# Infrared Interferometry in Flavor Analysis

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A multiple-scan interference spectrometer was used to record infrared spectra of samples deposited on salt plates or in solution. It was possible to obtain usable spectra in minutes with sample quantities of 0.5 and 1 μg.

The scientist engaged in flavor analysis research is faced with a formidable task in determining the composition of natural products. However, analytical difficulties arise not from the quantitative aspects of the work, but from the qualitative tests required for the characterization of complex mixtures. The gas chromatograph has proved eminently satisfactory for separation and detection, but the role of identification falls to spectral disciplines and often is complicated by the necessity of examining microsamples. Mass spectrometric examination of GLC fractions can be expected to result in the successful characterization of about half of the constituents of a complex natural product; additional spectral assistance is needed for the remainder. There is little difficulty in obtaining an infrared spectrum if 5 to 10 μg. of sample are available, but unfortunately there is still a disparity of some three or four orders of magnitude between the sensitivities of modern mass spectrometers and conventional infrared dispersion spectrometers, so that the full potential of the GLC-mass-infrared combination has not been realized. In trying to approach the sensitivity limitation set by the mass spectrometer, the authors are exploring the feasibility of using a multiple-scan interferometer to reduce the sample size requirements for infrared spectra into the submicrogram range. Some work on the infrared interferometric analysis of fractions eluted from a gas chromatograph using packed columns has been described (Low, 1966a; Low and Freeman, 1967) and trapped, gaseous samples in the order of 10 μg. were examined. The present paper is an interim report of an extension of such work, using different instrumentation and sampling techniques.

## INSTRUMENTATION

The general aspects of infrared multiple-scan interferometry, as well as some commercial instrumentation, have been described (Low, 1966b). The spectrometer used for the present experiments was modular and is shown schematically in Figure 1. The interferometer used (Block Engineering, Inc., Model 195T) consisted of a scanning Michelson interferometer optical system plus bolometer detector and an electronics package. Its various functions are shown schematically within the dashed rectangle in Figure 1. The instrument was set to sweep repetitively, every second producing an interferogram containing information concerning the spectral range 2500 to 250 cm.$^{-1}$ (4 to 40 microns) with a resolution of approximately 15 to 18 cm$^{-1}$. Successive interferograms were coherently added and stored with a 1024 channel time-averaging computer or Coadder (Block Engineering, Inc., Model 350). The digitizing and storage rate was controlled by trigger and clock signals from the interferometer. The Coadder, which functioned in add as well as subtract modes, contained a 10-bit A/D converter rather than the 8-bit one described previously (Low and Freeman, 1967). When operating in the output mode, the Coadder was triggered and clocked by Hewlett-Packard audio oscillators. The signal corresponding to stored interferograms was fed either to a prototype wave analyzer, or to a Block Engineering, Inc., Model 310 paper tape punch. The latter served as an interface between the Coadder and an IBM Model 301 digital computer used for data reduction (Low, 1966b). The output of the wave analyzer, which scanned in approximately 1 minute, was recorded on a Moseley X-Y plotter to yield a spectrum. The ordinates of the spectra shown in Figures 3 and 4 are displaced and are given in arbitrary units of transmission rather than transmittance.

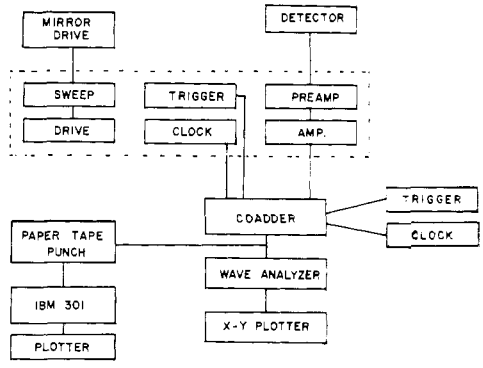


**Figure 1. Schematic of interference spectrometer**

## EXPERIMENTS AND RESULTS

The diagram appearing in Figure 2 depicts the experimental arrangement employed in this study. The globar

