required.

5.9 Disadvantages of Cars

1) Due to background, radiation detection is limited.
2) Probably not useful for opaque or Mie scattering conditions.
3) Equipment costs are high.
4) Many interactions occur which are not well known at this time.

5.10 Equipment and Sensitivity

The basic equipment required to obtain Raman spectra is a spectrophotometer, usually in the visible range, although some work has been done at both ends of the visible; a means of holding the sample; a capillary tube; and a source of illumination which for practical purposes is always one or more lasers.

There are at least 10 commercial companies which currently advertise Raman spectrometers and there are likely to be more as more methods are developed and applied. Microprobe Raman has been applied to defects and contaiminants in the manufacture of microelectronic elements and this field is expanding rapidly.

Sensitivity is sufficient to examine a single particle 1 micron in length or to obtain spectra from 0.1 microliter in a capillary tube. Concentrations of some materials as low as 30-50 ppb have been determined. The speed of running a spectrum is dependent on the spectrophotometer, as the output is almost instantaneous, so that a spectrogram can be made with existing equipment in less than a secnd to several minutes. Sensitivity for bacteria impinged into a solvent would be easily adequate.

5.11 Applications

Many applications relating to biological materials have appeared in the literature. Studies of protein structure are discussed by Gratzer (Gratzer, 1978) using Raman spectra to study peptide bonds. Resonance Raman has been used to study specific locations of chromophores located in regions of interest. If no chromophores are present where desired, Gratzer refers to the work of Carey (Carey, 1978) who uses suitable colored reporter molecules bound to the active sites of enzymes to acquire information. For an extension into UV laser excitation he referes to Tsuboi (Tsuboi, 1975) who examined nuclei and derivatives.

Van Haverbeke (Van Haverbeke, 1981) applied Resonance Raman spectroscopy to the detection and identification of pollutants in water. Straight Raman spectra previously applied to waters showed that, at best, benzene could be detected at the 50 ppm levels and 4 to 40 ppm for a series of other compounds. However, using Resonance Raman, Van Haverbeke was able to go to 30-50 ppb for the detection of industrial dyes in river streams. UV and visible absorption spectrometry had about the same sensitivity but the spectral curves obtained with Resonance Raman were better able to identify the molecules present. The same method was used to detect colored dyes in foods. It is interesting to note that natural food dyes did not give a significant Raman spectrum but that artificial dyes all did, and all were different, therefore identifiable.

For pollutants which were themselves not colored, it was found that non-fluorescing dyes could be reacted with the pollutants (phenolic pollutants using 4-nitroaniline in alkaline medium were first tried) to give absorption maxima close to the wavelength of the laser. Pesticides in natural water were also derivatized and determined by the same author.

Van Wart (Van Wart, 1981) studied enzyme active sites using Resonance Raman. A protein or enzyme may have a native chromophoric constituent such as a porphyrin which serves a Resonance Raman probe or one may be introduced into a specific site on the enzyme by covalent attachment to an amino acid side chain. Structure and bondings were investigated.

Single fine particle were examined by Rosasco (Rosasco, 1975) using Raman spectroscopy on particles 0.7 microns long. Both organic and inorganic compounds were looked at. This ability is considered to be the result of being able to focus lasers onto such small volumes and to deliver a tremendous power density to the small sample. Their approach was to mount the particle on a holder onto which the laser was focused.

Different appraoches to microfine Raman examinations of samples have been reported (Dhamelincourt, 1979, Anderson, 1981). They used the MOLE, the Molecular Optics Lasers Examiner Probe which couples a microscope and a spectrophotometer. The sample need only be visible under the microscope and be of any composition.

The ocular of the microscope is replaced by a beam-splitter at 45° so that a laser beam from one side hits the surface of the beam-splitter and is directed onto the slide with the sample. The Raman spectra than passes up, through the microscope, through the beam splitter and into the entry aperture of the spectrophotometer. Many types of smaples such as urban dust, defects in materials and industrial samples of integrated circuits were each analyzed with useful results.

Information about molecular symetry polarization studies have been undertaken using such a set-up. A single 100 micron polyethylene fiber was enough to obtain polarization data. Recently, Adar (Adar, 1982) gave descriptions for a number of applications of this technique on industrial products.

Raman spectroscopy has been an important tool for determining the conformational structure of proteins and nucleic acids. Peticolas (Peticolas, 1975) points out that these spectra arise from either the backbones or side chains of these macromolecules, with frequencies between 200 cm^{-1} and 3,000 cm^{-1}. From these one can determine base stacking and hydrogen bonding interactions.

Finally, Howard (Howard, 1980) published a paper entitled A Resonance Raman Method for the Rapid Detection and Identification of Bacteria in Water. The authors make the point that ordinary Raman provides too much information to be interpretable but that Resonance Raman could be simple and more sensitive. They examine the chromophore carotene which is common to many bacteria found in water and by using lasers at 514.5 and 488.0 nm and a standard spectrometer equipped with a photon counting detection system they obtained high quality reproducible spectra suitable for rapid identification.

5.12 Conclusion of the Raman Spectral Analysis

Raman spectral analysis is a highly probable technique for the detection and identification of aerosol-borne bacteria. Sensitivity is adequate and sample presentation should be reasonably easy.

At this point data is lacking to identify chromophores in bacteria which would specify laser wavelengths suitable for Resonance Raman. If these should be difficult to find, it would always be possible to stain the samples with acridine orange or other bacterial stains to serve as a bacterial probe.

6. PHOTOACOUSTICS

The previous sections discussed the interaction of electromagnetic radiation with molecular structures. To some extent they have each been applied in investigations relating to microorganisms sufficiently to at least give direction and background for further work. This section describes methods which

have not yet been tried for this purpose although photoacoustics has been suggested, no work has been reported.

6.1 History

The discovery of the photoacoustic effect is usually attributed to Alexander Graham Bell in 1881 who found that a gas in an enclosed cell illuminated by chopped light pulses, gives rise to pressure fluctuations in the cell (Bell, 1881). At that time these fluctuations were detected as audible sound using an ear-tube. In the same year Tyndall (Tyndall, 1881) and Rontgen (Rontgen, 1881) also noted this phenomena.

For about 90 years the technique was used exclusively for applications to gas analysis using a microphone, commercial developments concentrating on these applicaltions (Delaney, 1959). Until recently other gas spectroscopies were preferred as they were more sensitive and more informative, except for the relaxation phenomena which was better done by photoacoustics. In 1971, a tunable laser and a sensitive microphone were applied to extend the sensitivity to micro concentrations of gaseous constituents (Kreuzer and Patel, 1971).

The first attempt to use photoacoustic phenomena to obtain spectroscopic information from solids is reported by Rosencwaig at Bell Laboratories (Rosencwaig, 1973). In the following nine years to the present many applications have been reported in imaginative ways to attempt to exploit the unique spectral capabilities of photoacoustics. Open-ended devices which can be applied to the surface of skin or plant tissue or to a stain on paper have been described. Solid surfaces have been studied including thin coatings on solids. solutions have been analyzed and much use of this technique has been devoted to the study of polymers and plastics.

In spite of numerous published works it must be pointed out that the method is not yet widely used. Most reports use in-house constructions usually specifically designed for a particular application. Though there are a few commercial photoacoustic adapters to spectrophometers being manufactured they have not penetrated the potential user market very deeply. It is very probably that this will change during the coming decade.

6.2 Theory

If a sample of any sort is enclosed with a gas and a detector capable of acoustic detection, and the sample is exposed to radiant excitation, causing vibrators of any sort such as electrons or chemical bonds to be brought to a higher energy level, they will drop back to a base level releasing much of the energy as heat. This heat energy is picked up by the over-lying gas, increasing its pressure. If the incident illumination is varied across wavelengths ranging from IR, throuh the visible, and through UV the sample absorptions will be reflected in the curves generated by the detector. To a large extent such curves closely follow the

absorption curves obtained by transmission at the same wavelengths using much larger samples. A surface coating a few molecules deep is sufficient to provide well-defined curves. Samples may be solids, liquids, or gases and even granules.

Two excellent text books delve into theory (Pao, 1977, Rosencwaig, 1980), especially as regards the types of excitation and vibrational modes and the physics of energy interchanges.

Emphasis is placed on photoacoustic effects with gases, as most work in the past has been with gases. Theories, as they relate to condensed states of matter, are simpler and litte space is devoted to that area.

6.3 Advantages and Disadvantages of Photoacoustics

The main advantage is said to be that samples can be taken in any form, without further treatment and a spectral curve obtained. Any wavelength range for which a source is available is useable. The sample size can be quite small, below microgram range, possibly nanogram amounts and laser light can be used to enhance response. Light scatter present no problem.

The main disadvantage at this time is that the method has not gotten widely used, so that many of the potential applications have not been developed and therefore little experience has been reported. Doubtlessly in the next decade instruments and applications will become generally available but meanwhile most set-ups are home-made for specific applications.

Another disadvantage stems from its main advantage, that the sample can be taken as-is. If it is a mixture, the spectra may be too complex and may require considerable computer manipulation to give meaningful results.

6.4 Photoacoustic Equipment and Sensitivity

An instrumentation article in Analytical Chemistry (McClelland, 1983) describes some of the equipment used in photoacoustic spectroscopy for gases and condensed state samples. Basically all require an optical radiation source which is intensity-modulated, an enclosed cell which holds the sample, an acoustic transducer and some gas. In addition an electronic proceeding system to amplify and read out the signal from the transducer is needed. Specific details for each component vary with the wavelength desired and the anture of the sample. The few currently available cells are designed as add-ons to spectrophotometers to take advantage of a variable light source. many authors describe apparatus for specific purposes (Oda, 1978, 1981, Farrow, 1978, Somoano, 1978, Gra 1977, Low, 1980, Fishman, 1981).

Sensitivity of the method varies with the application. Thus, in a paper by Oda (Oda, 1978), cadmium in the fungus

Penicilium ochro-chloron was determined at the 0.02 ng/ml level, approximately 100 times more sensitive than calorimetric or flame atomic absorption methods, using laser-induced photoacoustic absorption spectrometers. Casteldan (Casteldan, 1979) determined fluorescein in the range 0.2-2 micrograms with a limit of detection of 20 ng on thin-layer chromatographic plates by photoacoustic spectroscopy.

6.5 Photoacoustic Applications

Photoacoustics has been applied to a wisde variety of sample types and a broad range of wavelengths. Bulk studies, surface studies, and vapor analysis have been made in materials such as coals, biological and agricultural products where reflection techniques were previously used, as well as metallic powders, various complex chemicals, bacterial growths, smears of blood in different oxidation states and skin before and after the application of various antibacterial agents. In addition photoacoustics has been applied to all of the chromatographies, vapor, liquid, thin-layer paper and plate as a means of detecting and identifying compounds. Theoretical studies involving the de-exitation process have been profitably studied by this technique. These studies are useful in understanding the fluorescence phenomena in which electrons return from excited states to a base level by emitting fluorescence or phosphorescence, whereby no photoacoustic signal is obtained, or they can be de-excited through heat loss processes detectible by photoacoustics, thus providing useful knowledge of photochemical processes and reaction rates.

As previously mentioned, cadmium has been determined with a detection limit of 0.02 ng/ml by extraction of a fungus with chloroform and measuring the solution at 514.5 nm (oda, 1978). Fluorescein was determined at the 20 ng level as a spot on thin-layer chromatography with minimal sample preparation and no solvent extraction (Castleden, 1979). The interaction of a carbonate insecticide with a clay carrier was studied by Lowry (Lowry, 1982). In this application he was able to avoid destroying weak bonds which would have been destroyed by sample gridning and thus demonstrated the nature of the bond with this technique. Oda (Oda, 1981) used laser-induced photoacoustic spectroscopy (LIPDAS) to monitor liquie chromatographic effects. he was able to show that this approach was 25 times more sensitive than the use of UV. An open-ended photoacoustic cell has been described which is applied directly over a variety of samples including spots on thin-layer chromatographic plates (Fishman, 1981). Volgtman (Volgtman, 1981) developed a simple photoacoustic detection system for highly sensitive liquid measurements. He gave limits of detection for various porphyrins, laser dyes and drugs all in the nanogram per ml range. The oldest application, gas analysis was recently worked on by Yip (Yip, 1983) who used wavelength modulation in a device to get around problems inherent in the absorptions of the cell itself. He adapted a commercial CO_2 laser and used a modulation frequency of 100 Hz to improve experimental results. Some detection limits wer ein the parts per billion level.

A paper published in April 1983 applied photoacoustic spectroscopy to an algae, the cyanabacteria anacystis Nidulans (Carpentier, 1983). The speciment were deposited on a nitrocellulose filter and the effect of sample thickness was studied. The authors found less than a 10% variation among samples which were thinner than 10 microns. They stated that filtration of small particles gave an optically homogenous deposited layer. They were able to successfully study the relative photosynthetic efficiency of algae pigments.

6.6 Conclusions of Photoacoustic Techniques

Photoacoustics would appear to be a suitable and rapid technique for detection as well as identification of air-borne bacteria. According to the papers described above it is indicated that somewhat between less than a nanogram to 20 nanograms were minimum samples needed. Assuming that bacteria are 75 percent water and using the higher value, about 80 thousand bacteria would be needed as a minimum. If these were impinged on a flat surface they could then be treated in the same manner as a spot in paper chromatography, about which several reports have been given. A photoacoustic curve obtained from such a speicmen, made in less than 1 minute could provide definitive information.

An alternative possibility suggested by the referenced material is to ignite the specimen in the absence of air and to use the vapor to run a photoacoustic spectral analysis as has been done for automobile exhaust fumes.

Some work done with bacterial colonies on the surface of the growth media were able to differentiate different states of growth.

7. DIFFUSE REFLECTANCE SPECTROSCOPY

Reflectance spectroscopy studies the spectral composition of radiation reflected from a surface as to its angular dependence and the composition of the incident radiation. Two types of reflection can be differentiated, speclar reflection from a smooth surface and diffuse reflection from a matte surface. Although all reflection contains some of each, two quite different methods are used to study the two extreme cases. We consider here only the diffuse reflectance.

It may be assumed that diffuse refllectance produces an angular reflectance that is isotropic i.e., that the reflectance is directionally independent.

In theory the nature of the reflectance is dependent on the relationship of particle size to wavelength. For particles of a size much larger than the wavelength there is reflection off the surfaces, plus some internal absorption, refraction and diffraction until finally it emerges diffusely from the surface. With small particles relative to the wavelengths, scatter occurs according to

the Mie theory, which is not isotropic. But with a sufficiently thick layer of closely packed particles this too yields an isotropic result.

Actual measurements of diffuse reflectance has been done over many decades but has seen a resurgance recently. Several textbooks on the topic (Kortum, 1969, Wendlandt and Hecht, 1966), and a number of papers describing applications have begun to appear in spectrographic and analytical journals. Experimental work by the author of this report directed to an investigation of the feasibility of this app' ach to bacterial detection will be described later.

For our purposes, in this study, the diffuse reflectance phenomena are similar to those found by photoacoustics discussed earlier. A number of papers have appeared comparing these two techniques and it has been found possible to make the same measurements on the smae samples in the same equipment using an adapter. Thus Rohl (Rohl, Childers and Palmer, 1982) go into some detail to describe the equipment to do both measurements on the same sample and present comparison results on two powder samples. The curves presented show an invrse relationship, that is, where peaks point upwards in the photoacoustic graphs, they point down in the diffuse reflectance curves. They conclude that the two techniques have complimentary advantages and disadvantages. The photoacoustic method is subject to thermal properties in the sample, especially if thick, whereas the diffuse reflectance may be affected by specular reflections which may be present.

7.1 Applications of Diffuse Reflectance Spectroscopy

Applications have been described for all wavelength bands from UV through the visible and through IR. The near-IR was recently discussed at some length in a report in Analytical Chemistry (Wetzel, 1983), in which he describes this method as a "sleeper among spectroscopic techniques". The author refers to work by Griffiths (Griffiths, 1978) on the use fo the technique with Fourier transform IR as having generated considerable attention. In the near-infrared paper the use of spectral subtraction serves to eliminate the need for chemical separation of otherwise interfering constituents. Applications are made to a wide variety of otherwise difficult materials, and with little sample preparation. Coals, textiles, pharmaceuticals and many agricultural products have been examined by this technique. The article by Wetzel gives a good summary of the pro's and con's of this method.

Ditzler (Ditzler et. al., 1983) describes UV-Visible spectroscopy by diffuse reflectance for a study of reagents attached to silica gel. In this paper a method of subtracting the effect of the substrate yields spectral data which resemble transmission data of model compounds. This is a useful application, particularly because in recent years many reagents have been immobilized onto silica absorbants for bonded chromatographic applications and Ditzler cnsiders that of all the available techniques used to

examine such samples, diffuse reflectance is the least expensive and is of comparable quality to the others.

Hannah (Hanah and Anacreon, 1983) describes a diffuse reflectance accessory to be used in an infrared dispersive spectrophotometer. He prepares his samples on potassium chloride pellets using 50 ng samples and obtained data which can be expanded to reveal highly discriminating peaks.

An interesting earlier article (Henderson, 1980) compares the use of near-infrared reflectance and photoacoustic spectra as applied to agricultural grains. He views the comparison from the point of view that the first method is well established and he wishes to compare the photoacoustic method to it. The curves shown are similar for both techniques with the sample types i.e agricultural grains, high in oil and moisture. Careful sample preparation is required so that particle size and condition is similar from sample to sample in order for comparison data to be meaningful for both techniques.

7.2 Conclusions of Diffuse Reflectance Spectroscopy Techniques

As will be discussed in the Experimental section later, it is possible to look at a surface nto which aerosol bacteria have been impacted, with either photoacoustic or diffuse reflectance spectroscopies and obtain comparably useful detection capabilities.

With sufficient basic data, not available at present, either of these techniques presents a god possibility for an instrumental method of rapid microbiological detection.

8. PIEZOELECTRIC DETECTION

The methods discussed previously involved interactions of radiant energy with the molecular structures found in microbiologicals. With piezoelectric detection we wil concern ourselves with physicochemical properties of a different kind.

8.1 History

Piezoelectricity is a property in which pressure on certain quartz crystals produces an electrical potential. When such a crystal is subjected to a specific electrical charge it will vibrate for a short time. This effect was first reported by Pierre and Jacques Curie in 1880. Advantage has been taken of this property in many ingenious applciations. Such quartz crystals are used in great numbers for regulating and controlling frequency in communication equipment and in filters in electrical networks. Among other applications are: use for clocks because of their accuracy to 1 part in 10^9; ultrasonic wave generation, measurement of temperature and thickness of evaporated films; dew point determination, and of special interest to us is its use to detect specific gases absorbed on coatings on the surface of the quartz.

8.2 Theory of Piezoelectric Gas Detection

Sauerbrey (Sauerbrey, 1959) reported the relationship between the frequency change and the change in mass occuring as a crystal is coated with a metal film. A brief description and some of its implications is given in an equation developed by King (King, 1964):

$$F = 2.3 \times 10^6 \times \frac{F}{T} \times \frac{W}{A}$$

In which F is frequency change in cps due to a coating.
F is frequency of the quartz crystal in MC.
T is the thickness of the quartz in cm.
W is weight of added coating in grams.
A is the area of the quartz in square cm.

King points out that a commercially available 15 MC crystal with electrodes 5 mm in diameter will have a frequency change of 2600 cps per microgram of coating. Thus, when in use as a weight indicator it has a detection limit of about 10^{-12} grams, about the weight of 1 bacteria.

Many authors have taken advantage of this property to develop sensitive vapor detectors. A coating of any of a variety of chemicals weighing approximately 40-100 micrograms and capable of absorbing only a specific compound is applied to the quartz surface. The frequency of vibration of the quartz crystal is measured before and after exposure to air containing the specific compound and the change in frequency then becomes a measure of the concentration of the compound in air. Such detectors are now in use as environmental protection devices.

8.3 Advantages and Disadvantages of Piezoelectric Detection

Very few methods exist which are capable of detecting such extremely small weights without using extreme precautions. The simplicity of the equipment combined with its extreme sensitivity is unique. The equipment is inexpensive and compact, making its use as field equipment convenient. Present coatings allow for a variety of atmospheric contaiminants to be detected. Many coatings have reversible properties so that the absorbed molecular species can be de-absorbed, permitting its continuous use. A disadvantage occurs with coatings that are not reversible, in which the absorbed species forms a strong chemical bond with the absorber. In such cases the "life" of the coated crystal is limited and it must be replaced or recoated periodically. There are limitations in the use of these detectors. The maximum load that can be applied, that is the heaviest coating is small and difficult to apply. The response is not linear outside of narrow limits, being smaller for a given increment as the total load is increased. Until now no one has proposed a way of applying these detectors to bacerial detection.

8.4 Piezoelectric Detector Equipment

The basic equipment requires, beside the coated quartz chip and its housing, an oscillator and power supply to vibrate the crystal and a frequency meter to measure the cycles per second. Also an air pump or other source of carrier gas, and a flow meter to help regulate the flow rate. Optionally, if a chart recording is desired, a digital to analog converter is also needed. Aside from the chart recorded all of the basic equipment can fit into 1 or 2 cigar boxes.

The design of the cell which carries the crystal can take a variety of forms depending on its intended application. For use in conjunction with a gas chromatograph where the gas samples pass in very small amounts and a minimum volume gas space is needed, King describes a holder with about a 0.02 cc gas chamber. On the other hand Guilbault (Ho and Guilbault, 1982) has described a larger volume cell for continuous monitoring of the atmosphere in which incoming air impinges onto both sides of a coated quartz crystal through two lead-in tubes with constricted apertures.

For some applications a variety of auxillary equipment might be needed to prepare or convert the compound desired to be detected into a form for which a coating has already been developed. Such a set-up will be discussed under the subject of applications.

8.5 Applications of Piezoelectric Detection

In an early report, King (1964) applied the piezoelectric detector to gas chromatography using a variety of coating materials and found that different carrier gases did not effect sensitivity. Starting about 1974 Professor Guilbault and a series of graduate students, over the years of the following decade produced numerous applications with various sensitivities to a range of constituents in the atmosphere from parts per million to parts per trillion (karmarker, 1974, 1975, Webber, 1976, 1978, Hlavay, 1978, and Ho, 1980). In a paper presented at the 10th Annual Symposium on the Analytical Chemistry of Pollutants, May 1980, Professor Guilbault (1981) summarized the work done up through 1980 n the use of piezoelectric detectors to determine environmental pollutants, in which he referenced 27 publications on this subject.

A series of papers were also issued from workers at the National Bureau of Standards (Scheide, 1974, 1975, 1976) dealing with the use of a gold coated piezoelectric crystal to monitor mercury in industrial environments. Mercury amalgamates with the gold coating and the detector serves as an indicator of the daily accumulation of exposure to mercury vapor. At the end of the day the electrode is baked to remove the mercury and re-new the electrode. In 1983 Guilbault reported (Kristoff and Guilbault, 1983) that the same gold electrode without any coating could be used to detect an organic phosphorate (DIMP) in the microgram per liter range.

A piezoelectric crystal detector for water in gas streams was reported by Lee (Lee and Fung, 1982) who found that gelatin was the best coating material, being subject to interference only by alcohols and formic acid. In this case the response time was unusually long, about 6-7 minutes compared with most other detectors (about 30 seconds).

An ingenious indirect procedure for the detection of carbon monoxide has been described by Ho (Ho, 1982). He used a pre-heater and passed the gas over mercuric oxide so that the carbon monoxide reacted to give elemental mercury. From that point on, the detector was similar to the previously described mercury detector. In a book on the subject, Ultra Micro Weight Determination in Controlled Environments (Wolsky, 1969) the authors referred to the determination of carbon monoxide by direct chemical absorption on gold surfaces at 40° C (page 237). In another unusual application Nomura and Maruyama (Nomura, 1983) found that the piezoelectric crystal could be submerged in aqueous solutions and still give useful readings. In this application they were able to calibrate, and thereafter determine the concentration of iron (111) b absorption as a phosphate in a solution.

8.6 Conclusions of Piezoelectric Detection Techniques

Piezoelectric detectors may be a simple and extremely sensitive means of detecting specific compounds in the atmosphere. If bacterial particulates in air are accumulated into a small recepticles and ignited in the presence of air the products from a small number of organisms will produce gases such as H_2S, NH_3 and other end products of combustion which could be detected. If the particles in the range 0.5-5 microns could be selectively isolated from air, and subsequently ignited, and if the products of ignition showed the presence of these gases, the result could be a probable indication of microorganisms.

9. CHROMATOGRAPHY

In the procedure previously described, until we had gotten to piezoelectric detectors, we were using interactions at the electronic level involving radiant energy. With piezoelectric detectors we began to look at molecular chemical reactions. In this section we are using physicochemical methods.

The use of chromatography in connection with microbiological detection and identification opens up as many diverse possibilities as there are forms of chromatography.

The original use of the term chromatography arose when plant pigments were extracted and poured through a chalk column, whereupon the colors separated into distinct visible bands. Since then the term has been generalized so that visible color is no longer required. It now refers to any procedure where a mixture of constituents, usually as a liquid or gas, is placed at one position, onto an absorber which is fixed in place. Another medium, the

mobile phase, to which the substances have a greater affinity, is passed over the fixed absorber so that the constituents of the sample washes n the another location. Usually, though not always, the fixed absorber is in a tube, usually narrow or even capillary in size, through which the mobile phase passes. During this process each molecular species migrates at a different rate related to their relative affinities to the two media. As a result, they emerge at the end of tube at different times. Development of detectors to identify when each species emerges has become a separate science, leading to many ingenious developments and innumeral publications describe new detectors almost each month.

Chromatographies have taken many physical forms. Diffusion on paper or through a gel medium, sometimes under the influence of an electrical field, has been widely used and might possibly be adapted to our purpose but the most highly instrumented methods have been developed for so-called high performance liquid chromatography and gas chromatography. We will look at the last two primarily, as to how they may be applied to analyze, and thereby detect bacteria.

9.1 High-Performance Liquid Chomatography (HPLC) and Gas Chromatography (GC)

A single bacterium contains many hundreds of different molecules, which can be broken down to thousands of different andpotentially identifiable fragments. To analyze them sufficiently for purposes of identification, separation of the interesting constituents is a critically important step.

It is of interest to those seeking improved methods of microbiological detection to note that during the past decade a great deal of attention has been paid to the analysis of exceedingly small samples dealing with biochemical studies, where many of the constituents present in bacteria, such as proteins and their constituent amino acids, sugars of various complexity, enzymes, etc., have been studied. To meet the requirements of these studies the major tool has been the development and requirement of both high-performance liquid chromatography (HPLC) and gas chromatography (GC). Special attention has been given to miniaturization down to capillary size for chromatographic columns and to the enhancement of the abilities of new detectors so that picogram (bacteria sized) samples can be detected routinely.

A joint U.S.-Japan seminar held August 1982 in Hawaii devoted to microcolumn separation methods was written up in a paper in Analytical Chemistry (Novotny, 1983) in which the recent advances in miniaturizatin and detection were elaborated. Modern detectors mentioned included laser-based devices, LC/Mass spectrometry, microcolumn chromatography/FT-IR spectroscopy, flame and plasma-based detection devices and electrochemical detectors with miniaturized electrodes.

In a paper in which Jennings (Jennings, 1984) refers to "a renaissance in analytical chromatography" he describes chromatography as the most powerful means of achieving analytical separations and finds that in most recent developments, LC and GC are particularly worthy. He feels that especially with the advent of rugged capillary systems, certain advantages such as improved separations, faster analyses, higher sensitivities and less expensive equipment points the future direction for applied analysis. Capillary columns compared to packed resin columns yield sharper peaks due to less heat differences throughout the system, less turbulence mixing and less time for side reactions to complicate the process.

In 1984 (Jinno, 1984) a paper describing the mating of microscale HPLC with FTIR points up the advantages of this combination. Most detectors used for HPLC have been able to detect the presence or absence of a constituent as it comes through a column. By graphic representation of time vs the emergence of constituents one obtains a pattern which serves very well if the constituents are reasonably known, but where only the relative amounts are to be determined. in this case a known mixture is passed through the column and the resultant pattern establishes a basis for comparison with the unknown sample mixture. A "fingerprint" represented by the pattern is characteristic for the unknown and a library of such fingerprints can be used to match new samples against.

In Jinno's paper he suggests that if the output of the column is looked at by FTIR each time that a constituent comes through the column a great deal of additional information becomes available. This procedure has been used with GC for some time, but not with the miniaturized HPLC which has gotten popular recently. Solvents which are highly transparent in the IR range tend to be expensive, but the very low consumption using microscale HPLC effectively reduces the cost.

The possible use of GC on the volatile products produced by pyrolysis of impacted bacteria may well be suitable for detection and/or identification purposes. However, if the bacteria were impacted into a liquid lysing agent capable of disrupting the cell walls the resulting solution of bacterial constituents might well be productively treated by HPLC. Pyrolysis produces fragments products which must then be interpreted as to origin, whereas a direct solution retains the original molecules intact.

9.2 Applications of Chromatography

An outstanding textbook entitled Methods of Detection and Identification of Bacteria (Mitruka and Bonner 1976) discussed the subject of primary interest in our study in great detail but from a hospital clinical point of view. Aside from the fact that they are not concerned with great speed of identification, as we are, the material presented is essential to a proper understanding of our subject matter. In this book, analysis only begins after cultures

of the microbiologicals have been made so that the available sample is already species separated and of good concentration. However, the techniques described, in considerable detail, can be up-dated using equipment developed since the described work was done, which may well give similar results on far smaller samples.

The authors of this book use a variety of chromatographies including paper, thin-layer and gas chromatography. Over 30 pages are devoted to gas chromatography as applied to bacterial identification and they apply mass spectrometry to the emerging cnstituents to identify the effluents as they emerge from the column.

Another, more recent textbook, closely related to the present study is Microbiological Applications of Gas chromatography by D. B. Drucker (Drucker, 1981). This book contains 800 references and the authors found it necessary to restrict their discussion to only the commonly used methods. One whole chapter is devoted to methods of detecting microorganisms by gas-liquid chromatography. This text deals with a number of examples of bacterial detection other than in a clinical setting, such as in foods and in planetary exploration. Specific curves are shown from pyrolysis-GLC for a number of bacteria.

As was mentioned earlier with the example of FTIR, the information on the effluent has in recent years been greatly enhanced by new detectors capable of spectral analysis in addition to mere detection. A recent addition is the use of photodiode arrays as detectors for LC, (Borman, 1983) using dispersion of a laser passed through the effluent of an LC column, either by prism or grating. The spectrum is impinged on an array of many detectors (a series of light-sensitive elements etched on a silicon chip) each of minute dimensions, so that the whole spectrum is recorded at once. A complete spectrum can be stopped. This detector can be used with microbore HPLC as well as more conventional HPLC.

Mass spectrometers as used for detectors for GC have begun to take many forms. Theoretically mass spectrometry may be the best way to identify every fragment of a molecule and in the most minute amount, but it is very expensive, running into hundred of thousands of dollars. A number of simplified less expensive designs have been developed. A typical variation is the ion trap detector (ITD), developed specifically for GC (Stafford, 1983). This detector is very compact and has less critical mechanical parameters and works with less than 1 ng of consituent.

Many recent papers on GC describes applications to environmental studies. For example Isodorov (Isodorov, 1983) discusses GC/MS determinations of volatile organic matter in the Leningrad atmosphere. The details given are a description of typical GC/MS practice with results showing the great number of organic molecules we breathe every day, although no specifically bacterial information is included. Some applications to bacteria is however, given by Tunlid (Tunlid, 1983) in a paper which studies the

specific components of the bacterial cell wall using capillary GC and selected ion monitoring detection for several compounds. It is indicated that the described procedure may be capable of detecting 3 x 10^5 E. coli cells. The basis for their calculations include determining the D and L ratio of alanine in bacteria.

An interesting detection method, laser-induced fluorescence was applied by Diebold (Diebold, 1977) to analyze for the very poisonious aflatoxins at less than picogram levels after HPLC. Using a He-Cd ion laser focused on a droplet of the effluent as it emerges from a column he avoids problems of fluorescence from cell walls and detects femtogram levels of the aflatoxin.

A number of interesting papers have appeared us.ng HPLC on separations involving proteins, peptides and polynucleotides, which are the basic molecular structures of bacteria. An early paper (Rittinghaus, 1980) presents HPLC for protein analysis as an alternative to gel-filtration or gel-electrophoresis. This worker presented basic parameters and also curves obtained in separating acidic, neutral and mildly basic proteins with molecular weights from 2000 to 80000 daltons. His separations required 6 minutes with a single column.

In a report by Regnier (Regnier, 1983) the separation of these biological constituents are discussed from the analytical requirements viewpoint. He elaborates the various chromatographies needed to separate these different classes of compounds. He includes in his discussion; size exclusion chromatography (SEC), ion exchange chromatography (IEC), reversed phase chromatography (RPC) and liquid affinity chromatography (LAC) at some length.

Chromatograms are often obtained after reacting proteins with a fluorescent marker which is used to detect the product after chromatography. Boykins (Boykins, 1982) describes the micro-analysis of amino acids after reacting with o-phthaldehyde in the presence of 2-mercaptoethanol. The automated instrument described produced routine analysis of amino acids at the picomole level.

A novel method of analyzing for trace organics in water uses gas chromatography with a pre-column consisting of a plastic tube called Nafion which allows water molecules to pass through, but retains the organics (Simmonds, 1979). The water sample is injected at one end of the set-up where it passes, first through the Nafion where it looses the water, then through the column and past an electron-capture detector to produce a GC curve.

9.3 Conclusions of Chromatography Techniques

It is clear that both gas and liquid chromatographies have been scaled down and perfected to a level whereby samples of aerosols impacted onto a surface which can be heated, such as a pyroprobe or other heatable surface, can be vaporized to produce sufficient product which can be identified by GC.

Hospital laboratories have used such techniques on larger samples for many years. Newer, more sensitive equipment can operate on much smaller samples. There are a great variety of new detectors giving considerable choice of properties which one may wish to select for purposes of detection and identification.

10. CELL SORTERS

The technique of flow cytometry has great potential for detecting and identifying bacteria and is in use now for a variety of cell measurements. The method uses the ability developed recently to make meaningful measurements at micro second speeds on cell-sized volumes with laser beams.

In the basic set-up a liquid sample containing particulates flows into a constricted tube which is in the center of and surrounded by a somewhat wider tube of clear, flowing liquid. The constricted tube terminates so that its contents flows down the wider tube but the surroundng clear fluid holds the particulates in the center by the nature of hydrodynamics. As the particles descend, they are hit by one or more laser beams and a variety of optical properties can be observed and utilized to acquire useful information about the particles.

In addition, the outer tube may be vibrated rapidly by being held against a piezoelectric crystal. The liquid emerges from the outlet of the outer tube, in the form of a stream of fine droplets due to this vibration. If desired, these droplets may pass charged plates which can divert those droplets which bear particles detected to have specific properities by the laser detectors. Thus if aerosol particles are impinged into a water carrier it would be possible to detect, separate and accumulate microbiologicals from other detritus and accumulate them for further study.

An excellent instrumentation article on the flow cytometer and sorting has been written (Pinkel, 1982) by Pinkel of the Lawrence Livermore National Laboratory, Biological Sciences Division, based on work in that laboratory.

Work has been done with this approach for about 15 years and such instruments can now measure a number of parameters on several thousand particles per second and to make decisions about them and thus sort based on pre-selected properties. Measurements of fluorescence, both intrinsic and a result of a specific stain, fluorescence depolarization, phosphorescence, light scatter, absorbance, electrical conductivity and capacitance are all possible.

Commercial manufacturers have made general-use flow cytometers, which are now in wide use, and many laboratories have built variations to suit individual needs and a textbook written on the subject (Melamed, Mullaney, Mendelsohn, 1979) gives many references.

10.1 Applications of Cell Sorting

An early paper by Bonner (Bonner, 1972) describes fluorescence activated cell sorting. Using an improved form of earlier-described equipment Bonner uses a set-up similar to that discussed in the previous section. Various body cells can be reacted so that only certain cells are tagged by such fluorescent compounds as fluorescein, acriding orange and quinacrine mustard. The device generates 40,000 drops per second and separates out 2000 cells per second.

The following year the same group (Hulett, 1973) extended their cell sorter so that they could sort by either different fluorescent intensities or light scattering characteristics or different combinations of these. Along with a description of the modified instrument they give biological and clinical applications. The described instrument used a neon laser to give signals to a scatter photodetector and an argon laser to activate a fluorescence detector. No bacterial applications were referred to.

In 1976 Cram and Salzman (Cram, 1976) at the Los Alamos Scientific Laboratory, Biophysics and Instrumentation Group described their instrumentation and applications in Chapter 13 of Developments in Industrial Microbiology. They illuminate the carrier cell with an argon-ion laser at the rate of 1000 cels per second. They call their instrument a flow microfluorimeter (FMF) as they use primarily fluorescent measurements and detect light scattered in up to 30 different angles simultaneously. They were able to detect infected vs non-infected cells by various viruses. No direct bacterial work was reported.

The following year (Horan, 1977) an informative review article was presented which both summarizes the stage of development of quantitative single cell analysis and sorting and also points forward to expected future developments which he anticipates. The article presents a clear description of each stage of the cell sorter and a detailed description of biological applications especially as they apply to DNA studies in connection with cancer and cancer therapy. He concludes by stating his expectation that the cell sorter will become as essential in research as the scintillation counter or the Autonalyzer.

Another report from the Los Alamos National Laboratory Group (Dovichi, 1983) described recent work on attogram (10^{-18} g) detection limits for aqueous dye samples using a modified flow cytometer and laser-induced fluorescence. This is a unique application which analyses fluorescence of a solution rather than a particle, using the flow cytometer as a convenient way of presenting microvolumes of liquid for fluorescence analysis.

Symposia on the subject of Pulse-Cytometry held in Belgium presented many discussions of equipment and applications from world-wide laboratories (Pulse-Cytometry, 1976, 1978).

A related method called the Coulter Counter has been used for bacterial applications particularly by clinicians in hospitals. This device, long used for various kinds of cell counting is quite different in principle from the cell sorter as described above. The Coulter Counter works on the principle that a partition with a small aperture separates a conductive fluid on both sides of the partition. As the fluid, bearing cells, flows through the apertrue, a cell passing through, blocks from fraction of the opening. When this occurs the electrical conductivity from one side to the other is reduced in proportion to the size of the particle. Cells may be passed through at a fair rate so that many can be counted and "sized" rapidly (Smathers, 1975, Human, 1978, Alexander, 1981). This procedure has been used world-wide in hospitals to detect bacteria in urine where at the $10^5/Cm^3$ level, over 90 percent of the tests agreed with more time-consuming standard procedures. Such applications go back to at least 1964 and are often reported in small company publications. Coulter Counter instrumentation has been continuously refined and 1984 instrument designs allow size range from 0.4-800 microns discrimination for one model and 0.003-3 microns for another model. The later model can be used to measure average particle sizes or molecular weight. Although these are sold only for particulates in water and the manufacturers state that it is not suitable for measurement of particles in an air medium, particles can be impinged from the air into water and can then be run through the Coulter Counter to obtain valuable information.

10.2 Conclusions of Cell Sorting Techniques

Aerosol particles impacted into water can probably be passed through a cel sorter to differentiate microbiologicals from other particles and it may be possible to obtain some detree of classification.

It also may be possible to impact aerosols into a dye solution and subsequently to do cell sorting using fluorescence of the dye as a means of microbiological detection. As cell sorters are available widely from commercial sources it would seem to be an obvious approach to attempt microbiological detection in this way.

11. ADDENOSINE TRIPHOSPLATE (ATP) AND CHEMILUMINESCENCE

In search for specific molecular components of bacterial cels hich could be tested for easily, and with great sensitivity, probably the brightest performer found has been adenosine triphosphate (ATP). Possibly the earliest report (Hinkkanen, 1960) mentions this compound, which, by virtue of a phosphorylating mechanism, enables energy to be stored and given up easily and is used for this purpose by all cells including bacteria.

In studies of the glow produced by fireflies it was found that the glow was produced by the enzyme luciferase acting on ATP. It was also found that magnesium was required to be present. In 1968 an assay for bacteria, using this reaction, was reported

(Chappelle, 1968) and the details of the mechanism for the reaction was further elaborated in the following year (McElroy, 1969).

The use of catalytic reactions to produce chemiluminescence with constituents of cells was not new. Neufeld (Neufeld, 1965) at Fort Detrick and described the use of luminol in an alkaline medium, in the presence of peroxide, to detect iron porphyrin, a common cellular constituent, by the flash of light produced. He refers to a review of this subject earlier (White, 1961) and to subsequent work by a number of others on this reaction.

Oleniacz, Pisano and insolera (Oleniacz, 1967) after having completed work the previous year using luminol to react with bacteria, looked at other chemiluminescent compounds. They examined lucigenin, which had been reported in 1935, using this compound in alkali and peroxide. They found that extracts and intact cells of Serratia marcescans activated lucigenin, even without peroxide and alkali, but in alcoholic solvents. The bacterial stimulation of the glow produced was found to be increased if fluorescein was present.

In the same year Chappelle (Chappelle, 1967) working on extraterrestrial life detection, used the previously mentioned luciferase extracted from fireflies, but they extracted luciferase from the photobacteria Achromobacter fischeri and applied the product to detect flavin mononucleotide (FMN). The later compound is widely present in living matter and the authors refer to a review of the subject (Hastings, 1966a, 1966) involving these reactions. A linear relationship was found between light intensity produced and FMN concentration. Hastings provides a table showing FMN concentrations in bacteria, urine and blood.

11.1 Applications of ATP and Chemiluminescence

In the early 1970's papers and instruments began to appear in substantial numbers applying chemiluminescence to practical applications. In 1973 Owens (Owens, 1973) describes the application of the Luminescent Biometer (DuPont) to monitor bacteria in process water. As the Food and Drug Administration includes bacteria and an adulterant, the water quality is required to be continuously monitored. This paper describes the use of the ATP-luciferase raction for quick monitoring.

The ATP procedure was extended by Kimmich (Kimmich, 1975) to ADP and AMP at picomole levels. As an instrument they utilized an unmodified scintillation detector. Their modifications included the use of calcium phosphate-arsenate extraction of firefly lanterns, low arsenate during the assay and pH 8.0. The sensitivity was said to be 5 times better than previously reported. The method was suitable for perchloric acid extracts of biological tissue including bacteria.

In the same year (Wettermark, 1975) the luciferase-ATP reaction was applied to ATP determiantions in single cells down to subfemtomole quantities. Microtechniques were described including

photon counting with multichannel techniques. They used the microorganism Paramecium Peridinium and also microphages from the abdominal cavity of mice for their assay. These cells ranged from 0.07 to 2.5 ng dry mass.

As the problem of stability of reagents was always present, the matter was studied in 1977 by Spiegel (Spiegel, 1977). This was important especially if the procedure is to be automated and run unattended for a period of time. The author felt that 1 week stability was essential for such applications. At that time, 1976, it was found that prepared liquid reagent had a maximum useable life of 2 days unless stored in a refrigerator. By the 1980's, using better purified reagents, manufacturers claimed an extended lifetime for their reagents to exceed 1 week stability, and some published data has been available to substantiate these claims.

As recently as 1983 (Hinkkanen, 1983) papers continue to appear, and will probably continue, determining FAD content of bacteria with the peroxidized luminol reaction. This luminometric method is preferred over other techniques due to its ability to respond with subpicomole quantities, a substantially lower level then otherwise attainable.

