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The wavelength or frequency derivative of an absorption spectrum often enhances the apparent resolution of the spectrum, a fact long exploited by workers in magnetic resonance. The time derivative of an absorption feature at fixed wavelength is frequently used to determine chemical or photochemical reaction rates.

For those instruments which generate an electrical signal proportional to the absorption, it is possible to produce the derivative by means of electronic differentiation.[1] The adjustment of such circuits usually demands considerable experimentation to insure that accurate differentiation is obtained for all features in the spectrum.

Some instruments have no electrical signal proportional to absorbance. For example, the Cary Model 14 spectrophotometer, a dual-beam instrument, employs a logarithmic slide-wire; the demodulated signal from the sample beam is compared with the wiper voltage; the resulting difference or error signal is used to drive the pen-wiper servomotor to produce a null difference. Accordingly, only the pen position indicates the logarithm of the ratio of beam intensities or absorbance.

Ganged with the servomotor is a tachometer generator whose output voltage is added to the pen amplifier in a manner to achieve critical damping of the pen servomechanism. The output voltage of this tachometer generator is proportional to its angular velocity and therefore to the instantaneous slope of absorbance. This signal may be rectified and filtered, giving the absolute value of the derivative. With little more complexity the signal may be synchronously rectified, yielding the sign as well as the magnitude of the slope.

In Figure 1 are shown the absorption spectrum, the wavelength derivative, and a "logarithmic" wavelength derivative spectrum of a bacteriochlorophyll-protein complex.[2] It is apparent that the derivative spectrum reveals features which are barely perceptible in the absorption spectrum. A long-wavelength shoulder is seen on the 810 mμ absorption derivative, and the 590 mμ band is obviously complex. Reducing the sample temperature to 77°K narrows the lines sufficiently to resolve these spectral features directly in the absorption spectrum, thus confirming their presence in the room temperature derivative spectra. To increase the dynamic range of the derivative recording we have found it convenient to interpose a nonlinear compression amplifier between the synchronous detector and the recorder.

One of our principal reasons for developing this technique was to provide a derivative spectrum for comparison with the magnetic circular dichroism (MCD) spectrum of the same compound. In certain instances the MCD spectra are expected to be magnetic derivatives of the normal absorption spectra,[3] and comparison of the MCD with the absorbance derivative is useful for establishing these cases.

Thus far we have discussed this addition to the Cary 14 instrument. It is similarly adaptable to the Varian A-60 NMR spectrometer, which produces an absorption spectrum. In this instrument there is indeed an electrical signal proportional to absorption intensity, but as stated earlier, electronic differentiation may be sufficiently troublesome to discourage its use. The pen servomechanism of this spectrometer also contains a tachometer generator. Accordingly we may produce a derivative spectrum in a manner completely analogous to that used in the Cary 14.

Figure 2 offers a comparison between the absorption and derivative spectra of the methyl and methylene protons of ethyl-benzene. The enhanced visibility of the second-order splittings is readily apparent in the derivative spectrum.

Any of the great number of lock-in detectors generally available may be used to synchronously demodulate the tachometer generator voltages, but since they produce signals in the 10-V range virtually no amplification need precede the detector. In Figure 3 is shown a diagram of the detector we have used. The transformers are used to isolate the spectrometers from the external circuitry, thus avoiding the introduction of hum and loading. The output amplifier provides 12 dB/octave filtering.

In a spectrometer which produces derivative spectra by frequency modulation and synchronous detection[4] the modulation amplitude must always be less than the width of any feature in order to preclude modulation broadening, although this constraint requires the sacrifice of signal-to-noise ratio of broad lines accompanying narrow lines. In the present system, by contrast, the derivative signal amplitude is dependent solely on the slope of absorbance. As the

velocity-sensitive device, the tachometer generator, is an optimally engineered adjunct to the servomotor, the "time constant" is always correct as long as the slope does not exceed the response of the servomechanism. The filter following the synchronous detector must, of course, be chosen so that it does not introduce distortion of the derivative.

Rather than scanning wavelength at a uniform rate to generate a wavelength derivative spectrum, one can use the time derivative at constant wavelength to determine reaction rates. This procedure offers the advantage that a more objective measure of slopes is available than is usually the case when the slopes are measured manually.

Since noise within the instruments will be accentuated in the process of obtaining the derivative spectra, it is to be expected that the latter will exhibit a poorer signal-to-noise ratio than the absorption spectra themselves. A noisy slide-wire, any tendency toward oscillation in the pen servo, or an extended dead zone due to low servo gain will strongly degrade the quality of the derivative signal. The signal-to-noise ratio will increase linearly with increasing scan rate so long as the lines (peaks) do not become too narrow for the response time of the pen servo.