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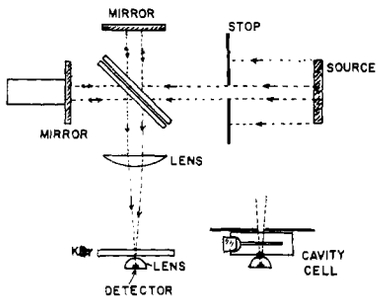


**Figure 2. Experimental arrangements**

and its power supply were similar to those used in a Perkin-Elmer Infracord. The beam emerging from the interferometer was converged by a KRS-5 lens, approximately *F*/2, and passed to the detector flake through a KRS-5 fish-eye lens. Because a small source image was desirable, and a suitable beam reducer or collimator was not available, the beam entering the interferometer was reduced with an aluminum stop. The relatively great radiation-gathering capability of the interferometer, one of its advantages over dispersion spectrometers, thus was not fully used. Materials were placed between the interferometer and the detector, at or near the focus as required by the size of the sample. An additional stop was used with microcells to define the beam, as shown in the insert of Figure 2. This design has the advantage of severely reducing the radiation emitted by the sample (or sample cells). The interferometer acts as optical chopper, and the electronics accept only a.c. signals; a steady d.c. detector signal produced by the unmodulated self-emission of the sample is therefore not amplified.

As described here, the interferometer is a single-beam device and the spectra suffer all the disadvantages of spectra from single-beam dispersion spectrometers—i.e., absorption bands appear on a slanting background, interfering absorptions are present due to atmospheric water and $CO_2$, etc. However, a correction for background can be made. An example is shown in Figure 3. The sample was 1 μl. of eugenol on the surface of a KBr plate. Trace *A* of Figure 3 is a spectrum of the globar modified by the instrument functions—e.g., detector response, with superimposed absorptions due to atmospheric gases. When eugenol is placed in the beam, some additional absorptions appear as in trace *B*, but the spectrum of the sample per se is obscured until a correction is made. This can be accomplished by using the Coadder in an add mode first to store signals of the composite sample-plus-background, and in a subtract mode to remove the background (see spectrum *C* of Figure 3). A pseudo-double-beam operation is thus effected.

However, spectra gathered by these means, although usable for identification, are not entirely satisfactory for rapid comparison with spectra recorded with conventional double-beam dispersion spectrometers because of differences in relative band intensities. These differences arise because the intensity of a particular absorption band in a subtraction spectrum, such as trace *C*, is a function of the energy distribution of the source itself. However, a

