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Raman scattering from bacteria suspensions

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SEZIONE II

(Fisica, chimica, geologia, paleontologia e mineralogia)

Fisica. — *Raman scattering from bacteria suspensions* (*). Nota di VITTORIO MAZZACURATI, GIAN LUCA ROMANI e GIOVANNI SIGNORELLI, presentata (**) dal Corrisp. G. CARERI.

RIASSUNTO. — È stata studiata la possibilità di rivelare l'esistenza di banda Raman in una sospensione di batteri, con particolare riguardo alla zona compresa tra 1–400 cm^{-1}.

La mancata rivelazione di bande ha permesso di dare un limite alla loro eventuale sezione d'urto, una volta ipotizzata una certa larghezza di riga. Sono spiegate in dettaglio le difficoltà dell'esperimento e la linea da seguire per migliorare questi risultati.

1. INTRODUCTION

Recently, it has been proposed that a long range order may take place both in simple liquids [1] and in living systems [2]; if this assumption should be true, we could suppose the existence of a somewhat ordered structure, over distances much greater than the intermolecular ones, in order to make all the soft motions condense into a single mode. If we neglect any selection rules arising from symmetry considerations, a sharp intense peak should be presented by the infrared and Raman spectra of these substances, in the low wavenumbers region. McTague *et al.* [3] have unsuccesfully tried to detect this mode in simple liquids, giving in this way an upper limit to the Raman intensity of the predicted band.

We have studied the Raman spectra in living systems, whose investigations involve a great deal of experimental problems, mainly due to the relevant turbidity of these kinds of samples. Virus and bacteria suspensions, indeed, are strongly opalescent even at very low concentrations of living matter (less than 1 %): this means that a big fraction of the incoming beam intensity is scattered elastically in all directions (turbidity or Tyndall effect), increasing the experimental background and decreasing the Raman light. As we will see in the following, both Raman light from living matter and turbidity depend in such a way on the bacteria concentration that the optimum signal to noise ratio may be obtained only for low values of the concentration itself: i.e. very low Raman intensities. That is why it is extremely hard to detect Raman bands from living matter, unless for very strong ones.

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(**) Nella seduta del 18 giugno 1971.

2. Experimental considerations

The possibility of detecting a Raman band in opalescent suspensions is limited by the monochromator's characteristic not to cancel out completely a very strong line, even for frequencies out of the line itself; the transmitted fraction of this line's intensity is called the " stray-light background " of the monochromator. In our case, the strong line is due to the high turbidity mentioned before, and the existence of Raman bands may be masked by this background. The stray-light experimental $\Gamma(\nu)$ curve is plotted in fig. 1 (contrast function) [4]: however we want to point out that this is an average behaviour. In fact fluctuations arise because of the equipment instabilities and the convective motions within the suspensions (in our case about 20 %).

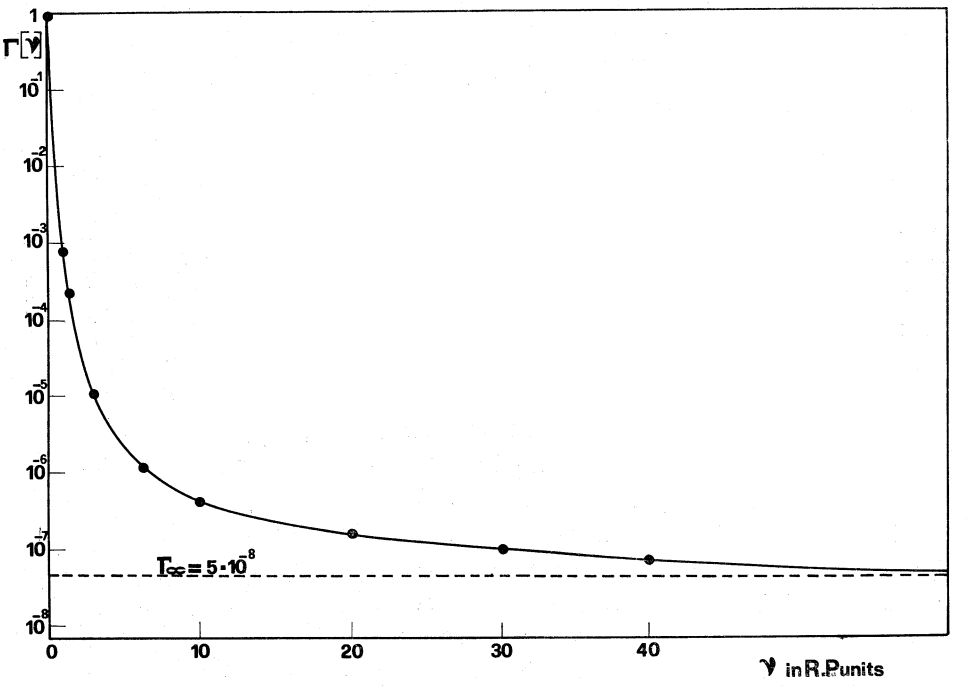

Fig. 1. – Experimental behaviour of the $\Gamma(\nu/\delta)$ function (monochromator contrast) for the used Jarrel Ash 25–100 dual Czerny–Turner scanning spectrometer. The wave number increment is given in resolving power (R.P.) units.