Instruments containing servos not equipped with tachometer generators may substitute for the tachometer generator signal the actual ac servomotor signal, with performance determined by the characteristics of the particular servomotor. Some instruments, e.g., the Cary Model 60 spectropolarimeter, employ dc rather than ac servomechanisms, and also may contain dc tachometer generators. The output of the dc tachometer generator is a measure of the derivative and may

be recorded directly. Small dc generators designed specifically for tachometer applications are available and may be mechanically coupled to any recording system to generate a derivative.

Addition of the derivative feature to commercial spectrophotometers lends added versatility to instruments of high photometric accuracy and obviates the need to construct separate instruments to enjoy the benefits of this technique.

Its use by workers in high resolution nuclear magnetic resonance will restore an advantage used by their colleagues in EPR and wide line NMR.

Footnotes and References

*Work performed under the auspices of the U.S. Atomic Energy Commission.

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2. J. M. Olsen in The Chlorophylls, L. P. Vernon and G. R. Seely, eds. (Academic Press, New York, 1966), p. 413.

3. Edward A. Dratz, The Geometry and Electronic Structure of Biologically Significant Molecules as Observed by Natural and Magnetic Optical Activity (Ph.D. Thesis), Lawrence Radiation Laboratory Report UCRL-17200, September 1966.

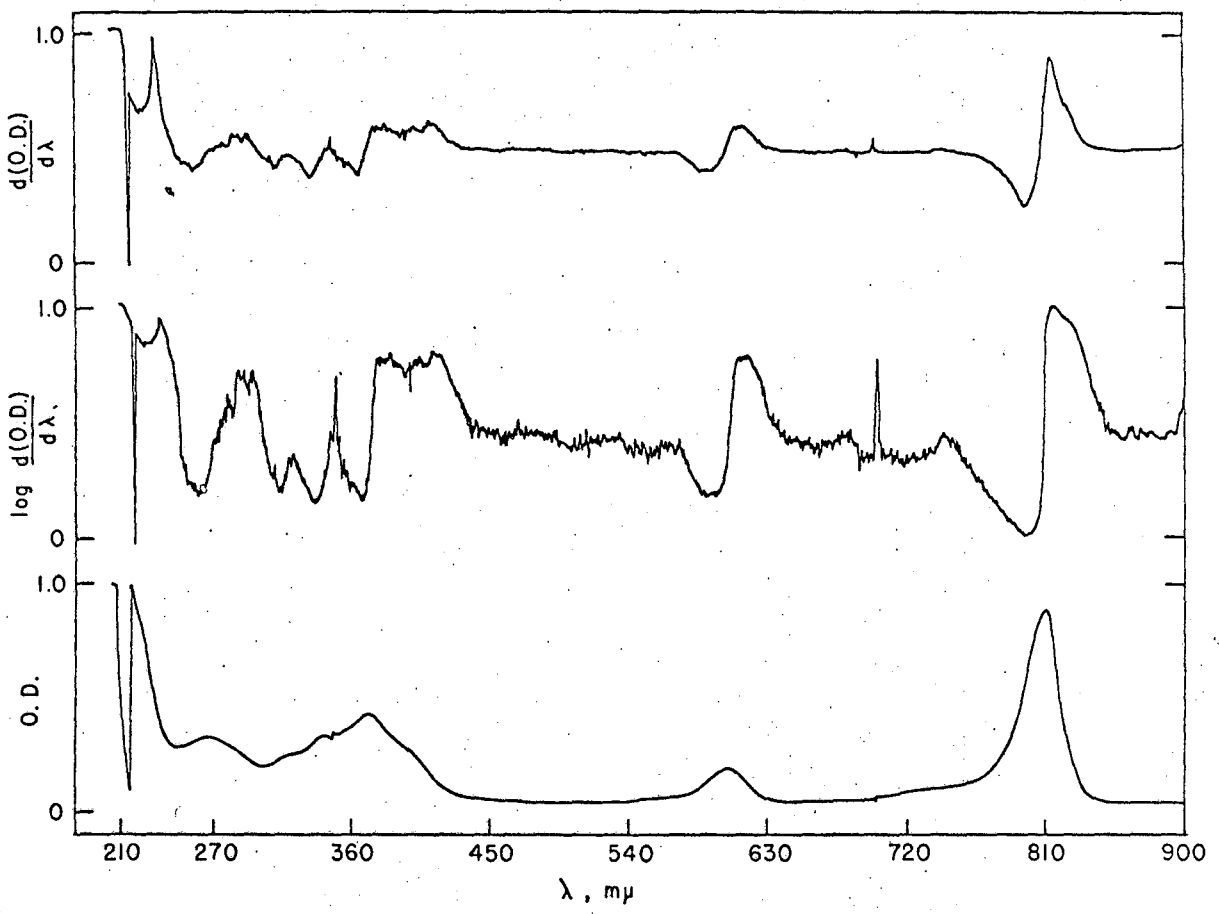
4. C. S. French and G. E. Harper, Carnegie Inst. Wash. Yearbook, 56, 281 (1956-7).