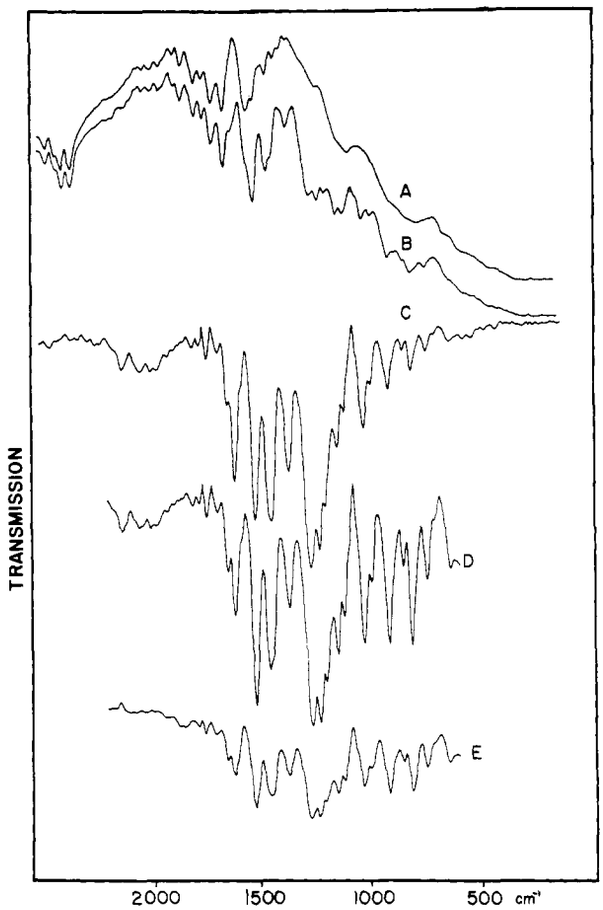


**Figure 3. Spectra of eugenol**

All spectra were produced by digital computation
*A*. Background (globar radiation passing through 2 KBr plates), 100 scans
*B*. 1 μl. of eugenol was placed between KBr plates, 100 scans
*C*. 100 scans of background as in *A*, above, were subtracted from 100 scans of sample plus background as in *B*, resulting in the difference spectrum of 1 μl. of eugenol
*D*. Ratio of spectra *A* and *B*
*E*. Ratio of spectra *A* and *C*

correction can be made by digital computation procedures by calculating the ratios of the absorptions. For example, spectrum *D* of Figure 3 was obtained from a computation of the ratio of spectra *A* and *B*. An alternative procedure involves taking the ratio of the background and difference spectra—e.g., spectra *A* and *C*—to result in a corrected spectrum such as *E* in Figure 3. The differences in line intensities produced by the correction procedures, particularly at lower wave numbers, is readily apparent when comparing the subtraction spectrum *C* with spectrum *D* or *E*.

Such digital computation is desirable but not essential. Similarly, digital computation of Fourier transforms is more precise and results in spectra of greater resolution than those from analog procedures using a wave analyzer. However, the procedure of background subtraction employing the Coadder with subsequent analog data reduction yields spectra which are useful and more readily and rapidly obtained. Consequently, little emphasis was placed on digital computer techniques at this time. The

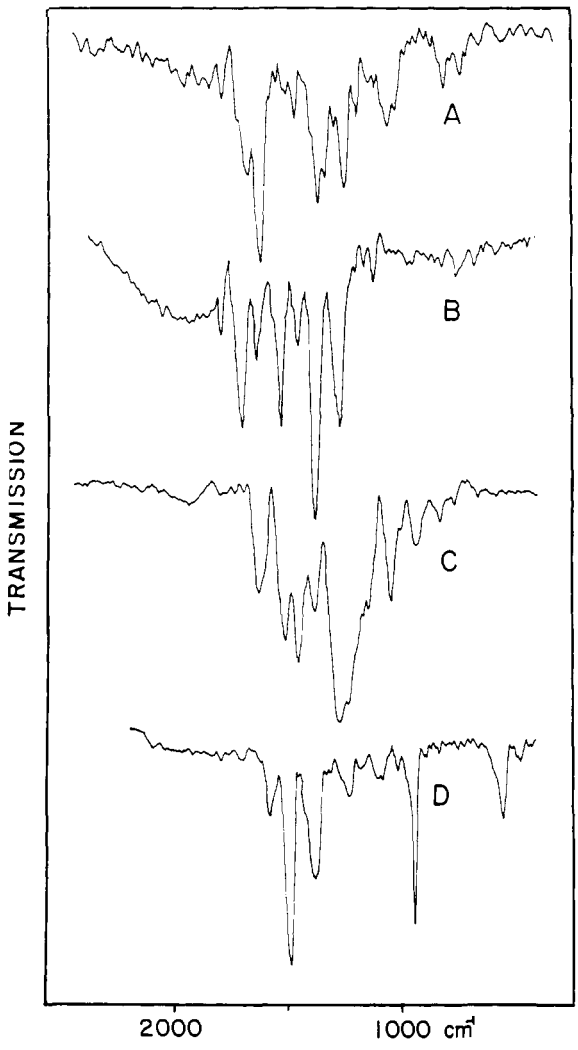


**Figure 4. Spectra of microgram quantities**

*A.* 0.5 μg. of chalcone, on KBr plate, 261 scans
*B.* 1 μg. of *m*-nitroacetophenone, on KBr plate, 200 scans
*C.* 1 μg. of longifolene, in solution, 250 scans
*D.* 2 μg. of eugenol, in solution, 300 scans
All spectra were corrected by background subtraction, and data reduction was carried out by wave analyzer. Ordinates are arbitrary