11.2 Clinical Applications of ATP and Chemiluminescence

A great deal of attention, and thus many papers, has been devoted by hospital laboratories to the detection of bacteria, especially at the level considered to be "infection", about 10^5 bacteria/ml in a variety of body fluids, especially urine and bood. The preferred method, aside from stains discussed later in this report, has been the detection of ATP. The approach used is clearly illustrated in an early paper on the subject by the Swedish investigator Thore (Thore, 1975). In body fluids one must differentiate between ATP in bacteria from ATP in other body cells. Therefore, the biological specimen is first mixed with a detergent, usually Triton-X-100, and an ATP destroying enzyme apyrase. This does not effect the ATP in the bacterial cells, but does eliminate the ATP from body cells which may be present. The mext step is to run the ATP in the bacterial cells by boiling briefly in buffer to extract the ATP which is then submitted to luciferase assay. This procedure gives results well correlated with results of the same sample after culturing. The author suggests the procedure for screening urine for bacteria.

A paper critical of this approach appeared in the same year (Conn, 1975) entitled "Limits of Applicability of the Firefly Luminescence ATP Assay for the Detection of Bacteria in Clinical Specimans". The author felt that the limitations of this approach sharply curtailed its applicability due to various inhibitors found in urine. Only after bacteria had been separated from the rest of the specimen did the authors feel that the method would be useable. An interesting observation to explain previously reported good correlations with cultures was that leukocytes (white blood cells) present where infection existed were not destroyed in the

pre-treatment thus releasing their ATP along with the bacteria giving a measure which correlated with infection. Also, the range of ATP in various microbial organisms ran from 12 to 200, from S. epidermidis to E. coli. They made the point that in this doctoral thesis J.S. Ames successfully used ATP to study the effectiveness of antimicrobial materials by following change of ATP in cultures and that this was a legitimate useage.

Several comparative studies made at a later date have been reported. A typical report was presented by a Finnish investigator (Ruskonen, 1982) of a detailed study which showed that normal urines and those with infection had approximately the same level of leucocytes, and in neither case gave significantly higher ATP results. They also pointed out the value of negative results of a screening procedure. Positive results could be further investigated for identification purposes, but the work load was greatly reduced by the negative results.

11.3 Automated Methods of ATP and Chemiluminescence

Because of the great number of assays conducted by clinical laboratories there has always been a great interest in automating the procedure used. ATP determinations have not been neglected by such interests. An early report, contributed as a by product of space studies was described (Picciolo, 1971) in a bulletin from the Goddard Space Flight Center. A great deal of detailed information is given on construction of the manual instrument and its use, as well as data obtained and compared to other methods for a number of bacterial species. Automated equipment, called the "FLASH" is then described in detail but at the time of publication had not been put into use.

In 1974 a Swedish report (Ewetz, 1974) described a procedure adapted to the Auto Analyzer (a commercial set-up for many varieties of automatic analysis). This report applied Luminol to determine iron-organic compounds, many of which are present in bacteria, rather than ATP. Perborate was used in place of hydrogen peroxide, but otherwise it was a standard Luminol procedure. An interesting procedure was used to bypass difficulties from inorganic iron compounds which cause a very rapid glow, but one which dissipates rapidly. The iron-organics of biological origin reach a peak after 15 seconds, then slowly decay, at which time the glow from inorganics is gone. Readings are therefore taken after 15 seconds. Blood present in poorly sampled speciment gave false positive, but 99 percent of the urine samples with infection gave positive results. Seven percent of false positives occurred with other types of samples, however other standard procedures also give their percentage of false positive and negative results. The authors considered the procedure to be suitable for further evaluation as a mass screening technique.

Two years later an English report (Johnston, 1976) entitled "An Automated Test For Detection of Significant

Bacteruria" described an ATP procedure which was claimed to be more sensitive than a conventional culture using a standardized loop. Results were obtained in 30 minutes and were not effected by non-bacterial elements. The equipment used was a Technicon, similar to that in the previously described paper, which runs samples continuously. Urine speciment (95% samples) were run directly as received in a routine way. It was pointed out that the high cell counts in the absence of bacteria did not give false positives, which is an intrinsic property when alkaline Lumionol is used as mentioned in the previous paper.

An exceptionally simplified automated system using a moving tape on which all of the reactions are conducted was described in 1977 (Piccolo, 1977). The paper described the performance characteristics of a new instrument manufactured by the Vitect Corporation of Alexandria, Virginia using the ATP reaction. Filter paper tape commercially available is used for sample processing and cellophane tape is used as a backing before adding reaction reagents. Automated hypodermic syringes are used for adding reagents and the published tables showed that good results could be obtained in 2 minutes from one ml of sample with 7×10^3 bacteria. The instrument appears to have excellent potential.

McWalter (McWalter, 1982) evaluated a commercially available semi-automated procedure using the ATP approach. The instrument was a Lumac Biocounter and they compared results from 2000 urine samples run by a routine method and the bioluminescent procedure. They catered to 10^4-10^5 bacteria per ml as being the cut-off for bacterial infection. No false negative results were obtained. High false positive were obtained from maternity specimen, about 3 times as many as in the general population, due to the presence of high leels of Lactobacilli and diptheroids in the maternity group. They also mentioned the helpful nature of rapid negative results and its dependenability.

An article which is almost exclusively an instrumental paper (Marino, 1981) entitled "A Microcomputer-controlled Intensified Diode Array Data Acquisition System for Chemiluminescence Spectra" was described. A 512 point spectrum of the flash of the chemiluminescent reaction from 200-840 nm was obtained in nanoseconds. Readings can be re-run to average them for better S/N ratios and background can be substracted while obtaining the spectral plots.

Because manufacturers of photometers are anxious to popularize appropriate methods employing their equipment some of them issue excellant research. ATP measurements are particularly well researched such as the results published by Turner Designs of Mountain View, California, which issues bulletins of an informative nature on ATP reagents and procedures and points out means of avoiding pitfalls. For example Bulletin No. 202, February 1981, compares the use of glass and plastic tubes in which ATP reactions are carried out and finds that the decay rate

of the glow is almost double in glass vs plastic. Bulletin No. 204, August 1982, gives useful information on reagent preparation. The statement is made that reagent diluted for use on Monday will be 25-50% effective by Friday, which is still sufficiently effective to be useful.

11.4 Conclusions of ATP and Chemiluminescent Techniques

Bacterial detection has been widely practiced using chemiluminescent techniques, especially in the detection of ATP in a hospital setting. Among other advantages, the presence of ATP shows viable, or very recently viable, organisms to be present, and therefore is more significant than some other rapid bacterial tests.

For purposes of detection on aerosol particulates it would be necessary only to impact the aerosols from a few cubic meters or less of air into a presently available phosphorimeter and allow routine determination of ATP to proceed. Such a procedure, combined with automated equipment presently available should yield definitive results every few minutes, or at any desired interval. It is unlikely that great difficulty should be encountered.

12. MASS SPECTROMETRY

Mass spectrometry is a theoretically simple but instrumentally complex approach to the analysis of volatile molecules. It is capable of detecting and quantifying all of the molecules and fragments of molecules, basically by separating and detecting them by molecular weight.

Although the design and properties of mass spectrometers has proliferated in the past decade the basic concept is the same. The sample is passed as a vapor into an evacuated chamber where it is ionized and movement imparted to the molecules of vapor. As it passes through electric and/or magnetic fields the trajectory of each molecule depends on its mass and charge. A detector, or detectors, are positioned so that each of the separated streams of molecules, as determined by their mass/charge ratio, is impacted precisely at one detector.

A considerable body of literature exists devoted to the use of such instrumentation to analyze atmospheric particles as well as directly on bacteria in a variety of modes. Some mass spectrophotometry has been done on bacteria by pyrolyzing the whole cells and sending the vapors directly into the spectrometer, others first do chromatography on the vapors before analyzing each fraction, but all are capable of providing definitive information on the molecules present. Another variation uses so-called microprobe technology where a single cell can be placed under the microscope, vaporized with a laser and a mass spectrmetric analysis of the resulting vapor is

achieved. A commercial instrument built for this purpose called LAMMA will be discussed later.

One drawback to the use of mass spectrometers as direct analyzers for natural samples is the enormous wealth of data which emerges. Everything present is recorded, so that any meaningful interpretation may be difficult. Therefore the technique is often coupled with a preliminary separation by gas chromatography, the so-called GC/MS which is widely used. This satisfies the needs for two types of analysis. Those who are primarily interested in chromatrography achieve a greater knowledge of each fraction as it emerges, and those whose primary interest is in the mass spectrometry acquire a simpler spectra to interpret.

In 1973 a book was published titled "Techniques of Combined Gas Chromatography Mass Spectrometry", (McFadden, 1973). Clinicians have a special interest in GC/MS systems and a report on such systems was presented by Gochman (Gochman, 1979). Among other uses is the rapid identification of bacteria from their characteristic molecular fragments to give a definitive identification of the microorganism. Goshman refers also to previous work on this use (Burlingame, 1978). A number of commercial systems are described suitable for this application.

12.1 Applications of Mass Spectrometry

Among the earliest works to be reported on whole bacterial organisms analyzed by mass spectrometry after pyrolysis and gas chromatography is one described by Simmonds of the Jet Propulsion Laboratory in connection with extraterrestrial life exploration (Simmonds, 1970). Recognizing that gas chromatography had already given "fingerprints" for bacteria (Reiner, 1965) Simmonds coupled the gas chromatograph to mass spectrometer and studied the results. He used micrococcus lutens and the common soil bacterium Bacillus subtilis var. niger, pyrolyzed at 500° C. He found that the products were primarily those obtained from pyrolysis of any proteins and charbohydrates. He identified 38 protein produced fragments, 15 produced from carbohydrates and about 15 from nucleic acids, lipids and porphyrin. These were based on a two second interval analysis during the elution of chromatographic peaks.

A direct insertion of bacteria into the mass spectrophotometer was described by Anbalt (Anblt, 1975). He felt that under the conditions of the pyrolysis, temperatures obtained by direct insertion into an ion source as well as under the high vacuum, the products would not be subject to thermal degradation. His results on two pyrolized staphlococcus speciments showed a substantial differenct. On five gram-negative bacteria spectra were more similar, still, specific differences could be seen. In working with cross-sections of bacterial types he concluded that different taxonometric

classifications gave significantly different spectra but species differences could only be defined by differences in the relative amounts of the molecules present.

Applications to aerosol particles have been given some attention. An interesting approach was described by Schulten (Schulten, 1975) who impacted aerosols directly into the field-emitter, which he then placed into the mass spectrometer. This provided a simple and rapid technique for aerosol analysis. Both inorganic and organic constituents could be identified. The instrument used was a field desorption mass spectrometer (FD-MS). This technique has been previously described by Schulten in 1973 in an application to deoxyribonucleic acid. It is interesting that the author found no particulates which had both inorganic and organic matter.

Schultz (Schultz, 1974) has described studies of airborne particulates and associated them with possible pollution sources, as did Voorhees (Voorhees, 1981) at a later date. Voorhees pyrolyzed samples at 450° C directly into a mass spectrometer. By combining the data obtained with pattern recorgnition procedures in some cases classification was possible, as well as characterization of a variety of complex molecules. Bacteria were not specifically addressed.

In a study at the Jet Propulsion Laboratory in an attempt to do mass spectrometry on single particles in the micron range, Sinha (Sinha, 1982) set up studies on PAMS (particle analysis by mass spectrometry). This work stemmed from an earlier work by Davis (Davis, 1977) who used a needle valve to impinge a jet onto a hole 0.05-0.07 nm in diameter which allowed single particles to impinge on a rhenium ribbon filament. The heated filament produced surface ionization the ions of which then passed into a mass spectrophotometer. Some of the parameters during this work were not well controlled. Sinha repeated this work but with increased control, with the result that he could do real-time continuous spectrometry on experimentally generated aerosols. Most of the described work dealt with inorganic particles.

In addition to GC application, liquid chromatography (LC) has also been coupled to mass spectrometry. An interesting review of recent developments in this field (Science Editor, 1983) has described how these techniques can be successfully coupled. Many molecules can not be handled successfully by GC and are better managed by LC, although the subsequent mass spectrometry is not as easy as with GC. The liquid may be sprayed into a chamber with a nozzle leading to the mass spectrometer inlet or onto a heated belt which passes into the ionization chamber and is then suitable for "soft-ionization" discussed below.

12.2 Instrumentation Used in Mass Spectrometry

Mass spectromety has been in use for a long tme and is an established approach to the analysis of volatile compounds. However, in recent years a great interest has developed in biological molecules of poor volatility and of difficult identification after fragmentation. To meet this new interest many instruments and methods have been devised to reduce these fragmentation problems under the general title of "soft ionization". A symposium held in London in 1980 on advances in soft ionization mass spectrometry was summarized in a book by morris (Morris, 1981). All of the modern MS methods discussed concerned biopolymers such as proteins and other bioorganics in submicrogram quantities.

In a book entitled Analytical Problems (Bahr, 1981), a large section (35 pages) is devoted to different instrumental approaches to mass spectrometry. Described are gas discharge mass spectrometry, plasma ionization mass spectrometry (PIMS), field desorption mass spectrometry (FDMS), and a short section on directly sampled natural aerosols.

Several review articles on molecular secondary ion mass spectrometry (SIMS) described this surface technique (Day, 1980), Scheifers, 1982), Laxhuber, 1983). SIMS is used for a wide variety of nonvolatile and thermally fragile molecules, especially biochemicals, organics and organometalic compounds.

The article by Scheifers particularly goes into the details in a clear, well illustrated description. In this technique on ion gun shoots primary ions onto the surface bearing the sample. Secndary ions released from the surface are then sent to the mass spectrometer. The method has been used on paper chromatograms and directly on plant surfaces, an example being shown of a fungus spectrogram. Applications to biological and environmental samples are well documented. Alkaloids, vitamins and nucleosides have been reported. It is expected that this technique will continue to develop.

An instrumental technique called the ion cyclotron resonance mass spectrometer has been improved and described recently in a paper by Kemper (Kemper, 1983). This instrument is more rugged and simpler than earlier mass spectrometers. It attempts to separate regions of ion formation and reaction from each other and thus is more selective. A greatly simplified explanation of ion cyclotron (ICR) is that it causes a spiral movement of ions to intersect a conductor causing a current to flow proportional to the ion flux intersecting the conductor. By expanding the spiral the resulting change in current recorded provides a spectrogram. The device has the advantage of being extremely sensitive, fast and inexpensive. At this time however it has been applied in a limited manner, primarily for basic theoretical studies, but its use will undoubtedly be further extended.

A special instrument referred to earlier, the laser microprobe mass analyzer (LAMMA) is especially suited for small particle analysis. In essence, it couples a laser for vaporization with a microscope for locating the specimen and a mass spectrometer. It is an ideal way to identify bacteria findable by a microscope and indeed this has been reported (Seydel, 1981). The Bureau of Standard has used this device and has issued a special bulletin (NBS Special Publication #533, 1980) entitled "Laser Microprobe Mass Analyzer-Lamma in Particle Analysis". This instrument is sold at this time only by the Leybold-Heraeus company at Export, Pennsylvania and a large bibliography is available on the subject from this manufacturer.

The basic principles of the instrument has been elaborated, along with some performance characteristics by Denoyer (Denoyer, 1982) and some applications to studies of molecular structure by Hercules (Hercules, 1982). The ability to analyze a single cell or even a portion of the cell makes the instrument unique for sample size requirements especially among surface analysis techniques.

A study of particulate matter in air was reported by Surkyn (Surkyn, 1983) recently for both organic and inorganic constituents. The study of individual particles, done in several areas of the world showed that the sources of the particles could be identified based on their composition.

Biomedical polymers using positive and negative ion mass spectra have been reported (Gardella, 1980, Graham, 1982) using the LAMMA. The literature using this instrument is substantial, only a small proportion has been mentioned.

12.3 Conclusions of Mass Spectrometry Techniques

Mass spectrometry can be useful in detection and identification of microbiological aerosols in any of several ways. It would be possible to impact the particulates onto a small surface which would then be looked at by the SIMS procedure for surfaces described earlier.

Samples may be accumulated into a receiver enclosed in a mass spectrometer then periodically vaporized and run directly or first passed through a gas chromatographic column and a mass spectrographic curve run on each fraction as it emerges.

As far as the LAMMA instrument goes the possibility of automating the sample handling in a meaningful way would seem to be difficult. However, because one can obtain mass spectral data from single cells it would seem to be an ideal research instrument for the accumulation of a library of spectra useable to make the above two approaches most meaningful.

13. POLARIZED LIGHT

It is well established since the early 1800's that certain atomic architecture, where carbon atoms have four different structures attached, are capable of rotating light in one direction or another. Furthermore, it is also well known that living organisms produce only those forms of molecules which rotate light to the left. It would therefore be reasonable to suppose that bacterial detection might be accomplished by observing the presence or absence of this rotatory ability.

Because polarized light vibrates undirectionally it is well suited to measurement of optical rotation. Furthermore polarized light is capable of vibrating in a plane whose angle can be measured, or in a circular direction, right or left. The whole spectrum of wavelengths can be used and either of the above modes so that a variety of spectral studies are possible. For each it is possible to show a spectral curve containing useful but different shapes wich can serve as fingerprints for identification.

An early text on the subject, (actually the proceedings of a NATO summer school at Bonn, September, 1965), gives an excellent discussion of optical rotatory dispersion (ORD) and circular dichroism (CD), (Snatzke, 1965). These, along with UV spectral curves provide the organic chemist considerable structural information and considerable related work has been published.

As with all techniques the methods developed most rapidly when convenient instruments are designed and become easily available. A book on instrumentation titled "Polarization Interferometers", (Francon, 1971) published in Paris, France helped considerably by describing applications in microscopy.

By 1973 some work had begun to appear on applications to bacterial identification (Torten, 1973). The authors used five different species of bacteria and presented spectral curves of UV absorption from 185-310 nm as well as circular dichroism curves (CD) across the same range. In the latter case circularly polarized light is measured at each wavelength as the difference in absorption of right and left circularly polarized light as the angular rotation changes. The result may be positive or negative depending on which is the preferred absorbance. The CD curves were seen to be quite different and the UV curves were somewhat different so that these five species could clearly be differentiated. The concentration of bacteria was 10^8-10^9 cells per ml. The light patch was 1.0 mm and measurement was done in a Cary model 60 spectropolarimeter. When plane polarized light is scanned across the spectrum an optical rotatory dispersion (ORD) curve is obtained. ORD and CD curves together provide a good informative data base often used for optically active molecules. These are well described by Morris (Morris, 1978) in a chapter on carbohydrate metabolism. As carbohydrates enter to

a significant degree into bacterial wall structure it is of special interent here.

A recent paper that was particularly suited to our study, with the title "Rapid Identification of Microorganisms by Circular Intensity Differential Scattering" is reported by Los Alamos workers (Salzman, 1982). they made a scan of the differential scattering of left and right circularly polarized light (CIDS) measured at 90°. The scan took 4 minutes on a slightly modified recording spectropolarimeter. In their discussion they state that either intact bacterial cells or virus suspensions provide a unique signature to identify the microorganisms. The virus signals were 10 times stronger than those from bacteria. They claim that DNA contributes significantly to the signal although other factors do not enter into the overall effect. At the stage of instrument development of the report sensitivity was not adequate for concentrations normally found in body fluids but further work was being carried on.

An illustration of the practical use of the technique of circular dichroism is given in a paper describing its use to detect the drug Heroin (Bowen, 1982). It was found possible to take a sample directly without checmical separation. The author compares the technique favourably with mass spectrometry or gas chromatography. They make their measurements at 253 nm in an alkaline solution and have the cmpleted results in 20 minutes.

13.1 Conclusions of Polarized Light Techniques

At this stage of development one can not expect to apply circular dichroism or its related rotational techniques to aerosols to detect microrganisms due to the present lack of sensitivity.

However, should instrumentation be developed specifically for small samples with increased sensitivity it is theoretically a direct and logical approach.

It is particularly interesting for virus detection as the measurements respond to DNA unusually strongly, 10 times more than for bacterial contents.

14. STAINS

The reaction of various proteinaceous structures with dye materials has been used for a long time particularly in cellular studies done under the microscope. Bacterial cells have long been identified and divided into Gram positive and negative types by appropriate stains on microscope slides. Dyes capable of fluorescence added specificity, where certain structures reacted with the fluorescent dye could be pin-pointed among non-reactive materials. The history of stains has always been intimately tied to microorganism detection and identification.

An early report of direct interest to our project was given by Pital (Pital, 1966) of Fort Detrick, Frederick, Maryland. In this work Pital concerned himself with the possibility of using fluorescent dye labelling of microorganisms with a view to detection under extraterrestrial conditions. He reacted the sample with the dye fluorescein isothiocyanate which stained protein, but was easily washed from non-protein matter. Previously, reactions involving specific antibody stains had been reported, but the investigated dye was chosen because it was rapid and non-specific. He cites two possible sources for false positives. If self-fluorescent materials are present or if some non-positive materials might unexpectedly give fluorescence an error may occur. However, the first problem can easily be by-passed by running a sample blank and the second can be studied as its real significance.

Many workers have applied a variety of fluorescent dyes since then. A particularly good one, O-phthaldehyde was reported by the Swiss chemist Roth (Roth, 1971). He reported that in alkaline solutions, when mixed with a reducing agent such as 2-mercaptoethanol and any amino acid, strongly fluorescent compounds were produced, especially at 340 and 455 nm. Nanomole quantities could be detected in 5 minutes after adding reagents. He compared this dye with ninhydrin, a frequently used stain, but found O-phthaldehyde to be much better.

Experiments to investigate specific structural sites pushed fluorescent markers still further. Kanaoka (Kanaoka, 1973, working in Tokyo, was interested in the sulfur bearing amino acids in proteins and reported on N-(1-Anilinonaphthyl-4-) malemide (ANM) which had the interesting property that it was not fluorescent itself but produced a strongly fluorescent product with sulfur groups in amino acids but not with non-sulfur amino acids. Because ANM was fairly stable at room temperature in solutions he expected it to become a useful reagent.

As time progressed some earlier reported work was nvestigated for shortcomings and possible improvements. In 1975 O-phthaldehyde referred to earlier, which was found to be poor for primary amines was re-worked by Benson (Benson, 1975). This worker reported that by increasing the reducing agent 2-mercaptoethanol by a factor of 10 and adding a more suitable buffer, most of the shortcomings were overcome while retaining its high sensitivity. He claims that picomole amounts of amino acids, peptides and proteins could be easily detected.

The existence of the newer stains did not seem to deter some workers from publishing papers using old stains. Lewis (Lewis, 1976) evaluated the Gram stain as applied to smears from urine and arrived, not unexpectedly, to the conclusion that smears on centrifugal samples stained by this techniques correlated well with cultures.

One of the dye stains in use, Bromosulphothalein (BSP) was looked at (Wallace, 1978) from a different point of view. If the protein molecule reacts with a sufficient number of dye molecules it can be measured by light scatter due to the insolubility of the complex. However, it was found that collagen does not react with sufficient dye to make it insoluble. Therefore it scatters very little light. As collagen is often used in media it is a usefule technique to test for protein without interference from collagen. Light scatter techniques after dye addition became popular in hospital urine analysis and automated analyzers used this method. In the 1980's (Jenkins, 1980) a paper titled "Rapid Semiautomated Screening and Processing of Urine Specimens" compared a number of methods in use.

Another popular stain, acriding orange, was looked at for the detection of microorganisms in blood (McCarty, 1980). With this dye it was found that microorganisms were detected that were often not detected after 1 days incubation in a culture, and therefore was a rapid inexpensive alternative to older culturing techniques.

Wallis (Wallis, 1981) used the dye safranine in a very efficient rapid test, to determine if bacteria were more or less than 10^5 per ml. The test could be done in one minute using 1 ml of urine. The 1 ml sample is passed through a paper filter, the safranine dye is passed through, and then a decolorixer is passed through. If the filter paper is pink there is a bacterial infection. If was interesting that patients taking antibiotics, but still giving positive tests truly had bacteria present, but cultures made at the same time were negative due to the inhibitory effect on growth by the antibiotic present.

Two papers published by Paul (Paul, 1982, 1982a) both deal with Hoechst produced dyes, #33258 and #33342. Both of these are specific for DNA and were used to count bacteria directly in natural waters by the fluorescence produced. These dyes are proposed to replace acridine orange, which, in natural present. The proposed dyes, it is claimed, bypass this difficulty.

14.1 Conclusions of Staining Techniques

Staining bacteria to detect and identify them is a time honored practice. If aerosol particulates are impacted onto a surface they can doubtless be usefully detected and can be available for identification.

With pattern recognition techniques, applied after staining, it should be possible to automate a one minute detector. The chemistry and microbiological interpretation is well established at this time.

15. EXPERIMENTAL WORK

As a result of the library work required in the contract, some experimental work of an exploratory nature was suggested and performed to evaluate the feasibility of leads developed which were new and sufficiently simple for possible field use. The two sets of experiments are described here.

15.1 Photoacoustic and Diffuse Reflectance Spectroscopy

Photoacoustic spectroscopy (PAS) discussed earlier is a technique for obtaining a spectral curve similar to an absorption curve but using much less material than needed for absorption. It is used to examine the composition of surfaces. If microbiologicals are impacted onto a surface, they become amenable to this technique, which might then serve as a means of detection. A complimentary technique, diffuse reflectance spectroscopy (DRS) also previously described is used for the same purpose and is done with similar equipment, therefore both techniques were used and compared in our experiments.

Briefly, in photoacoustic measurements, the sample to be examined is placed inside an enclosure with a cover transparent to the wavelengths to be studied. The sample is enclosed in a small volume of any gas or air with a microphone or a piezoelectric detector to serve as a microphone. The whole chamber is usually mounted in a spectrophotometer which serves to provide illumination of narrow wavelength range to illuminate the sample. This incident light is chopped or modulated at audio frequency, which is required for microphone or piezoelectric detection. As the spectrophotometer scans across its range of wavelengths illuminating the sample, the sample absorbs those wavelengths which are characteristic of it, heating the gas above it, changing its pressure which is sensed by the microphone. The output of the microphone is scanned at the same rate that the spectrum is scanned and the result is a spectrogram for the sample which is essentially identical to an absorption curve. Thse peake ont he curves can be compared to the extensive literature on absorption curves.

The diffuse refectance technique uses the same spectrophotometer set-up except that the sample is not enclosed and the microphone is replaced with a photedetector. This technique has been in use for a long time but instrumental improvements have occurred over the years. The curves produced appear inverted relative to the photoacoustic curves because reflected light is reduced when the sample absorbs more, whereas with photoacoustics the response increases. However, the information is nearly the same, the diffuse reflectance resporses being somewhat greater. It should be noted that the graphs bear the notation transmittance on the ordinate but they are in reality reflectance measurements.

Both techniques were applied over two wavelength ranges 200-2600 nm (0.2-2.6mu) and 4000-500 cm^{-1} (2.5-20mu), which covers from UV into IR.

The curves were normalized to a standard material in both cases. For photoacoustics (PAS) the spectra were referenced to carbon black for all readings. For diffuse reflectance spectroscopy (DRS) the IR curves were referenced to KCl but for the UV-Visible - Near-IR, the samples were referenced to the appropriate blanks. All IR was done as Fourier transform IR (FTIR).

15.1.1 Experimental Design

The investigation of the photoacoustic and diffuse reflectance spectroscopy was conducted using the materials indicated here.

All substances were placed on Whatman glass microphore filters with 2.1 cm diatmeter. The organism, Lypholized Bacillus subtilis var niger spores, was used. For a chemical 0.1% detergent, Zwittergent, said to be able to dissolve cell walls, was used. This was 0.1% Acridine Orange in ammonium acetate adjusted to pH 4.

15.1.2 Equipment and Parameters

The equipment used in the photoacoustic and diffuse reflectance spectroscopy equipment is indicated here.

- FTIR-PAS: IBM 9195 FTIR spectrometer

  Princeton Applied Research 6003 photoacoustic cell and peripheral electronics.

  mirror velocity: 0.059 cm/sec
  resolution: 8/cm
  number of scans: 128
  all spectra referenced to carbon black unless otherwise noted.

- FTIR-DRS: IBM 9195 FTIR spectrometer

  Harrick Scientific Model DRA-2CN DRS Attachment.

  mirror velocity: 0.396 cm/sec
  resolution: 4/cm
  number of scans: 128
  detector: LN2 cooled MCT
  All spectra referenced to KCl

- UV-VIS-NIR PAS: PAR 6001 photoacoustic spectrometer equipped with PAR 6003 PAS cell.

  modulation frequency: 20 Hz
  resolution: 8 nm in UV-VIS
  32 nm in NIR
  number of scans: 1 scan at 100 nm/min
  All spectra referenced to carbon black.

- UV-VIS-NIR DRS: PAR 6001 spectrophotometer equipped with specially built DRS attachment (see Rohl, 1982 for description).

  modulation frequency: 20 Hz
  resolution: 8 nm in UV-VIS
  32 nm in NIR
  number of scans: 1 scan at 100 nm/min
  spectra referenced as indicated on individual plots.

15.1.3 Experiment Procedures

The experiments were set up in 5 batches as follows:

- BATCH 1

  Blank paper
  Bacillus 100 ng
  Bacillus 1 ug

The bacillus 3Bacillus subtilis var niger lypholized spores) was a fine powder and was placed onto the filter paper with a small spatula and then smeared across the filter paper disc. The weight is a visual estimate based on previous experience and is only to be considered nominal.

- BATCH 2

  H_2O Blank
  H_2O plus bacillus 10 ng
  H_2O plus bacillus 100 ng
  H_2O plus bacillus 1 ug

The second bach was intended to be a possible way of spreading the bacillus more uniformly then in the first batch. The bacillus was mixed with 0.2 ml H_2O stirred, then poured onto the filter paper held in a small funnel.

- BATCH 3

  Detergen Blank
  Detergent and bacillus 100 ng
  Detergent and bacillus 1 ug

The third batch was prepared like the second batch except that the water contained a detergent. The thought was that if cell walls could be dissolved the cell contents would be more accessible to the measurements.

o BATCH 4

Dye Blank
Dye plus bacillus 10 ng
Dye plus bacillus 100 ng
Dye plus bacillus 1 ng

The fourth batch was based on the idea that if the cells were stained they might give a more sensitive response to the two techniques employed.

o BATCH 5

The fifth batch was a repeat of Batch 1 intended as an indication of reproducibility.

After the samples were prepared it was found necessary to cut the filter paper carriers down to 12 mm diameter to fit into the instrument. The spectrometric measurements were done at the Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina under the supervision of Professor C.H. Lochmuller.

After the curves were obtained some data manipulation was performed. For all of the FTIR data by PAS, sample curves were divided by the blank curve data. For all of the UV-Visible-Near IR by PAS, blanks were substracted from samples and for the UV-Visible-Near IR by DRS, samples were divided by blanks. The purpose of these manipulations was to bring out significant features and were limited to the above by the nature of the data.

15.1.4 Results

The graphs presented for samples are almost all based on the comparison of 100 ng of bacillus subtilis.Interpreting spectral data requires an understanding and expectation for what might be present, as the possibilities for inbterpreting any given peak are many. Even when one is working a purified single compound, care must be exercised not to over interpret a single peak or even a few. Therefore, in this section it will suffice to indicate what lines and peaks were found. The later Discussion will attempt to interpret them.

In Figure 1 and 2, for dry paper and bacteria, the curve with the bacteria shows a peak at 3260 cm^{-1} which is 30 units above a baseline at 4000 cm^{-1}. Both curves have a near zero base line in the range 3750-4000 cm^{-1}. There is a middle-sized double peak centered around 2937 cm^{-1}. The sample has a

peak at 1630 cm^{-1} on a sharply rising slope. The blank also has a small peak at this point. When the bacterial curve is divided by the paper blank in Figure 13 a sharp peak is seen at 1630 cm^{-1} and two large bands at 2937 cm^{-1} and 3255 cm^{-1}.

Results with the water blank and detergent experiment were similar to the dry sample Figures 3 and 4 vs. Figure 1 and 2. Thus, the photoacoustic (PAS) experiment in the IR range can be said to show three potential peaks in the 1500 cm^{-1} to 4000 cm^{-1} range. From 500 cm^{-1} to 1500 cm^{-1} the paper blank absorption is so high that any contribution by bacteria would have been obscurred.

Looking at the UV-visible with PAS there is a peak at 300 nm which is clearly visible in Figure 26 and 27 and in the difference Figure 36. All of the other experiments with H_2O and detergent are repeats of a similar curve except in the case of the dyed smaple which contains an additional peak at just below 600 nm. All curves show a low flat base line from 800 nm to 2200 nm and beyond. We can conclude from the above that PAS shows only one strong peak in the UV at a little below 300 nm.

As regards the diffuse reflectance spectroscopy (DRS), which presents itself in an inverted form compared to PAS, with peaks pointing down, the IR range, Figures 16 and 17 show a clear set of peaks at about 3300 cm^{-1} and at 2900^{-1}. The blank rises to a maximum at 1500 cm^{-1} and also remains high. All of the other curves in the 500-4000 cm^{-1} range were similar.

The UV-visible range done by DRS showed only the same peak at approximately 300 nm as was seen by PAS, except that the dye sample, Figure 45 showed a peak near 500 nm. Thus the DRS showed three peaks in the UV for the bacterial samples at about the same locations as was seen for the PAS.

15.1.5 Discussion

The objectives of the study were threefold. First, to determine whether these techniques could differentiate blanks from samples at some practical level (nanogram range). The answer to this was apparently yes for the given sample. Secondly, to see if specific lines or bands could be identified. Four were found. Third, to compare the two techniques to see if one was clearly better than the other. As the instrumental set-ups used the same intensity of light source and the same samples, it was an unusual chance to make comparability studies. The results showed them to be comparable in producing needed measurements. However, the device required for DRS was simpler than the PAS cell and therefore has an edge for further development.

What do the peaks we observed tell us as they relate to reported IR curves? In the literature on UV-Visible spectra relating to proteins, it is stated that "once you have seen one

protein curve you have seen them all". They all have a peak at 280 nm which is their main and almost only feature. This agrees with the one peak we found in our sample in the UV. In the IR the two peaks at our near 3000 cm^{-1} are known to be characteristic of amines which is an essential part of the structure of proteins. At 1640 cm^{-1} many ketones have peaks as well as do other structures.

15.1.6 Conclusions

It can be seen from the curves that the results obtained using the equivalent of directly impacted particles on a paper strip do not require any of the pre-treatments investigated, using either PAS or DRS.

The study, which ranged from 0.2 m in the ultraviolet to 20 m in the infrared found 4 peaks which discriminated the microbiological specimen from the glass-fiber paper.

However, two of the peaks in the infrared near 3000 cm^{-1} are in the same range as is published for ammonium sulfate which is the most likely molecule to be found in atmospheric particulates. This is not true for the peak at 6.25 m (1600 cm^{-1}) in the IR or the one at 0.28 m (35,715 cm^{-1}) in the UV. For the purpose of designing a detector, a choice between these two wavelengths would favor the one in the UV because the light source and the optics would be simpler. Furthermore, choosing between PAS and DRS, there would be a preference for DRS, as it also requires simpler equipment.

The information developed here is suggestive. It should be further developed using specimens of blanks, simulants actually impacted onto paper and likely materials normally found in aerosol particulates. Should these additional experiments confirm the applicability of this technique, a simple device can be easily designed.

Such a device would take the following form:

A small vacuum pump would suck a few cubic meters of air through an impactor to selectively impact 0.5-5 m particles onto a strip of paper about 1 cm wide. The strip woudl then rotate into a dark chamber where the area impacted would then be illuminated by a source of UV. The reflected light would be passed through two filters to select light at 280 nm and 800 nm and their intensities compared. The difference would be proportional to the protein concentration. Both bacteria and virus should give positive results.

We can conclude that our sample of bacteria was detectable on glass fiber paper by either of the above methods. We have not established what might also give positive results. The most likely contaminant in particulates in the size range bacteria occur in air is ammonium sulfate. This does have a

