Title
THE ABSENCE OF COHERENT VIBRATIONS IN THE RAMAN SPECTRA OF LIVING CELLS

Authors
Cooper, M.S.
Amer, N.M.

Publication Date
1983-05-01

LBL-16389
Preprint

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

ENERGY & ENVIRONMENT DIVISION

Submitted to Physics Letters A

THE ABSENCE OF COHERENT VIBRATIONS IN THE RAMAN SPECTRA OF LIVING CELLS

M.S. Cooper and N.M. Amer

May 1983

ENERGY
AND ENVIRONMENT
DIVISION

Prepared for the U.S. Department of Energy under Contract DE-AC03-76SF00098

DISCLAIMER

The Absence of Coherent Vibrations in the Raman Spectra of Living Cells

M.S. Cooper and N.M. Amer

Applied Physics and Laser Spectroscopy Group
Lawrence Berkeley Laboratory
University of California
Berkeley, CA 94720
USA

May 1983

This work was supported by the Office of Energy Research, Physical & Technological Research Division, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098 and National Research Service Award No. 1-T32-GM07379.

The Absence of Coherent Vibrations in the Raman Spectra of Living Cells

M.S. Cooper and N.M. Amer

Applied Physics and Laser Spectroscopy Group

Lawrence Berkeley Laboratory

University of California

Berkeley, CA 94720, USA.

Abstract

Mie scattering from cell density fluctuations reproduces the majority of reported spectra from a Raman study of living cell suspensions. These previous spectra do not represent evidence for coherent vibrations of intracellular macromolecules predicted by Fröhlich.

ICSU classification: 8A (Biophysics)

Fifteen years ago, Fröhlich proposed that longitudinal electric vibrations in the 10^{11} to 10^{12} Hz frequency region might be coherently excited in living systems with metabolic energy [1,2]. Fröhlich showed theoretically that when a critical rate of energy is supplied to a group of dipolar oscillators, such as the hydrogen bonds in a macromolecule, a Bose-like condensation of phonons can occur, resulting in the coherent excitation of the lowest longitudinal electric vibration of the system.

A variety of experiments have been interpreted as giving support to Fröhlich's theory [3]. Among these is a set of Raman spectra taken by Webb et al. from bacterial and mammalian cell suspensions [4-10]. In these Raman scattering experiments, strong lines are reported to occur when cell populations are metabolically synchronized. This activity is reported to be absent in metabolically inactive or dead cells. Above 200 cm^{-1}, transient and often narrow lines are observed. The lines are claimed to follow regular temporal sequences, and have been attributed to strongly excited vibrations of macromolecules involved with metabolism and activities of the cell cycle [4,9,10]. In addition, non-thermal anti-Stokes to Stokes intensity ratios have been observed for low frequency lines (< 200 cm^{-1}) in the spectra of synchronized bacteria. These ratios are considered to be evidence for the coherently excited macromolecular vibrations predicted by Fröhlich [6]. To date, however, a molecular basis has not been established for any of the above spectra.

Sullivan and Santo have reported that there are several effects which can produce spurious Raman lines when scattering from cell suspensions [11], including Mie scattering from settling cell clumps. In this paper we demonstrate that much of the spectra recorded in [4-10] can be

closely reproduced by this effect. Furthermore, we argue that these spectra cannot be explained in terms of Raman scattering from coherently vibrating intracellular molecules.

Raman spectra were recorded at 5 cm^{-1} resolution using F/8 optics, a 1-m focal length double monochromator (Jobin-Yvon, ISA Ramanor HG2-S) with holographic gratings and convential photon counting detection. The 4880 $\mathring{A}$ line of an Ar^{+} laser (Spectra-Physics 165-03) , polarized parallel to the scattering plane, was used for excitation. All spectra were recorded using 90^{o} scattering, from samples at room temperature. For each spectrum shown, the intensity of a standard benzene line at 992 cm^{-1} is included to reference the throughput of the optical system.

