Application of Infrared Fourier Transform Spectroscopy to Analysis of Micro Samples

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A Fourier transform mid-infrared spectrometer equipped with a 6X beam condenser has been used to record infrared spectra of samples in submicrogram quantities. This setup is ideal for the identification of small particles which can be as small as 0.05 mm in diameter. If the sample is in a dilute solution, it is possible to obtain a usable infrared spectrum of 0.05 μg sample

Infrared spectroscopic analysis is a very useful method to solve many analytical problems. However, the sensitivity of a conventional infrared spectrometer for analysis of micro samples is far below the sensitivity of some other analytical instruments, such as a mass spectrometer. The demands for infrared analysis of very small quantities of samples have increased rapidly in recent years. The newly developed Fourier transform infrared (ft-ir) spectrometer has certain advantages over the grating or prism infrared spectrometers (Horlick, 1968), and the present study describes several situations where it can be used advantageously for microanalysis.

Low and Freeman (1968) performed some experiments on an early model of a mid-infrared interferometer (Block Engineering, Inc., Model 195T) in application to micro work. They did not use a beam condenser in their study. Instead, the sample was placed very close to the detector in order to take advantage of the small beam size. They found it possible to obtain usable infrared spectra with sample quantities of ~1 μg, although it was very difficult to manipulate such small quantities of samples onto KBr plates. Since many significant improvements of the mid-ft-ir spectrometer have been made during the past 5 years, higher sensitivity and better quality spectra can be obtained from the newer instruments. Griffiths and Block (1972) have shown that the infrared spectra of a few nanograms of samples can be obtained from their Digilab model FTS-14 infrared Fourier transform spectrometer equipped with a 4X beam condenser. These authors achieved this high sensitivity by using a large quantity of samples in a 13-mm diameter KBr disk which was attenuated with a 0.5-mm aperture. However, when they tried to make a 0.5-mm micro KBr disk from a 0.3-mg aliquot of KBr which contained 1 μg of *o*-ethyl-*p*-methyl phosphonate, they found that the sample evaporated very rapidly from the KBr. Their data tended to indicate that the micro KBr disk technique is impractical for analyzing submicrogram quantities of organic materials, even if their vapor pressure at ambient temperature is very low.

The present study had been carried out before Griffiths and Block (1972) published their results. Several micro sampling techniques were tried for different type samples. It seems that the loss of sample from KBr powders depends not only on the volatility of the sample but also on its physical state. The rapid loss of the methyl phosphonate monoester from KBr powder observed by Griffiths and Block (1972) may be attributed to the fact that this liquid sample was thinly coated on the large surface area of fine KBr powder and its evaporation rate could be much higher than a solid sample, even it had the same vapor pressure as the liquid sample, probably because the solid compound tended to form crystals and had much less surface area exposed to air.

This report emphasizes the study of micro sampling techniques, since a major problem in micro infrared work is to transfer submicrogram quantities of samples from a practical container to the small focal point of the beam in the spectrometer. By improving the sample handling techniques and by utilizing the advantages of an ft-ir spectrometer, it was found that analysis of submicrogram quantities of samples by this method is practical.

EXPERIMENTAL SECTION

The ft-ir spectrometer used in this study was a Digilab Model FTS-14 IR spectrometer, equipped with a TGS detector. A 6X Perkin-Elmer off-axis elliptical mirror beam condenser was carefully aligned with the help of two laser beams. The aligned beam condenser was mounted on the FTS-14 spectrometer. With this arrangement, energy throughput of the system was actually slightly higher than when the beam condenser was not in place. This surprising result may be attributed either to the possible improvement of the focus at the detector or the correction of some optical mismatch in the spectrometer when the beam condenser is in place. If the beam at the focus of the beam condenser was masked with a 0.5-mm aperture, about 8–10% energy transmission of the unattenuated beam was obtained.

The solid particle samples were mounted on a sample holder made from a thin brass plate. A small pinhole was drilled through the brass plate and the sample was fixed at the pinhole by using double-face adhesive tape.

Several techniques were tried to transfer micro quantities of samples from dilute solution to KBr substrate. The most efficient method found was to transfer a few microliters of the solution onto 0.1–0.4 mg of KBr powder by using a 10-μl microsyringe. The solvent was evaporated slowly on the KBr powder, which was attached to the end of the syringe needle. The KBr powder was then pressed into a 0.5-mm diameter disk.