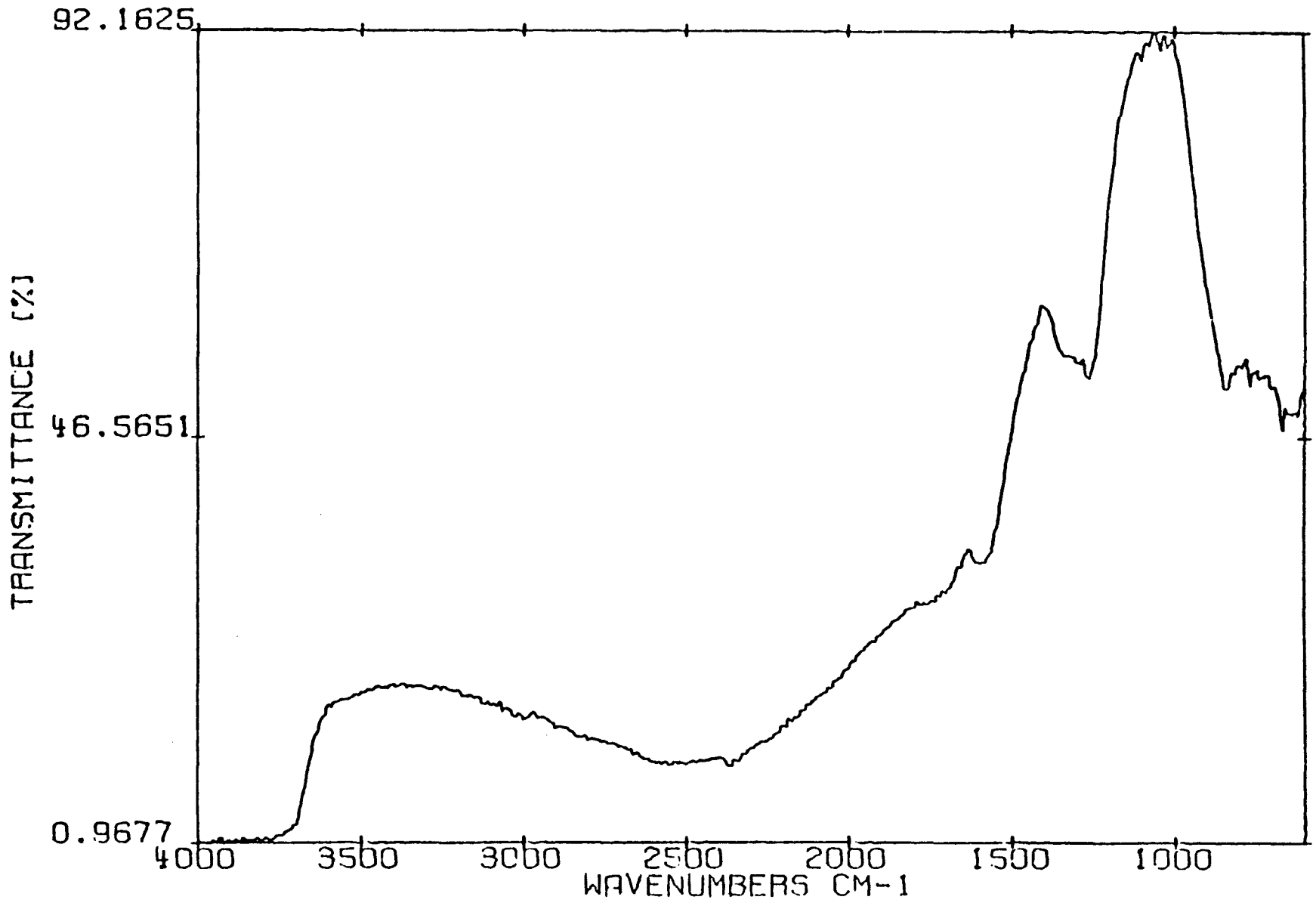


Figure 1 FTIR-PAS of blank paper

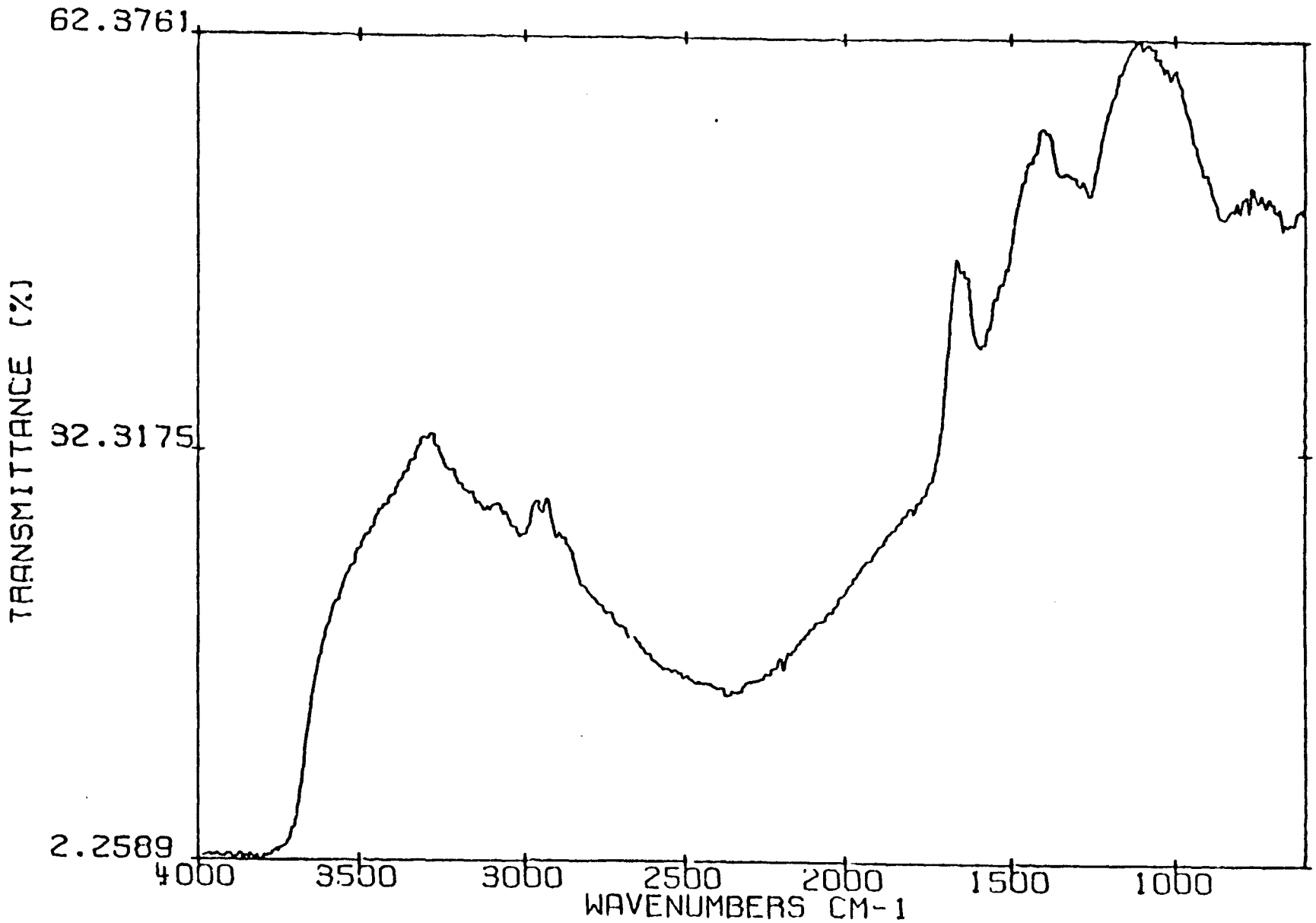


Figure 2 FTIR-PAS of protein A 100 ng

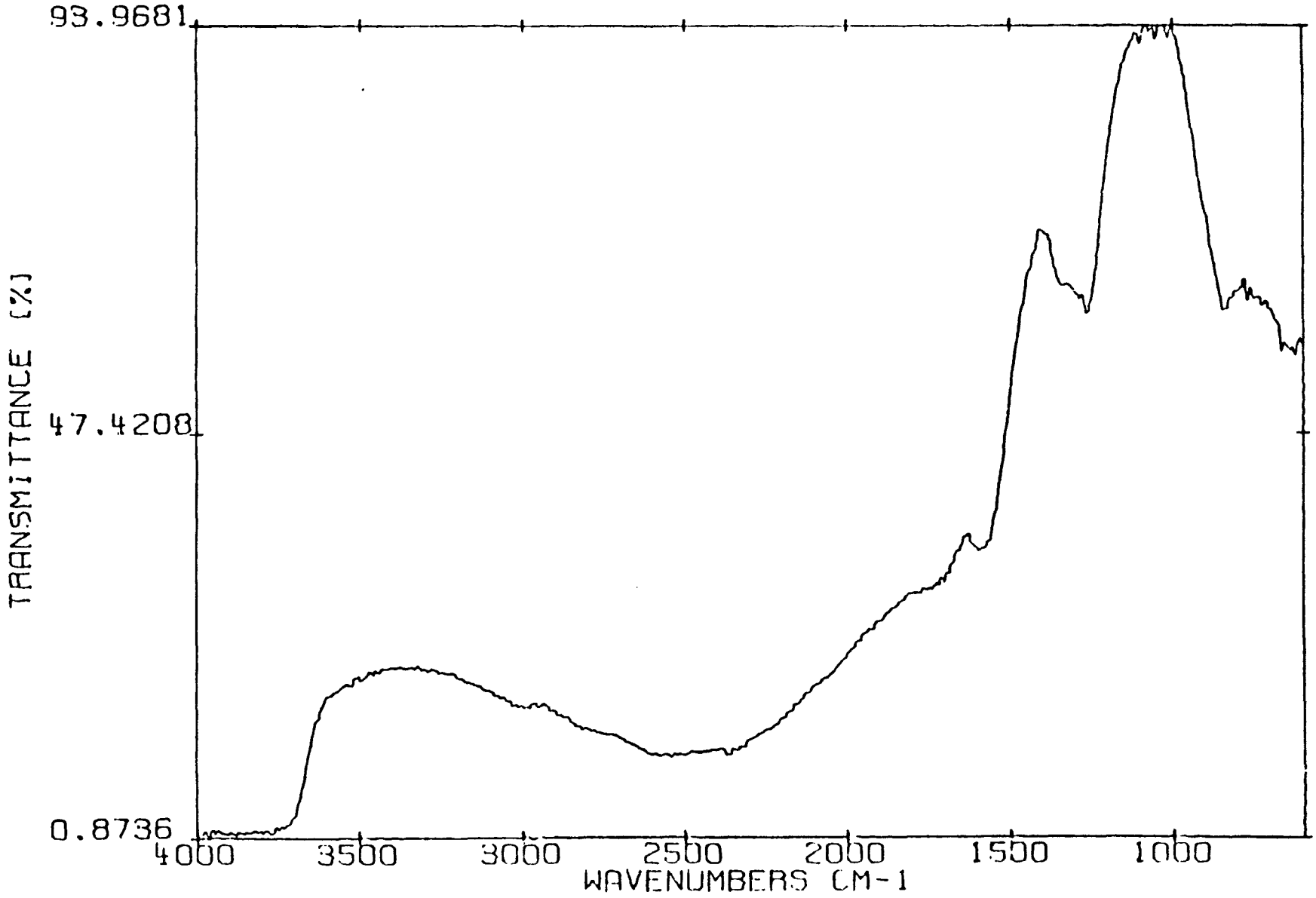


Figure 3 FTIR-PAS of H_2O blank

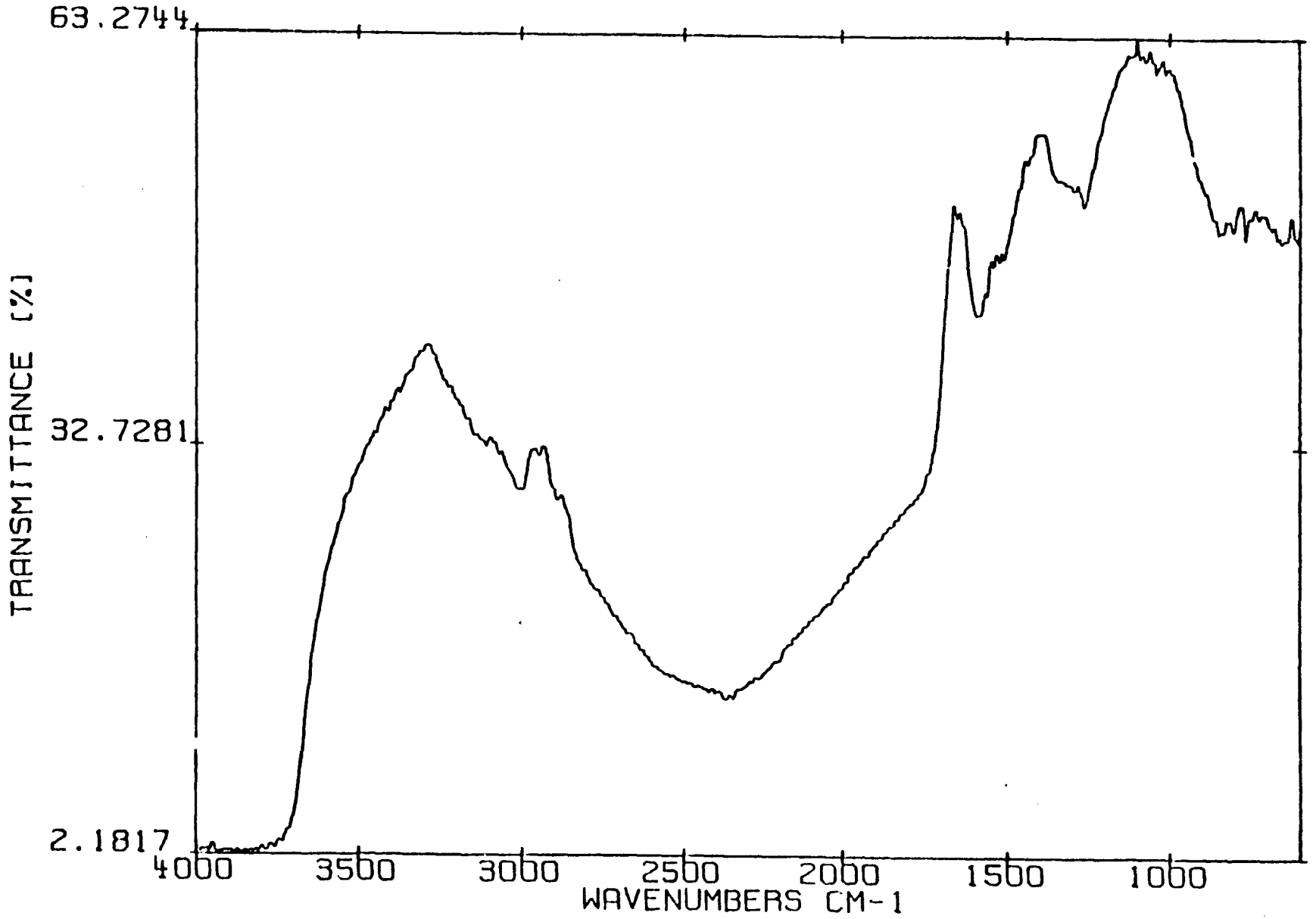


Figure 4 FTIR-PAS of H_2O blank and protein A 100 ng

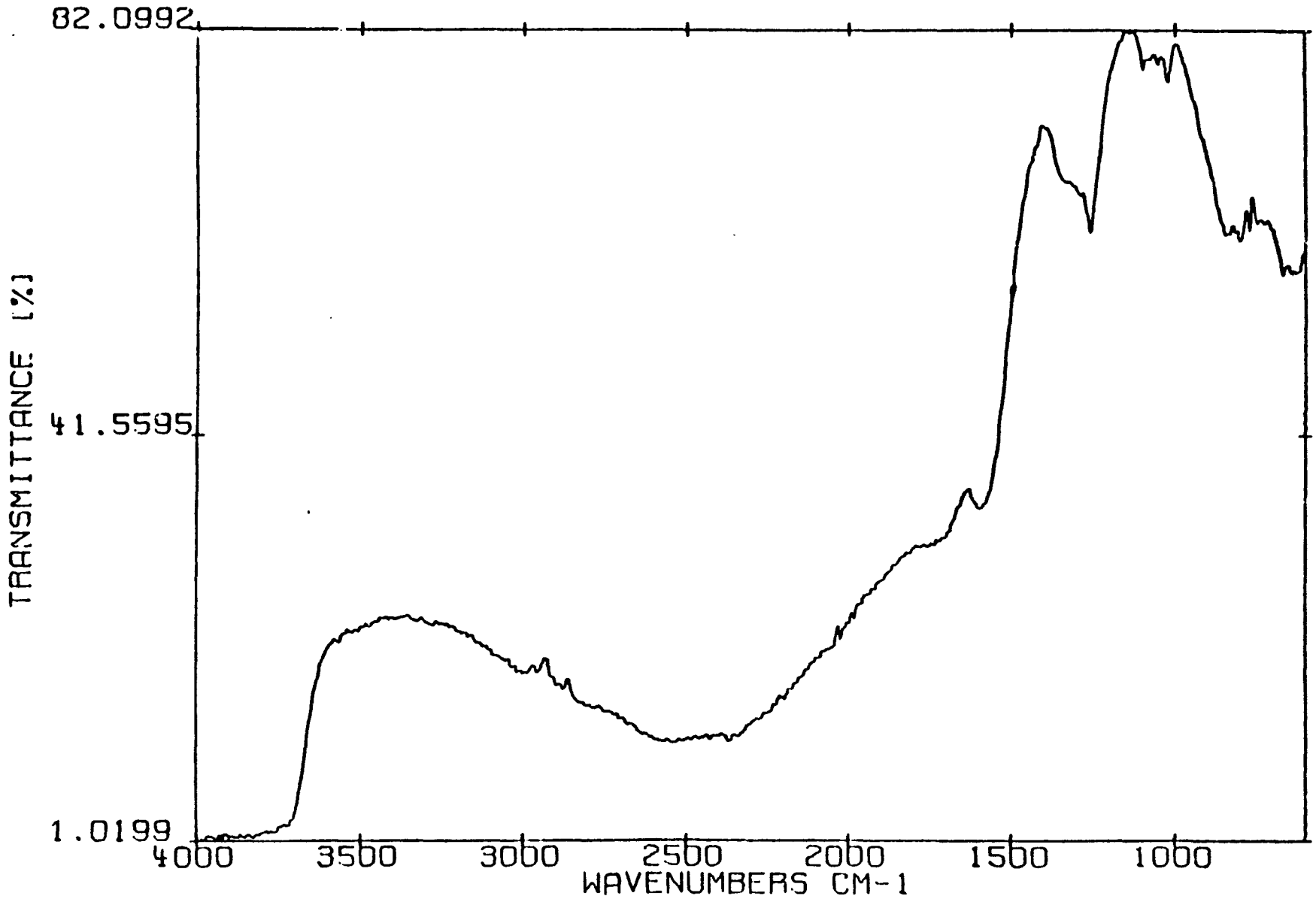


Figure 5 FTIR-PAS of .1% detergent

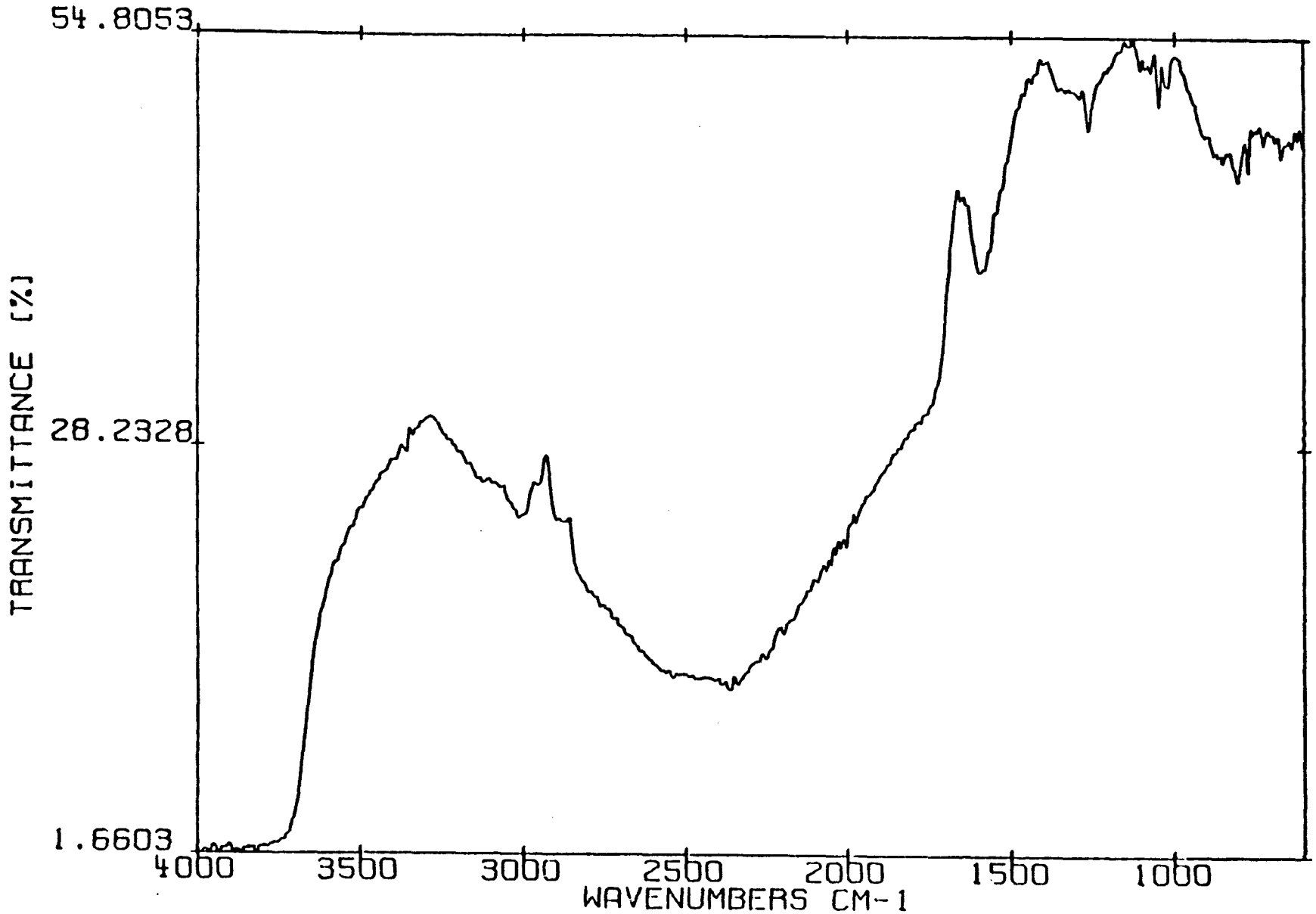


Figure 6 FTIR-PAS of .1% detergent and protein A 100 ng

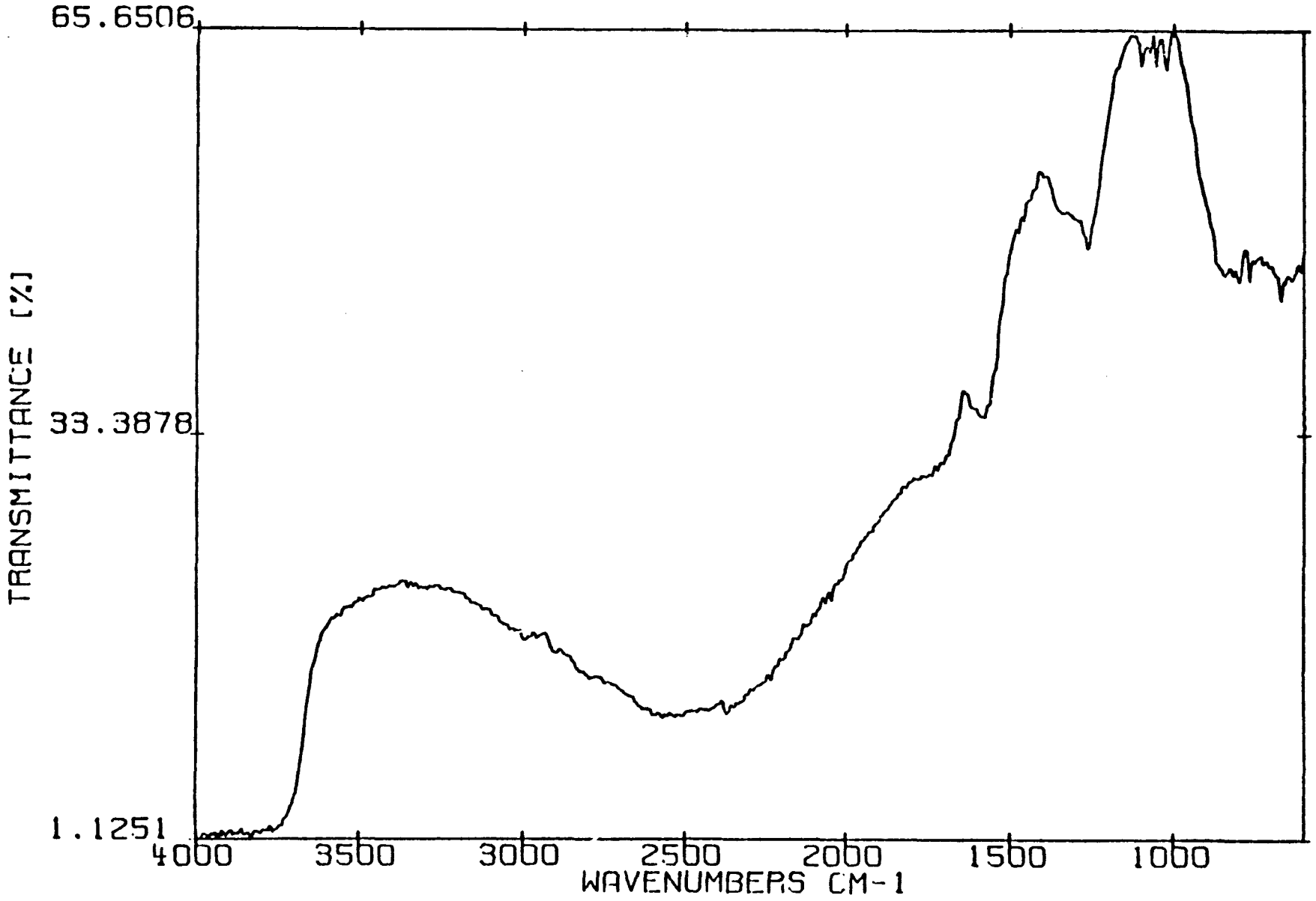


Figure 7 FTIR-PAS of .1% dye blank

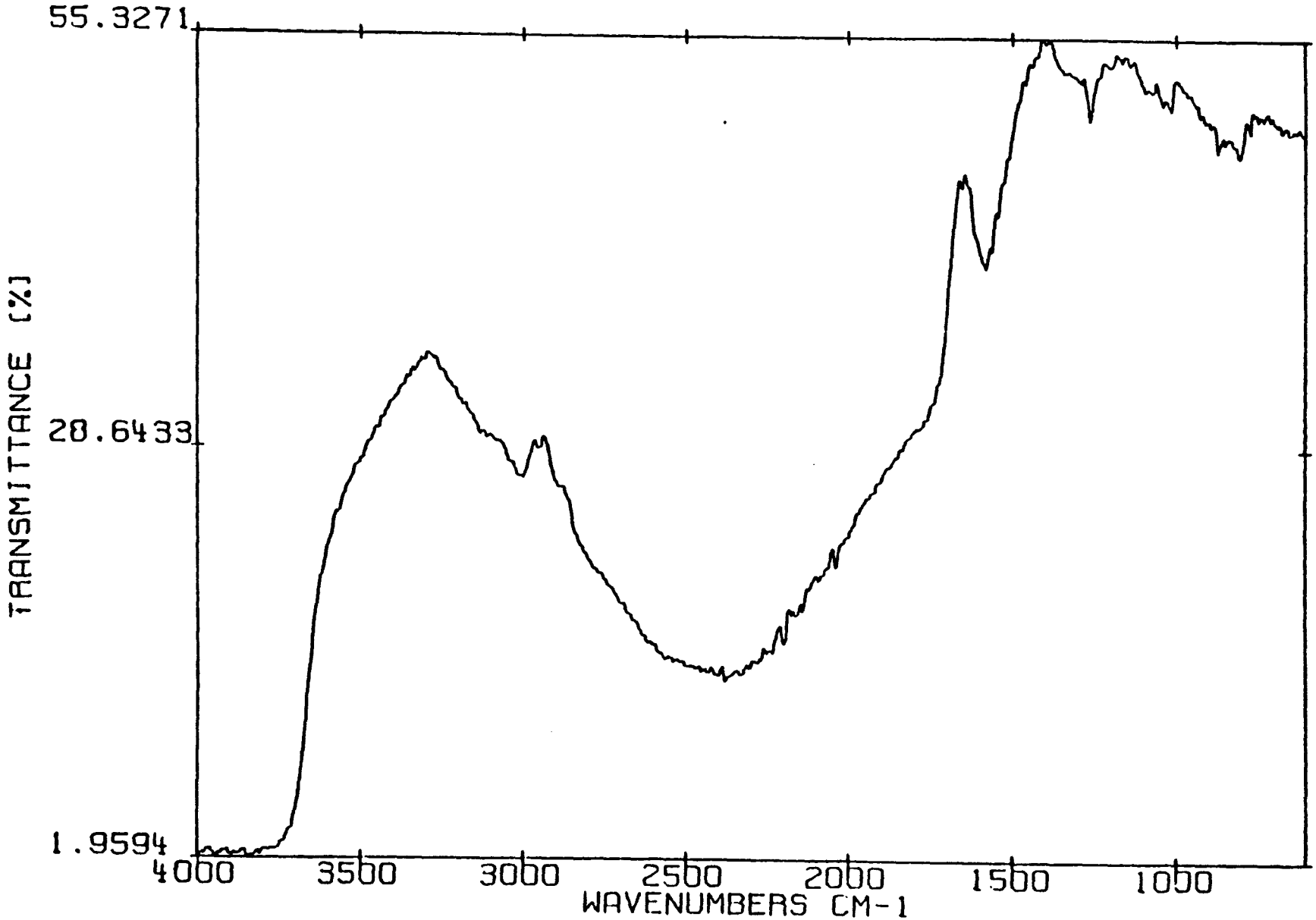


Figure 8 FTIR-PAS of .1% dye blank and 100 ng protein A

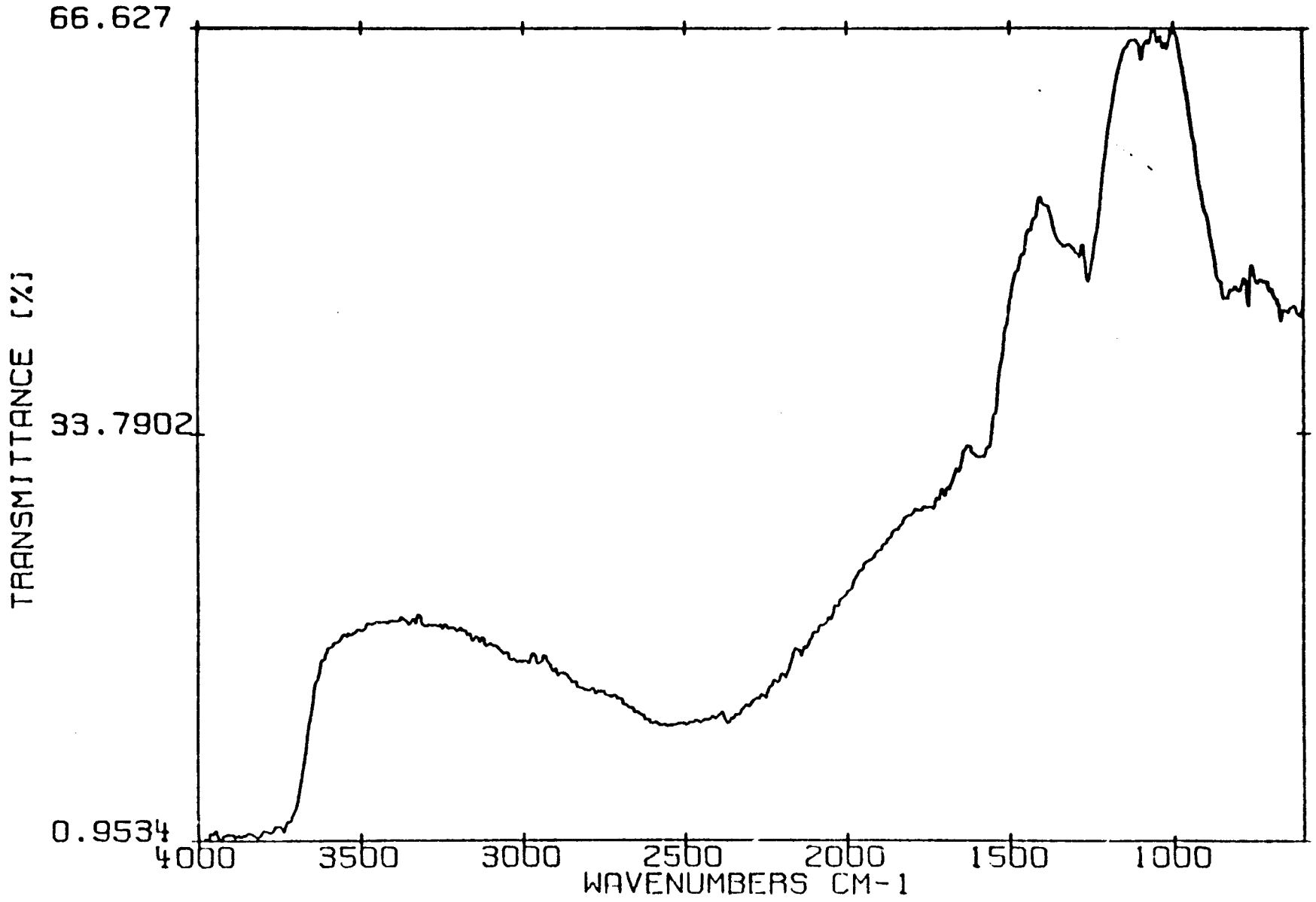


Figure 9 FTIR-PAS of blank paper (set #5)

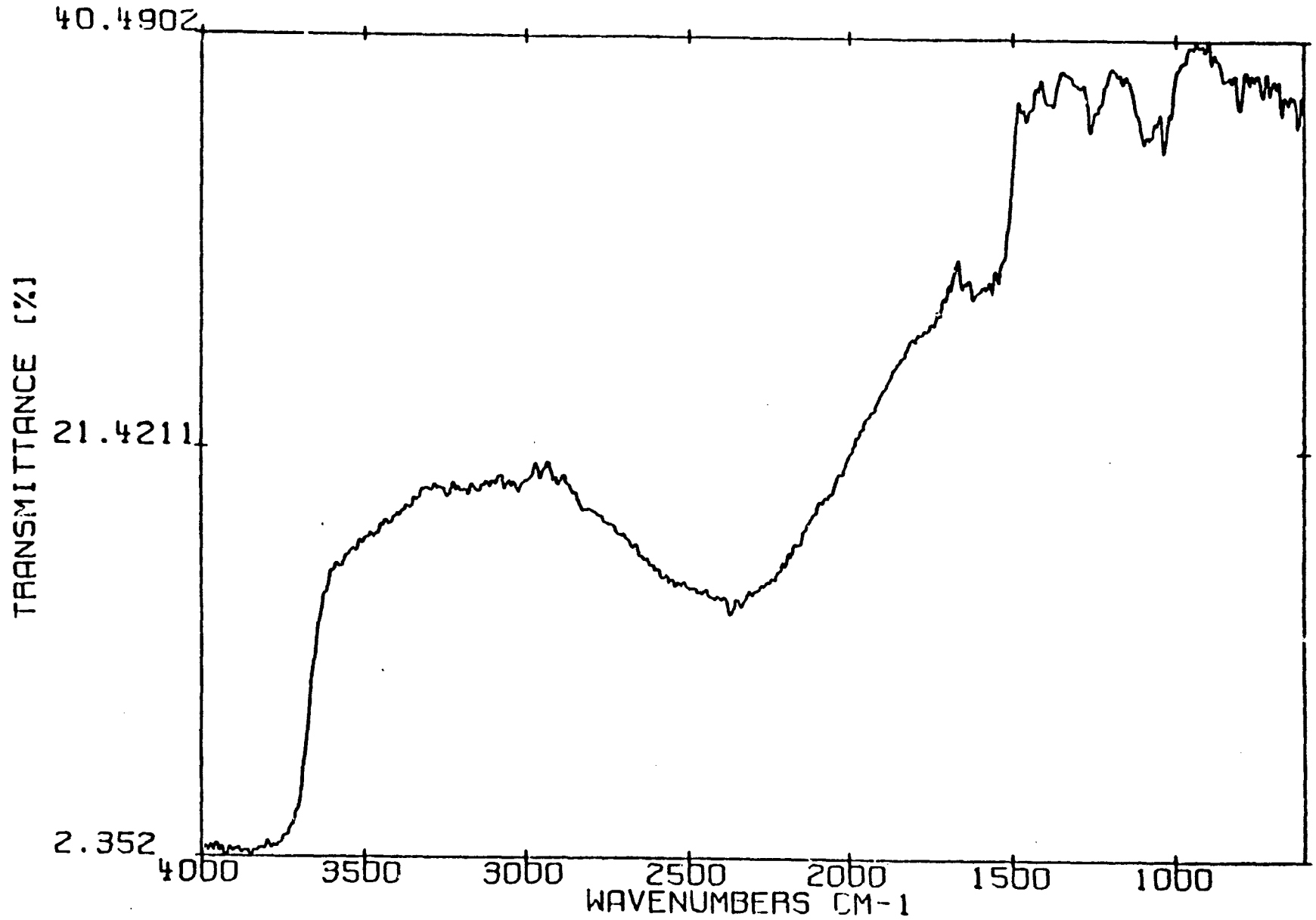


Figure 10 FTIR-PAS of protein A 100 ng (set #5)

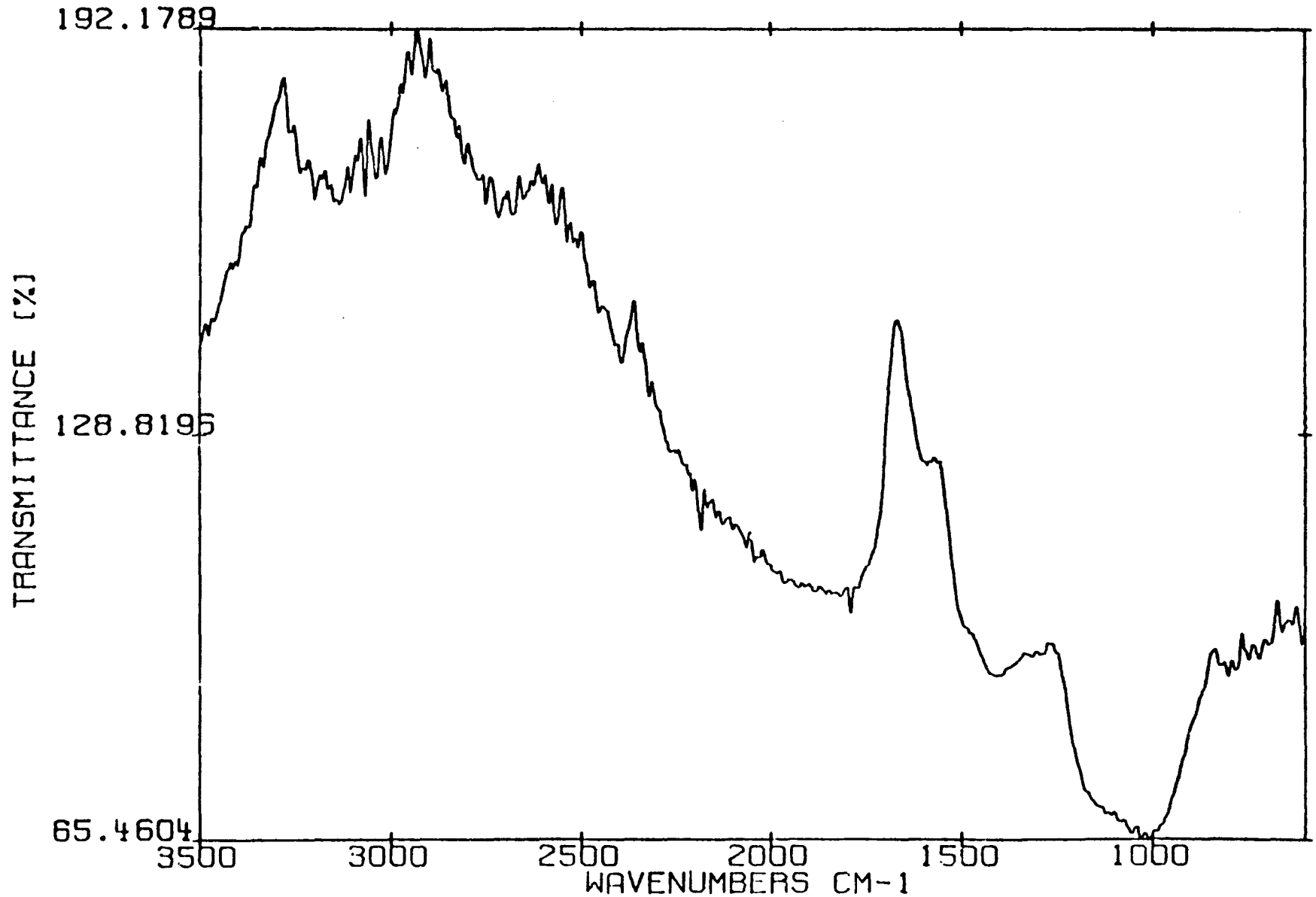


Figure 11 FTIR-PAS (sample #2)/(sample #1)

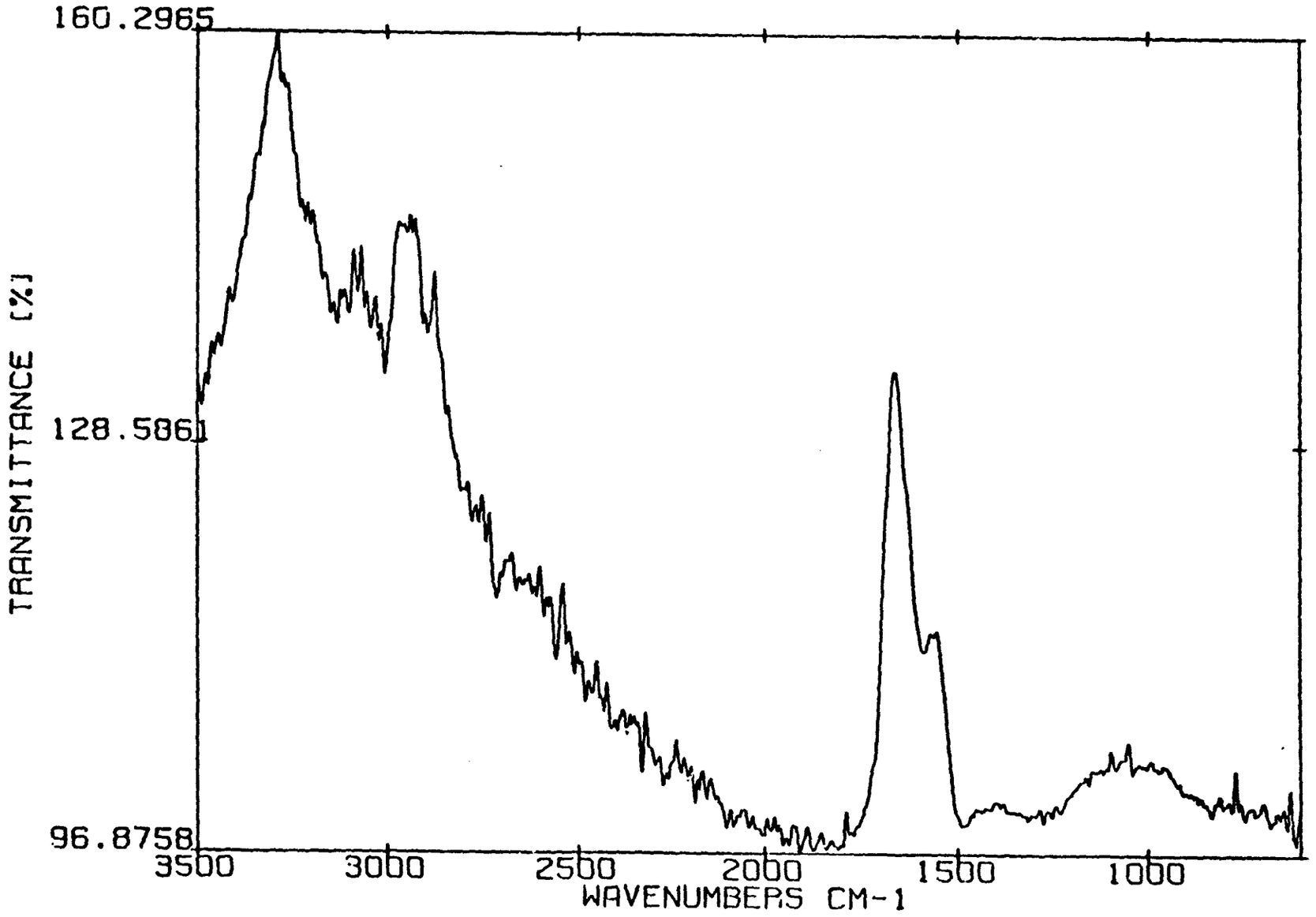


Figure 12 FTIR-PAS (sample #5)/(sample #4)

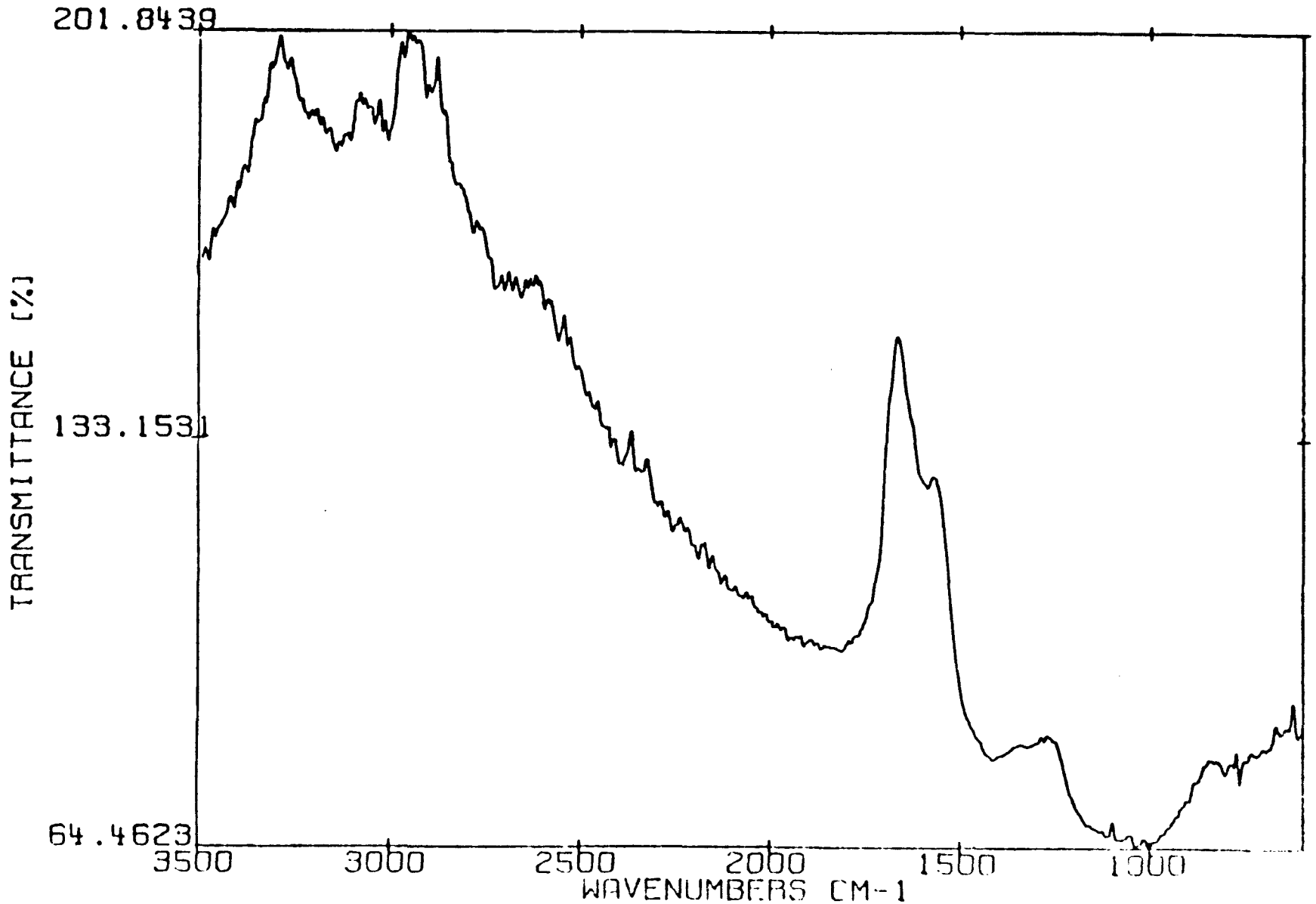


Figure 13 FTIR-PAS (sample #6)/(sample #4)