As suggested in [11], an auxillary detector was included to record elastic Mie scattering from cell suspensions. Total light entering the monochromator was monitored by inserting a glass plate between the scattering collection lens and the monochromator entrance slit. The reflected light was imaged on an EG&G SGD-100A photodiode by use of a second lens. A 2 X 2 mm aperture was constructed 1 cm from the sample to restrict the light mointored simulataneously by the monochromator and the photodiode to the same scattering volume.

Fig. 1 shows such a simultaneous recording at a fixed wavenumber setting of the monochromator. 50 µl of a 100 mg/ml suspension of commericial yeast in deionized water was added to the open top of 1 mm X 1 cm X 4.5 cm cuvette already filled with deionized water, and allowed to settle. Numerous intense lines were recorded from the monochromator's output as cell density fluctuations occurred at the laser beam. These lines were exactly correlated in time with the elastic light monitored

by the photodiode. This Mie scattering declined in intensity with time as the settling and mixing of the yeast suspension proceeded. This pattern matches the temporal activity recorded from resuspended E. coli cells under similar optical conditions [ref. 4, Fig. 12 & ref. 8].

Webb et al. utilized an experimental procedure in most of their work which greatly enhances the probability of Mie scattering artifacts in their recorded spectra. After they determined that spectra could only be produced for about 6 minutes when a cell suspension was placed in the laser beam, they adopted a technique by which they scanned a given sample for only 3 minutes, then changed to a fresh sample of cells from a master culture [4]. This procedure would systematically introduce settling and mixing artifacts for each scan.

In several spectra of [4] (Fig. 1c, Fig. 5, Fig. 7), there are very narrow lines, with linewidths smaller than the instrumental resolution. These spikes are of comparable amplitude to neighboring lines in the same spectra with normal linewidths. Such narrow lines have been previously described in [11] as transient cell clumps passing through the laser beam. It is our experience that during a Raman scan, a clump of cells may cause either a noticeably false narrow line or a seemingly real line, of comparable amplitude and normal linewidth, depending upon the clump's transist time through the laser beam.

Another characteristic of elastic Mie scattering resulting from changes in cell density is baseline fluctuation in spectral scans. Such fluctuations can be seen in Fig. 1b between 1300 and 1600 cm^{-1} and Fig. 8 of [4]. To illustrate this, we recorded a Raman spectrum of a settling yeast suspension (see Fig. 2a). False lines and baseline

fluctuations are again well correlated with the light fluctuations seen in the photodiode output. This scan resembles those of E. coli suspensions in [4], Fig. 2. When the same sample of yeast is well mixed, these false lines are no longer observed (Fig. 2b).

Figs. 1 and 2 illustrate that Mie scattering can easily produce large false Raman lines throughout the range of the monochromator, including the 0-200 cm^{-1} region. Many of the low frequency (< 200 cm^{-1}) lines reported in [4-10] could therefore also be due to Mie scattering. For example, numerous lines from an E. coli suspension are seen with large baseline changes, indicative of cell density fluctuations, in Fig. 8 of [4].

The lines observed in [4-10] have been attributed to coherently vibrating intracellular molecules. We now examine the extent to which Fröhlich's theoretical coherent vibrations could increase the cross-section for Raman scattering of intracellular molecules. For non-resonant scattering, the power radiated from a molecule due to a Raman process is given classically by

$$I = \frac{16\pi^4\nu^4}{3c^4} \cdot \left(\frac{\partial\alpha}{\partial Q}\right)^2 Q^2 I_o \qquad (1)$$

where I_o is the incident power per sq cm, ν the exciting light frequency, α the molecular polarizability, and Q the normal mode coordinate [12]. $\partial\alpha/\partial Q$ is essentially constant for small displacements from equilibrium and actually decreases at large displacements when the molecular vibration is strongly excited [12]. Thus, $\partial\alpha/\partial Q$ cannot contribute to large increases in Raman scattering from a coherently excited molecular vibration.

The quantum value for Q^2 is given by

$$Q^2 = \frac{(n + \frac{1}{2})h}{2\pi^2 \nu_m} \qquad (2)$$

where ν_m is the molecular vibration frequency, and n the excitation number [12]. The intensity of Raman scattering is therefore linear in n.