Ir spectra of solutions were obtained by placing the samples in a special 1-mm path microcavity cell. The cavity was drilled very close to the front surface and was masked with aluminum foil having a 0.5 mm × 1 mm hole. It was found easier to focus the beam, which has a large aperture angle in the beam condenser, on to the cavity of this new cell than in the case of the regular microcavity cell. About 2–3 μl of solution was required to fill this cell.

All spectra recorded in this study were obtained at a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

There are several advantages in using the ft-ir spectrometer for analyzing micro samples. The multiplex and throughput advantages for this type of application have been discussed (Horlick, 1968), which allow an infrared spectrum with very good signal-to-noise ratio to be obtained within a short time. Large ordinate scale expansion can be applied to measure the weak absorption bands due to the small quantities of samples used.

To illustrate how small a sample can yield useful data, Figure 3 gives the infrared spectrum of a polyethylene film which was masked by a 0.05-mm aperture. The spec-

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trum was co-added from 300 scans on the FTS-14 ir spectrometer equipped with the 6X beam condenser. The amount of sample in the beam is about 40 ng. No ordinate scale expansion was used. Since most organic compounds have stronger infrared absorptions than polyethylene, Figure 3 indicates that a usable infrared spectrum may be

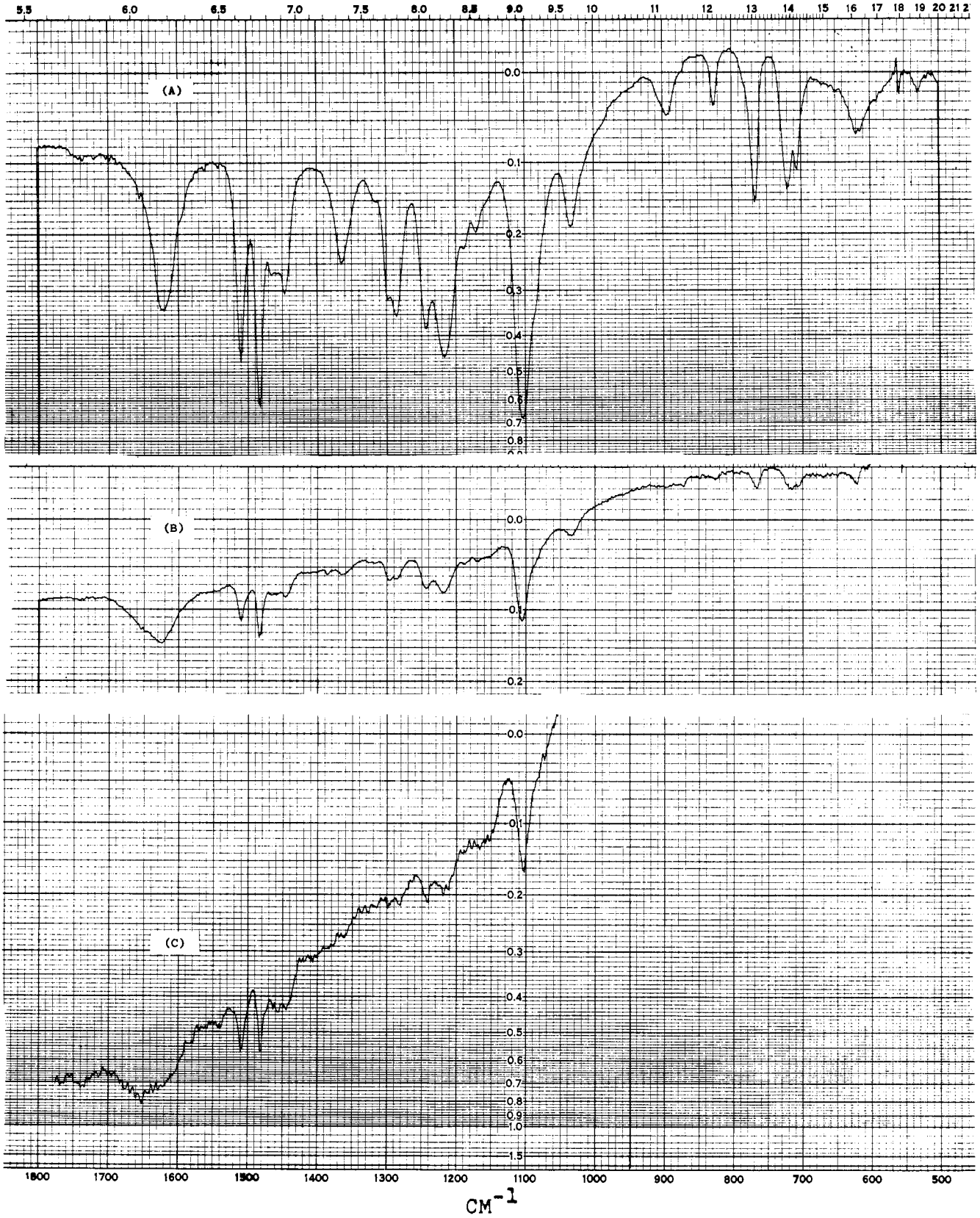

Figure 1. The ir spectra of 2,6-dimethoxyphenol in the micro KBr disks (0.5 mm in diameter). (A) Sample in 0.5 μl of 0.1% CS_2 solution (0.5-μg sample) transferred to 0.2 mg KBr, 300 scans, no-scale expansion. (B) Sample in 5.0 μl of 0.01% CS_2 solution (0.5-μg sample) transferred to 0.2 mg KBr, 300 scans, no-scale expansion. (C) Sample in 0.5 μl of 0.01% CS_2 solution (0.05-μg sample) transferred to 0.2 mg KBr, 400 scans, 7X scale expansion.