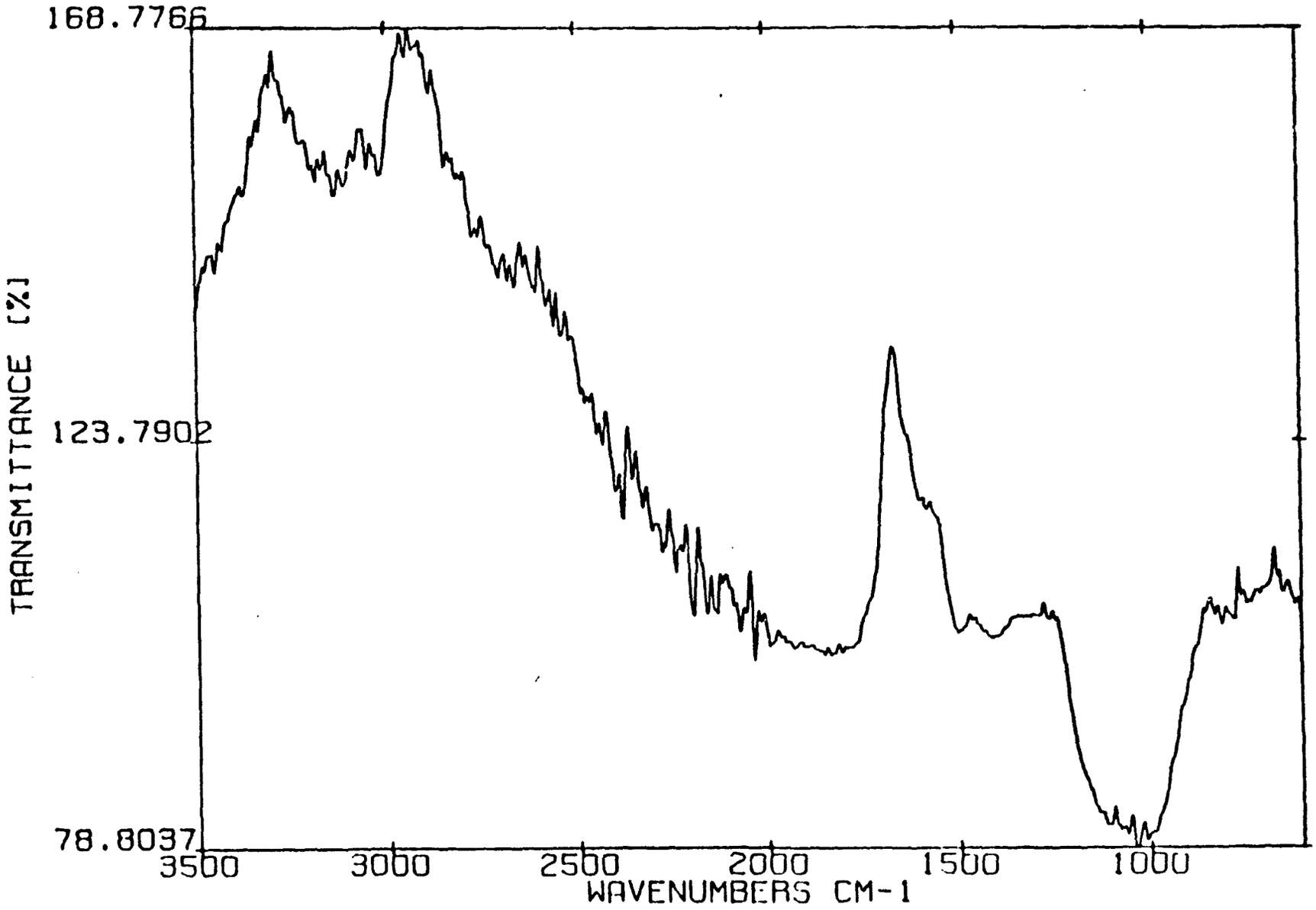


Figure 14 FTIR-PAS duplicate 100 ng A/dye blank

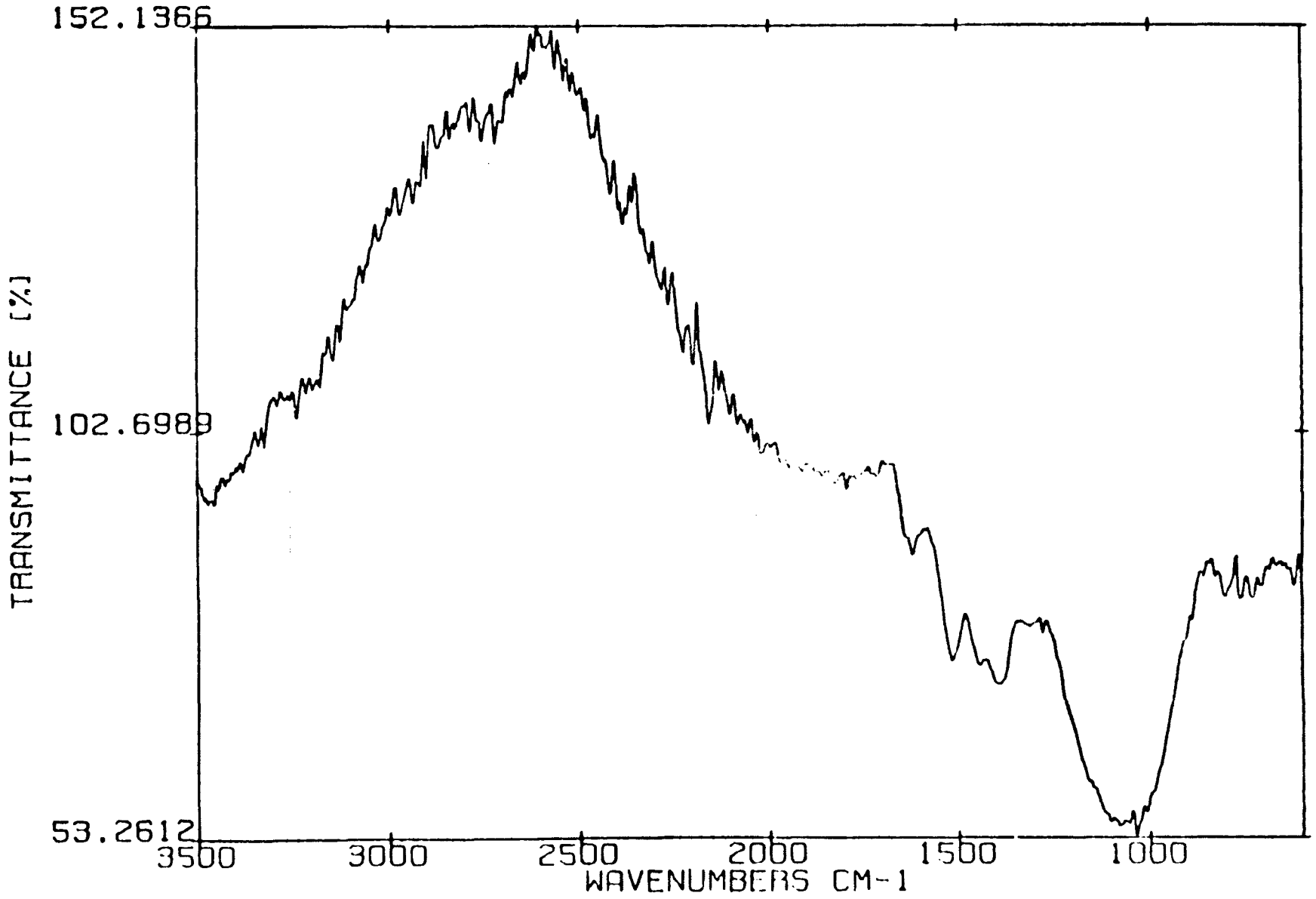


Figure 15 FTIR-PAS (sample #19)/(sample #18)

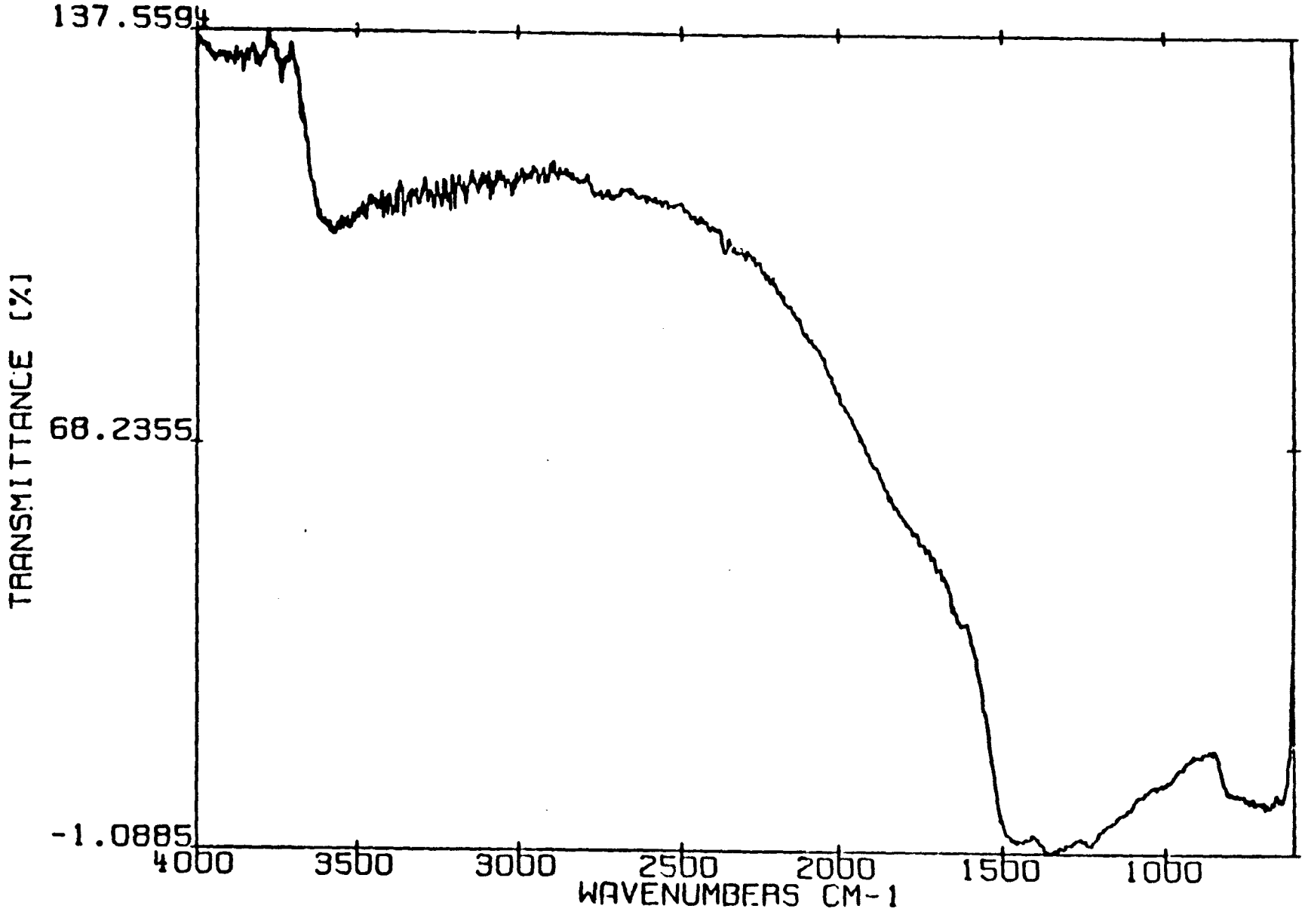


Figure 16 FTIR-DRS of blank paper

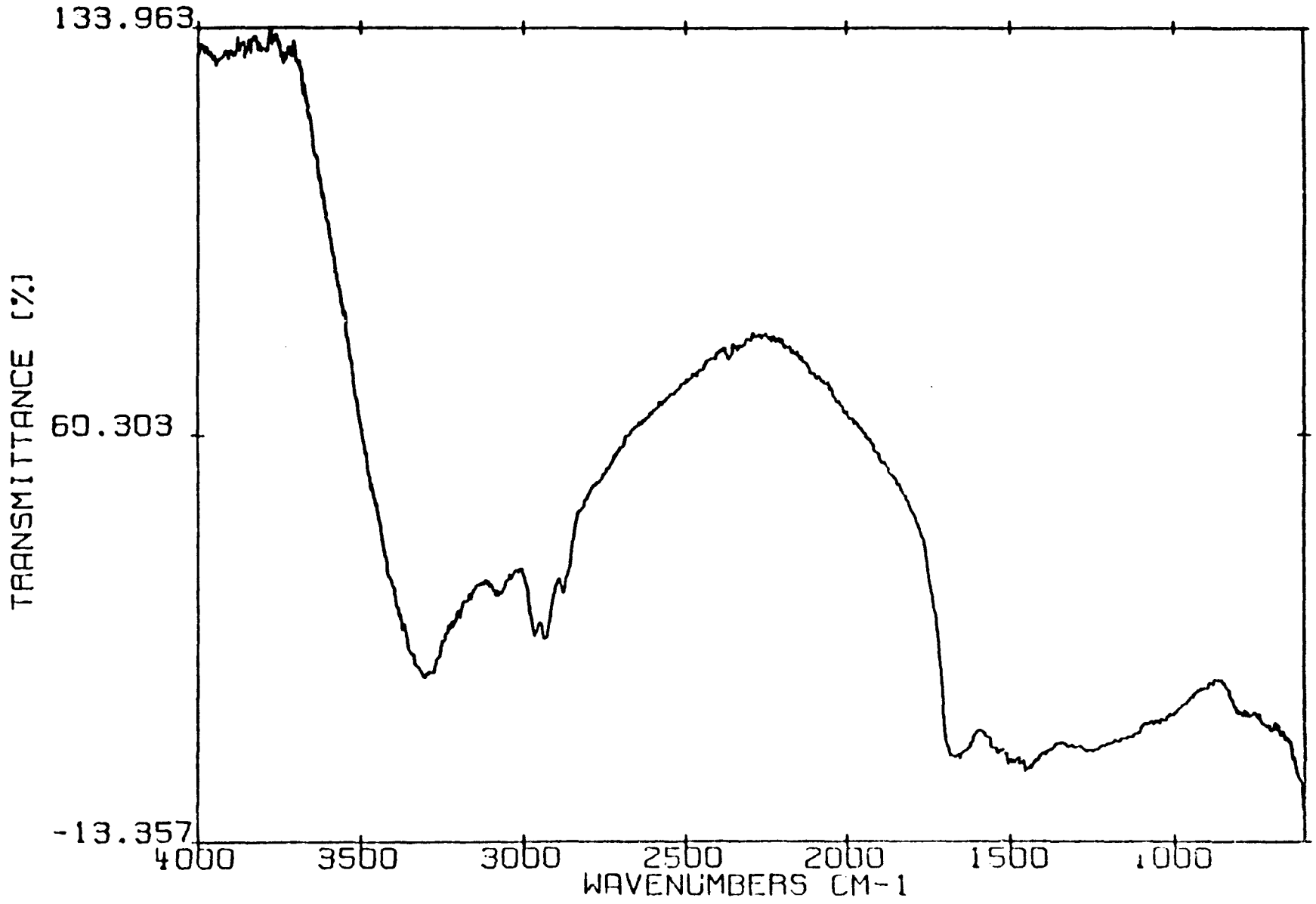


Figure 17 FTIR-DRS of protein A 100 mg

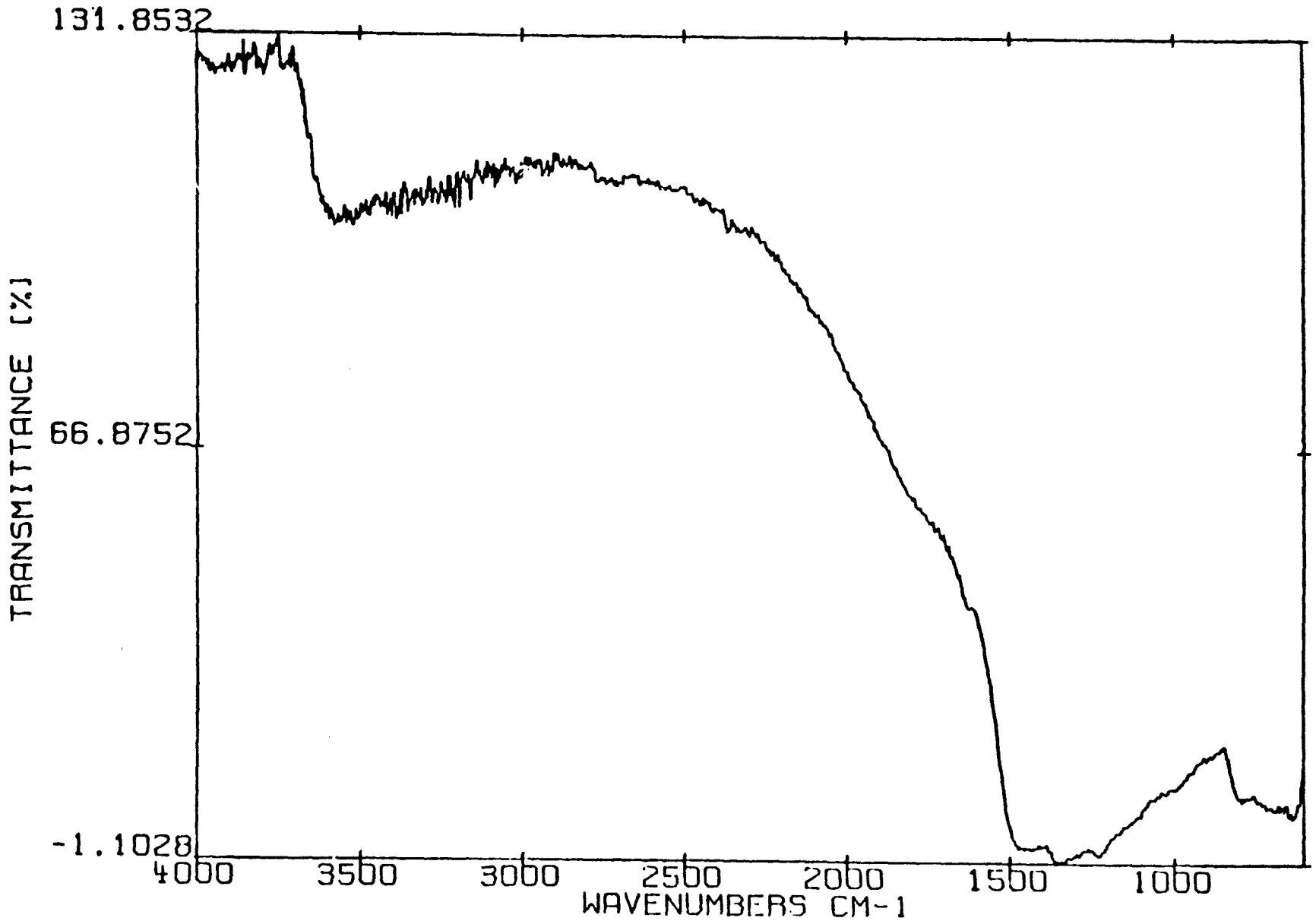


Figure 18 FTIR-DRS of H_2O blank

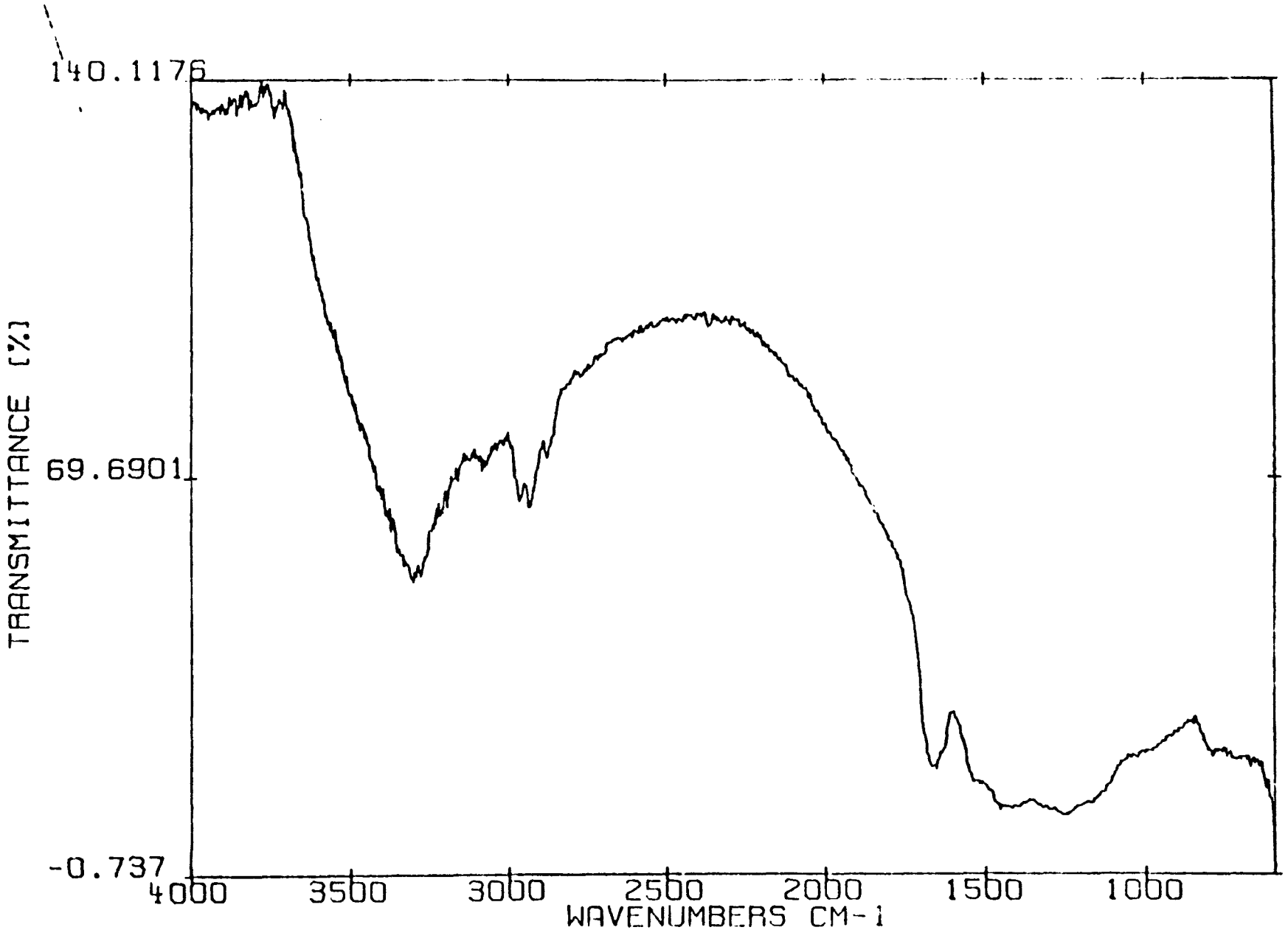


Figure 19 FTIR-DRS of H_2O blank and protein A 100 ng

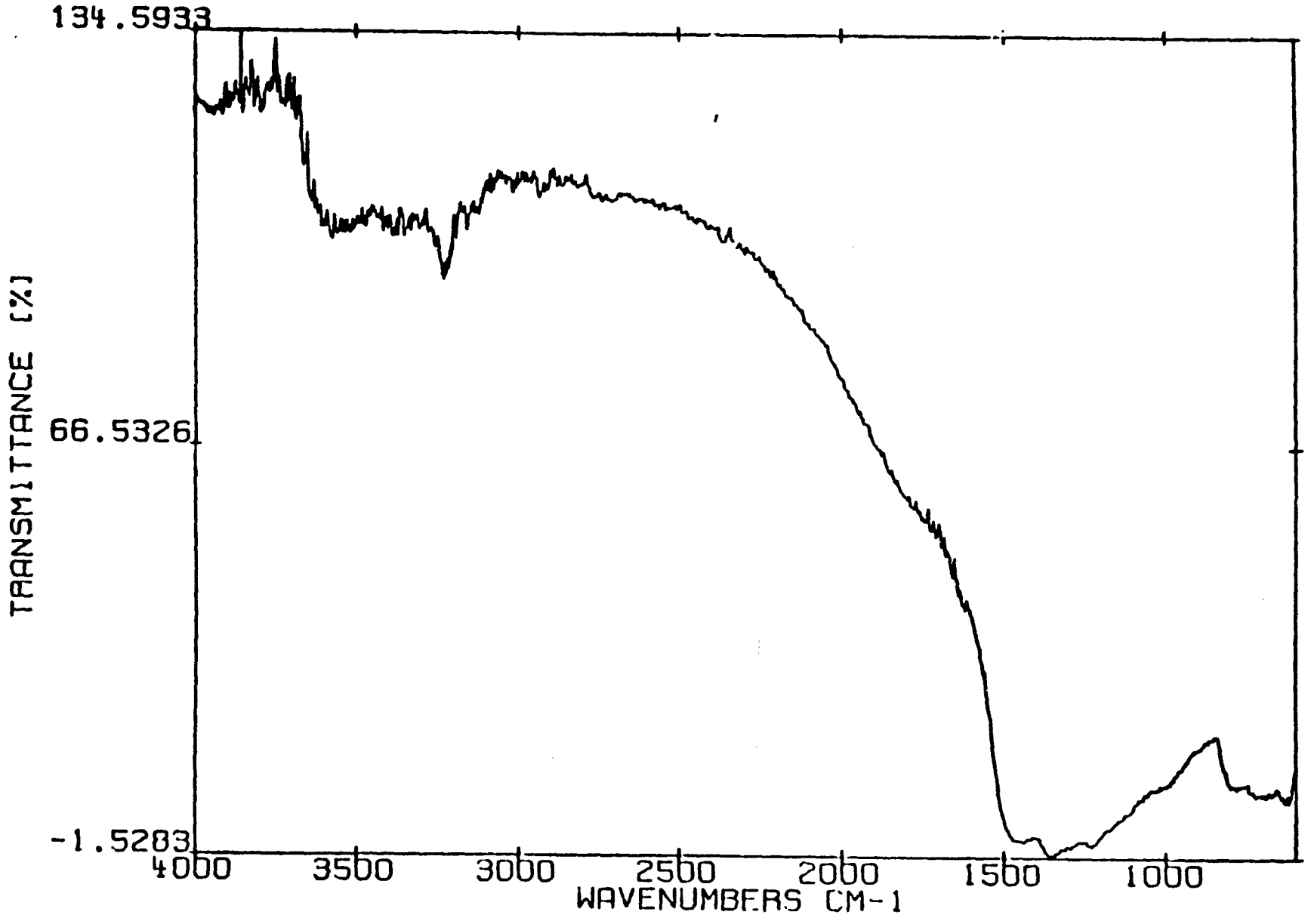


Figure 20 FTIR-DRS of 1% detergent

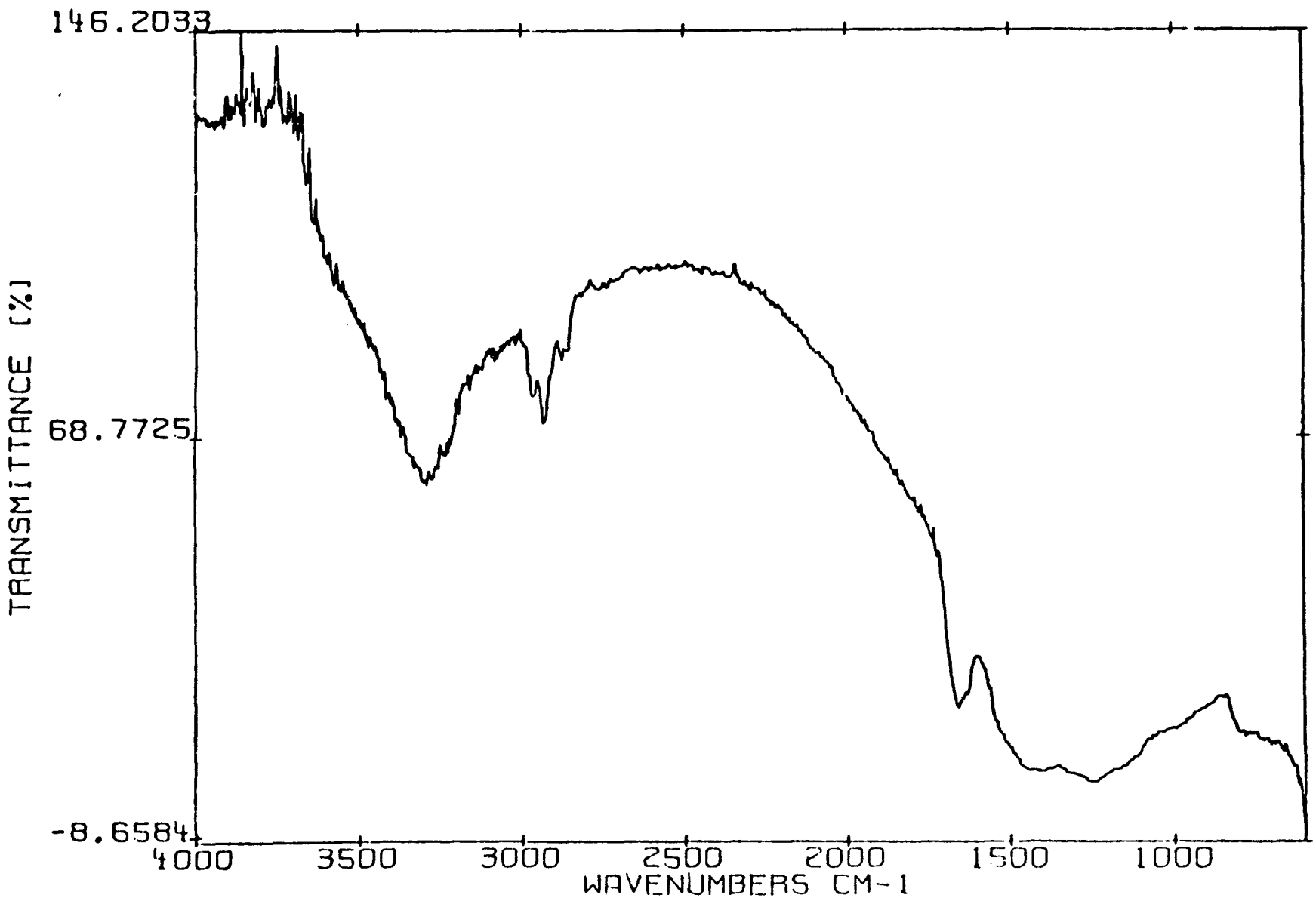


Figure 21 FTIR-DRS of 1% detergent and protein A 100 ng

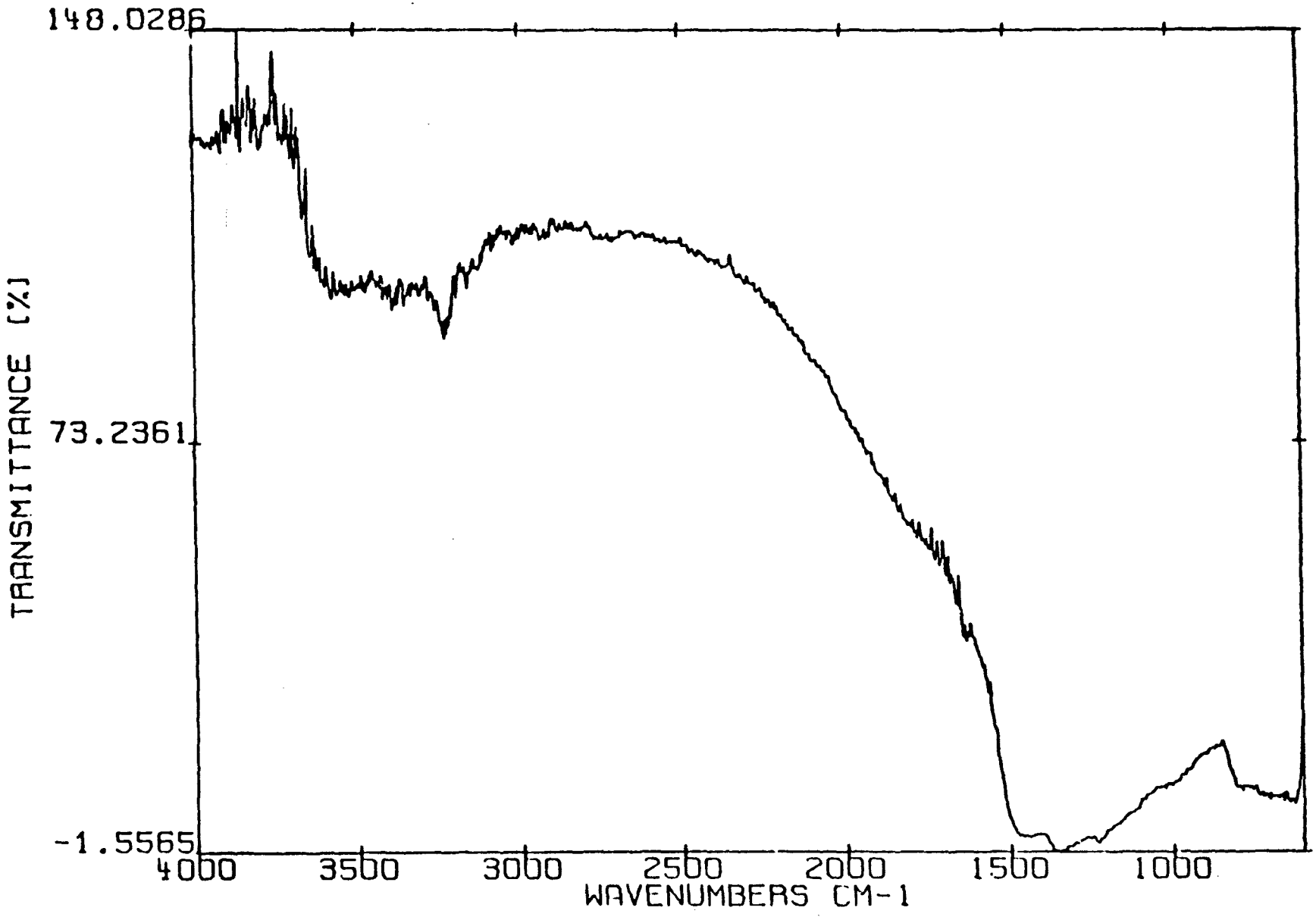


Figure 22 FTIR-DRS of 1% dye blank

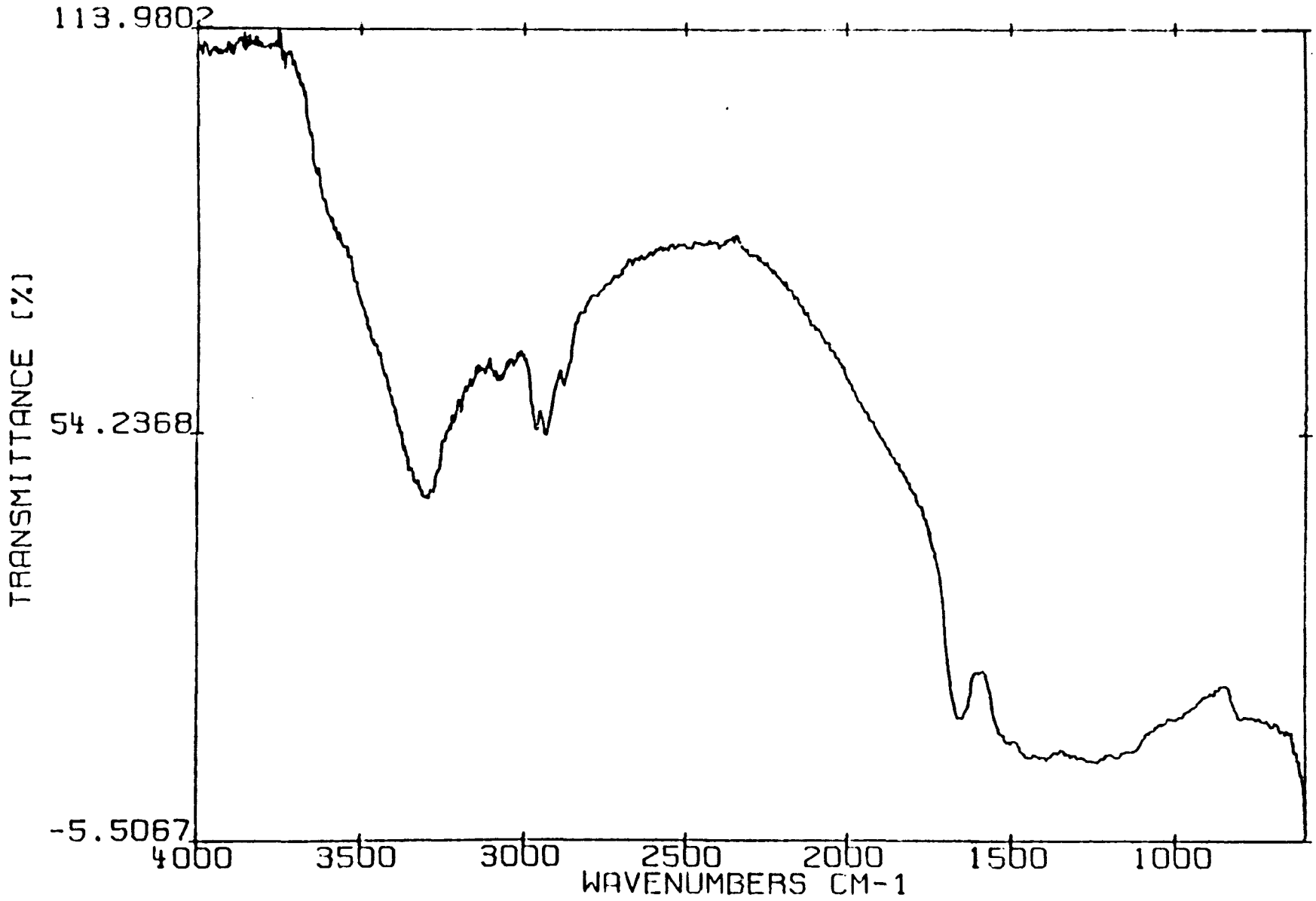


Figure 23 FTIR-DRS of 1% dye blank and 100 μg protein A

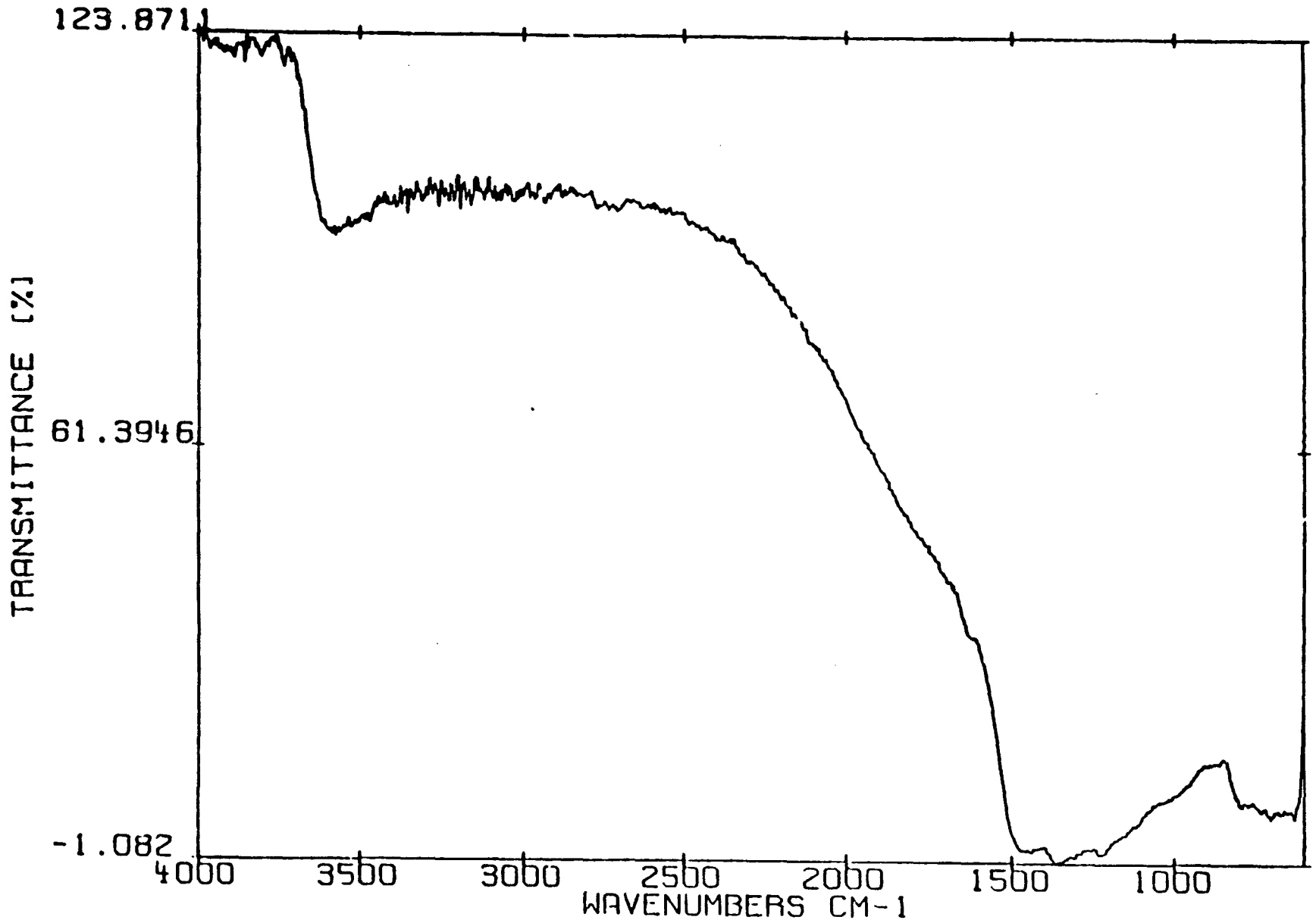


Figure 24 FTIR-DRS of blank paper (set #6)

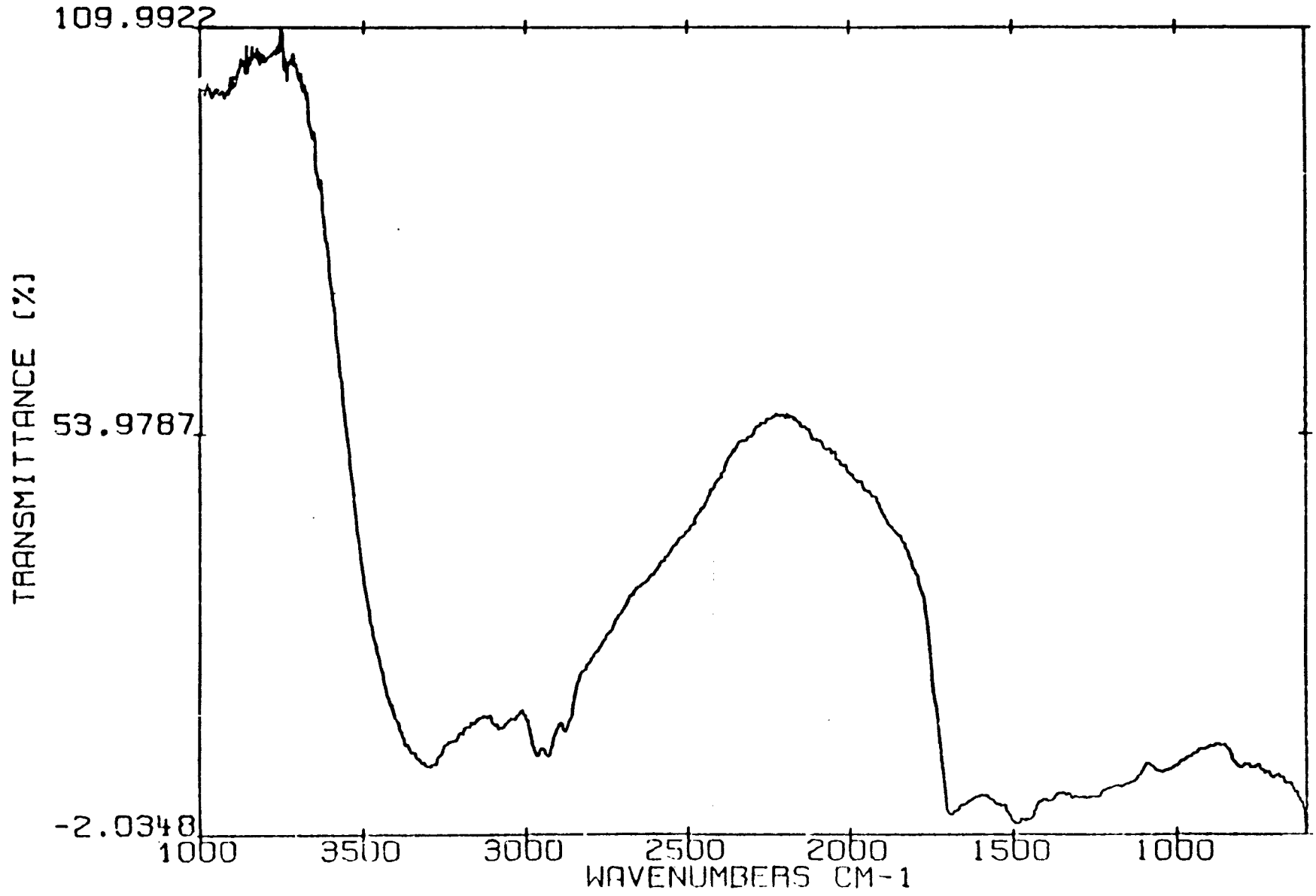


Figure 25 FTIR-DRS of protein A 100 ng (set #6)