Figure Captions:

Figure 1. The absorption spectrum of a bacteriochlorophyll-protein complex (bottom), the wavelength derivative spectrum (top) and a "logarithmic" derivative spectrum (middle). The spikes at 700 and 350 mμ occur when the wavelength range of the spectrophotometer is changed.

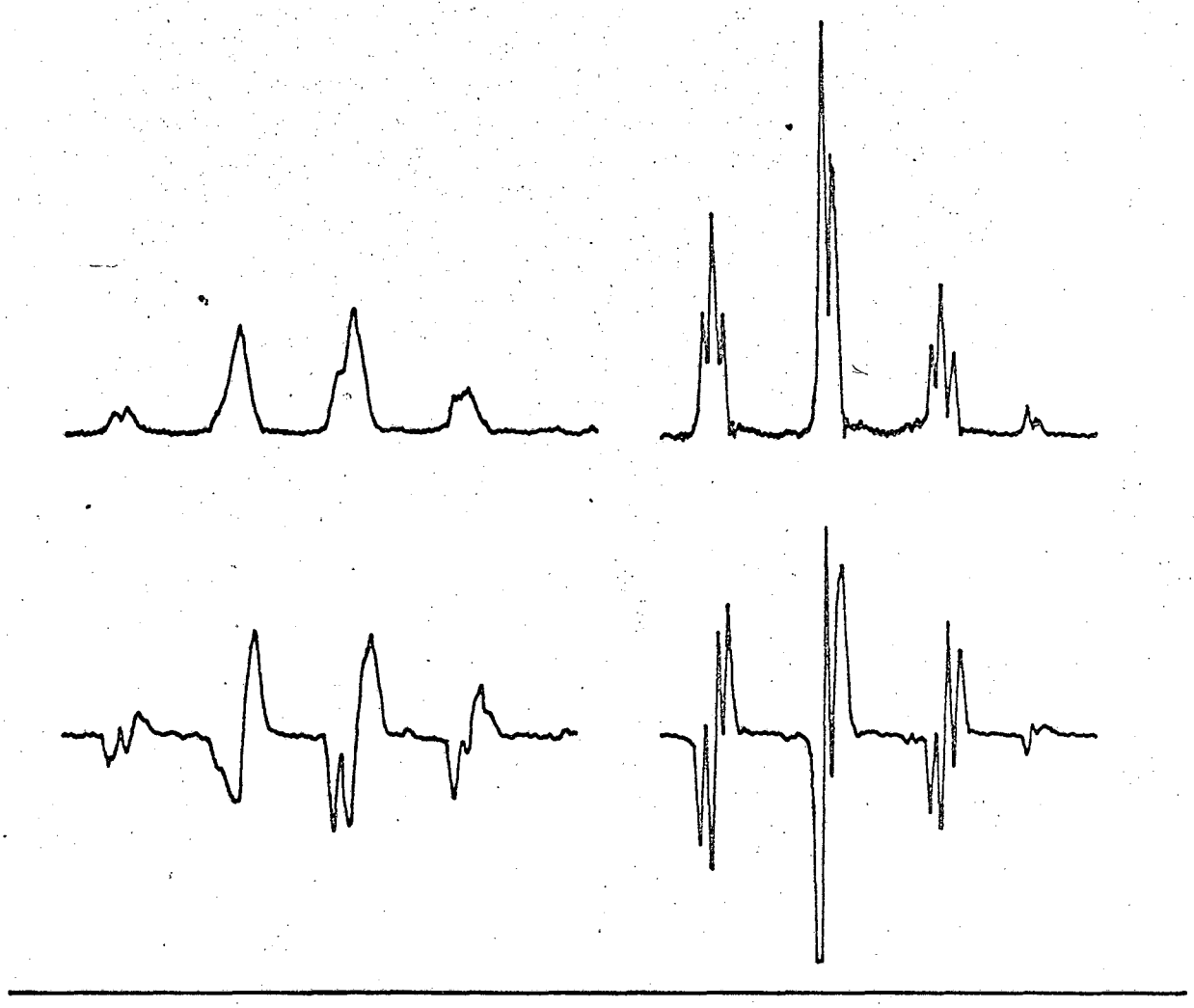
Figure 2. The proton magnetic resonance of the methylene (left) and methyl (right) groups of ethylbenzene. The upper curves are the direct absorption spectra and the lower curves are the absorption derivatives.