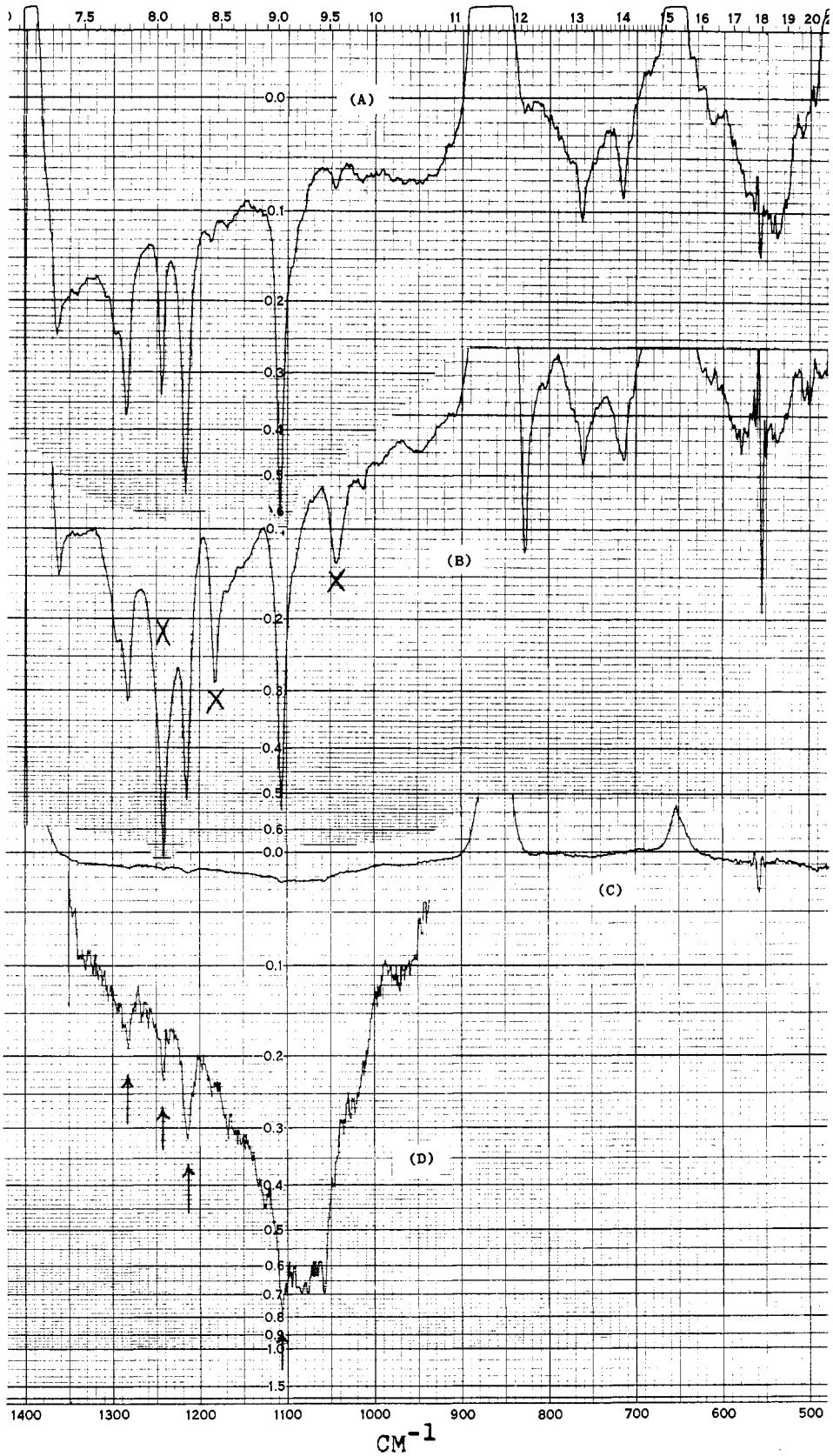

Figure 2. The ir spectra of 2,6-dimethoxyphenol in CS_2 solution. The solvent absorptions were compensated by the solvent spectrum. 1-mm path cavity cell was used. The negative absorptions at 860 and 650 cm^{-1} are due to the unbalanced CS_2 bands. (A) 3 μl, 0.01% (0.3 μg) in cavity cell, 500 scans, 5X scale expansion. (B) 200 μl of 0.00025% CS_2 solution was evaporated to 5 μl, 500 scans, 5X scale expansion. The bands marked with X's are due to the concentrated impurity in the solvent. (C) 5 μg of sample on a silica gel tlc plate was developed with CH_2Cl_2 for 35 min. The sample moved from the origin about 1 in. The sample was extracted with 50 μl of CS_2 twice and the CS_2 solution was concentrated to 5 μl. 1000 scans, no-scale expansion. (D) 30X scale expansion of curve (C). The arrows indicate the absorption bands of 2,6-dimethoxyphenol.

obtained for less than 10 ng of sample if the sample can be concentrated into the appropriate small area (~0.05-mm diameter).

This technique is ideal for analyzing small solid or semi-solid contaminants in plastics. The plastic can be microtomed and mounted on a micro holder under a microscope, and then mounted in the 6X beam condenser. Identification of the impurity particles can then be easily made from their infrared spectra, and this technique has now become routine in our laboratory. Curve (A) in Figure 4 is the infrared spectrum of a black speck (~0.25 mm in diameter) which was found in a molded plastic plate. The curve (B) is the infrared spectrum taken directly from a portion of a dead insect found in the storage bin of the polymer feedstock. The obvious similarity between the two curves indicates the source of the contamination.