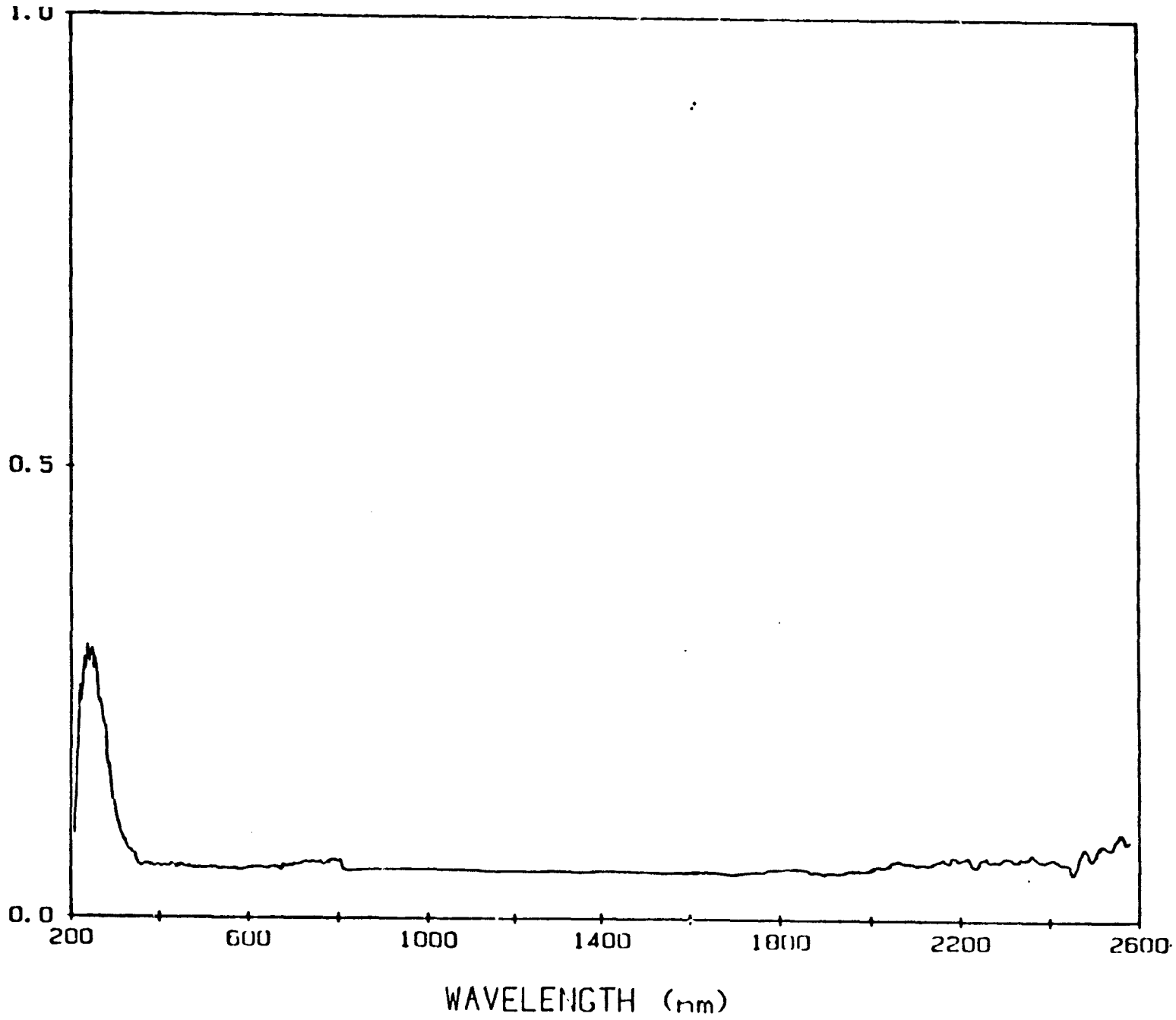
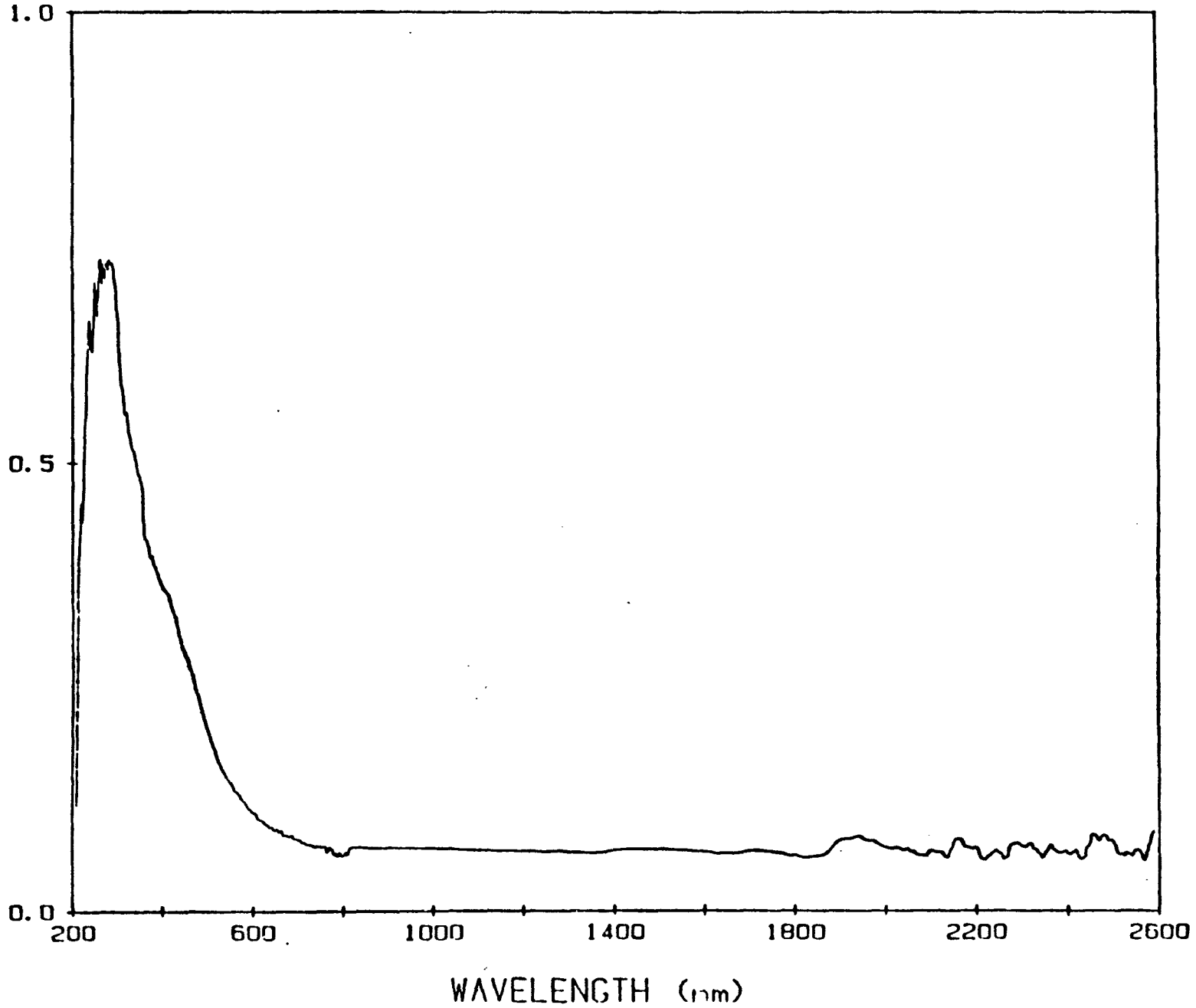


Figure 26 UV-VIS-NIR PAS of blank paper


1.0
0.5
0.0
200
600
1000
1400
1800
2200
2600
WAVELENGTH (nm)

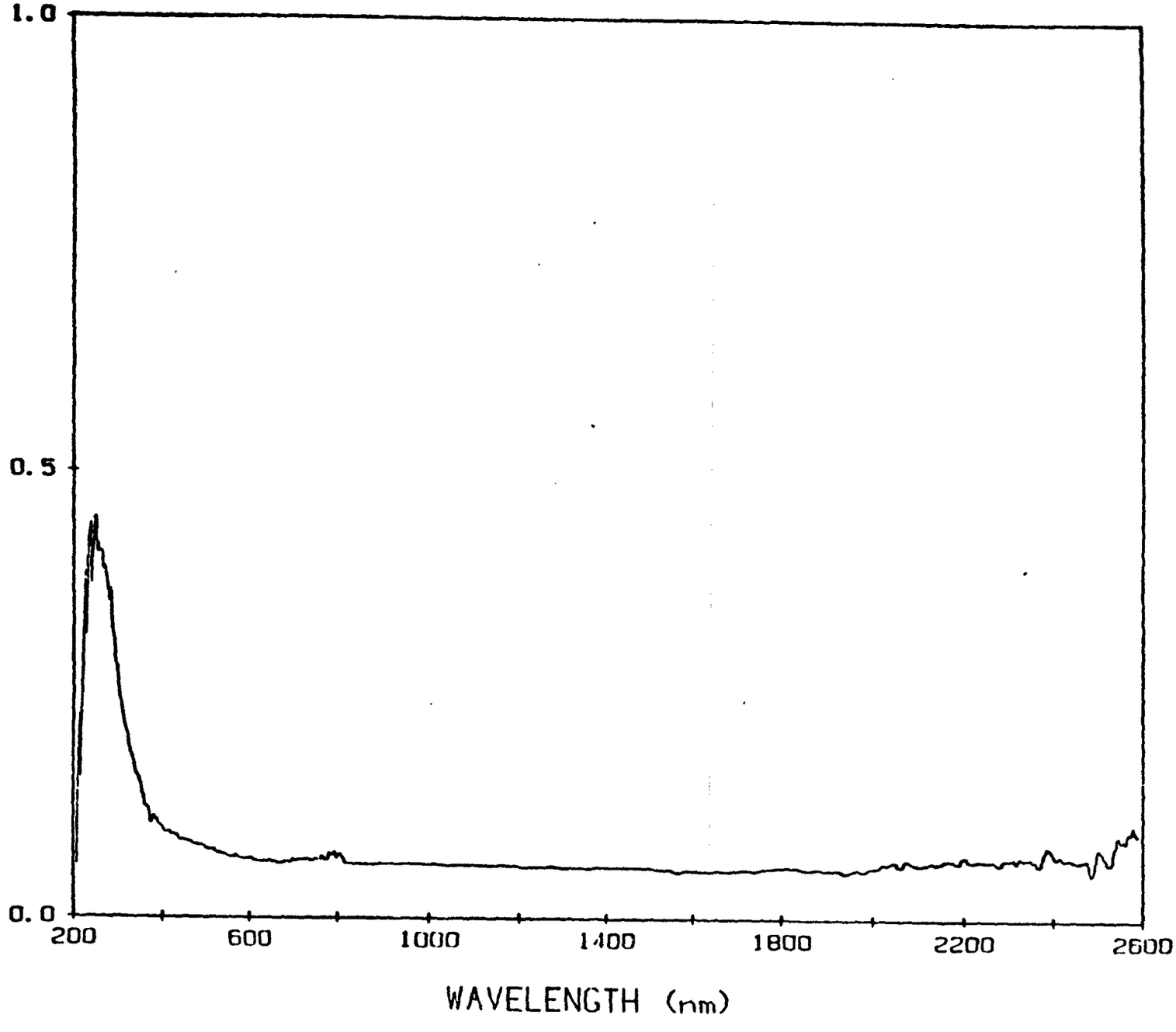


Figure 28 UV-VIS-NIR PAS of H_2O blank

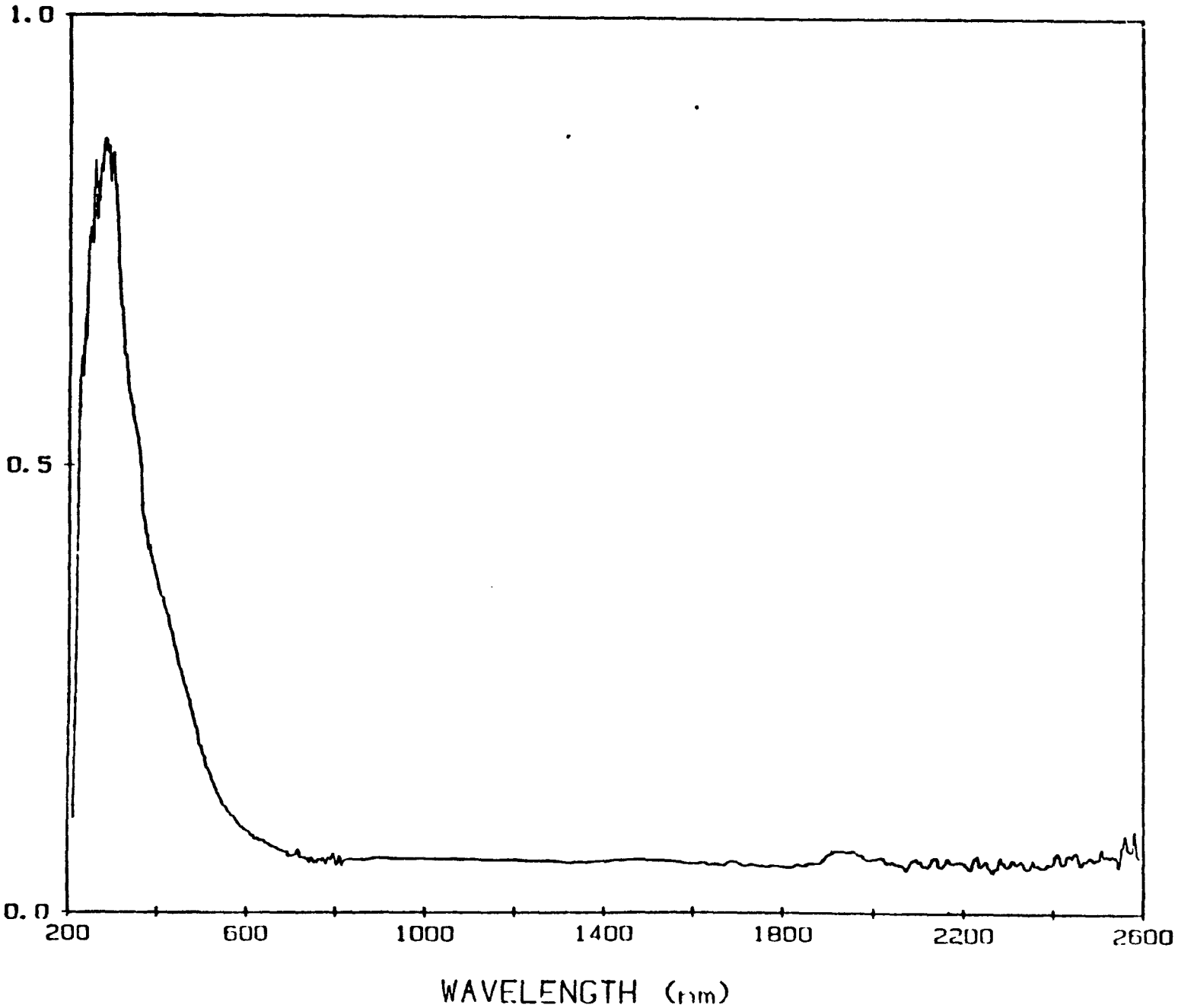


Figure 29 UV-VIS-NIR PAS of H_2O blank and protein A 100 ng

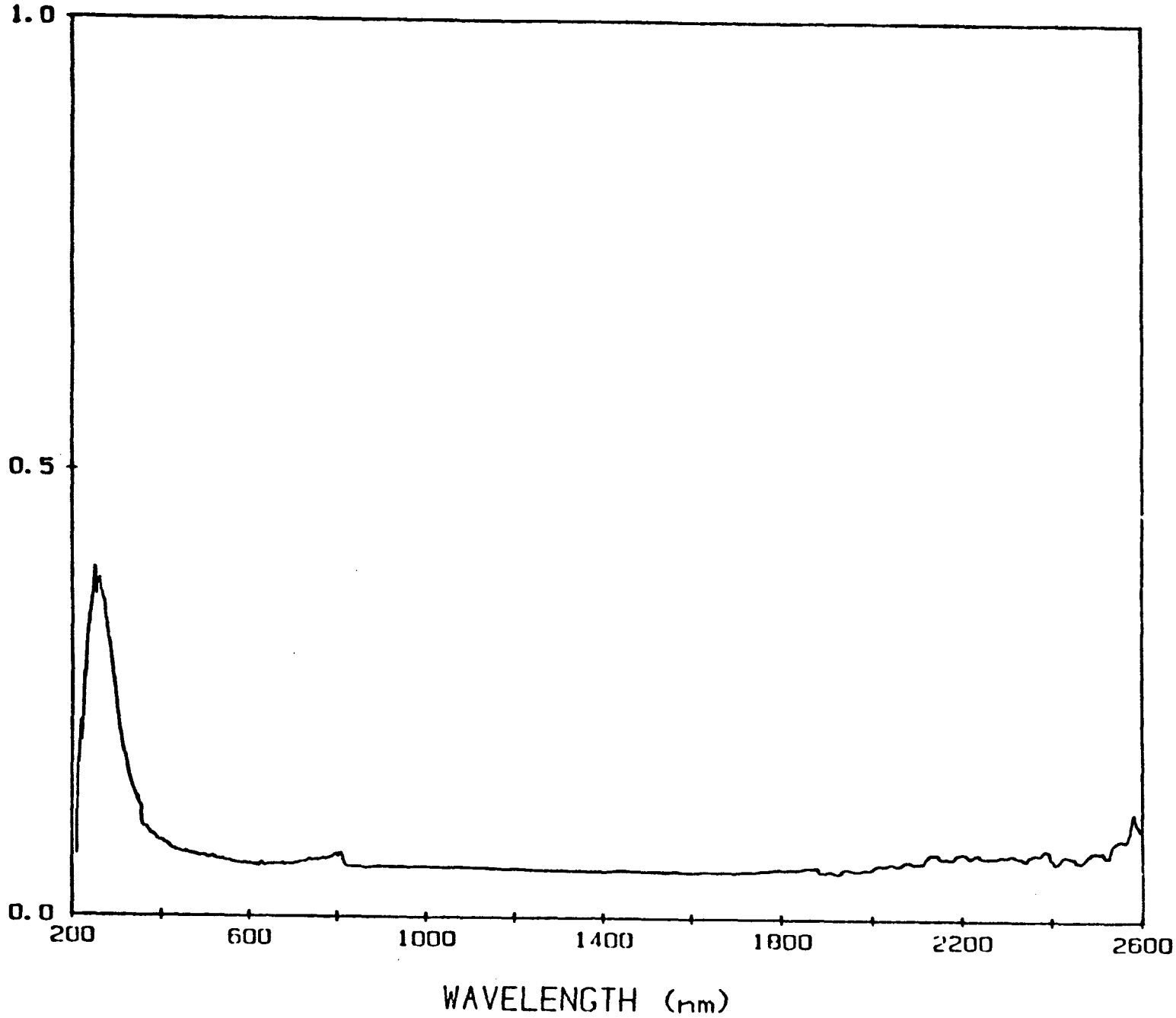


Figure 30 UV-VIS-NIR PAS of /1% detergent

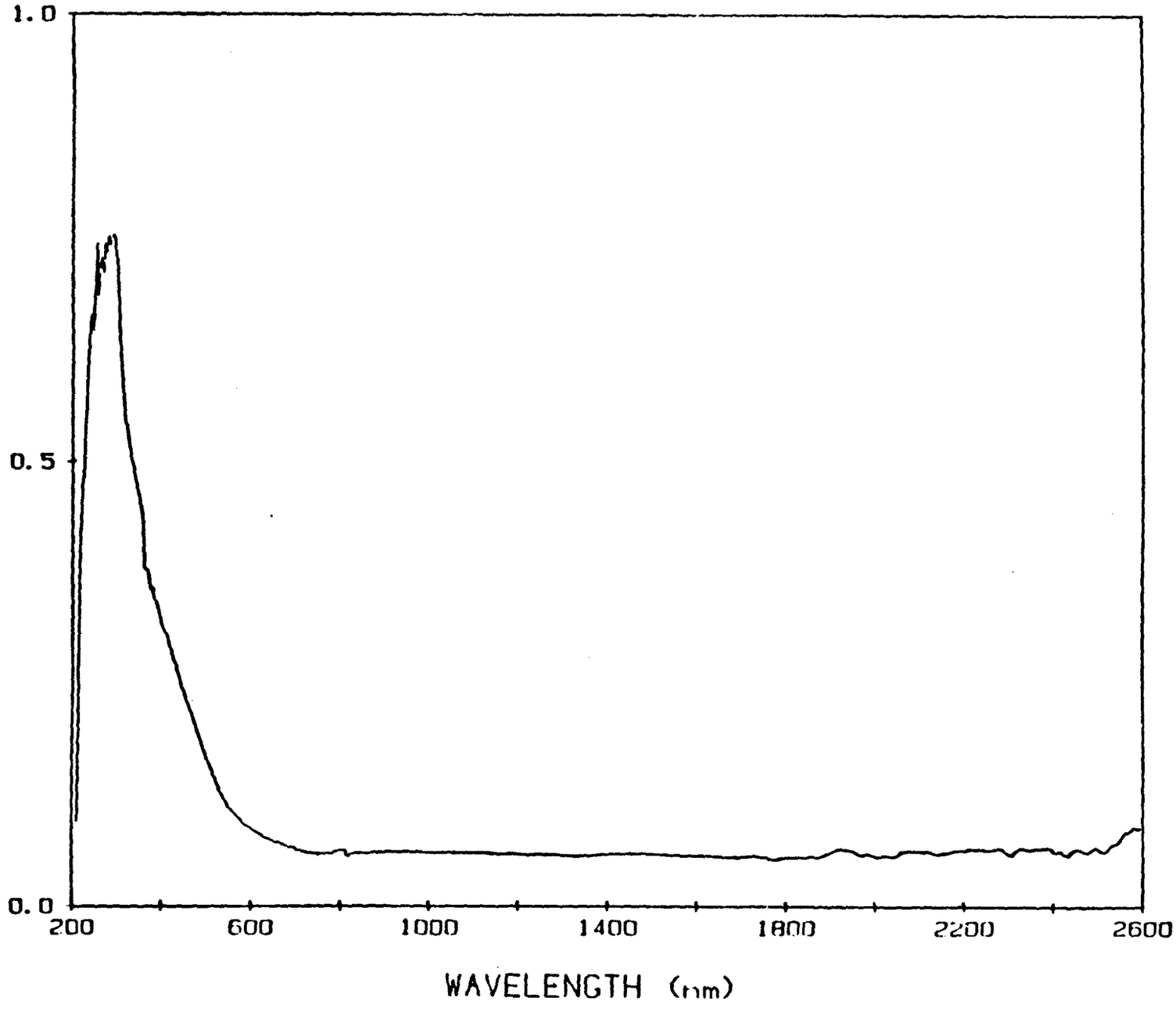


Figure 31 UV-VIS-NIR PAS of .1% detergent and protein A 100 ng

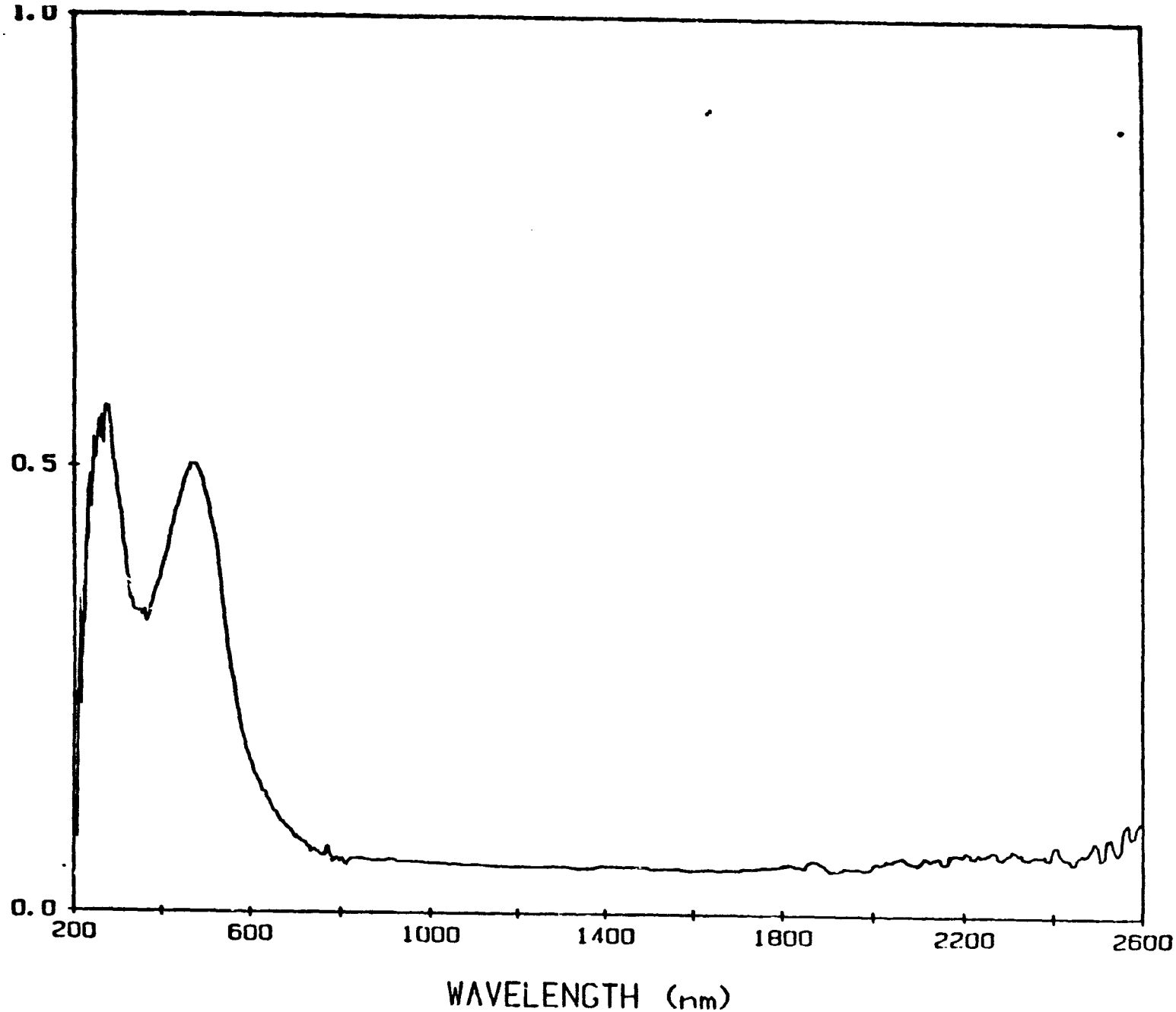


Figure 32 UV-VIS-NIR PAS of .1% dye blank

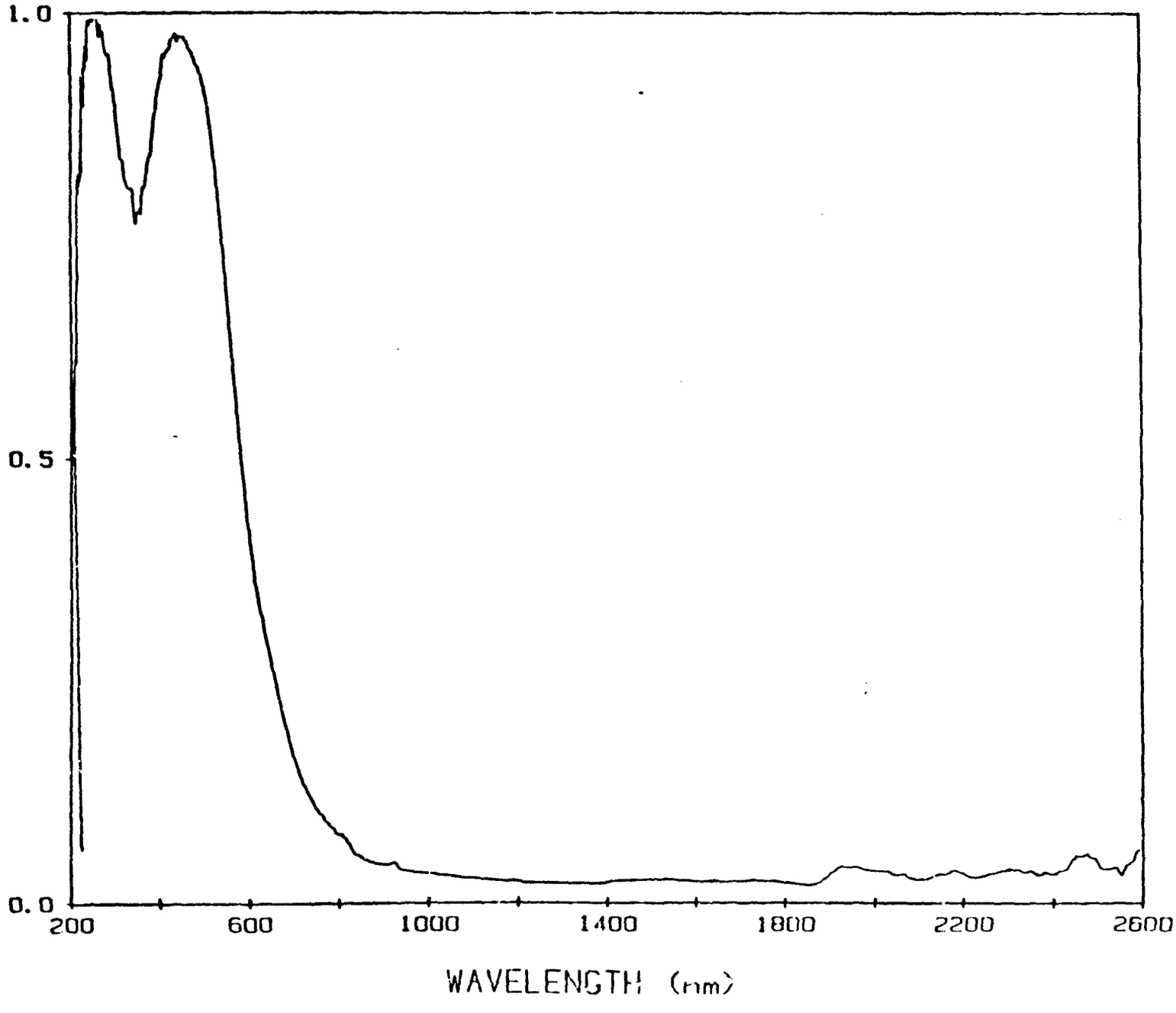


Figure 33 UV-VIS-NIR PAS of .1% dye blank and 100 ng protein A

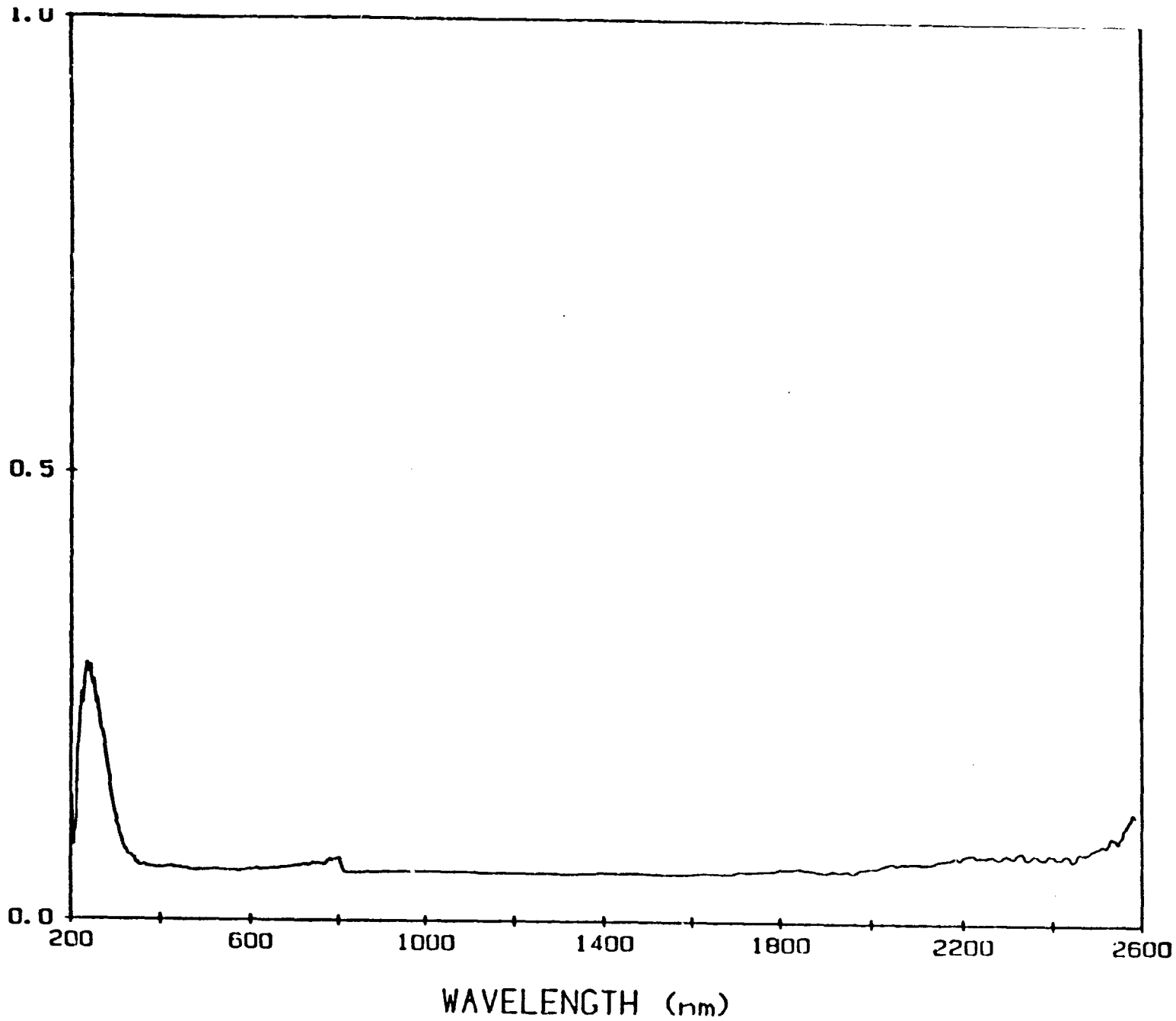


Figure 34 UV-VIS-NIR PAS of blank paper (set #6)

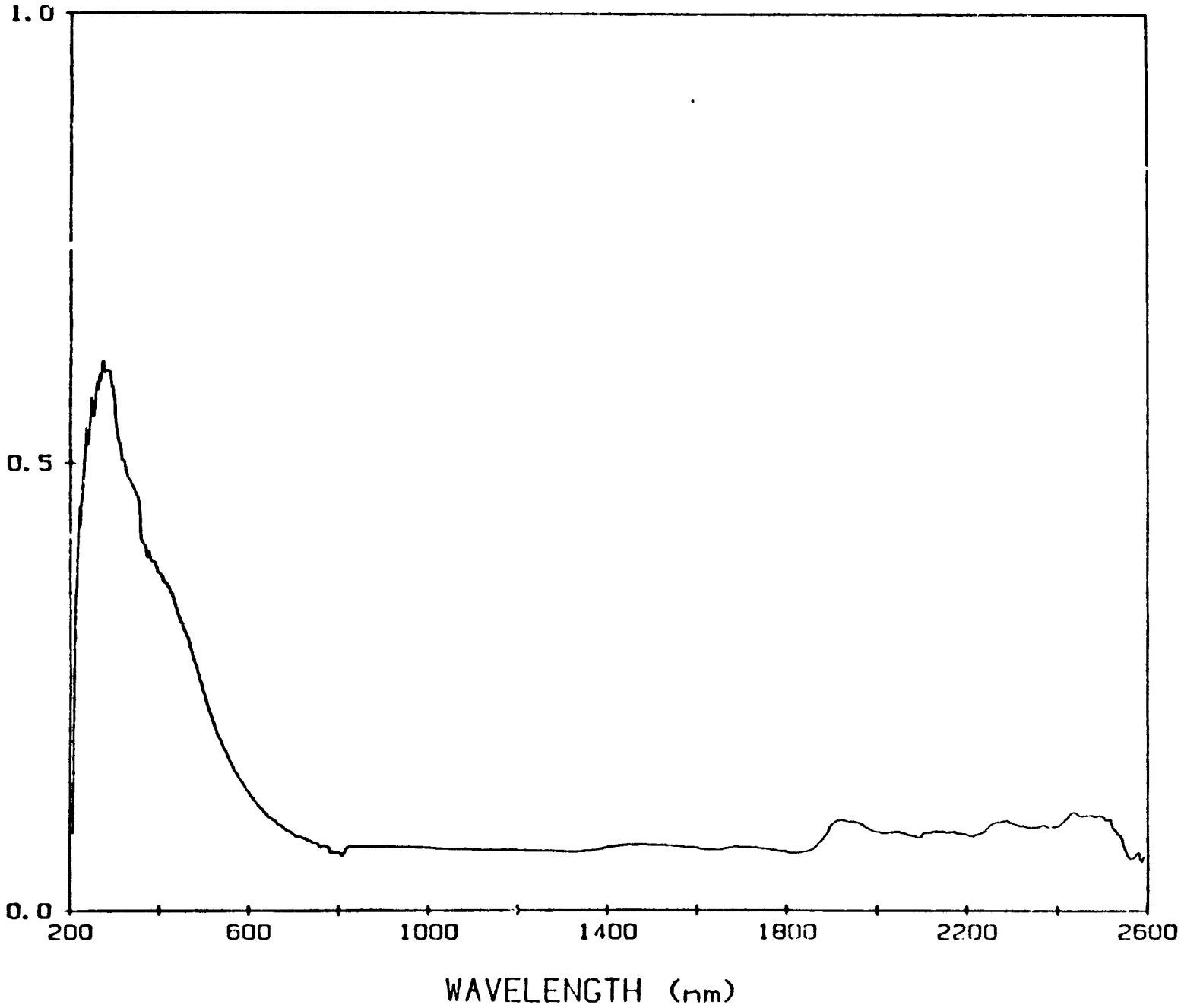


Figure 35 UV-VIS-NIR PAS of protein A 100 ng (set #6)