The lines above 200 cm^{-1} in [4-10] are in range of local intramolecular vibrations, such as C-C streching and bending modes. For such covalent bonds, the vibrational excitation number, n, cannot reach values on the order of 100 without the bond rupturing. With this rough limit on possible enhanced scattering due to an excited vibration, we point out that the concentrations of specific components in the cell suspensions used in [4-10] would be too small to produce detectable lines, even if the molecules were coherently vibrating.

In order to obtain non-resonant Raman spectra of pure biomolecules in aqueous solution, 1-10% (by weight) solutions must usually be employed [13-16]. Bacteria and mammalian tissue cells are an extremely heterogeneous mixture of materials. At all times the concentration of specific cellular components within a suspension of 5×10^7 cells/ml (concentrations used in [4-10]), will be below the limits of detection. This is true regardless of any metabolic synchrony in the cell suspension.

It is therefore important to reexamine the experiments in which non-thermal anti-Stokes to Stokes intensity ratios were observed for low frequency lines from metabolically synchronized E. coli suspensions [6]. A line near 120 cm^{-1} was reported to range from 118 cm^{-1} to 125 cm^{-1}

depending on the age of the cells used. It is also reported that when a Stokes line reached its maximum intensity so did the corresponding Anti-Stokes line, but detailed analysis of the intensity ratio proved difficult because of the need to change the cell sample between successive runs [4]. The ratio R of the intensity of Anti-Stokes:Stokes line was found to be $R = 1.01 \pm 0.10$ at 118 cm^{-1} and $R = 0.93 \pm 0.13$ at 125 cm^{-1}. At these frequencies, the thermal equilibrium value of $R = \exp(-h\nu_m/kT)$ is 0.55 and 0.57, respectively. The excitation number of a vibrational mode can be calculated from the line intensity ratio [3] by

$$n/(1 + n) = R \tag{3}$$

For the observed values in [6], the vibrational excitation number could range anywhere from $n = 4$ for $R = 0.8$ to $n = \infty$ for $R = 1.0$. However, we point out again that for a vibrational mode at 120 cm^{-1}, n cannot exceed approximately 10^2 due to the constraint on bond dissociation energy discussed above. With this limit on possible scattering enhancement, it is highly unlikely that the observed lines in [6] are due to a coherently vibrating intracellular component. Since there was no monitoring of total light entering the monochromator in these experiments, it is impossible to tell whether the lines are due to Mie scattering or some other mechanism.

Non-thermal anti-Stokes to Stokes intensity ratios of the magnitude reported in [6] have not been reproduced or established by others. A Raman study of active algae found that most of the enhancement of anti-Stokes lines of intracellular carotenoids was due to resonance enhancement effects although some additional non-thermal population of the vibrational states might have been present [17]. A subsequent study,

however, found that carotenoid line intensities from living algae are given by a normal Boltzman distribution [18]. In addition, it was determined that deviations from expected thermal equilibrium values could be produced by experimental factors such as laser heating of the cell suspension.

In conclusion, we have shown that Mie scattering from cell density fluctuations provides a plausible explanation for the majority of the spectra reported in [4-10], whereas Raman scattering from strongly vibrating molecules do not. Various theories of coherent macromolecular vibrations [1-3,19-23]. and solitons [24] in cells therefore can not be supported on the basis of these experiments.

This work was supported by the Office of Energy Research, Physical & Technological Research Division, of the US Department of Energy under Contract No. DE-AC03-76-SF00098 and National Research Service Award No. 1-T32-GM07379.

References

[1] H. Fröhlich, Phys. Lett. 26A (1968) 402.

[2] H. Fröhlich, Int. J. Quant. Chem. 2 (1968) 641.

[3] H. Fröhlich, Adv. Electron. Electron Phys. 53 (1980) 85.

[4] S.J. Webb, Phys. Rep. 60 (1980) 201.