In most cases of microanalysis of organic compounds, the actual compounds to be identified are usually obtained in the form of very dilute solutions. We have found that it is nearly impossible to concentrate a sample from a large amount of dilute solution directly into a very small pinpointed area. For example, 200 μl of 0.001% CS_2 or CH_2Cl_2 solution (2-μg sample) was transferred onto 0.2–5.0 mg of KBr powder. The KBr powder was pressed into either a 0.5- or a 1.5-mm diameter disk, but no useful spectra resulted in these attempts. Much better results are obtained if we first concentrate the dilute solution using a micro distillation apparatus and then transfer the last drop (<10 μl) of the concentrated solution to 0.2–0.4 mg of KBr powder by using a microsyringe. Figure 1 shows some infrared spectra of 2,6-dimethoxyphenol (mp 55°, bp 262.7°) in 0.5-mm KBr disks, obtained by starting with equivalent amounts of material, but in solutions of different concentrations. The KBr disk used for curve (A) was prepared by evaporating 0.5 μl of 0.1% CS_2 solution (0.5-μg sample) onto 0.2 mg of KBr powder. The spectrum was scanned 300 times, but no ordinate scale expansion was used. The KBr disk for curve (B) was prepared by evaporating 5 μl of 0.01% CS_2 solution (0.5-μg sample) onto 0.2 mg of KBr powder. In curve (B) only about one-fifth as much sample as observed in curve (A) apparently reached the sample beam. The sample loss may be attributed to evaporation of the sample itself, uneven distribution of the sample on the KBr, and tendency of a solution to creep up the outside of the syringe needle. However, no continuous sample loss from the KBr was observed after the deposition. Curve (C) was recorded by using a 0.05-μg tion techniques. Figure 2 shows the infrared spectra of mg of KBr powder). This curve is a 7X expansion of the original ordinate scale. Other samples have also been tried. It appears that 0.05 μg is about the smallest amount of sample that can be transferred and observed by this method. The ill-defined base line in curve (C) is due to scattering and impurities in the KBr disk, and is another drawback for micro KBr disk technique.