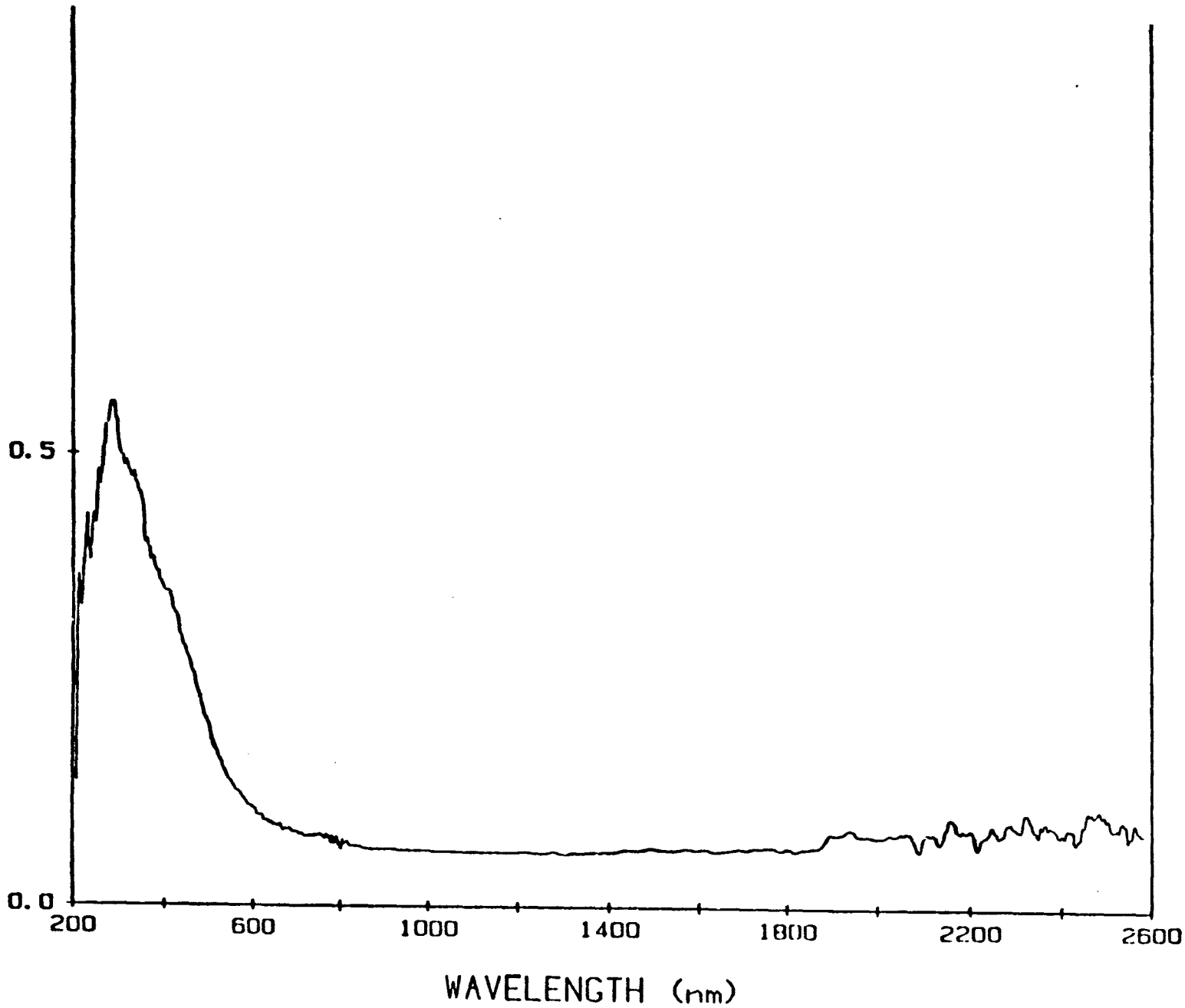


Figure 36 UV-VIS-NIR PAS 100 ng A paper blank

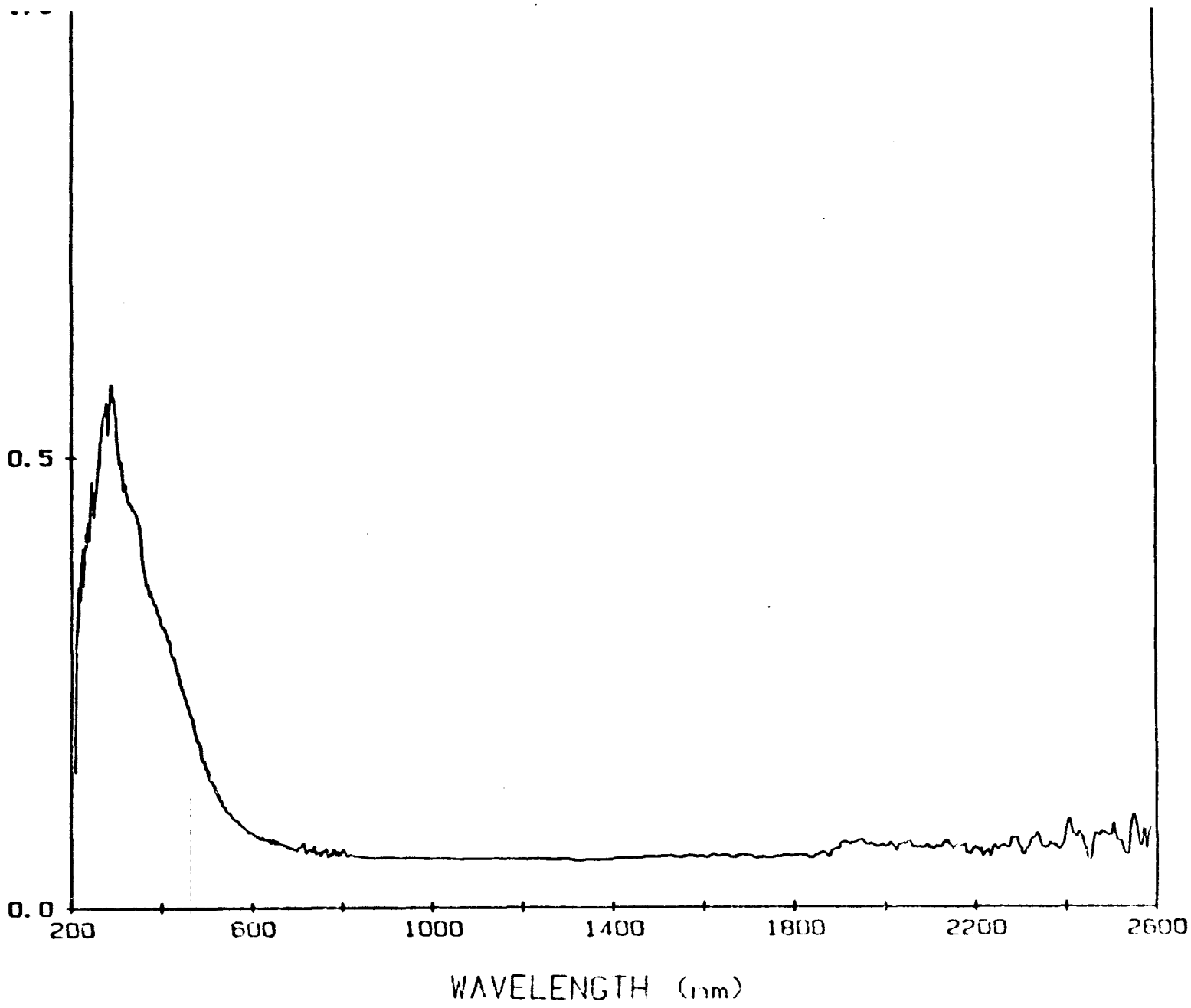


Figure 37 UV-VIS-NIR PAS 100 ng A H_2O blank

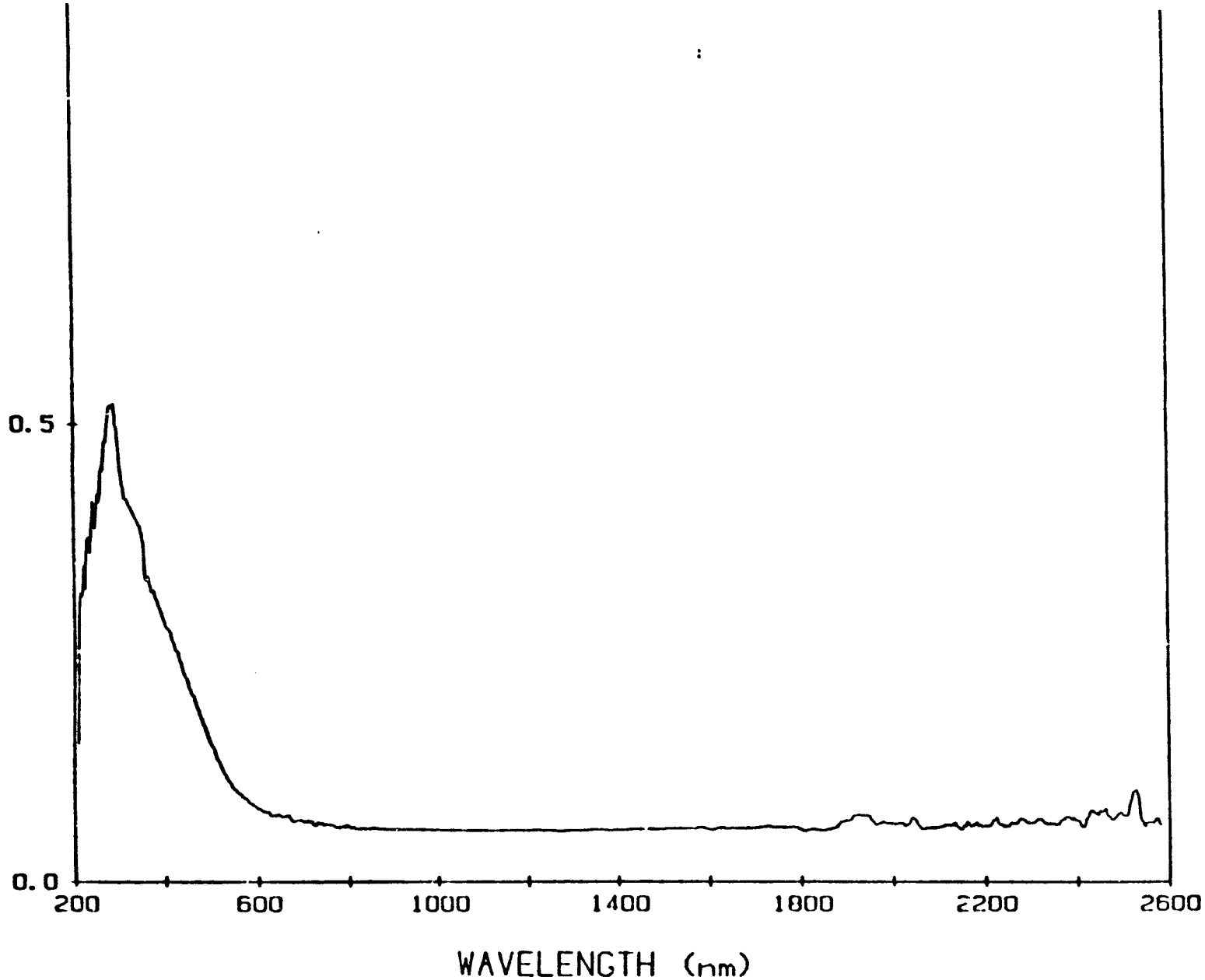
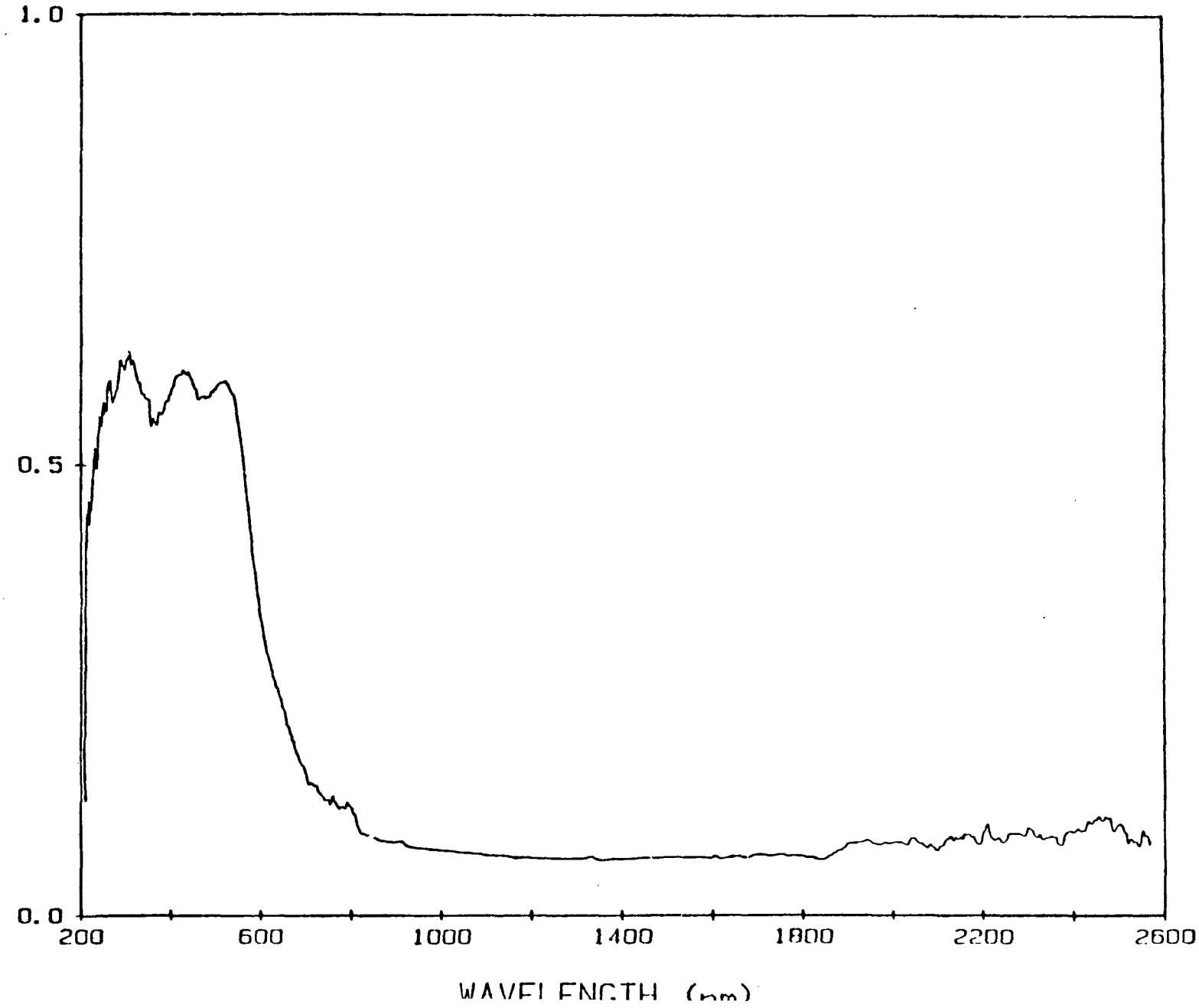


Figure 38 UV-VIS-NIR PAS 100 ng A detergent blank


1.0
0.5
0.0
200
600
1000
1400
1800
2200
2600
WAVELENGTH (nm)

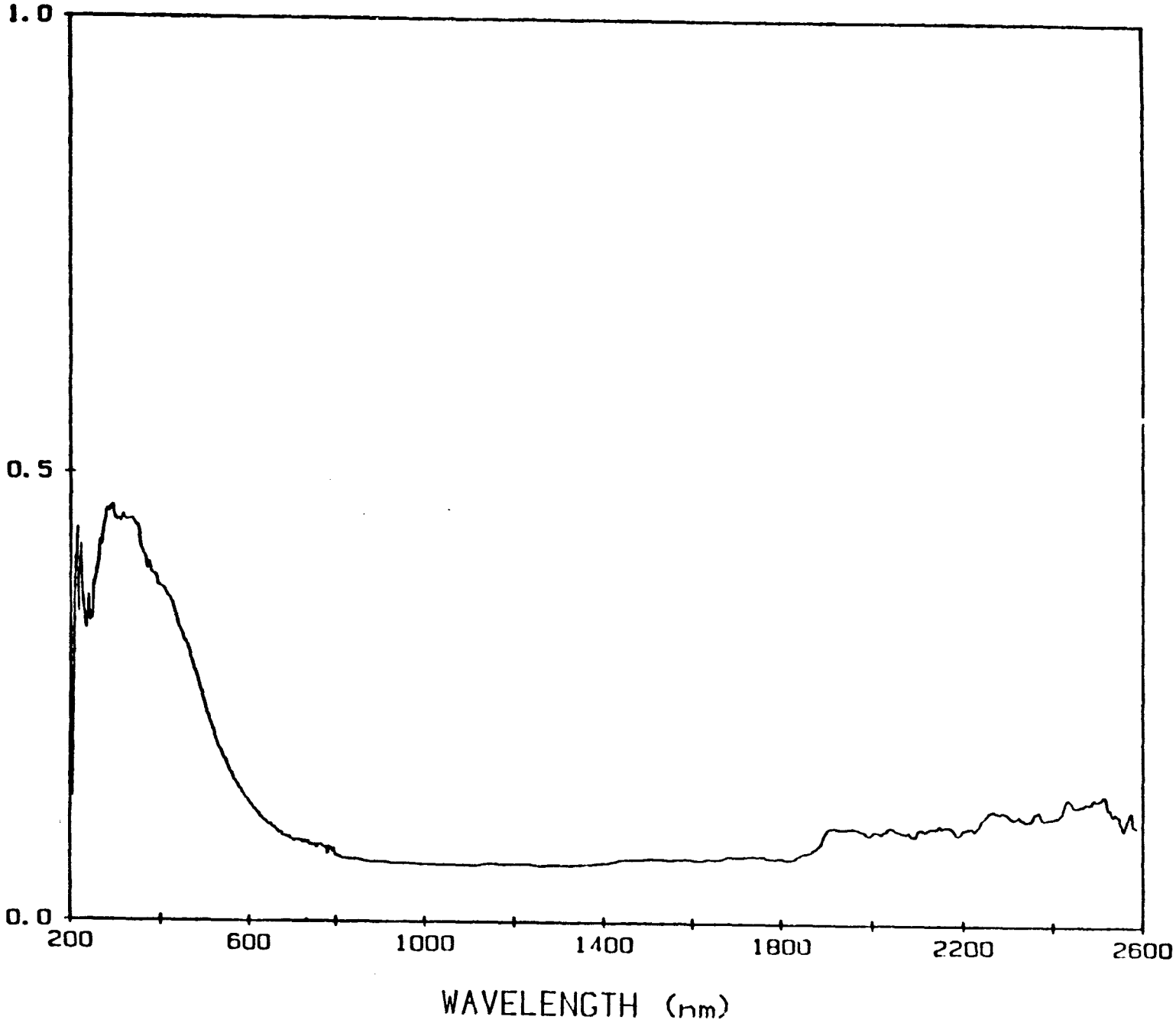
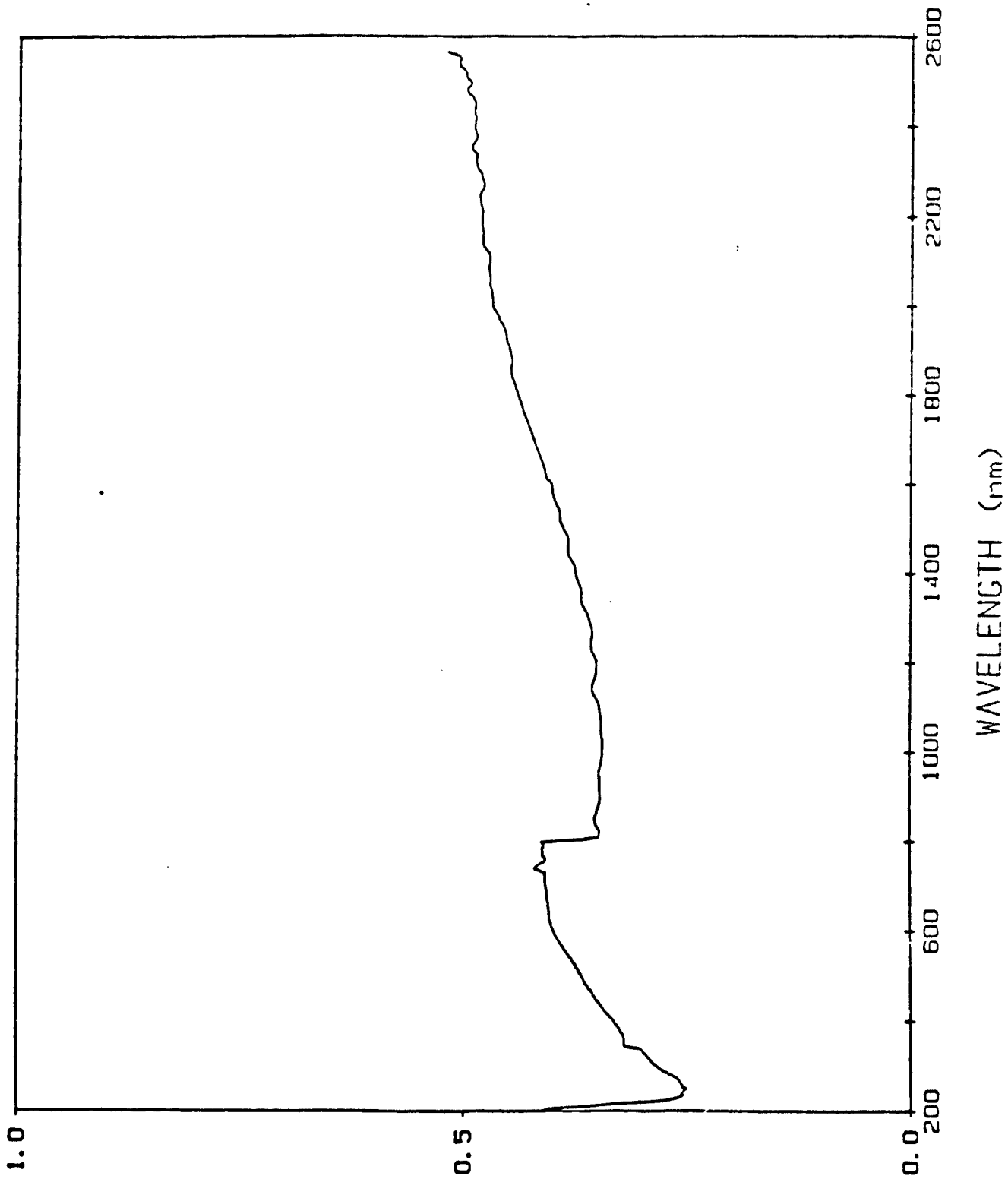


Figure 40 UV-VIS-NIR PAS duplicate 100 ng A paper blank


1.0
0.5
0.0
200
600
1000
1400
1800
2200
2600
WAVELENGTH (nm)

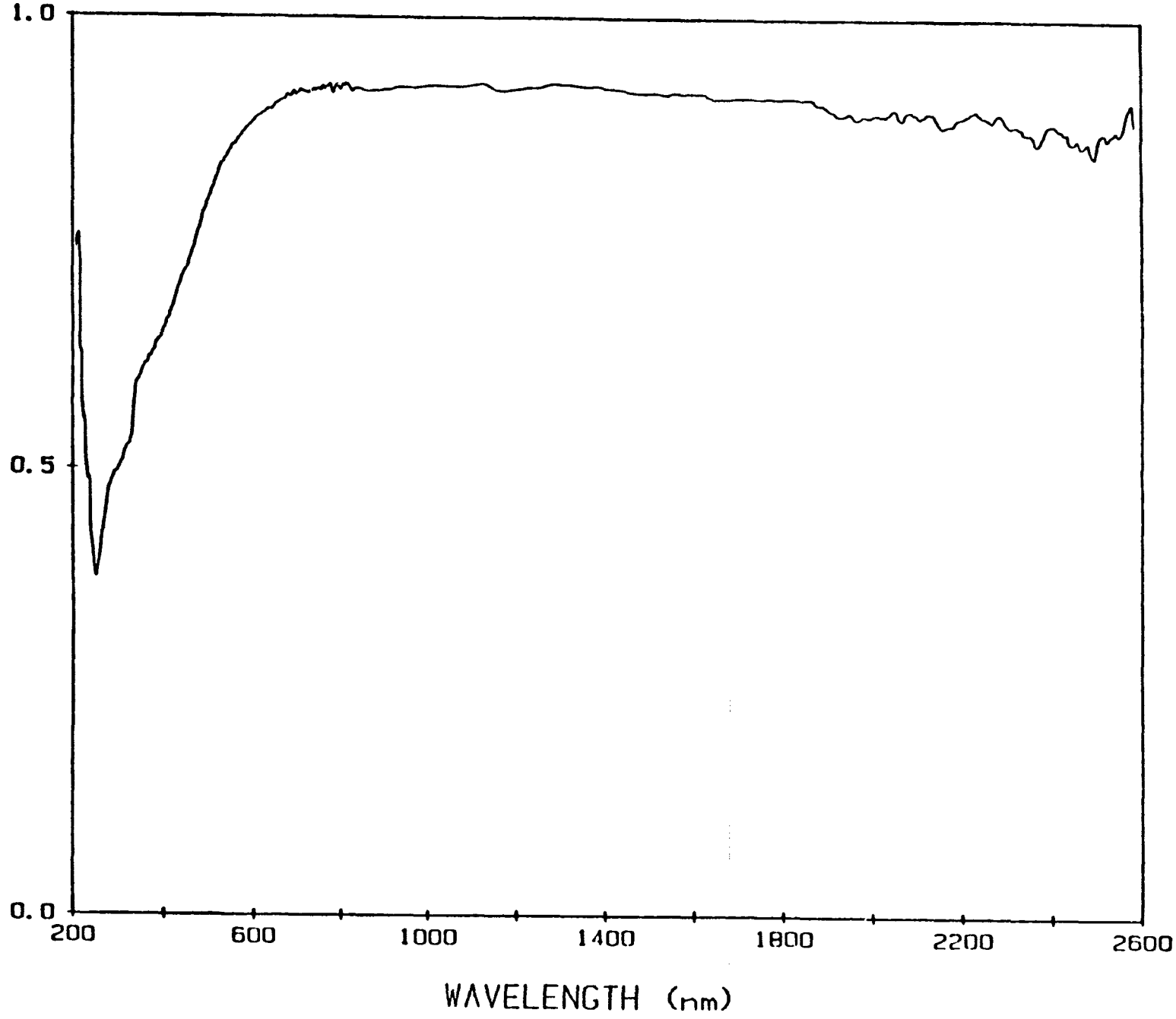


Figure 42 UV-VIS-NIR DRS of protein A 100 ng 100 ng A/paper blank

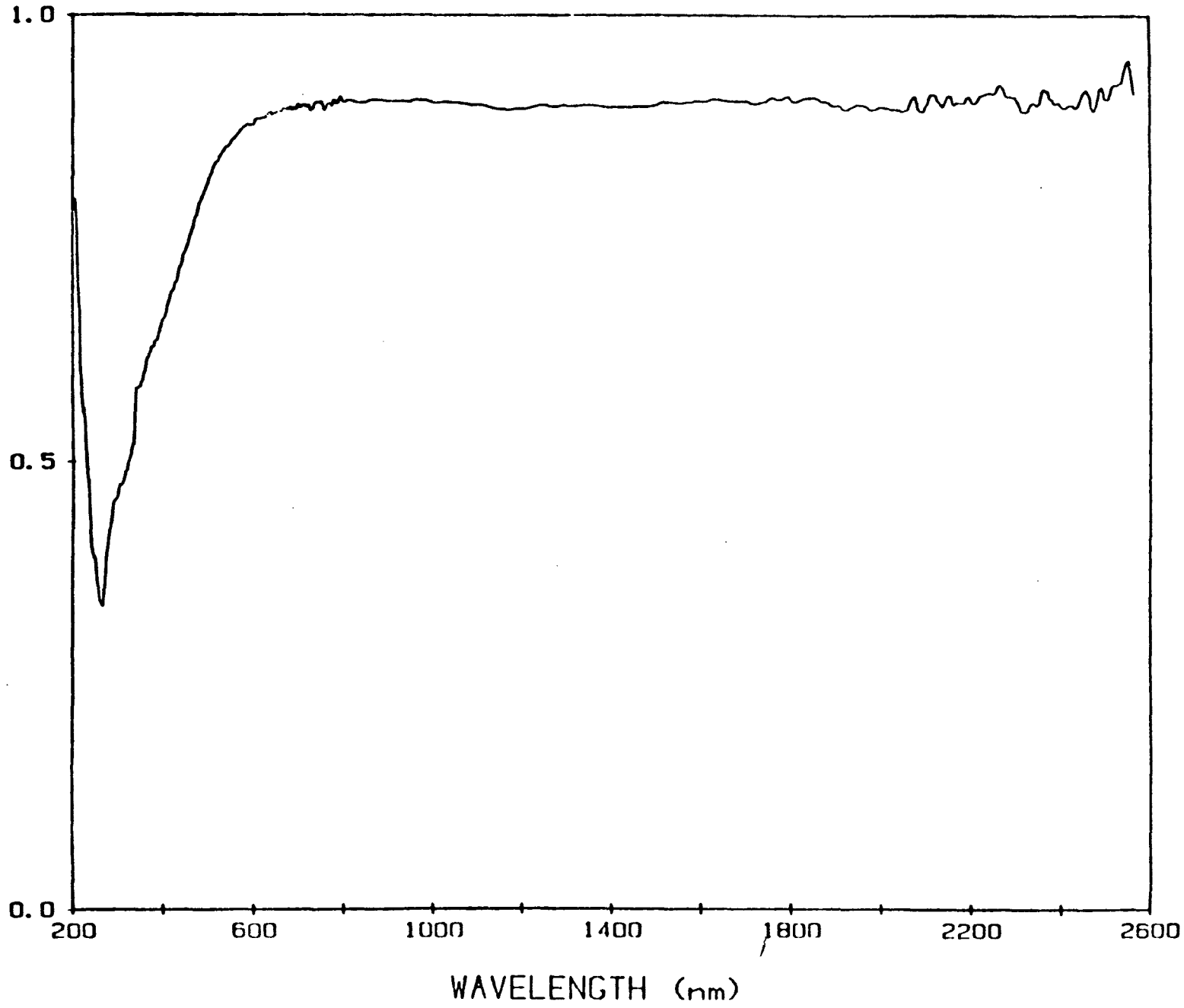


Figure 43 UV-VIS-NIR DRS of H_2O blank and protein A 100 ng 100 ng A/water blank

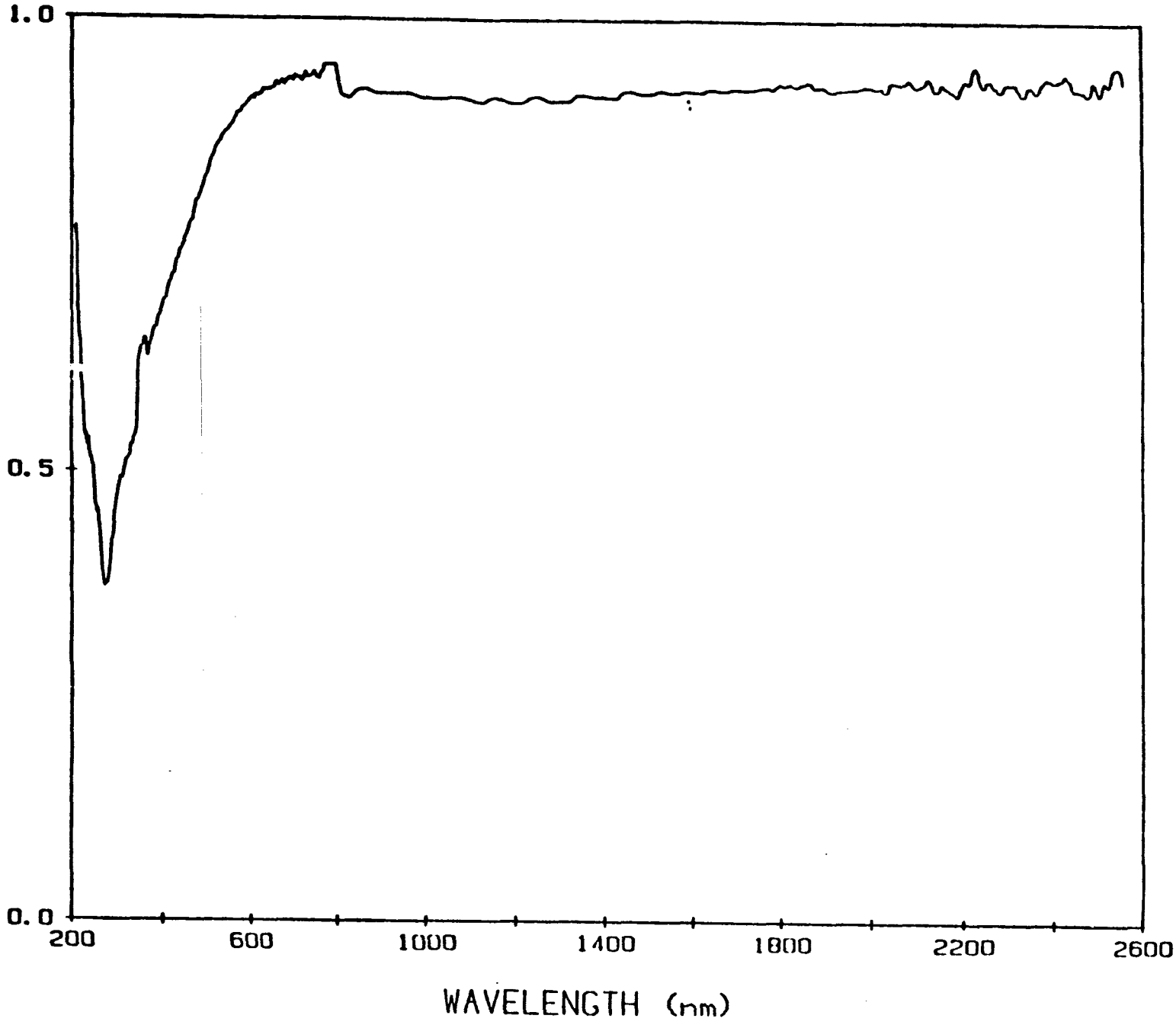


Figure 44 UV-VIS-NIR DRS of .1% detergenct and protein A 100 ng 100 ng A/dye blank

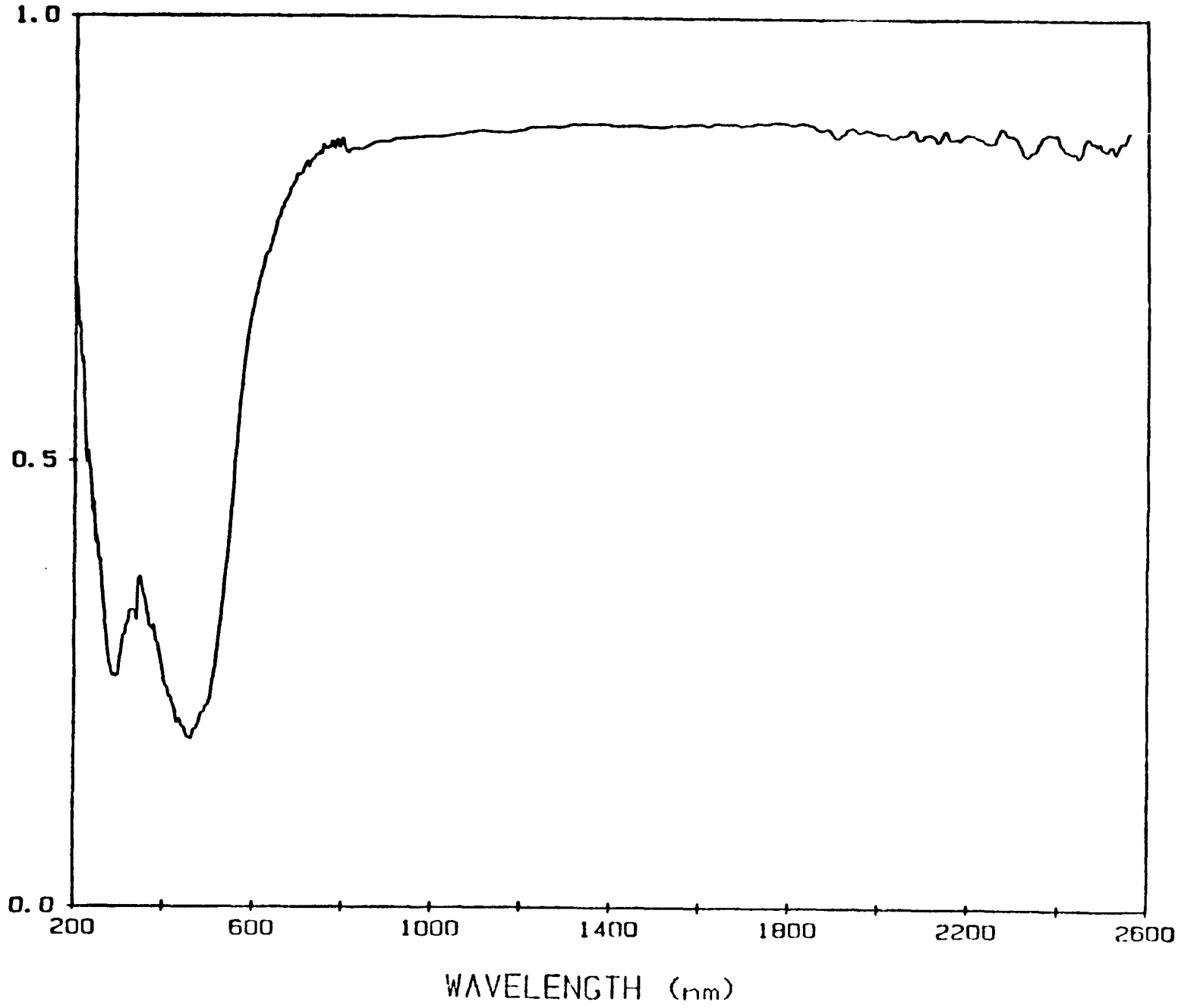


Figure 45 UV-VIS-NIR DRS of .1% dye blank and 100 ng protein A 100 ng A/dye blank

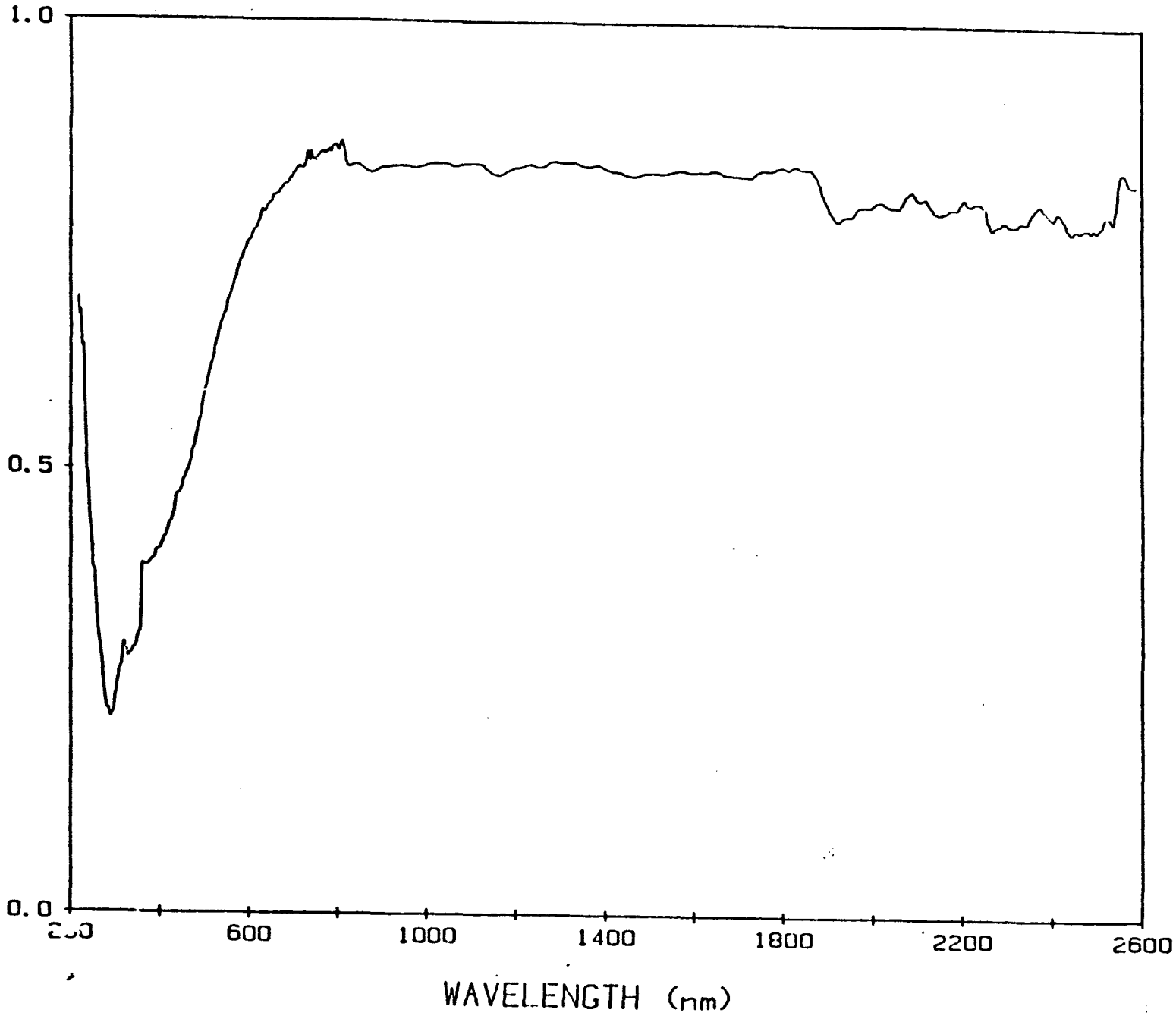


Figure 46 UV-VIS-NIR DRS of protein A 100 ng duplicate 100 ng A/paper blank

strong absorption in the 300 cm^{-1} region but not in the 1600 cm^{-1} in the IR, or 280 nm in the UV. Therefore, these two wavelengths remain the most likely for further investigation.

15.2 Piezoelectric Detection

A study was made to detect hydrogen sulfide (H_2S) and ammonia (NH_3) in the gas produced by pyrolysis of bacteria using a silver acetate ($AgC_2H_3O_2$) coated crystal and a chlorobenzoic acid coated crystal respectively. The set-up used was the simplest possible arrangement including a 5 inch glass tube with 1/4" ID to serve as sample carrier, connected at both ends with tygon tubing. A stopcock was attached at one end and the detector attached to the other. The sample was heated, the stopcock was opened, and air pulled through by the detectors pump, swept the gases produced over the coated crystal in the detector. When the H_2S detector crystal was in place any H_2S in the gas prodcued a decrease in the weight of the coating probably due to a loss of acetic acid which is produced and was greater than any increase caused by water absorbed by the crystal. The net effect of increase in frequency of vibration in the presence of H_2S showed that it was a feasible detector for H_2S.

The NH_3 detector showed a gain in weight for both NH_3 and H_2O and therefore would be impractical to use as a detector without additional stages to remove water. Water removal would be perfectly feasible but would add somewhat to the complexity of the set-up.

15.2.1 Purpose

The purpose of the piezoelectric detection experiment was to extend basic information previously obtained by experiments at University of New Orleans during the period May 25-27, 1983. It was found in New Orleans that bacteria, when ignited, yielded H_2S and possibly ammonia which could be detected by absorption on coated piezoelectric crystals causing a change in vibration frequency. The significance of temperature, time, and the effect of H_2O (formed when bacteria are ignited) required investigation.

15.2.2 Coatings and Their Mode of Behaviour

Some coatings were prepared in advance, others were made as needed. Although it became simpler after some practice, the first few were done poorly. It was found that thin coating followed by drying over a hot surface and repeating this process 2 or 3 times gave good results. The coating for NH_3 detection was chlorobenzoic acid dissolved in water, about .1 g in 5 cc of water. For H_2S silver acetate was used in the same proportions except that .1 M nitric acid replaced the water. A micro droplet was placed on each side, on the gold electrode resting on the crystal surface. The droplet was dried over a hot surface (a soldiering iron was used). This was repeated two more times.

The reaction between the H_2S and the silver acetate ($AgC_2H_3O_2$) is probably as follows:

$$\underset{34}{H_2S} + 2\ \underset{333.8}{AgC_2H_3O_2} \qquad \underset{247.8}{Ag_2S} + 2\ \underset{120}{CH_3COOH}$$

The numbers represent molecular weights. Before the H_2S is passed over the electrode $AgC_2H_3O_2$ is the coating on it. After the reaction the acetic acid (CH_3COOH) evaporates and only silver sulfide (Ag_2S) is left. As a consequence the mass represented by 333.8 is replaced by 247.8 i.e. a net loss in mass occurs and the vibration frequency is increased. It must be noted that the reaction is only in one direction, leading to the loss of the product. As a result the coating is consumed, and therefore, must be recoated or replaced once it has reacted with sufficient H_2S. However, in its intended use, if it has detected a significant influxof bacteria and emitted its warning, it would be demanding attention adn would presumably have served its purpose. In any case then be re-set with a replacement coated-crystal easily.

Reversible coatings for H_2S have been reported, for example one made from the soot produced by igniting chlorobenzoic acid, which is then extracted with acetone. Other coatings also have been reported, but have not tried any of them.

The NH_3 sensitive crystal, coated with chlorobenzoic acid, is however, reversible. It's reaction produces a loosely bound complex which adds to the mass on the coated-crystal, lowering its frequency of vibration.

Both of these coatings were used because they were provided by the vendor of the detector.

A good coating amount is one which changes the frequency by about 5,000 cycles/sec. The unloaded crystals were cut to vibrate at about 9 MCycles but only the 4 least significant digits were visible on the digital readout.

15.2.3 Experimental Procedures

The experimental set-up was very simple. A glass tube about 5 inches long and 1/4" I.D. was used to hold the bacteria sample (bacillus subtilus var niger, lypholized spores) which was inserted by a narrow spatula to the middle of the tube. The sample tube was connected with tygon tubing, at one end to a stopcock and at the other end to the inlet of the detector.

The detector (a commercial device) consisted of a small pump, pulling about 100 cc/minute of air across the surface of the piezoelectric coated-crystal; the crystal; and a digital readout showing the right-most 4 digits of the vibrational frequency of the crystal. When the above-mentioned stopcock was opened, the air path was thru the sample tube, over the detector.

The procedure was to place a small sample (upper nanogram range) into the sample tube, close the stopcock, record the reading, heat the tube at the location of the sample for a chosen number of seconds; using a blow torch, remove the heat, open the stopcock to air and start recording the reading every minute or two until the readings stopped changing.

15.2.3.1 Experiments to Detect H_2S

1) A small sample (we had no way to measure sample size, but all samples were probably below 1 microgram) was placed into the sample tube and run as above. Heat was for 5 seconds and, as the tube glowed red, the temperature attained was assumed to be 800°C. It was found that the readings required 10-14 minutes to reach a maximum after which it declined slightly. The reading increased about 400 counts.
2) A blank was runa s above with no sample. The reading showed a decrease of 50 counts after about 10 minutes, which on further counting to 20 minutes was restored to its original reading.
3) A small sample as in experiment 1 was run with similar results and an increase in 12-16 minutes of about 1200 counts.
4) Another blank was run with a decrease of 200 counts.
5) Another small sample was run, heating for 10 seconds. The increase in count was 300. At this point it was clear that the coating was blackened. Therefore, a fresh coating was prepared on a new crystal for the following runs.
6) This run used a sample about 3 times larger than the previous ones. This time the detector started to change rapidly and increased about 2200 counts within 2 seconds after opening the stopcock. It attained a maximum in about 10 minutes but only added an additional 300 counts. However, the coating had turned black with this one use.
7) Using a newly coated crystal small amount of water was used in place of the sample. The amount of water was a good deal greater than the sample size of the bacteria. The count declined by about 2200.
8) A small sample was run again with an increase of 1500.
9) Finally, a larger sample was used with the above detector. An increase of 1500 was found and the detector was blackened at this time.