Figure 3. Synchronous detector to demodulate tachometer generator signals to produce a derivative spectrum from a recording spectrometer. The output amplifier employs 12 dB/ octave filtering.



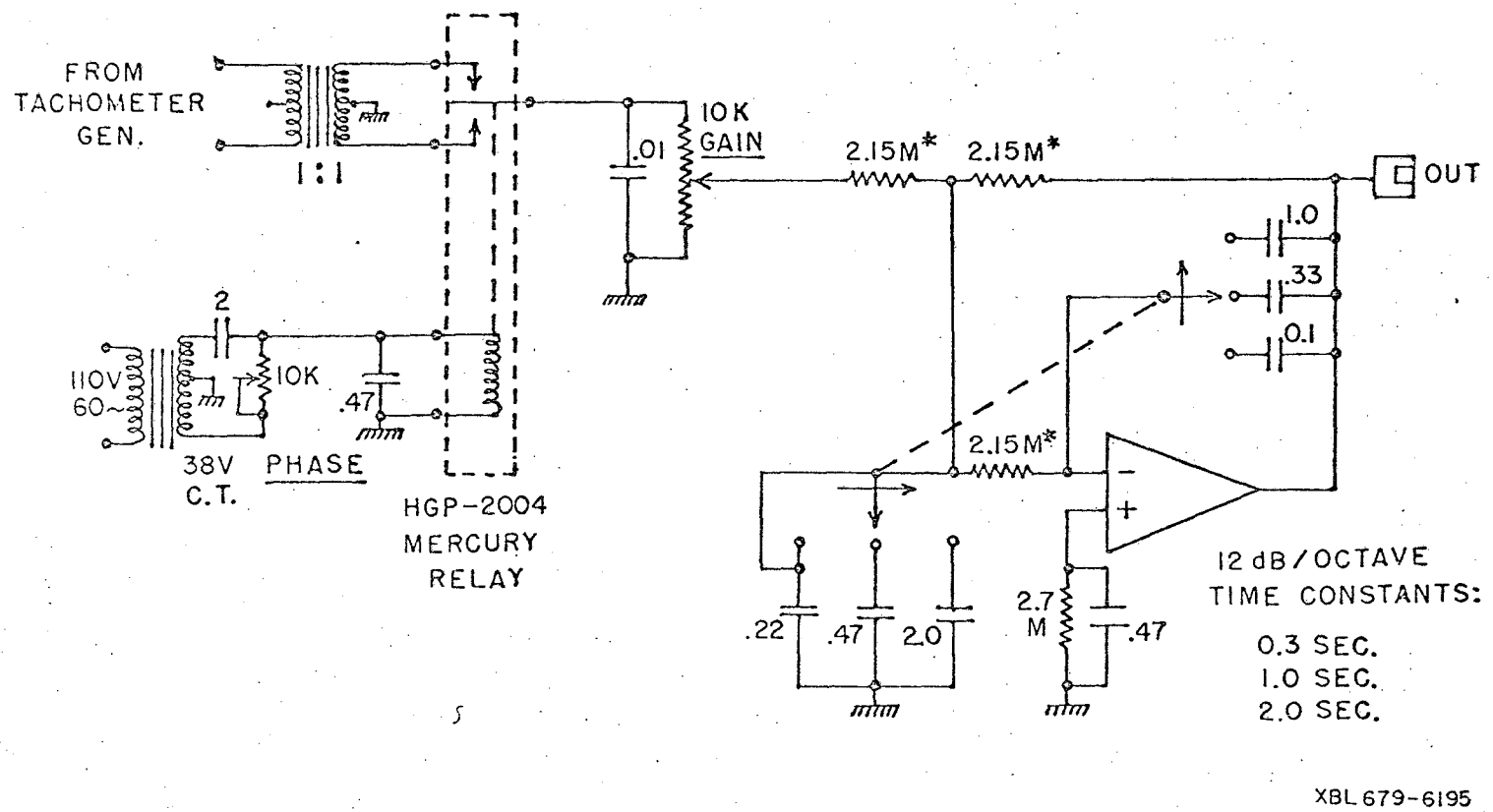
XBL 676-1133

Fig. 1



XBL 676-1134

Fig. 2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60~
10K
.47
38V
C.T.
PHASE
HGP-2004
MERCURY
RELAY
.01
10K
GAIN
2.15M*
2.15M*
OUT
1.0
.33
0.1
2.15M*
.22
.47
2.0
2.7
M
.47
12 dB/OCTAVE
TIME CONSTANTS:
0.3 SEC.
1.0 SEC.
2.0 SEC.
XBL 679-6195

Fig. 3