The base line problem is reduced and the reproducibility of the spectrum is considerably improved by using solution techniques. Figure 2 shows the infrared spectra of 2,6-dimethoxyphenol as obtained in CS_2 solutions. About 3 μl of CS_2 solution was placed into the 1-mm path cavity cell described above. The beam splitter in the ft–ir spectrometer filters out most of the radiation of shorter wavelength than 2.5 μ and thus the heating effect on the sample is reduced (as compared to conventional grating or prism spectrometers, which place the micro sample at a small focus between source and entrance slit, and thus tend to overheat the micro sample, with the result that a larger volume of solution is required to keep the cell full while measurements are being made).

Curve (A) in Figure 2 was recorded from 3 μl of CS_2 solution which contained 0.3 μg of 2,6-dimethoxyphenol (0.01%); about half of the solution in the cell was in the

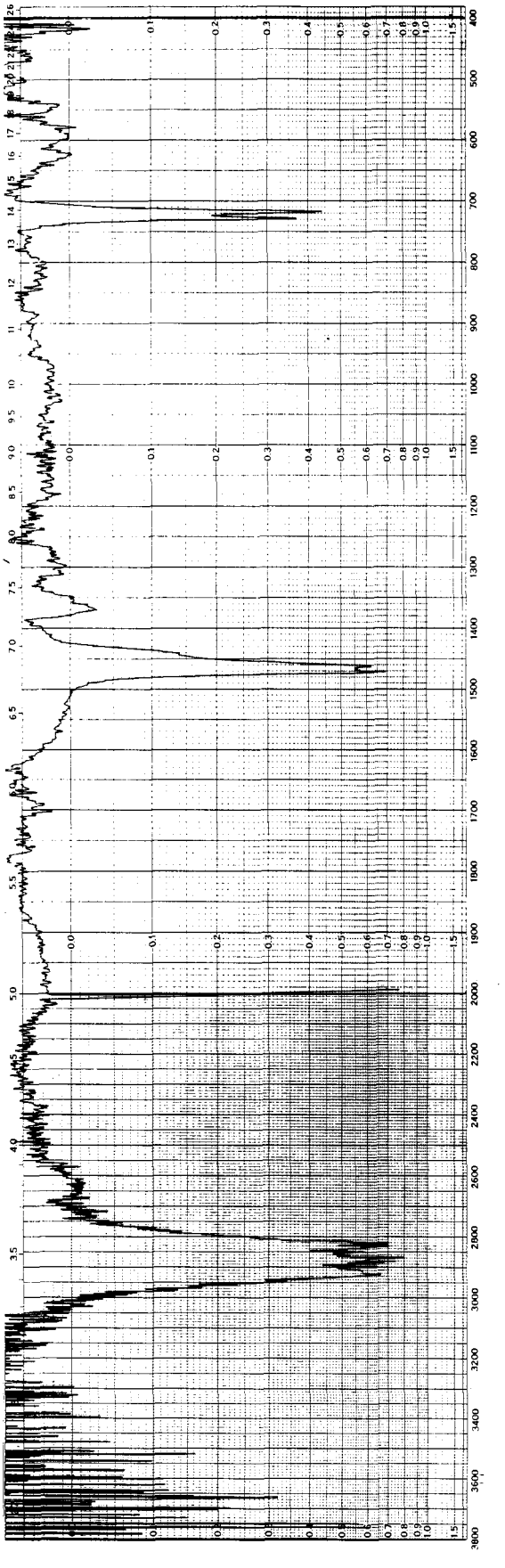

Figure 3. The infrared spectrum of the polyethylene film attenuated with 0.05-mm aperture. The spectrum of 300 scans of the sample was ratioed against the stored 300 scans of the background.