15.2.3.2 Experiments to detect NH_3

1) A small amount of water resulted in a hugh change in reading with the count decreasing beyond 10,000 counts.
2) A small sample likewise, reduced the count greatly.
3) A very small amount of water still gave a large reduction in the count

15.2.4 Conclusions

The silver acetate - coated crystal reacts much more to H_2S than to water and in opposite directions. It is clear that the net results of the products of pyrolysis of the bacteria tested provides a favourable mixture for H_2S detection. The reduction in count due to water is probably a reversible effect whereas the increase in count due to H_2S is not. This may account for the time delay of about 10 minutes before the increase is seen with small samples. It is also noted that with larger samples, after an immediate increase and a time delay of about 10 minutes an additional increase is observed.

Under conditions of the experiment it was seen that glass, when heated to about 800° C, required a minimum of 5 seconds to char the bacteria, and the results after 5 seconds and 10 seconds of heating were similar.

It is clear that the use of a silver acetate-coated piezoelectric crystal for the detection of H_2S after the pyrolysis of bacteria is feasible with a simple set-up. A shortcoming is that it is an irreversible coating, once having signalled the existance of bacteria the crystal must be replaced. Although the crystals are inexpensive, about $10.00 each, and are simple to replace, it requires that someone be present to do so. Other coatings have been reported which are reversible and may therefore, satisfy our requirements better.

The ammonia experiments using chlorobenzoic acid-coated crystals were seen to also react to water as well as ammonia but in the same direction, that is both ammonia and water cause a decrease in count. It would be possible to remove the water by passing the vapor produced through silica or other absorbents but we were not in a position to undertake such a study.

We can only conclude that, using the simplest set-up, heating bacteria and passing the gas directly to a chlorobenzoic acid coated crystal will not be suitable to detect NH_3. Modifications resulting in the removal of watr or the use of coatings less sensitive to water might well change the picture.

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1984 Lovborg U., Amer. Clin. Products., January, p26-28: Monoclonal Antibodies.
[This article provides a short review of the field.]

1983 Navarrete P. and Serrano R., Biochimica et Biophysica Acta., v.728, p403-408: Solubilization of Yeast Plasma Membranes and Mitochondria by Different Types of Non-Denaturing Detergents.
[These include solubilization methods for bacterial cell membranes.]

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1976 Guschlbauer W., Nucleic Acid Structure - An Introduction. Springer-Verlag Publ.

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[This paper describes a limulus endotoxin assay.]

1973 Daniels S.L., Develop. Indust. Microbiol., v.13, Chap.19: The Absorption of Microorganisms onto Solid Surfaces - A Review.

1973 Gregory P.H., The Microbiology of the Atmosphere. Second Edition. A Plant Science Monograph. Leonard Hill - an Intertect publisher.

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1970 Jost R. and Fey H., Applied Microbiol., v.20, no.6, p861-865: Rapid Detection of Small Numbers of Airborne Bacteria by a Membrane Filter Fluorescence Antibody Technique.
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1970 Graber C.D., Editor; Rapid Diagnostic Methods in Medical Microbiology. Williams and Wilkens Publ.
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1969 Mitz M.A., Annals of the New York Acad. Sci., v.158, p651-664: The Detection of Bacteria and Viruses in Liquids.
[This is work done for NASA. Various automated methods are discussed.]

1969 May M.A., Applied Microbiol., v.18, no.3, p513-514: Prolongation of Microbial Air Sampling by a Monolayer on Agar Gel.
[The author reports that oxethylene docosanol reduces surface evaporation.]

1969 Perfil'ev B.V. and Gabe D.R., Capillary Methods of Investigating Micro-Organisms, translated by Shewan J.W., University of Toronto Press.
[The authors use capillary tubes to culture and examine bacterial specimens.]

1967 Bowman R.L., Blume P. and Vurek G.G., Science, October, p78-83: Capillary-Tube Scanner for Mechanized Microbiology. A Photoelectric Scanner Scans Agar - Filled Capillaries to Count Viable Colonies.

1951 Army Contract W-18-064-CM-207, Early Detection of Bacterial Growth.

[A study done by the California Institute of Technology similar to the present study.]

CELL SORTER

1983 Dovichi N.J., Martin J.C. and Keller R.A., Science, v.219, p845-847: Attogram Detection Limit by Laser-Induced Fluorescence.
[The authors use flow cytometers to detect dye molecules down to 35,000 molecule levels.]

1982 Lasers and Applications - October, p12-13.
[A news item about a laser activated cell sorter developed at Livermore National Laboratory and the University of California.]

1982 Pinkel D., Analy. Chem., v.54, no.3, p503A-519A: Flow Cytometry.
[This is an instrumentation article.]

1981 Alexander M.K., Khan M.S. and Dow C.S., Jour. Clin. Pathol., v.34, p194-198: Rapid Screenign for Bacteriuria using a Particle Counter, Pulse Height Analyzer and Computer.

1981 Pintino F. and Findl E., Naval Research Contract No. N00014-78-C-0713, Task No. NR 133-994: Development of a Methodology for the Rapid Detection of Coliform Bacteria - Final Report.
[In this description a flow cytometer and fluorescent enzyme were used.]

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[These are techniques applicable to biomedical problems.]

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[Cells can be made to have different fluorescent intensities o different light scattering characteristics; they can then b separated as they pass two laser beams.]

1973 Kaye W., Jour. Colloid and Interface Sci., v.44, no.2 p384-386: Low Angle Light Scattering - Particle Measurement. Note.

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1984 Jinno K., Fujimoto C. and Uematsu G., Amer. Labor., February, p39-45: Micro-HPCL/FTIR.
[The authors points out that where compounds cannot be separated by GC due to low volatility, HPCL can still provide an excellent analytical separation. In this article they mate the FTIR with a micro-HPCL to study complex mixtures.]

1984 Milby K.H. and Zare R.N., Amer. Clin. Products, January, p12-19: Antibodies, Lasers and Chromatography.
[This article discusses laser fluorimetry as a detector for HPCL.]

1984 Jennings W., Amer. Labor., January, p14-32: The Renaissance in Analytical Chromatography.
[Discusses up to the minute developments, especially microbore columns and newer "bonded phase" columns.]

1983 Stafford D., Kelley P. and Finnigan R.E., Amer. Labor., June, p51-57: Advanced Ion Trap Technology, An Economical Detector for GC.

1983 Borman S.A., Analy. Chem., v.55, no.8, p836A-842A: Photodiode Array Detectors for Liquid Chromatography.

[This type of detector can give a complete spectrum in 0.01 seconds, yielding a reduction in time or an improvement in S/N. The authors compare the use of these detectors with LC to the use of mass spectrophotometeric detection for GC.]

1983 Horvath E.C., High Performance Liquid Chromatograhy, vol.3, Advances and Perspectives. Academic Press.

1983 Regnier F., Analy. Chem., v.55, no.13, p1298A-1306A: HPCL of Proteins, Peptides and Polynucleotides.
[Because samples of biological origin may contain 2000 or more constituents, the author states that multiple columns of different type are required to separate the various classes. He discusses size exclusion (SEC), ion exchange (IEC), reversed phse (RPC) and liquid affinity (LAC) chromatographies only.]

1983 Novotny M., Analy. Chem., v.55, no.13, p1308A-1314A: Microcolumn Separation Methods and their Ancillary Techniques: A Joint U.S.-Japan Seminar.
[Much information is presented on the rapidly changing fields of miniaturized instrumentation in modern chromatography, spectroscopy and electrochemical detection.]

1983 Isidorov V.A., Zenkevich I.G. and Ioffe B.V., Atmos. Environ., v.17, no.7. p1347-1353: Methods and Results of Gas Chromatographic Mass Spectrophotometric Determination of Volatile Organic Substances in an Urban Atmosphere.
[Leningrad is studied for average maximum and minimums for a large number of organics.]

1983 Attygalle A.B. and Morgan E.D., Analy. Chem., v.55, no.8, p1379-1384: Reaction GC without Solvent for Identification of Nanogram Quantities of Natural Products.
[Uses 200 ng samples to determine the position of double bonds in alkenes.]

1983 Tunlid A. and Odhai G., Jour. of Microbiol. Methods, v.1, p63-76: Capillary Gas Chromatography using Electron Capture of Selected Ion Monitoring Detection for the Determination of Muramic and Diamino Pimelic Acid and the Ratio D/L Alanine in Bacteria.

1983 Tracor Northern (an advertisement), Analy. Chem., v55., no.8, p837A: The Power of Spectral Detection.

1982 Boykins, R.A. and Liu T.Y., Jour. of Biochem. Biophys. Methods, v.7, p55-65: Microanalysis of Amino Acids.
[This is an automatic procedure that uses 10-100 picomoles of sample.]

1981 Drucker D.B., Microbial Applications of Gas Chromatography. Cambridge University Press.
[Describes mass spectroscopy of bacteria and parts of bacteria such as cell walls as well as gas chromatography after pyrolysis.]

1981 Robey F.A. and Liu T.Y., Jour. of Biol. Chem., v.256, no.2, p969-975: Lumulin: A C-Reactive Protein from Limulus Polyphemus.
[The authors separates Lumulin from horseshoe crab Limulus and shows this to be the active precipitant for bacterial cells. This is used in a test for bacteria in which the occurance of a precipitate indicates the presence of bacteria.]

1980 Rittinghaus K. and Franzen K.H., Fresenius Zeit. Analy. Chem., v.301, p144: HPCL in Protein Analysis: An Alternative to Gel-Filtration and Gel-Electrophoresis.
[Separates acidic neutral and basic proteins with molecular weights 2000-80000 daltons on the basis of effective molecular weight in solution.]

1979 Simmonds P.G. and Kerns E., Jour. of Chromatog., v.186, p785-794: Direct Aqueous Injection Gas Chromatography for the Analysis of Trace Organics in Water.
[A novel and simple method of selectively removing the water from the vapor prior to entrance to the chromatographic columns, based on the use of a plastic tube, Nafion, which is permeable to water only. The water diffuses out but the organics continue on to the column.]

1976 Mitruka B.M. and Bonner M.J., Editors; Methods of Detection and Identification of Bacteria. CRC Press.

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1965 Reiner E., Nature, v.206, p1272: Fingerprinting Bacteria using Gas Chromatography.

DIFFUSE REFLECTANCE

1983 Ditzler M.A., Allston R.A., Casey T.J., Spellman N.T. and Willis K.A., Applied Spectros., v.37, no.3, p269-272: Diffuse Reflectance UV-VIS Spectroscopy, An Economical Method for Studying Reagents Immobilized on Silica Gel.
[Discusses the elimination of the contribution of the substrate and demonstrates that reflectance curves are similar to absorption curves.]

1983 Hannah R.H. and Anacreon R.E., Applied Spectros., v.37, no.1, p75-77: Diffuse Reflectance using Infrared Dispersive Spectrophotometers.
[Uses the Perkin Elmer model 983 and 1420 spectrophotometers with diffuse reflectance accessories from Harrick Scientific Co. Powdered samples were mixed with powdered potassium bromide.]

1983 Wetzel D.L., Analy. Chem., v.55, no.12, p1165A-1176A: Near-Infrared Analysis - A Sleeper among Spectroscopic Techniques.
[This report gives a list of advantages and some applications, especially in agricultural commodities and pharmecuticals. They point out that subtle differences, not clear in curves, are well handled by computer comparisons.]

1982 Rohl R., Childers J.W. and Palmer R.A., Analy. Chem., v.54, p1234-1236: Diffuse Reflectance Attachment for a Photoacoustic Spectrophotometer.
[Converts a PAR 6001 UV-VIS-NIR spectrophotometer by adding a DRS attachment with a Laser Precision Corp. Model KT-2230 detector.]

1980 Henderson G. and Bryant M.F., Analy. Chem., v.52, no.11, p1787-1789: Comparison of Near-Infrared Reflectance and Photoacoustic Spectra of Grain Samples.
[Finds that where samples are thick, thermal properties enter into photoacoustic measurements, but not with thin samples.]

1978 Griffiths P.R. and Fuller M.P., Analy. Chem., v.50, p1906: Diffuse Reflectance Infrared Fourier Transform Spectroscopy.

1969 Kortum G., Reflectance Spectroscopy: Principles, Methods, Applications. Springer-Verlag Publ.

1966 Wendlandt W.W. and Hecht H.G., Reflectance Spectroscopy, (Volume 21 of Chemical Analysis; Koltoff E., Editor). Interscience Publ.

1983 Trulson M.O. and Munk M.E., Analy. Chem., v.55, no.13, p2137-2142: Table-Driven Procedure for Infrared Spectrum Interpretation.

[A program is given, requiring peak position, intensity and shape. By comparison with an experimental data file 18 classes of compounds can be identified.]

1983 Griffiths P.R., deHaseth J.A. and Azarraga L.V., Analy. Chem., v.55, no.13, p1361A-1387A: Capillary GC/FT-IR: Instrumentation.

[Because of its great speed and the capability to do repeat scans hundreds of times in seconds, thus enhancing S/N, the procedure can be applied to small samples carried in capillaries. Much of the instrumental development is described here.]

1982 Vanasse G.A., Applied Optics, v.21, no.2, p188-195: Infrared Spectrometry.

[A general description.]

1982 Lam R.B., Sparks D.T. and Isenhour T.C., Analy. Chem., v54, p1927-1931: Cross Correlation of Signal to Noise Ratio Enhancement to Quantitative GC/FR IR.

[This technique improves S/N by as much as three times when applied to gas chromatography.]

1982 Hanst P.L., Wong N.W. and Bragin J., Atmos. Environ., v.16, no.5, p969-981: A Long-Path IR Study of Los Angeles Smog. [Gaseous pollutants were followed in June for two days, down to parts per billion.]

1982 Marshall A.G., Editor; Fourier, Hadamard and Hilbert Transforms in Chemistry. Plenum Press.

1981 Aaronson J., Chemical Communications at the Microbial Level, v.1. CRC Press.

1980 Low M.J and Parodi G.A., Applied Spec., v.34, no.1, p76-80: Infrared Photoacoustic Spectroscopy of Solids and Surface Species.

1980 Gandy A.F. and Gandy F.T., Microbiology for Environmental Scientists and Engineers. McGraw Hill Publ.

1978 Mattson J.S., Mark H.B. and MacDonald J., Editors; Infrared, Correlation and Fourier Transform Spectroscopy. Marcel Dekker Publ.
[An excellent textbook, describing theory and instrumentation.]

1978 Powell L.A. and Hiefje P., Analy. Chem. Actu, v.10, p313-318: Identification of Infrared Spectra by Correlation-Based File Searching.

1978 Ferraro J.R. and Basile L.J., Editors; Fourier Transform IR Spectroscopic Applications and Chemical Systems, vol.2. Academic Press.

1978 Griffiths P.R., Editor; Transform Techniques in Chemistry. Plenum Press.

1977 Dolphin D. and Wick A., Tabulation of Infrared Spectral Data. John Wiley Publ.

1976 Mitruka B.M. and Bonner M.J., Methods of Detection and Identification of Bacteria. CRC Press.

1976 Jentzen E., Froholm L.O., Hutta R. and Bovre K., Acta. Pathol. Microbiol. Scand., Sect. B. vol.80, p660: Gas Chromatography of Bacterial Whole-Cell Methanolysates.

1975 Anderson D. and Wilson T.E., Analy. Chem., v.47, no.14, p2482-2483: Novel Approach to Micro Infrared Preparation. [This sample of less than 0.5 ug is ground with KBr and then the pellet is pressed into an aperature of a 0.25 jewel and is then scanned in a Digilab Model FTS-14 FT/IR spectrophotometer.]

1975 Mitruka B.M., Gas Chromatographic Applications in Microbiology and Medicine. John Wiley Publ.

1972 Miller R.G. and Stace B.C., Laboratory Methods in Infrared Spectroscopy. Heydon and Son Publ.
[A good section on tracking down spurious bands in infrared analysis.]

1971 Nyquist R.A. and Kagel R.O., Infrared Spectra of Inorganic Compounds (3800-45 cm^{-1}). A Collection of IR Curves. Academic Press.

1971 Parker F.S., Editor; Application of IR Spectroscopy in Biochemistry, Biology and Medicine. Plenum Press.
[See especially Chapter 17 Microbiology which is primarily on identification of metabolic products upon culturing microorganisms.]

1970 Yamaguchi K., Spectral Data of Natural Products, vol.1. Elevier Press.
[Gives UV, IR and Mass Spectra of natural products classified by chemical structure.]

1967 Reynolds, R.J., Hedrick H.J. and Crum M.J., Develop. Indust. Microbiol., v.8, chapt.27, p246-252: Application of IR Spectroscopy to Rapid Identification of Hydrocarbon Fuel Contaminating Microorganisms.
[After culturing, identification was possible if all parameters were carefully controlled. Due to complexity of spectra and overlap, all spectra were found to be similar but with subtle differences.]

1966 Kendall D.N., Editor; Applied Infrared Spectroscopy Reinhold Publ.
[This was an excellent general textbook.]

1964 White R.G., Handbook of Industrial Infrared Analysis Plenum Press.
[Gives the major absorption wavelengths of compounds classified as to chemical type.]

1952 Stevenson H.J.R. and Bolduan O.E.A., Science, v.116 p111-113: Infrared Spectroscopy as a Means for Identification of Bacteria.

LASERS

1983 Mims III, F.M., Computers and Electronics, October, p99-102: Visible Light Laser Diodes.

1983 Green R., Analy. Chem., v.55, no.1, p20A-32A: Report - Lasers: Practical Detection for Chromatography?

1983 Patel B., Photonics Spectra, February, p33-34: The Helium-Neon Laser: Applications.
[A short description of four applications of a cheap, reliable visible light red laser at 632.8 nm.]

1983 Sepaniak M.J., Tromberg B.J. and Eastam J.F., Clin. Chem., v.29, no.9, p1678-1682: Optical Fiber Fluoroprobes in Clinical Analysis.
[Used to do in vivo molecular fluorescence measurements.]

1983 Higashi T., Imashka T. and Ishibashi N., Analy. Chem., v.55, no.12, p1907-1910: Thermal Lens Spectrophotometry with Argon Laser Excitation Sources for Determination of Nitrogen Dioxide.
[Detection limit for NO_2 was 5 ppb using the 488 nm argon laser with a dynamic linear curve over four orders or magnitude. Had advantages over photoacoustic monitoring of atmospheric NO_2.]

1983 Yamada S., Hino A., Kano K. and Ogawa T., Analy. Chem., v.55, no.12, p1914-1917: Laser Two-Photon Ionization for Determination of Aromatic Molecules in Solution.
[This is particularly useful for weakly or non-fluorescent molecules.]

1983 Jeannesson P., Manfait M. and Jardillier J.C., Analy. Biochem., v.129, p305-309: A Technique for Laser Raman Spectroscopy: Studies of Isolated Cell Populations.
[This study gives information on the reaction of cells to laser radiation, but is primarily concerned with cell reaction to drug therapy.]

1983 Leybold-Heraeus Corp., Laser Microprobe Mass Analysis.
The LAMMA is a microprobe mass analyzer made by the Leybold-Heraeus Corporation which puts out many brochures describing applications and a large reference list.

1983 Surkyn P., DeWaele J. and Adams F., Internat. Jour. Environ. Analy. Chem., v.13, p257-274: Laser Microprobe Mass Analysis for Source Identification of Air Particulate Matter.

1983 Rhodes C.K., Kodak Labor. Chem. Bull., v.54, no.2, p1-6: Dye, Vacuum Ultraviolet and Extreme Ultraviolet Lasers.
[Discusses laser technology to produce radiation at less than 100 nm and is expected to reach 1 nm when fully developed.]

1982 Whitten W.B., Analy. Chem., v.54, p1026-1028: Time of Flight Optical Spectrometry with Fiber Optic Waveguides.
[Provides an absorption spectra in a few microseconds.]

1982 Carey P.R., Biochemical Applications of Raman and Resonance Raman Spectroscopies. Academic Press.

1982 Hercules D.M., Day R.J., Balasanmugam K., Dang T.A. and Li C.P., Analy. Chem., v.54, p280A-290A: Laser Microprobe Mass Spectrometry 2. Applicatoins to Structural Analysis.
[Discusses the mechanism of laser ionization/volatilization and describes information obtained from a single bacterial cell on inorganic matter.]

1981 Demtroder W., Laser Spectroscopy. Springer-Verlag Publ.
[This textbook gives the basic concepts and instrumentation in considerable detail as regards the analytical use of lasers. Does not delve into the theory of lasers per se.]

1981 Denoyer E., Van Grieken R., Adams F. and Natusch D.F.S., Analy. Chem., v.53, p26A-32A: Laser Microprobe Mass Spectrometry 1: Basic Principles and Performance Characteristics.
[The technique uses a high intensity laser pulse to vaporize and ionize a solid sample and the elemental and molecular ions produced are then mass analyzed in a time-of-flight (TOF) mass spectrometer. Its most important aspects are high sensitivity approximately 10-20 ug, speed. Useful for inorganic and organics and its small sample capacity, 1 ug.]

1981 Hieftje G.M., Travis F.E. and Lytle F.E., Editors; Lasers in Chemical Analysis. Human Press.

1980 Gardella J.A., Hercules D.M. and H.J. Heinen Spec. Letters, v.13, no.6, p347-360: Mass Spectroscopy of Molecular Solids.
[Describes positive and negative ion mass spectroscopy of organic polymers using LAMMA.]

1979 Omenetto N., Editor; Analytical Laser Spectroscopy. John Wiley and Sons Publ.
[Contains an excellent section on fluorescence and microfluorescence.]

1979 Seidel U. and Heinen H.J., Sixth International Symposium on Mass Spectrometry in Biochemistry and Medicine. Venice, Italy, June 1979. First Results on Fingerprinting of Single Mycobacteria Cells with LAMMA.

1979 Dhamelincourt L. and Wallart F., Analy. Chem., v.51, no.3, p414A-421A: Laser Raman Molecular Microprobe (MOLE).

1976 Hinkey E.D., Laser Monitoring of the Atmosphere. Springer-Verlag Publ.
[Describes various Lidar measurements to obtain size, distribution and refractive index.]

1975 Rosasco G.J., Etz E.S. and Cassett W.A., Appl. Spectros., v.29, no.5, p396-404: The Analysis of Descrite Fine Particles by Raman Spectroscopy.

1974 Moore C.B., Editor; Chemical and Biochemical Applications of Lasers. Academic Press.

1974 Marchand A., Piand J.J. and DuPasquier P., Applied Microbiol., v.27, p874-877: A High Precision and Large Capacity Laser Photometer.
[Describes a circular carrier with 32 cultures in depressions. Reads each every three seconds. Claims that because of its coherent light, the laser readings are linear with growth. Says results are meaningful in three minutes.]

MASS SPECTRA

1983 Laxhiuber L. and Mohwald H., Inter. Jour. Mass Spec. and Ion Physics, v.51, p93-110: Secondary Ion Mass Spectrometry of Organic Model Systems.

1983 Kemper P.R. and Bowers M.T., Inter. Journ. of Mass Spec. and Ion Physics, v.52, p1-24: An Improved Tandem Mass Spectrometer-Ion-Cyclotron Resonance Spectrometer.

1983 IBID, v.51, p93-110: Secondary-Ion Mass Spectrometry of Organized Organic Model Systems.
[Uses monomolecular layers of fatty acids as model systems to study biological processes. Shows that chemical composition can be determined with a depth resolution of better than 25 A.]

1983 Science Editor, Chem. and Eng. News, September, p19-20: Progress in Coupling Systems Boosts LC/MS Prospects.

1983 Worthy W., Chem. and Engin. News, September, p19-10: Progress in Coupling Systems Boosts LC/MS Prospects.
[Describes new ion sources, microbore columns, better nozzles and the supersonic jet method for purposes of interfacing LC and Ms.]

1982 Graham S.W. and Hercules D.M., Spectros. Letters., v.15, no.1, p1-19: Laser Desorption Mass Spectra of Biomedical Polymers.

1982 Sinha M.P., Griffith C.E., Morris D.D., Estes T.J. and Vilker V.L., Jour. of Colloid and Interface Sci., v.87, no.1, p140-153: Particle Analysis by Mass Spectroscopy.
[Most of the article describes the equipment to do single particle analysis in the micron range in real time continuous mode.]

1982 Scheifers S.M., Hollar R.C., Busch K.L. and Crooks R.G., Amer. Lab., March, p19-33: Molecular Secondary Ion Mass Spectroscopy.
[This article is a well-illustrated pictorial review of a surface analysis technique. It can be used to analyse directly off a material such as a plant or a substance placed on a surface.]

1982 Denoyer F., Natuse L.F.S, Adams F. and Van Grieken R., Analy. Chem., v.54, no.1, p26A-41A: Basic Principles and Performance Characteristics (LAMMA).

1981 Voorhees K.J. and Hileman F.D., Jour. Analy. and Applied Pyrolysis, v.3, p151-160: Pyrolysis-MS Study of Atmospheric Particle.
[Samples to distinguish various particulate sources.]

1982 Hercules D.M., Day R.G., Balasanmugan K. and L.C.P. Anal. Chem., v.54, p280A-300A: Laser Microprobe Mass Spectrometry, Applications to Structural Analysis.

1981 Bahr U., Bocek P., Gieck R., Schlunegger U.P. and Schulten H.R., Contributors, Analytical Problems. Springer-Verlag Publ.
[Contains an interesting chapter on mass spectrometry as well as a section on directly sampled natural aerosols.]

1981 Morris H.R., Editor; Proceedings of the Chemical Society on Advances in Mass Spectrometry Soft Ionization Methods. London, July 1980. Soft Ionization Biological Mass Spectrometry. Heyden Publ.

1980 Gardella J.A. and Hercules D.M., Spectros. Letters, v 13, no.6, p347-360: Laser Microprobe Mass Analysis (LAMMA) of Selected Polymers.

1980 Day R.J., Unger S.E. and Cooks R.J., Analy. Chem., v.52, no.4, p557A-572A: Molecular Secondary Ion Mass Spectrometry (SIMS).
[Provides a means of doing mass spectrometry of surface layers.]

1979 Gochman N., Bowie L.J. and Bailey D.N., Analy. Chem., v.51, no.4, p525A-542A: Specialized GC/MS for Clinical Chemistry.
[This article refers to rapid identification of bacteria by their characteristic molecular fragments. Identification is made the same day.]

1978 Burlingame A.L., Shackleton C.H.L., Howe I. and Chizhov, Analy. Chem., v.50, p346R, A Review. Also see: Analy. Chem., v.50, p371R, A paragraph of the review section which describes linear programmed thermal degradation MS.

1975 Schulten H.R. and Schurath U., Atmos. Environ., v.9, p1107-1112: Aerosol Analysis by Field Desorption Mass Spectrometry Combined with a New Sampling Technique.
[Sub-microgram quantities of aerosols are impacted directly on a field emmitter which is then transferred into the mass spectrometer.]

1975 Anbalt P. and Fenselau C.C., Analy. Chem., v.47, no.2, p219-225: Identification of Bacteria Using Mass Spectrometry.
[Suggests direct insertion of a bacterial specimen into the ion source of an MS.]

1970 Simmonds P.G., Applied Microbiol., v.20, no.4, p567-572: Whole Microorganisms Studied by Pyrolysis-GC/MS: Significance for Extra-Terrestrial Life Detection Experiments.
[During the work with micrococcus luteus and bacillus subtilis var niger much similarity is found with other sources of proteins and carbohydrates, and a great difference between these and other types of materials.]

1982 Hirose S., Hayashi M., Tamura N. and Kamidate T., Analy. Chem., v.54, p1690-1692: Determination of Ammonia with a Microwave Stark Cavity Resonator.
[This article demonstrates an application of microwave analysis.]

1981 Kitchin R.G., Willis R.E. and Cook R.L., Analy. Chem., v.53, p1190-1192: Detection of Methanol in Wine by Microwave Spectroscopy.
[Used 26.5-40 GHz. Moderate amounts of methanol were detected in a rapid scan.]

1980 Swicord M.L., Ph.D. Thesis, Maryland University: Studies of Microwave Absorption in Liquids by Optical Heterodyne Detection of Thermally Induced Refractive Index Fluctuations.

1980 Ghandhi O.P., Hagman M.J., Hill D.W., Partlow L.M. and Bush L., Bioelectromagnetics, v.1, no.3, p285-298: Millimeter Wave Absorption Spectra of Biological Samples.
[The authors tested cellular, viral, biological and pharmacological suspensions in water and found that they could not be differentiated by microwave absorption.]

1980 Bainikov V.S. and Roshkov S.B., Dokl. Akad. Nauk., SSSR, v.255, no.3, p746-748: Absorption of Microwaves by Bacterial Cells.
[Studies E. Coli and K-12 (Lamda) at 4126 MHz. Refers to the possibility of identifying bacterial cells by a combination of dispersed light in the range 30-200 cm^{-1} and microwave absorption at 65-95 GHz.]

1979 Chantry W., Editor; Modern Aspects of Microwave Spectroscopy. Academic Press.
[Has a 405-item reference list.]

1979 Varma R. and Hurbesh L.W., Chemical Analysis by Microwave Rotational Spectroscopy, from the series "Chemical Analysis", v.52. John Wiley Publ.
[An excellent textbook, including a section on pyrolysis and the use of high temperature cells to observe species in situ.]

1979 Reporter, Design News, February 5, 1979, Radio-Sized Tools Detect Pollution.
[This news article refers to a patent of the Lawerence Berkeley Labs which uses a concave wall moved back and forth by a piezoelectric tuner to keep the cavity in resonance with the microwave frequency.]

1978 Kadaba P.K., Bhugat P.R. and Goldberger G.N., Bull. Environ. Contam. and Tox., v.19, p104-111: Application of Microwave Spectroscopy for the Simultaneous Detection of Toxic Constituents in Tobacco Smoke.
[The authors demonstrate that vapors can be well characterized by microwave absorption.]

1977 Rinehart E.A., Analy. Chem., v.49, no.2, p249A-262A: Analytical Microwave Spectroscopy: Instrumentation.

1976 Jones R.C.E. and Cramers C.A., Analy. Pyrol., September. Proceedings of the Third International Symposium on Analytical Pyrolysis.

1975 Mishuck E. and Roberts M., Automation in Microbiology and Immunology (Hedin and Illeni, Editors; John Wiley Publ.).
See especially Chapter 28, p493-504.

1975 Svehla G., Editor; Comprehensive Analytical Chemistry. Elsevier Scientific Publ.
[Volume V, Chapter 2, Analytical Microwave Spectroscopy has some clear discussions.]

1975 Townes C.H. and Schawlow A.L., Microwave Spectroscopy, Chapter 18, p486-498: The Use of Microwave Spectroscopy for Chemical Analysis. Dover Publ.

1972 Graff R.W. and Suffet I.H., Intern. Jour. Environ. Analy. Chem., v.1, p327-342: Diagnosis of Air Pollution by Microwave Spectroscopy.

[The authors point out that the results at atmospheric pressure are fraught with trouble due to water vapor. However, at very low pressures the results are meaningful.]

1969 Webb S.J. and Booth A.D., Nature, v.222, p1199-1200: Absorption of Microwaves by Microorganisms.

[The authors exposed cells of E. Coli in films dried at 80% relative humidity to microwaves. They claimed that IR spectra differentiate microbes very little but microwave spectra between 65 and 75 GHz are clearly very different.]

MISCELLANEOUS

1984 Wohltjen H., Analy. Chem., v.56, no.1, p87A-100A
Chemical Microsensors and Microinstrumentation.
[The article especially stresses microfabrication an microlithography as the means to close the gap betwee microelectronics advances and instrumentation. Excellent revie of state-of-the-art of gas sensors of microscope slide-size devices. The author points out that all chemical microsenso devices fall into two categories. The first senses chemica species which effect the transport of electronic charge in th device. Examples are CHEMFET's, ion-controlled diodes, Schottsk diodes, thin film tin oxide gas sensors, chemiresistors an microdielectrometers. The second category is "all others" including surface acoustic devices, potentiometric gas sensor and pyroelectric enthalpimetric sensors.]

1984 Novitsky T.L., Medical Device and Diagnostic Industry v.6, no.1, p49-53: LAL Methodology - The Choice is Yours.
[Describes four ways of applying the Limulus Amebocyte Lysat (LAL) test for the presence of bacteria. This is a quantitativ (or qualitative) test in which, if bacteria are present, a clo forms.]

1983 Natusch D.F.S. and Hopke P.K., Analy. Aspects of Environ Chem., v.64 of the series Chemical Analysis. Elving Winefordner, Koltoff; Editors. John Wiley and Sons Publ.

1983 Webster C.R. and Rettner C.T., Laser Focus, February, p41-46: Laser Optogalvanic Spectroscopy of Molecules - A Novel Technique.

[The technique is used to study exotic molecular species present in flames.]

1983 Abromowitz M., Amer. Lab., April, p38-41: Rheinberg Illumination.

[This is a microscopy illumination technique to reveal details otherwise requiring staining. One can use multicolor to better bring out microstructure.]

1983 Good D.W. and Vurek G.G., Analy. Biochem., v.130, p199-202: Picomole Quantitation of Ammonia by Flow-through Fluorimetry.

[This is a fiber optics fluorimeter. It measures the decrease in fluorescence and can detect a difference of 0.3 pmole of ammonia content.]

1983 Dessy R., Analy. Chem., v.55, no.6, p650A-662A: Languages for the Laboratory - Part 1.

1983 IBID, no.7, p756A-766A, Part 2.

1983 Stafford D., Kelley P. and Finnigan R.E., Amer. Lab., June, p51-57: An Advanced Ion Trap Technology Detector for Gas Chromatography.

1983 Kawakubo S. and Fukasawa T., Analy. Chem. Acta., v.149 p363-367: Rapid Microdiffusion-Photometric Determination o Nanogram Quantities of Ammonia.
[A simple method used on crystalline particulates in an Andersc sampler. Requires thirty minutes.]

1983 Kawabata Y. and Ishibashi N., Analy. Chem. Acta., v.163 p261-263: Ultratrace Photometric Determination of Phosphate wit a Solid State Emitter as a Light Source.
[The authors state that because of the extreme stability of th battery-powered solid state emitter, sensitivity can be amplifie greatly.]

1983 Stewart K.K., Analy. Chem., v.55, no.9, p931A-940A: Flc Inject Analysis. A Report.
[This is a new approach to analytical mesurements suitable fc automated routine repititious analysis.]

1983 Harder M.E. and Koski P.A., Amer. Lab., September p27-32: A Microprocessor-based Data Base Management System.

1982 Reporter, New Scientist, v.24, November, p578: New Ligh on Bacteria from Space.
[This is a news article on the use of an IR telescope. They fai to find a "Wiggle" at 3.52 microns in the space observations therefore suggesting the absence of bacteria in space.]

1982 MacFie H.J.H., Analy. Proceed., November, p529-530: Pattern Recognition in Practice-Differentiation of Microorganisms by Pyrolysis-Gas Chromatography.
[The patterns are the spectrum of the individual ion masses.]

1982 Eschenbruch M. and Burk R.R., Analy. Biochem., v.125, p96-99: Experimentally Improved Reliability of Ultrasensitive Silver Staining of Protein in Polyacrylamide Gels.

1982 O'Haver T.C., Analy. Proceed., January, p22-33: Derivative Spectroscopy and its Applications in Analysis.
[This is a Plenary Lecture discussing the techniques and its application to UV-VIS-IR spectrophotometry.]

1982 Volgtman E. and Winefordner J.D., Analy. Chem., v.54, p1834-1839: Two Photon Photoionization Detection of Polycyclic Automatic Hydrocarbons and Drugs in a Windowless Flow Cell.
[Uses laser-excited flow to simultaneously measure fluorescence, photoacoustics and 2-photon ionization.]

1982 Plembeck J.A., Electroanalytical Chemistry, Basic Principles and Applications. John Wiley and Sons Publ.
[Includes information on gas sensing electrodes.]

1982 Tabaqchali S., Scand. Jour. Infect. Disease, 14/Supp. 35, p23-30: Rapid Techniques for the Identification of Anaerobic Bacteria and Presumptive Diagnosis (excerpt from Excerpta Medica, September 4, 1983).
[Uses gas/liquid chromatography (GLC) and indirect immunofluorescence (IFA) and require less than two hours.]

1982 Chabay A., Analy. Chem., v.54, no.9, p1071A-1080A: Optical Waveguides: Photon Plumnbing for the Chemistry Laboratory.
[Discusses fiber optics, waveguides and evanescent waves as tools for chemical analysis.]

1981 Seydel U. and Lindner B., Fresenius Zeit. Analy. Chem., v.308, no.3, p253-258: Qualitative and Quantitative Investigations on Mycobacteria with LAMMA.

1981 Dutka B.J., Editor; Membrane Filtration. Marcel Dekker Publ.
[Has a section on aerobiology.]

1980 Covington A.K., Editor; Ion-Selective Electrode Methodology, v.2. CRC Press.
[Has a good section (p17-20) on gas-sensing probes.]

1980 Gaudy A.F. and Gaudy E.T., Microbiology for Environmental Scientists and Engineers. McGraw Hill Publ.
[A good chemistry section on microorganisms.]

1980 Allington W.B., ISCO Applications Bulletin No. 33: Centrifugation, Photometric Scanning and Fractionation of Density Gradients.
[Gradients in glycerol or other liquids yield bands at different densities.]

1979 Briggs, D., Editor; Handbook of X-ray and Ultraviolet Photoelectron Spectroscopy. Heyden Publ.

1979 Rytel M.W., Editor; Rapid Diagnosis in Infectious Diseases. CRC Press.

1979 Peterkin P.I. and Malik N., Applied and Environ. Microbiol., v.38, no.3, p431-435: Improved Detection of Coliform and E. Coli in Foods by a Membrane Filter Method.

1978 Freiser H., Editor; Ion-Sensitive Electrodes in Analytical Chemistry, Volume 1. Plenum Press.
[Among the applications are included the use of these electrodes for constituents in cigarette smoke and airborne paticulates.]

1977 Hardy D., Kaeger S.J., Dufour S.W. and Cady P., Applied and Environ. Microbiol., v.34, no.1, p14-17: Rapid Detection of Microbial Contamination in Frozen Vegetables by Automatic Impedance Measurement.
[Required five hours using a "Bactometer".]

1977 Wideman A., Citronbaum B. and Sutter G., Jour. Clin. Microbiol., p315-319: Simple Disk Technique for Detection of Nitrate Production by Anerobic Bacteria.
[Requires a 48-hour incubation.]

1976 Mitruka B.M. and Bonner M.J., Methods of Detection and Identification of Bacteria. CRC Press.
[An outstanding summary, up to that date, outlining the problems and solutions.]

1976 Allington R.W., Brakke M.K., Nelson J.W., Aaron C.G. and Larkins B.A., Analy. Biochem., v.73, p78-92: Optimum Conditions for High Resolution Gradient Analysis.

1974 Sharp A.N. and Michand G.L., Applied Microbiol., v.28, no.2, p223-225: Hydrophobic Grid-Membrane Filters: New Approach to Microbial Enumeration.
[A grid separates the colonies making them more easily countable.]

1974 Merchand A., Piaud J.J. and DuParqvier P., Applied Microbiol., v.27, p874-877: A High Precision and Large Capacity Laser Biophotometer.
[Grows thirty-two cultures in small cells and reads absorption every three seconds.]

1974 Wilkens J.R., Stoner G.E. and Boykin E.H., Applied Microbiol., v.27, p949-957: Microbial Detection Method based on Sensing Molecular Hydrogen.
[Using electrodes over a solution of bacteria it requires about eight hours until bacteria are detected.]

1973 Hedin C.G. and Illeni T., Editors; Automation in Microbiology and Immunology. Wiley Publ.
[Has a section on fluorescent antibody (FA) techniques , including automated FA and the FAST system with a tape roll for airborne bacteria.]

1972 Strange R.E., Advances in Microbial Physiology, v.8, p105-137: Rapid Detection and Assessment of Sparse Microbial Populations.
[Describes a whole collection of procedures including ATP, immunofluorescence and gas chromatography, among others for both bacteria and viruses.]

1970 Bailive A. and Gilbert R.J., Editors; Automation, Mechanization and Data Handling in Microbiology. Academic Press.

1970 Jost R. and Fly H., Applied Microbiol., v.20, no.6, p861-865: Rapid Detection of Small Numbers of Airborne Bacteria by a Membrane Filter Fluorescent-Antibody Technique.
[After a short incubation on a solid nutrient, the author looks for individual cells using a high-power UV microscope.]

1969 Mitz M.A., Annals New York Academy of Science - Part 2, p651-664: New Developments in Techniques for Particle Measurements. The Detection of Bacteria and Viruses in Liquids.
[The article clearly describes instrumentation developed at NASA, such as the well-known Wolf Trap and Gulliver systems. Both work on cultures, the former detects change in pH, the latter uses radioisotopes which are looked for after metabolism has resulted in gaseous products.]

1968 Casida Jr. L.E., Science, v.159, p199-200: Infrared Color Photography-Selective Demonstration.
[This is a unique observation. Only unstained bacteria shows red when photographed with Kodak Ektachrome infrared film.]

1967 Truper H.G. and Yentsch C.S., Jour. Bacteriol., v.94, p1255-1256: Use of Glass Fiber Filters for the Rapid Preparation of In vivo Absorption Spectra of Photosynthetic Bacteria.
[Measures photosynthetic bacteria in water after filtration. Runs a transmission curve right through the filter versus a blank filter at 400-1200 mu.]

1966 MacLeod R.A., Light M., White L.A. and Currie J.F., Applied Microbiol., v.14, p979-984: Sensitive Rapid Detection for Viable Bacterial Cells.
[Uses radioactive P_{23} and detects twenty-three cells after one hour of incubation.]

1966 Brewer J.H., Allgeir D.L. and McLaughlin C.B., Applied Microbiol., v.14, no.1, p135-136: Improved Anaerobic Indicator. [A small plastic bag containing indicator.]

1962 Nelson S.S. and Boldman O.E.A., Annals New York Acad. Sci., v.99, p290-297: The Partichrome Analyzer for the Detection and Enumeration of Bacteria.
[This report from workers at Fort Detrick detects bacteria in air by staining and recognizing stained versus unstained particles.]

1983 Soini E. and Kojola H., Clin. Chem., v.29, no.1, p65-68: Time Resolved Fluorometer for Lanthanide Chelates - A New Generation of Non-Isotopic Immunoassays.
[Describes a manually-operated fluorometer with a xenon flash tube. Sensitivity for a one-second determination is similar to that of radioisotopic methods.]

1983 Hurtubise R.J., Analy. Chem., v.55, p669A-680A: Phosphorimetry: Instrumentation Section.
[New developments including solid surfaces, micelle-stabilized and solution-sensitized room temperature phosphorescence (RTP).]

1983 Froelich P.M., Perkin-Elmer Applications Data Bulletin, Energy Sources and Fluorescence Spectroscopy.
[Discusses fluorescence and phosphorescence spectroscopy, with specific applications to polycyclic aromatic hydrocarbons.]

1982 Kricka L.J. and Carter T.J., Editors; Clinical and Biochemical Luminescence. Marcel Dekker Publ.
[Includes some application to bacteria.]

1982 Pauls J.H. and Myers B., Applied Environ. Microbiol., v.43, no.6, p1393-1399: Fluorimetric Determination of DNA in Aquatic Microorganisms using the Dye Hoechst 33258.

1982 IBID, no.4, p939-944: Use of Hoescst Dyes 33258 and 33342 for the Enumeration of Planktonic Bacteria.

1982 Singh G.N. and Hinze W.L., Analyst, v.107, p1073-1080: Micellar Enhanced Fluorimetric Determination of 1-NN-Dimethylamine Napthalene-5-Sulphonyl Chloride and O-Phthaldehyde-2-Mercaptoethanol Derivatives of Amino Acids.
[This paper bears on the applicability of micelles to enhance fluorimetric methods.]