[5] S.J. Webb and M.E. Stoneham, Phys. Lett. 60A (1977) 267.

[6] S.J. Webb, M.E. Stoneham and H. Fröhlich, Phys. Lett. 63A (1977) 407.

[7] S.J. Webb and M.E. Stoneham, Int. J. Quant. Chem.: Quant. Biol. Symp. 2 (1975) 339.

[8] M.E. Stoneham and S.J. Webb, IRCS J. Med. Sci. UK 4 (1976) 520.

[9] S.J. Webb, M.E. Stoneham and J. Montgomery, IRCS J. Med. Sci. UK 4 (1976) 8.

[10] S.J. Webb, J. Montgomery and M.E. Stoneham, IRCS J. Med. Sci. UK 4 (1976) 10.

[11] R.A. O'Sullivan and L. Santo, Can. J. Spec. 26 (1981) 143.

[12] G.W. Chantry, in: The Raman Effect, Vol.1, ed. A. Anderson (M. Dekker, New York, 1971) p.49.

[13] P.R. Carey and V.R. Salares,in: Advances in Infrared & Raman Spectroscopy, Vol.7, ed. R.J.H. Clark and R.E.Hester (Heyden, London, 1980) p.1.

[14] B.G. Frushour and J.L. Koenig, in: Advances in Infared and Raman Spetroscopy, Vol.1, ed. R.J.H. Clark and R.E. Hester (Heyden, London, 1975) p.35.

[15] K.G. Brown, W.L. Peticolas and E. Brown, Biochim. Biophys. Res. Comm. 54 (1973) 358.

[16] J.L. Lippert and W.L. Peticolas, Biochim. Biophys. Acta 282 (1972) 8.

[17] F. Drissler and R.M. MacFarlane, Phys. Lett. 69A (1978) 65.

[18] S. Kinoshita, K. Hirata and T. Kushida, J. Phys. Soc. Japan 49 (1980) 314.

[19] E. Del Giudice, S. Doglia and M. Milani, Phys. Lett. 85A (1981) 402.

[20] E. Del Giudice, S. Doglia, M. Milani, and S.J. Webb, Phys. Lett. 91A (1982) 257.

[21] M.S. Cooper, Physiol. Chem. Phys. 11 (1979) 435.

[22] M.S. Cooper and O. Theimer, Physiol. Chem. Phys. 12 (1980) 249.

[23] M.S. Cooper, Collective Phen. 3 (1981) 273.

[24] P.S. Lomdahl, L. MacNeil, A.C. Scott, M.E. Stoneham and S.J. Webb, Phys. Lett. 92A (1982) 207.

Figures

Fig. 1. Mie scattering from a settling cell suspension. 50 µl of a 100 mg/ml yeast suspension is added to a 0.45 ml cuvette filled with deionized water (arrows). Changing cell density in the laser beam results in large elastic (Mie) scattering fluctuations.

A. Photodiode record of elastic light intensity entering monochromator.

B. Output of monochromator held at a fixed 1273 cm^{-1} setting. 5 cm^{-1} instrumental bandwidth, sensitivity 10^3 cps, 0.4 second time constant.

C. Optical configuration. Single pass laser beam, 400 mW, 4880 Å. The peak height of the 992 cm^{-1} benzene line under the same scattering conditions is 1.6 X 10^4 cps.

Fig. 2. Raman scan of a settling cell suspension. Same conditions as Fig. 1 except monochromator is scanned at 200 cm^{-1}/min.

A. False Raman lines and background fluctuations (lower trace) are correlated with photodiode record of elastic light fluctuations (upper trace).