spectra shown in Figure 4 are difference spectra, similar to spectrum *C* of Figure 3, but produced by analog means. A series of experiments was conducted with samples of pure compounds on the surfaces of KBr crystals. A suitable number (*N*) of interferograms of the background of the clean plate were first recorded with the Coadder in the subtract mode. A known volume of solution containing the sample was then deposited with a microsyringe on the same small area of the plate used for the background measurement. After the spectrally pure solvent had evaporated, *N* interferograms of sample plus background were added to the *N* interferograms of background stored in the Coadder memory. The residual stored interferograms were then processed by means of the wave analyzer to gain a spectrum. Using such procedures, spectra of liquid or solid samples of approximately 20 μg. were easily obtained in about 1 minute. However, difficulties were encountered with much smaller samples, particularly liquids. The deposition of several microliters of solution on one small area, the unpredictable spreading of the solution, the uncontrollable evaporation of the solvent, and the beading of liquids prevented control of samples in the range of 10 to 1 μg. However, with careful sample manipulation, some spectra of 1- and of 0.5-μg. quantities were obtained in 100 and 261 seconds, respectively. The intensities of spectral bands were erratic because of uneven deposition of the sample. Sometimes there occurred a migration of the sample from the small area traversed by the infrared beam, leading to an almost "perfect" subtraction and resulting only in a spectrum of residual noise, while at other times a small sample of solid could scatter sufficient radiation so that the subtraction procedure was not precise. Attempts at confining samples within small cavities in the crystal were not successful, probably because the sample coated the cavity walls. A series of experiments was carried out with dilute solution confined within a cavity cell. The following sequence was typical. A cell was rigidly clamped in place in order to avoid positioning errors. Pure solvent was introduced, and *N* interferograms were recorded. The solvent was removed by means of a syringe without moving the cell. A solution of known concentration was introduced into the dry cell, and *N* interferograms were recorded. The resultant cumulative interferogram was then reduced. From the cell-beam geometry it is estimated that the infrared beam passed through less than 1 μg. of sample. Some spectra are shown in Figure 4.

Good spectra on quantities less than approximately 20 μg. were more difficult to obtain than in the case of samples on KBr plates because in many cases the subtraction procedure did not compensate precisely for the absorption bands of the solvent compound which were several orders of magnitude larger than those of the sample. Such spectra were frequently of high quality in terms of signal-to-noise ratio but were meaningless in terms of spectral structure because of the summation of solvent and solute bands. Since use of a single, rigidly mounted cell would minimize such compensation effects and the electronics used were stable and functioned reproducibly, the effects are ascribed to fluctuations in the temperature of the source and detector.

## DISCUSSION

The present exploratory study has shown that infrared spectra can be recorded in 100 seconds with a resolution of 15 to 18 $cm.^{-1}$ on 1 μg. of sample in solution or the neat state. Difficulties were encountered in the control and positioning of very small samples such as materials deposited on KBr plates, and with solvent band compensation due to inadequate background subtraction for solutions. However, the results are intriguing and valuable because the potential for significantly improving the instrument appears to be relatively great.

An advantage of interferometric infrared spectrometry lies in the capability of swift scanning. It is this feature,

coupled with the instrument's sensitivity, which prompted our initial work pertaining to recording of flowing GLC effluent. Because of recent improvements in instrumental design, we decided first to examine solution and solid spectra. The anticipated increase in signal to noise ratio resulting from recent instrumental improvements (stabilizing the source and detector fluctuations) should permit the generation of interpretable spectra from a 1-$\mu$g. gas sample contained in a light pipe cell within 15 seconds. This period is compatible with the time required for elution from the gas chromatographic detector of approximately 80% of constituents encountered in essential oils and related natural products.

ACKNOWLEDGMENT

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