1982 Dalterio R.A. and Hurtibuse R.J., Analy. Chem., v.54, p224-228: Room Temperature Phosphorescence of Hydroxyl Substituted Aromatics Absorbed on Solid Surfaces.
[Among other information, the article lists many organic compounds and how they respond with this technique.]

1982 Siegel J.I., Amer. Lab., p65-69: Fluorescence Microscopy.
[This article describes the principles of this subject.]

1982 Cline-Love L.J. and Skrilec M., Amer. Lab., p103-107: Room Temperature Phosphorescence in Micellar Solution.
[Describes the principles and advantages and gives some limits of detection.]

1981 Ortner M.J., Galvin M.J., McRee D.I. and Chignell C.F., Jour. Biochem. and Biophys. Methods, v.5, no.3, p157-167: A Novel Method for the Study of Fluorescent Probes in Biological Materials During Exposure to Microwave Radiation.
[The cell is placed in a fluid-filled waveguide and is subjected to 2450 MHz. A fluorescent probe of 1-anilo-8 napthalene sulfonate (ANS) is used to monitor the effect on calcium binding.]

1981 Hurtibuse R.J., Solid Surface Luminescence Analysis. Marcel Dekker Publ.
[Excellent description of apparatus potentially useful for examining aerosols impacted on surfaces.]

1981 Perlman D. and Laskin A.I., Editors; Applied Microbiology. Volume 27. Academic Press.
[Chemiluminescence for the rapid counting of coliforms is discussed on p178-183.]

1981 Rhys W.A.T., An Introduction to Phosphorescence Spectroscopy - A Perkin-Elmer publication.

1981 Armstrong D.W., Hinze W.L., Bui K.H. and Singh H.N., Analy. Letters, v.14, (A19), p1659-1667: Enhanced Fluorescence and Room Temperature Liquid Phosphorescence Detection in Pseudophase Liquid Chromatography (PLC).
[Note that the micellar sodium dodecylsulfate mobile phase enhances fluorescent peaks ten times.]

1981 Marino D.F. and Ingle J.D., Analy. Chem., v.53, p645-650: Microcomputer-Controlled Intensified Diode Array Data Acquisition System for Chemiluminescence Spectra.

1981 Hieftje G.M. and Haugen G.R., Analy. Chem., v.53, no.6, p755A-765A: Correlation-Based Approaches to Time-Resolved Fluorimetry: Instrumentation.

1980 Skrilec M. and Cline-Love L.J., Analy. Chem., v.52, no.11, p1559-1564: Room Temperature Phosphorescent Characteristics of Substituted Arenes in Aqueous Thallium Lauryl Sulfate Micelles.
[Compares results favourably with published values obtained at 77K°.]

1980 Cline-Love L.J., Skrlic M. and Habarta J.G., Analy. Chem., v.52, p754-759: Analysis by Micelle-Stabilzed Room Temperture Phosphorescence in Solution.
[Note that it requires a heavy metal/Na ratio of 10-20% heavy atoms to have a dramatic effect on relative luminescence intensity.]

1980 McAleese D.L., Freedlander R.S. and Dunlap R.B., Analy. Chem., v.52, p2442-2443: Elimination of Moisture and Oxygen Quenching for Room Temperature Phosphorescence.
[A troublesome interference is handled using sodium citrate saturated paper.]

1980 Yen-Bower E.L., Ward J.L., Walden C. and Wineforder J.D., Talanta, v.27, p380-382: The Present Status and Future of Room Temperture Phosphorescence.
[Gives the advantages and limitations of room temperature phosphorescence.]

1980 Parker R.T., Freedlander R.S. and Dunlap R.B., Analy. Chim. Acta, v.119, p189-205, Part 1. Also v.120, p1-17, Part 2.
[The development of room temperature phosphorescence into a new technique for chemical determinations is described in these two articles.]

1980 Shelley D.C., Quarles J.M. and Warner I.M., Clin. Chem., v.26, no.8, p1127-1132: Identification of Fluorescent Pseudomonas Species.
[Obtains fluorescent profiles of diffusible pigments diffused into the media after growth. Uses multiple exitation and emitting wavelengths.]

1980 American Instrument Company Publication. Fluorescence and Phosphorescence: A Data Compendium.
[Contains data on biologically related compounds, among others.]

1979 Ford C.D. and Hurtibuse R.J., Anal. Chem., v.51, no.6, p659-653: Design of a Phosphoroscope and the Examination of Room Temperature Phosphorescence of Nitrogen Heterocycles.
[The analytical potential of this approach, using a base of dried silica gel and paper is considered.]

1979 Yen-Bower E.L. and Winefordner J.D., Applied Spectro., v.33, no.1, p9-12: A Continuous Sampling System for Room Temperature Phosphorescence Analysis.
[The procedure is done on a filter paper guide. It employs phosphorometric time resolution with an analogue switch. The phosphoroscope is suitable for routine analysis.

1979 Richardson J.H., George S.M. and Ando M.E., National Bureau of Standards, Special Publication 519: Trace Organic Analysis: Subparts per Trillion Detection of Organics in Aqueous Solution by Laser-Induced Molecular Fluorescence.
[Pulsed laser is shown to be a superior excitation source for fluorescence analysis.]

1979 Soini E. and Hemmila I., Clin. Chem., v.25, no.3, p353-361: Fluroimmunoassay: Present State and Key Problems.
[A review of its uses on biological fluids.]

1978 Ulitzer S. and Heller M., Anal. Biochem., v.91, p421-431: A New, Fast and Very Sensitive Bioluminescent Assay of Phospholipases A and C.
[Uses a culture of certain light forming bacteria (Beneckeii harveyi) to detect very small concentrations of bacterial content.]

1978 Miller J.N., Phillips D.L. and Burns D.T., Anal. Chem., v.50, no.4, p613-616: Solvent Enhancement Effects on Thin-Layer Phosphorimetry.

[The authors find that spraying the proper solvent on th chromatogram immediately before examination greatly enhance phosphorescence.]

1978 Vo-Dinh T. and Gammage R.B., Analy. Chem., v.50, no.14, p2054-2058: Singlet-Triplet Energy Difference as a Parameter of Selectivity in Synchronous Phosphorimetry.
[This procedure is offered as a means of greatly enhancing selectivity in phosphorescence analysis.]

1978 Vo-Dinh T., Analy. Chem., v.50, no.3, p396-401: Multi-component Analysis by Synchronous Luminescence Spectroscopy.
[A selection advantage occurs because both excitation and emission wavelengths are scanned at a fixed difference and is therefore faster.]

1977 Vo-Dinh T., Walden G.L. and Winefordner J.D., Anal. Chem., v.49, no.8, p1126-1130: Instrument for the Facilitation of Room Temperature Phosphorescence with a Continuous Filter Tape Device.
[An automatic device suggested for clinical and environmental phosphorescent analysis of molecules on filter paper.]

1977 Schulman S.G., Fluorescence and Phosphorescence Spectroscopy: Physicochemical Principles and Practices. Pergamon Press.
[Gives a clear summary of electronic levels. Has tables of wavelengths for many compounds and 430 references.]

1977 Lief R.C., et al., Clin. Chem., v.23, no.8, p1492-1498: Development of Instrumentation and Fluorochromes for Automated Multiparameter Analysis of Cells.
[An automated instrument (AMACIII) interfaced with a computer records several parameters: cell volume, opacity, fluorescence of stains and light scatter.]

1977 Halmann M., Velan B. and Sery T., Applied and Environ. Microbiol., p473-477: Rapid Identification and Quantification of Small Numbers of Microorganisms by a Chemiluminescent Immunoreaction.
[This method (patent pending) claims to detect 30-300 bacterial cells.]

1976 Vo-Dinh T., Yen E.L. and Winefordner J.D., Anal. Chem., v.48, no.8, p1186-1188: Heavy Atom Effects on Room Temperature Phosphorimetry.
[Sodium iodide significantly increases limits of detection of room temperature phosphorescence.]

1976 Chen R.F. and Edelhoch H., Biochemical Fluorescence: Concepts, Volume 2. Marcel Drekker Publ.
[Section on protein emission spectra.]

1976 Karasek F.W., Research/Development, v.27, no.1, p28-34: Fluorescence Spectroscopy.]

1975 Berman M.R. and Zare R.N., Anal. Chem., v.47 [illegible] p1200-1201: Laser Fluorescence Analysis of Chromatog[illegible] Subnanogram Detection of Aflatoxins.

1974 Kasatiya N.G., Lambert N.G. and Laurence R.A., Ap[illegible] Microbiol., May, p838-843: Use of a Tunable Dye Laser [illegible] Quantitative Fluorescence in Syphilis Serology.
[This is a FTA-ABS test with a 4 mus pulse. Repeated puls[illegible] eliminates the fading which occurs with continuous radi[illegible] therefore gives more reproducible readings.]

1974 Wellons S.L., Paynter R.A. and Winefordner J.D., Sp[illegible] Chim. Acta, Part A, v.30, p2133-2140: Room Temper[illegible] Phosphorimetry of Biologically Important Compounds Absorbed o[illegible] Paper.

1973 Bergquist N.R., Scand. Jour. Immunol., v.2, p[illegible] Pulsed Dye Laser as a Light Source for the Fluorescent A[illegible] Technique.
[A photorecording done in 10 μsec. No significant bl[illegible] after 50 μsec exposure.]

1973 Owen E.M., Scarry P.C. and Vasers M.L., Develop[illegible] Microbiol., v.13, p257-260: Use of the Biometer for [illegible] of Process Waters.

1972 Fisher R.P. and Winefordner J.D., Analy. Chem., v.44, no.6, p948-956: Pulsed-Source Time-Resolved Phosphorimetry.
[Principles of time resolution phosphorimetry are described in multicompound mixtures. Has advantages over conventional continuous rotating can phosphorimeters.]

1972 Winefordner J.D., Schulman S.G. and O'Haver T.C., Luminescence Spectrometry in Analytical Chemistry. Wiley Interscience Publ.
[This is Volume 38 of Chemical Analysis. Elving and Koltoff; Editors.]

1972 Strange R.E., Microbiol. Physiol., v.8, p105-108: Rapid Detection and Assessment of Sparse Microbial Populations.

1969 Mitz M.A., Annals of New York Acad. Sci., Part II. New Developments in Techniques for Particle Measurements: The Detection of Bacteria and Viruses in Liquids.

1969 Sawicki E., Talanta, v.16, p1231-1265: Fluorescence Analysis in Air Pollution Research.
[Fluorescent assay methods are discussed in terms of selectivity, speed, simplicity, accuracy, precision, interferences, etc.]

1969 Becker R.S., Theory and Interpretation of Fluorescence and Phosphorescence. John Wiley and Sons Publ.

1968 Picciolo G.L., Chappelle E.W. and Rich E. Jr., Bioscience, v.18, no.5, p427-428: Research Report: Use of an Assay for Flavin Nucleotides as a Technique for Detecting Extraterrestrial Life.
[The flavin family of compounds are present in all organisms. These have been measured in bacteria. An automated instrument for this purpose is described.]

1967 Chappelle E.W., Picciolo G.L. and Altand R.H., Biochem. Med., v.1, p252-260: A Sensitive Assay for Flavin Mononucleotide Using the Bacterial Bioluminescence Reaction.
[Cells are extracted in buffered butanol for two minutes. A positive reaction indicates bacteria.]

1967 Konev S.V. (translated from the Russian by Udenfriend), Fluorescence and Phosphorescence of Proteins and Nucleic Acids. Plenum Press.
[Note that the tryptophane absorption maximum at 280 mu is a major contibutor.]

1966 Pital A., Janowitz S.L. and Hudak C.E., Applied Microbiol., v.14, no.1, p119-123: Direct Fluorescence Labelling of Microorganisms as a possible life-detection technique.]

1962 Udenfriend S., Fluorescence Assay in Biology and Medicine. Academic Press.
[Has a chapter on Public Health and Sanitation with an interesting section on Chemical Warfare.]

1957 Keirs R.J., Britt R.D. Jr. and Wentworth W.E., Analy. Chem , v.29, no.2, p202-209: Phosphorimetry - A New Method of Analysis.

[This seems to be the first paper on the use of phosphorescence as a method of chemical analysis.]

1944 Lewis G.N. and Kasha M.J., Jour. Amer. Chem. Soc., v.66, p2100-2115: Phosphorescence and the Triplett State.

1983 Rutan S.C. and Brown S.D., Analy. Chem., v.55, no.11, p1707-1710: Pulsed Photoacoustic Spectroscopy and Spectral Deconvolution with the Kalman Filter for Determination of Metal Complexation Parameters.

1983 McClelland J.F., Analy. Chem., v.55, no.1, p89A-105A, Photoacoustic Spectroscopy.
[A general review article. Concludes that the PAS technique is now practical as a qualitative mid-IR method, applicable where it is too difficult for conventional IR.]

1983 O'Conner M.T. and Diebold G.J., Nature, v.301, p321-322: Chemical Amplification of Photoacoustic Signals.
[Uses the photoacoustic effect to follow a chemical reaction between H_2-Cl_2 and can follow the reaction rate versus time.]

1983 Ferguson A.I., Nature, v.301, p286: Rvival of an Old Technique.

1983 Volgtman E. and Winefordner J.D., Talanta, v.30, no.3, p75-80: Simultaneous Fluorescence, Photoacoustics and 2 Photon Photoionization Detection for Liquids in a Cuvette.

1983 Yip B.C. and Yeung E.S., Analy. Chem., v.55, no.6, p978-971: Photoacoustic Spectroscopy in Gases based on Wavelength Modulation.
[A method of reducing background contributions due to cell walls, etc.]

1983 Kawabata Y., Samikubo T., Inasaka T. and Ishibashi N., Analy. Chem., v.55, no.8, p1419-1420: Semiconductor Lasers and Solid-State Emitters as Near-IR Light Sources for PHotoacoustic Spectroscopy.
[Because of low cost, small size and stability when operated from a battery, it can give great sensitivity.]

1983 Carpentier R., LaRue B. and LeBlanc R.M., Archives Biochem. and Biophys., v.222, p403-410: Photoacoustic Spectroscopy of Anacystis Nidulans (an Algae).
[Studied the effect of various thicknesses of samples and some means to overcome limitations found.]

1982 Volgtman E., Jurgensen S. and Winefordner J.D., Analyst, v.107, p408-413: Comparison of Laser-Excited Fluorescence and Photoacoustic Limits of Detection of Polynuclear Aromatic Hydrocarbons.

1982 Helander P., Jour. Photoacoustics, v.1, p103-120: An Open Photoacoustic Cell.
[Presents a so-called acoustic window cell which is designed primarily for liquids.]

1982 Poizat O. and Atkinson G.H., Analy. Chem., v.54, no.9, p1486-1489: Determination of NO_2 by Visible Photoacoustic Spectra.
[Mathematical derivations given.]

1982 Lloyd L.B., Yeatis R.C. and Eyring E.M., Analy. Chem., v.54, p549-552: Fourier Transform IR Photoacoustic Spectroscopy in Thin-Layer Chromatography.

1982 Lowry S.R., Mead D.G. and Vldrine D.W., Analy. Chem., v.54, p546-548: Photoacoustic IR Spectra in the Investigation of Bonding Effects in Formulated Pesticides.
[Used PAS because sample grinding required for IR would have destroyed bonds.]

1981 Fishman V.A. and Bard A.J., Analy. Chem., v.53, p102-105: An Open-Ended Photoacoustic Spectroscopic Cell for Thin-Layer Chromatography and other Applications.
[Used solid, liquid and gel samples. Did spectra of spots on TLC plates.]

1981 Sawada T. and Oda S., Analy. Chem., v.53, p539-540: Double-Beam Laser-Induced Photoacoustic Spectrometer.
[The purpose was to eliminate background PA signals due to solvent.]

1981 Oda S. and Sawada T., Analy. Chem., v.53, p471-474: Laser-Induced Photoacoustic Detector for High Performance Liquid Chromatography.
[Used a piezoelectric tansducer as part of the flow cell. Approximately 20 microliters volume gave a 25-fold increase in sensitivity over UV.]

1981 Volgtman E., Jurgensen A. and Winefordner J.D., Analy. Chem., v.53, p1442-1446: Condensed Phase Photoacoustic Spectroscopic Detection of Porphyrins and Dyes.
[Designed for highly sensitive liquid phase PAS. Gives limits of detection for dyes, drugs and some porphyrins.]

1981 IBID, p1921-1923: Comparison of Laser-Excited Fluorescence and Photoacoustic Limits of Detection for Static and Flow Cells.

1981 Ashworth C.M., Castleden S.L. and Kirkbright G.F., Analy. Proceed., v.18, p14-16: Some Novel Applications of Optoacoustic Spectroscopy to Real Samples.
[Gives studies in visible and near IR using thin-layer chromatographic plates.]

1981 Bults G., Horowitz B.A., Malkin S. and Cahen D., FEBS Letters, v.129, no.1, p44-46: Frequency-Dependent Photoacoustic Signals from Leaves and their Photosynthesis.

1980 Lasser-Ross N., Malkin S. and Cahen D., Biochem. et Biophys. Acta., v.593, p330-341: Photoacoustic Detection of Photosynthetic Activities in Isolated Broken Chloroplasts.

1980 Garty H., Cahen D. and Caplan S.R., Biochem. and Biophys. Research Comm., v.97, no.1, p200-206: Photoacoustic Calorimetry of Halobacterium Halobuin Photocycle.
[Studies enthalpy changes in a millisecond time scale.]

1980 Low , M.J. and Parodi G.A., Applied Spectros., v.34, no.1, p76-80: IR Photoacoustic Spectroscopy of Solids and Solid Surfaces.

1980 Brandis E. and Rosencweig A., Microbeam Analysis, p93-94: Thermal Wave Microscopy in a Scanning Electron Microscope.
[Thermal wave or photoacoustic microscope is used to find defects on surfaces.]

1980 Rosencwaig A., Photoacoustics and Photoacoustic Spectroscopy. John Wiley and Sons Publ.
[This book is in the series Chemical Analysis, Volume 57. Elving, Winefordner and Kolthof; Editors. The book is by an early developer of the technique at the Bell Laboratories Among other interesting features it describes the piezoelectric transducer in some detail.]

1980 Crosley D.R.; Editor, Laser Probes for Combustion Chemistry. Amer. Chem. Soc. Publ.
[Chapter 40 describes the use of photoacoustic spectroscopy to characterize and monitor soot in combustion processes.]

1980 Lloyd L.B., Burnham R.K., Chandler W.L. and Eyring E., Analy. Chem., v.52, p1595-1598: Fourier Transform Photoacoustic Visible Spectroscopy of Solids and Liquids.

1980 Low M.J.D. and Parodi G.A., Applied Spectra, v.34, no.1, p76-77: Infrared Photoacoustic Spectra of Solids and Surface Species.
[Used to observe chemical changes on surfaces.]

1980 Lochmuller C.H., Marshall S.F. and Wilder D.R., Analy. Chem., v.52, p19-23: Photoacoustic Spectra of Chemically Bonded Chromatographic Stationary Phases.
[Found that polymerization on a surface could be followed with PAS.]

1979 Castledon S.L., Elliot C.M., Kirkbright G.F. and Spillane D.E.M., Analy. Chem., v.51, no.13, p2152-2153: Quantitative Examination of Thin-Layer Chromatography Plates by Photoacoustic Spectra.
[Detected 20 ng of fluorescein in 4.5 mg of silica gel.]

1979 Fuchsman W.H., Analy. Chem., v.51, p589-590: A General Method for Overcoming Photoacoustic Saturation in Highly Colored Organic and Inorganic Solids.
[Solves the problem by either increasing modulation frequency or the use of very thin layers of sample.]

1979 Blank R.E. and Wakefield T., Analy. Chem., v.51, no.1, p50-54: Double-Beam Photoacoustic Spectrometer for Use in the Ultraviolet-Visible and Near Infrared Regions.

1978 Eaton H.E. and Stuart J.D., Analy. Chem., v.50, no.4, p587-591: Microcomputer Assisted Single Beam Photoacoustic Spectrometer System for the Study of Solids.
[Designed for general use in most laboratories with easily constructed components.]

1978 Somoano R.B., Angew. Chem. Inst. Edit. Eng., v.17, p238-245: Photoacoustic Spectra of Condensed Matter.
[The author refers to the use of PAS in the identification of different states of bacterial cultures. For example, Bacillus subtilis in the spore state absorbs strongly at 410 nm but not at all in the vegetative state.]

1978 Oda S., Sawada T. and Kamada H., Analy. Chem. v.50, no.7, p865-867: Determination of Ultratrace Cd by Laser-Induced Photoabsorption Spectra.
[Determines 0.02 ng Cd/ml (in ppt) in Penicilliumochloron.]

1978 Farrow M.M. et al., Applied Optics, v.17, no.7, p1093-1097: Piezoelectric Detection of Photoacoustic Signals.
[Describes the advantage over the use of other microphones.]

1977 Adams M.J., Beadle B.C. and Kirkbright G.F., Analyst, v.102, p569-575: A Double-Beam Photoacoustic Spectrometer for Use in Solid and Liquid Samples in the UV, Vis, and Near IR Regions of the Spectrum.

1977 Gray H.C., Fishman V.A. and Bard A.J., Analy. Chem., v.49, no.6, p697-700: Simple Sample Cell for Examination of Solids and Liquids, by Photoacoustic Spectroscopy.
[Gives a number of representative spectra and details of the cell construction.]

1977 Pao Y.H.; Editor, Optoacoustic Spectroscopy and Detection. Academic Press.
[This is a fine textbook with some biological applications.]

1976 Adams M.J., Beadle B.C., King A.A. and Kirkbright G.F., Analyst, v.101, p553-561: Analytical Optoacoustic Spectrometry.
[Part II, gives UV and Vis optoacoustic spectra of some biochemical and phytochemical samples.]

1975 Rosencwaig A. and Hall S.S., Analy. Chem., v.47, p548-549: Thin-Layer Chromatography and Photoacoustic Spectrometry.

1975 Rosencwaig A., Analy. Chem., v.47, p592A-604A: Photoacoustic Spectroscopy - A New Tool for Investigating Solids.
[Refers to its recent development at the Bell Laboratories.]

1973 Rosencwaig A., Science, v.181, p657-658: Photoacoustic Spectroscopy of Biological Materials.
[Presents curves for whole blood, red blood cells and hemoglobin from 200-800 nm to show that hemoglobin can be monitored without interference from other UV absorbing species.]

1971 Kruezer L.B. and Patel C.K.N., Science, v.173, p45-46. Nitric Oxide Pollution: Detection by Photoacoustic Spectroscopy.

1959 Delaney M.E., Science Progress, v.47, p459: The Optic-Acoustic Effect in Gases.

1881 Tyndall J., Proc. Roy. Soc., v.31, p307: Action of an Intermittant Beam of Radiant Heat upon Gaseous Matter.

1881 Rontgen W.C., Phil. Mag., v.11, p308.

1881 Bell A.G., Phil. Mag., v.11, p510.

PIEZOELECTRIC

1983 Ho M.H., Guilbault G.G. and Rietz B., Analy. Chem., v.55, no.11, p1830-1832: Portable Piezoelectric Crystal Detector for Field Monitoring of Environmental Pollutants.
[A detector was developed for toluene and demonstrated in a Danish printing plant.]

1983 Alder J.F. and McCallum J.J., Analyst, v.108, p1169-1189: Piezoelectric Crystals for Mass and Chemical Measurements.
[This is a detailed review, including a section on bacterial and fungal growth which led to unsatisfactory results.]

1983 Guilbault G.G., Analy. Chem., v.55, no.11, p1682-1684: Determination of Formaldehyde with an Enzyme-Coated Piezoelectric Detector.
[This detector was specific to formaldehyde and was little effected by other aldehydes or alcohols.]

1983 Kristoff J. and Guilbault G.G., Analy. Chim. Acta, v.149, p337-341: Application of Uncoated Piezoelectric Crystals for the Detection of Organic Phosphonate.
[Gold, silver and nickel electrodes were used on these detectors.]

1983 Nomura T. and Maruyama M., Analy, Chim. Acta, v.147, p365-369: Effect of Metal Ion on a Piezoelectric Quartz Crystal in Aqueous Solution and the Absorptive Determination of Iron III as Phosphate.

[At 1.5 V applied between the electrodes on the crystal many metals electrodeposited to change its frequency.]

1982 Lee C.W., Fung Y.S. and Fung K.W., Analy. Chim. Acta, v.135, p277-283: A Piezoelectric Crystal Detector for Water in Gases.

[A gelatin coating was found best for this application.]

1982 Ho M.H. and Guilbault G.G., Analy. Chem., p1998-2002: Detection of Carbon Monoxide in Ambient Air with a Piezoelectric Crystal.

[The procedure is: first react the carbon monoxide with mercuric oxide to produce mercury, which is then absorbed on the gold electrode. Other interferences are removed by passing the vapors through a pre-column.]

1981 Guilbault G.G., Intern. Jour. Environ. Anal. Chem., v.10, p89-98: Analysis of Environmental Pollutants using a Piezoelectric Detector.

[Gives sensitivities, interferences and drawbacks (the most important being moisture). Methods are given for CO, SO_2 and NOx.]

1980 Ho M.H. and Guilbault G.G., Analy. Chem., v.52, p1489-1922: Continuous Detection of Toluene in Ambient Air with a Coated Piezoelectric Crystal.
[Using carbowax 550, toluene is detected in the 30-300 ppm range in 30 seconds. Reversal requires 40 seconds. A Nafion tube is used to remove the moisture.]

1978 Webber L.M., Karmarkar K.H. and Guilbault G.G., Analy. Chim. Acta, v.97, p29-35: A Coated Piezoelectric Crystal Detector for the Selective Detection and Determination of Hydrogen Sulfide in the Atmosphere.
[Uses an acetone extract of soots from burning organochlorine compounds (chlorobenzoic acid was best). Range was 1-60 ppm of H_2S. Recovery when detecting 60 ppm was about 30 minutes.]

1978 Scheide S.P. and Warner R.B.J., Amer. Indust. Hygiene Assoc. Jour., p745-748: A Piezoelectric Crystal Mercury Monitor.
[The detector was hand calculator size and used gold electrodes.]

1978 Heavay J. and Guilbault G.G., Analy. Chem., v.50, no.8, p1044-1046: Detection of Ammonia in Ambient Air with a Coated Piezoelectric Crystal Detector.
[Describes improved coatings with 1-minute response and 5-minute recovery. Moisture is eliminated with a silica gel precolumn.]

1976 Webber L.M. and Guilbault G.G., Analy. Chem., v.48, p2244-2247: Coated Piezoelectric Crystal Detector for Selective Detection of Ammonia in the Atmosphere.
[The coatings were capiscum annum pods, ascorbic acid and ascorbic acid plus silver nitrate, the latter being best.]

1976 Scheide E.P. and Taylor J.K., Amer. Indust. Hyg. Assoc. Jour., v.37, p897-901: A Piezoelectric Crystal Dosimeter for Mercury Vapor Monitoring in Industrial Atmospheres.

1975 Karmarker K.H. and Guilbault G.G., Analy. Chim. Acta, v.75, p111-117: Detection of Ammonia and NO_2 at the ppb Level with a Coated Piezoelectric Crystal Detectors.

1974 Karmarker K.H. and Guilbault G.G., Analy. Chim. Acta, v.71, p419-424: A New Design and Coatings for Piezoelectric Crystals for Measurements of Trace Amounts of Sulfur Dioxide.

1974 Scheide E.P. and Taylor J.K., Environ. Sci. and Tech., v.8, p1097-1099: Piezoelectric Sensor for Mercury in Air.
[A gold-coated electrode, reversed by placing in an oven at 150°.]

1969 Wolsky S.P. and Zdanuk E.J., Ultramicro Weight Determinations in Controlled Environments. Interscience Publ.
[Chapter 7 is on the use of the quartz crystal oscillator for micro weighing.]

1969 Holland R. and Ecernisse E.P., Design of Resonant Piezoelectric Devices. MIT Press.

1964 King W.H. Jr., Analy. Chem., v.36, p1735-1739: Piezoelectric Sorption Detector.
[First suggested use of a coated piezoelectric detector.]

1982 Bowen J.M., Crone T.A., Kennedy R.K. and Purdle N., Analy. Chem., v.54, no.1, p66-68: Determination of Heroin by Circular Dichroism.
[This paper demonstrates the use of this technique where the matrix is non-interfering to the determination of the desired constituent. Separations and purifications are not needed. The procedure is faster and easier than gas chromatography or mass spectrophotometry. Total time is about 20 minutes.]

1982 Salzman G.C., Griffith J.K. and Gregg C.T., Applied and Environ. Microbiol., v.44, no.5, p1081-1085: Rapid Identification of Microorganisms by Circular-Intensity Differential Scattering. (CIDS).
[Using a scanning time of four minutes, five different crude influenza viruses were discriminated.]

1978 Morris E.R. and Frangon S.O., Techniques in the Life Sciences. Elsevier-Holland Publ.
[Sections B308-B309 are on circular dichroism and optical rotatory dispersion techniques with carbohydrate systems. Used to fingerprint related compounds differing only in stereo structures.]

1973 Torten M. and Schneider A.S., Jour. Infect. Diseases, v.127, no.3, p319-320: Characterization of Bacteria by Ultraviolet Spectroscopy.
[Each of five bacterial species gave a distinctive set of spectral patterns. Suggested as an additional tool for the identification and characterization of bacteria.]

1972 Kirschenbaum D.M.; Editor, Atlas of Protein Spectra in the Ultraviolet and Visible Regions. IFI/Plenum Press.
[This is a compendium of absorption curves of proteins from many sources including bacteria.]

1971 Francon M. and Mallick S., Polarization Interferometers: Applications in Microscopy and Macroscopy. Wiley Interscience Publ.

1965 Snatzke G.; Editor, Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry. Proceedings of a NATO summer school. Bonn September-October 1965. Sadtler Research Laboratories Publ.

PYROLYSIS

1980 Chemical Systems Data Inc., "The Applications Laboratory". A selected bibliography on analytical pyrolysis.

1979 Gutteridge C.S. and Norris J.R., Jour. Applied Bacteriol., v.47, p5-43: The Application of Pyrolysis Techniques to the Identification of Microorganisms.
[This is an excellent review.]

1978 Daucelsom N.D. and Rogers L.B., Analy. Chem., v.50, no.12, p1680-1683: Determination of Tryptophane in Proteins by Pyrolysis Gas Chromatography.
[Pyrolysis done at 850° shows that skatole is a unique product of tryptophane pyrolysis.]

1977 Jones C.E.R. and Cramers C.A.; Editors, Analytical Pyrolysis Proceedings of the Third International Symposium on Analytical Pyrolysis. Amsterdam, September 1976. Elsevier Scientific Publ.
[Several papers deal with bacteria.]

1977 Laitenin H.A., Analy. Chem., v.49, no.2, p193: Automated Identification of Microbes.
[This is an editorial commenting on the subject matter.]

1976 Maugh T.H. II, Science, v.194, p1403-1407: Pyrolysis-Spectrometry: Automated Identification of Microbes.

1970 Buchler C. and Simon W., Jour. Chromat. Sci., v.8, p323-324: Curie Point Pyrolysis Gas Chromatography.
[A ferro-magnetic conductor in contact with sample is heated in an RF coil with the carrier gas flowing. The properties of the alloy produce a self-regulated temperature.]

1983 Ishida H. and Isitani A., Applied Spectro., v.37, no.5, p450-455: Raman Microprobe Analysis of Thin Films Formed on the Surface of Silver Electrical Contacts Utilizing the Surface-Enhanced Raman Scattering Effect (SERS).

1983 Simpson S.F., Kincaid J.R. and Hodder F.D., Analy. Chem., v.55, no.8, p1420-1422: Microdroplet Mixing for Rapid Kinetics in Raman Spectrometric Detection.

1983 Fleischmann M. and Hendra P., Analy. Chem., v.55, no.1, p146-148: Fiber Optic Probe for Remote Raman Spectrometry.
[A simple rugged probe suitable for routine work is described for UV-Vis spectrophotometry. It uses 200 micron diameter multimode fibers commonly used in communication applications. It takes advantage of Raman to fingerprint species present.]

1983 Baum R.M., Chem. and Engineer. News, October 3, p21-24: Surface-Enhanced Raman Scattering Begins to Yield to Research.
[Explains the SERS effect on metal-molecule interactions as submicron sized particle levels, inducing electromagnetic fields.]

1982 Owen J.F., Chang R.K. and Barber P.W., Aerosol Sci. and Technol., v.1, no.3, p293-302: Morphology-Dependent Resonance in Raman Scattering Fluorescence Emission and Elastic Scattering from Micro Particles.
[The microparticles used in the article are glass fibers and are suitable only for theoretical studies.]

1982 Kerker M., Aerosol Sci. and Technol., v.1, no.3, p285-291: Raman Scattering by Molecules Adsorbed on Colloidal Spheres.
[Discusses surface-enhanced Raman scattering (SERS) and the factors not yet understood but requiring clarification.]

1982 Carey P.R., Biochemical Applications of Raman and Resonance Raman Spectroscopies. Academic Press.

1982 McCreery R.L., Fleischmann M. and Hendra P., Analy. Chem., v.55, p146-148: Fiber Optic Probe for Remote Raman Spectroscopy.

1982 Borman S.A., Analy. Chem., v.54, no.9, p1021A-1026A: Non-Linear Raman Spectroscopy.

1982 Adar F., LeClerq M. and Grayzel R.E., Amer. Lab., March, p59-65: Industrial Applications of Micro Raman Analysis.
[Demonstrates the use of samples one micron in size, done through the microscope.]

1981 Andersen M.E. and Muggle R.Z., Analy. Chem., v.53, p1772-1777: Microscopical Techniques with the Molecular Optics Laser Examiner Raman Microprobe.
[No bacterial applications are discussed.]

1981 Van Wart H.E., Vallee B.L., Scheule R.K. and Scheaga H.A., Trends in Biochemical Science (TIBS): Resonance Raman Probe of Enzyme Active Sites.
[This is a review article. It emphasizes the structural information obtained for proteins and structure-function relationships.]

1981 Van Haverbeke L., Janssens J.F. and Herman M.A., Internat. Jour. Environ. Analy. Chem., v.10, p205-218: Resonance Raman Spectroscopy as a Tool for the Detection of Pollutants in Water.
[Both colored and non-colored compounds are examined in distilled and natural waters. Problems of fluorescence interference and their elimination are discussed.]

1981 Strommen D.P. and Nakamoto K., Amer. Lab., v.13, no.10, p70-78: Industrial Applications of Laser Raman Spectroscopy.
[Applications described include: trace analysis in the environment, quantitative analysis, remote temperature sensing and the Raman Microprobe.]

1981 Barrett J. in Chemical Applications of Non-Linear Raman Spectroscopy. Harvey B.; Editor, Academic Press.

1981 Wright H. in Lasers in Chemical Analysis. Hieftje G.M.; Editor, Humana Press.

1981 Carrera L.A., et al., in Chemical Applications of Non-Linear Raman Spectroscopy. Chapter 8. Academic Press.

1981 Grasselli J.G., Snaveley M.K. and Bulkin B.J., Chemical Applications of Raman Spectroscopy. John Wiley and Sons Publ. [A clear textbook. Compares Raman and infrared applications.]

1981 Eesley G.L., Coherent Raman Spectroscopy. Pergamon Press.

1980 Howard W.F. Jr., Nelson W.H. and Sperry J.F., Applied Spectro., v.34, no.1, p72-75: A Resonance Raman Method for the Rapid Detection and Identification of Bacteria in Water. [Specimen were cultured then centrifuged. Sixteen species of bacteria and algae containing carotene were used.]

1980 Howard W.F., Nelson W.H. and Sperry J.F., Applied Spectro., v.34, no.1, p72: A Resonance Raman Method for the Rapid Detection and Identification of Bacteria in Water.

1979 Morris M.D. and Wallen D.J., Analy. Chem., v.51, no.2, p182A-192A: Resonance Raman Spectroscopy - A Discussion.

1978 Harvey A.B., Analy. Chem., v.50, no.9, p905A-912A: Coherent Anti-Stokes Raman Spectroscopy - A Brief Discussion of CARS.

1978 Etz E.S., Wise S.A. and Heirich K.F.J., National Bureau of Standards, Special Publication No. 519, Trace Organic Analysis.
[Treats the analytical potential of micro-Raman spectroscopy in the trace characterization of polynuclear aromatic hydrocarbons. No bacterial material.]

1978 Gratzer W.B. and Mendelschn R., Techniques in Life Sciences, B108 and B109. Elsevier/North-Holland Publ.

1978 Van Haverbeke L., Analy. Chem., v.50, p315-318: Determination of Industrial Dyes in Water by Laser Excitation with Resonance Raman Spectrometry.

1977 Ewing E.W.; Editor, Environmental Analysis. Academic Press.
[Contains a chapter entitled, "The Chemical Identification of Airborne Particles by Laser Raman Spectroscopy", p295-340.]

1977 Hudson B.S., Ann. Rev. Biophys. Bioeng., v.6, p135-150: New Laser Techniques for Biophysical Studies.

1977 Tolles W.M., Nibler J.W., McDonald J.R. and Harvey A.B., Applied Spectro., v.31, no.4, p253-271: A Review of the Theory and Application of Coherent Anti-Stokes Raman (CARS).
[This is the feature article which outlines the basic theory behind CARS, describing its unusual effects and drawbacks.]

1977 Melveger A.J.; Editor, Resonance Raman Spectra as an Analytical Tool. Franklin Institute Press.

1976 Carey P.R. and Schneider G., Jour. Molec. Biol., v.102, p679-693: Evidence for Structural Change in the Substrate Preceeding Hydrolysis of a Chymotrypsin Acyl Enzyme: Application of the Resonance Raman Labelling Technique to a Dynamic Biochemical System.

1975 Peticolas W.L., Biochemie, v.57, p417-428: Application of Raman Spectroscopy to Biological Macromolecules.
[The backbone chains of such molecules give Raman vibrations between $200cm^{-1}$-$3000-cm^{-1}$ which are useful to determine conformational structure of proteins and nucleic acids.]

1974 Freeman S.K., Applications of Laser Raman Spectroscopy. John Wiley and Sons Publ.
[Has a good section on biological applications.]

1970 Gilson T.R. and Hendra P.J., Laser Raman Spectroscopy. Wiley-Interscience Publ.

1959 Brandmuller J., Zeit. Analy. Chemie., v.170, p29-43: Neuer Gesichtspunkte Zur Analytischen Anwendung des Raman Effektes.

1928 Raman C.V. and Krishnan K.S., Nature, v.121, p501: A New Type of Secondary Radiation. See also p619: A Change in Wavelength in Light Scattering.

1922 Brillioun J., Ann. Physik (Paris), v.88, p17.

SPECTRAL MATHEMATICS

1983 Hecht J., High Technol., October, p55-61: Processing Signals the Optical Way.
[Describes the use of lasers and lenses to perform complex operations usually done by digital electronics. Advantage is an instantaneous response.]

1982 Button K.J.; Editor, Infrared and Millimeter Waves, v.6. Academic Press.
[Good section on interferometry and Fourier transform spectroscopy.]

1982 Proceedings of the Indust. Elect. and Electron. Eng. (IEEE), v.70, no.9, p887-1128:
[Entire special issue on spectral estimation.]

1975 Marshall A.G. and Comisarow M.B., Analy. Chem., v.47, no.4, p491A-504A: Fourier and Hadamard Transform Methods in Spectroscopy.
[A review article with a clear explanation of these concepts.]

1975 Geddes L.A. and Baker L.E., Principles of Applied Biomedical Instrumentation. Wiley Publ.
[Chapter 13 has a discussion on criteria for the faithful reproduction of an event.]

1972 Horlick G., Analy. Chem., v.44, no.6, p943-947: Digital Data Handling of Spectra Utilizing Fourier Transformations.
[Smoothing, differentiating and resolution enhancement maximizes signal/noise.]

1970 Blackburn J.A., Spectral Analysis Methods and Techniques. Marcel Dekker Publ.
[This is a basic book on the subject.]

1968 Geddes L.A. and Baker L.E., Principles of Applied Biomedical Instrumentation. Wiley Publ.

1966 Lanczos C., Discourse of Fourier Series. Hafner Publ.

STAINS

1982 Paul J.H. and Myers B., Applied and Environ. Microbiol., v.43, no.6, p1393-1399: Fluorimetric Determination of DNA in Aquatic Microorganisms by the Use of Hoecht 33258.
[A simple extraction detects 0.05-10 mug of DNA in bacterial cultures.]

1982 IBID, v.43, no.4, p939-944: Use of Hoecht Dyes 33258 and 33342 for Enumeration of Attached and Planktonic Bacteria.

1981 Wallis C., Melnick J.L. and Longoria C.J., Jour. Clin. Microbiol., v.14, p342-346: Colorimetric Method for the Rapid Determination of Bacteriuria.
[Using the dye safranine the procedure quantifies bacteria without culturing. Filter 1 ml of urine; dye; decolorize paper with a decolorizer. If the paper is pink, there is more than 10-5 cfu/ml. It takes less than one minute, and one can do a gram positive or negative stain on a second sample.]

1980 McCarthy L.R. and Senne J.E., Jour. Clin. Microbiol., v.11, no.3, p281-285: Evaluation of Acridine Orange Stain for the Detection of Microorganisms in Blood Cultures.
[This procedure has been used to detect bacteria where 24-hour growth failed to be positive.]

1980 Jenkins R.D., Hale D.C., and Matsen J.M., Jour. Clin. Microbiol., v.11, no.3, p220-225: Rapid Semiautomatic Screening and Processing of Urine Specimens.
[Used a light-scatter photometer, (Autobac) and 3-, 5-, and 6-hour cultures.]

1978 Wallace L.J. and Partlos L.M., Analy. Biochem., v.87, no.1, p1-10: A Sensitive Microassay for Cells Cultured on Collagen.
[Using bromosulphophthalein, forms a precipitate with most proteins but not with collagen.]

1976 Lewis J.F. and Alexander J., Jour. Clin. Microbiol., v.4, no.4, p372-374: Microscopy of Stained Urine Smears to Determine the Need for Quantitative Cultures.
[Found that gram-stained centrifuged specimen were as definitive as cultured specimen.]

1975 Benson J.R. and Hare P.E., Proc. Nat. Acad. Sci., v.76, no.2, p619-622: O-Phthaldehyde-Fluorogenic Detection of Primary Amines in the Picomole Range.

1973 Kanaoka Y., Machida M. and Sekina T., Biochem. et. Biophys. Acta, p563-568: Fluorescent Thiol Reagents.
[Used to detect sulfur groups in proteins.]

1971 Roth M., Anal. Chem., v.43, no.7, p880-882: Fluorescence Reaction for Amino Acids.
[O-phthaldehyde reacts with amino acids in alkaline medium in the presence of reducing agents to give strongly fluorescent compounds. Can go down to nanogram range. Said to be much better than ninhydrin procedures.]

1966 Pital A., Janowitz S.L., Hudak C.E. and Lewis E.E., Applied Microbiol., v.14, no.1, p119-123: Direct Fluorescent Labelling of Microorganisms as a Possible Life Detection Technique.
[Developed at Fort Detrick as a possible approach for the detection of extraterrestrial life. Proteins are stained, non-proteins are readily destained.]

VIRUS

1982 Conrat H.F. and Kimball P.C., Virology. Prentice Hall Publ.

1980 Bitton G., Introduction to Environmental Virology. John Wiley and Sons Publ.

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