B. Same sample is well mixed and scanned. False lines disappear. Benzene 992 cm^{-1} standard, 2 X 10^4 cps.

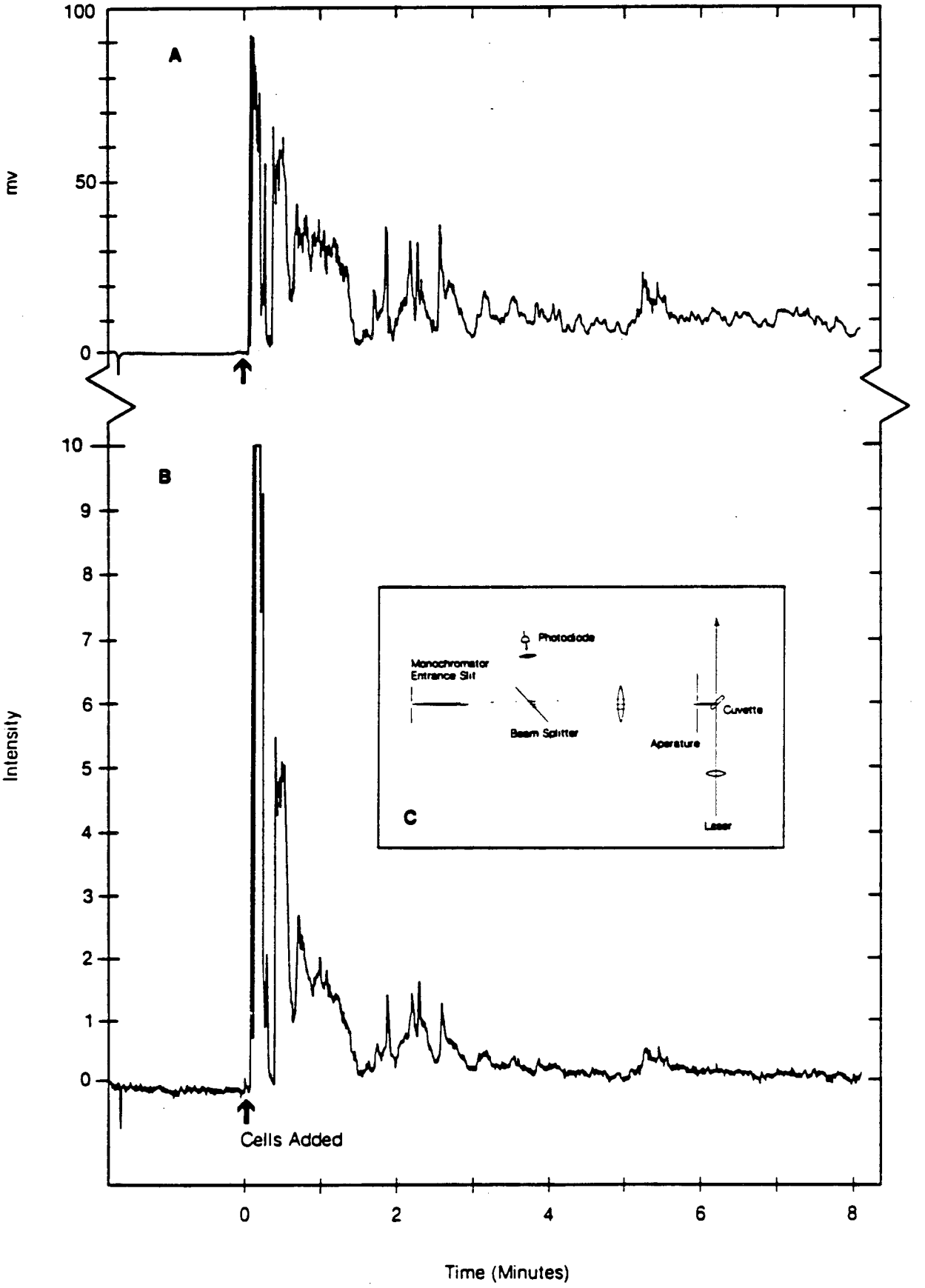
100
50
0
mv
A
10
9
8
7
6
5
4
3
2
1
0
Intensity
B
Cells Added
0
2
4
6
8
Time (Minutes)
Photodiode
Monochromator
Entrance Slit
Beam Splitter
Aperature
Cuvette
Laser
C