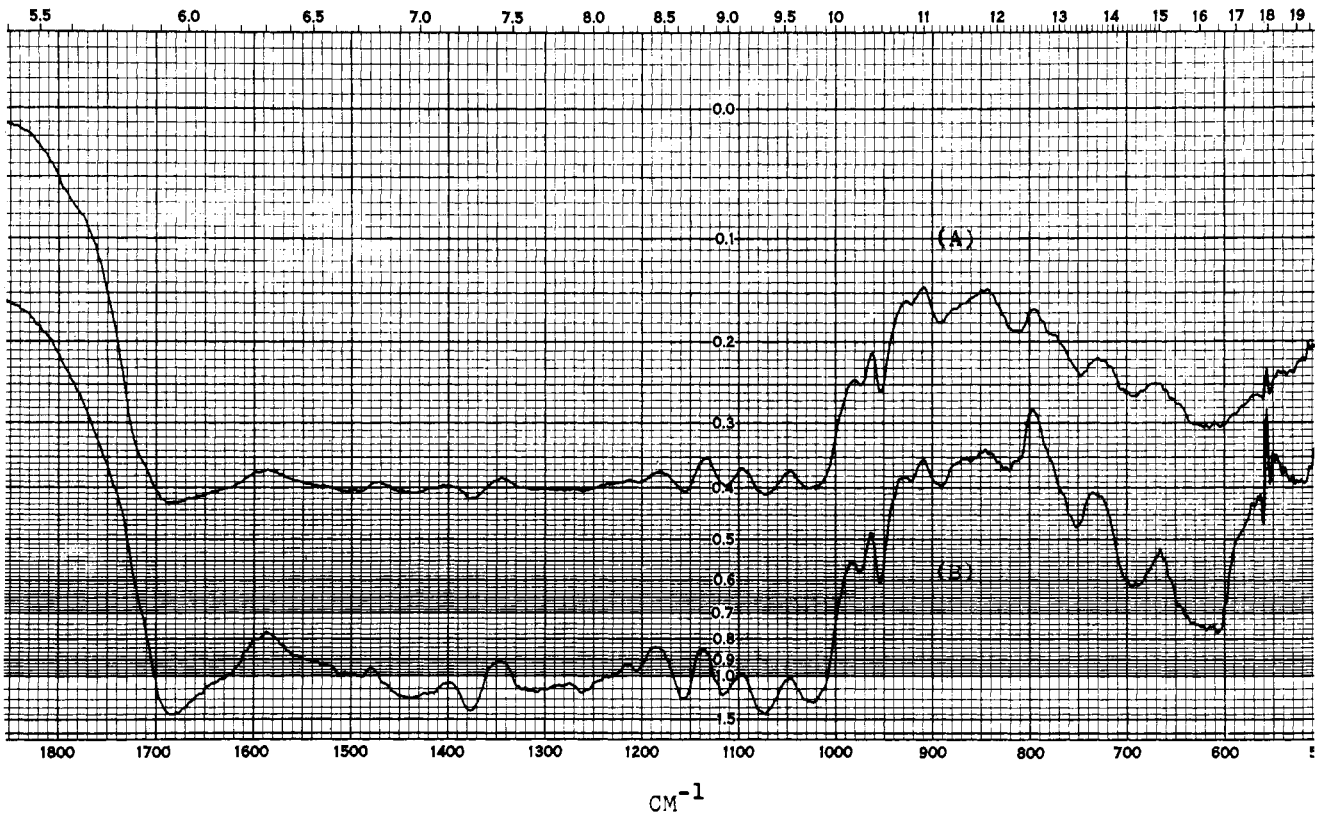

Figure 4. (A) The ir spectrum of a black speck (0.25 mm in diameter) which was found in a molded plastic sample, 300 scans. (B) The ir spectrum of a portion from a dead insect which was found in the storage bin of the granulated polymer feed stock, 300 scans.

path of the beam. Curve (B) is the spectrum of 5 μl of CS_2 solution in the same cavity cell, but this 5-μl solution was obtained by evaporating 200 μl of 0.00025% (0.5-μg sample) CS_2 solution in a centrifuge tube. This spectrum indicates that about 80% sample was recorded. The bands marked with X's in this curve result from impurity concentrated from the CS_2.

Identification of samples isolated on a thin-layer chromatographic (tlc) plate is useful in many analytical problems. The success of transferring the sample from the tlc plate to an ir cell or a KBr disk depends largely on the volatility of the sample and the effectiveness of the solvent in removing the sample from the substrate. If the sample occupies a 1 cm^2 area on a 0.2-mm thick tlc plate, the substrate can be scraped off and extracted with 50–200 μl of solvent. The solution is concentrated into a volume of a few microliters and the ir spectrum is recorded by the methods mentioned above. Figure 2, (C) and (D), demonstrates the application of this procedure: 5 μg of 2,6-dimethoxyphenol in CS_2 solution was transferred to a 0.2-mm thick silica gel plate. The plate was developed with CH_2Cl_2 for 35 min. The sample moved about 1 in. from the origin. The silica gel at the sample spot was scraped off and was extracted twice with 50 μl of CS_2. The combined CS_2 extracts were evaporated down to 5 μl and placed in the 1-mm cavity cell. Figure 2 (C) is the spectrum of the solution without scale expansion and curve (D) is with 30X scale expansion. About 20 ng of sample is present. The large sample loss may be attributed to evaporation on the CS_2. Clearly, further work is needed in technique development for handling tlc cuts.

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