Fig. 1

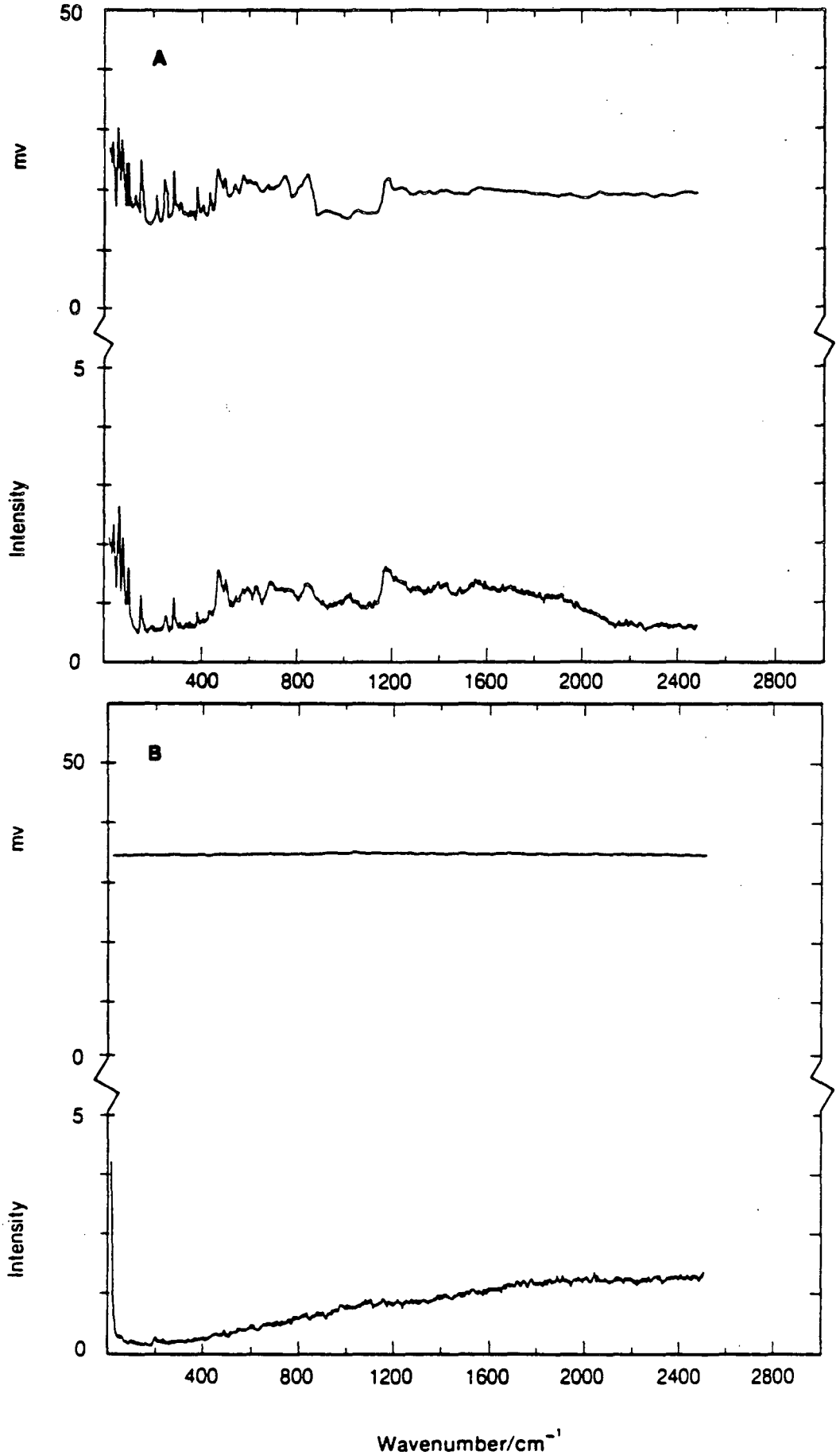
A
B
mv
Intensity
50
0
5
400
800
1200
1600
2000
2400
2800
Wavenumber/cm⁻¹

Fig. 2

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

TECHNICAL INFORMATION DEPARTMENT
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720