On the basis of this function's characteristic shape we want to find a relation which connects the possibility to detect a Raman band of a given integrated intensity with both its bandwidth and frequency shift from the exciting line.

We call δ the resolving power (R.P.), i.e. the wavenumber instrumental width used to analyse a spectrum; Γ is a function only of the ν/δ ratio [4]. Let I_T be the intensity of the elastic scattered radiation from the sample,

and I_S the stray-light intensity troughout the monochromator for a given δ; we have by definition

$$\Gamma(\nu/\delta) = I_S(\nu/\delta)/I_T . \tag{2.1}$$

This function for our dual monochromator [5] sharply decreases in the $0 \div 10$ R.P. range, achieving afterwards a smooth shape between $10 \div 50$ R.P.; for 50 R.P. it assumes its asymptotic value $\Gamma_\infty = 5 \times 10^{-8}$ (see fig. 1).

The Raman intensity collected at the monochromator output is

$$I_R \simeq I\,\delta/\gamma_R \qquad \text{for} \quad \delta \leq \gamma_R \tag{2.2}$$

where I is the integrated intensity of the whole Raman band, γ_R its bandwidth and δ is the chosen R.P.

We will show that if the Raman shift ν_R of the requested band is

$$\nu_R > 10\,\gamma_R$$

we can get the maximum value of the ratio I_R/I_S for $\delta = \gamma_R$, this ratio becoming smaller as δ decreases. Indeed the smooth behaviour of $\Gamma(\nu/\delta)$ for $\nu > 10\,\delta$ makes little difference in the value of the contrast function Γ, for instance between $10\,\delta$ and $100\,\delta$, and if we observe the band using two different R.P. δ and $\delta' = 0.1\,\delta$, we have ten times smaller Raman intensity collected, while the decrease in the stray-light is only a factor of 5. In this way the previous value of I_R/I_S is reduced to one half. If, on the contrary, the Raman shift of the band is

$$\nu_R < 10\,\gamma_R$$

the dramatic change of the Γ–function in the first region makes it possible to have better values for the ratio I_R/I_S, using $\delta < \gamma_R$. In other words, if the Raman frequency shift is ten times bigger than the bandwidth, the I_S value at that frequency will be the one limiting factor on the minimum detectable I value; in this case the best R.P. to be used will be the value of the bandwidth itself. But if we are dealing with very broad Raman bands ($\nu_R < 10\,\gamma_R$) we should be able to gain sensitivity by reducing the R.P. below the bandwidth.

The above statement may be established in the following way: we define the requested band as " detectable " if the ratio

$$I_R(\delta)/\sqrt{\langle \Delta I_S^2(\nu_R)\rangle} \geq 5 \tag{2.3}$$

where $\langle \Delta I_S^2(\nu_R)\rangle$ is the stray-light fluctuations mean square value. In our experiments, being $\sqrt{\langle \Delta I_S^2(\nu)\rangle} = 0.2\,I_S(\nu)$, the detecting condition (2.3) becomes:

$$I_R(\delta)/I_S(\nu_R) \geq 1 \tag{2.4}$$

Two different situations arise:

a) $\nu_R > 10\,\gamma_R$ (sharp Raman bands): the detecting condition (2.4) together with (2.1) and (2.2) yields

$$I \geq I_T\,\Gamma(\nu_R)$$

b) $\nu_R < 10\,\gamma_R$ (broad bands): the detecting condition becomes

$$I \geq I_T\,\alpha\Gamma(10\,\gamma_R)$$

where α ($\geq$ 1) measures how close to the exciting line we can reach the condition (2.4) by decreasing δ [4].

Both the above conditions give lower limits to the ratio I/I_T; this ratio, as we already said, depends on the bacteria concentration C_B in the sample.

The amount of turbidity is proportional to bacteria concentration (experimental result); then we can find a value of the concentration which optimizes the I/I_T ratio. This means that the ratio I/I_T is lowered when the concentration is increased over a certain value. In order to prove this statement, we introduce the integrated Raman scattering coefficient μ:

$$I = I_0 (1 - \exp(-\mu LC)) \cong I_0 \mu LC$$

where I_0 = intensity of the exciting line, L = scattering length (cm), μ = integrated scattering coefficient (cm^{-1}) and C = percent concentration of the species. In analogy with the above, we write down the following expression for I_T

$$I_T = I_0 (1 - \exp(-\mu_T C_B L))$$

where now μ_T is the Tyndall effect scattering coefficient.

Due to the presence of high turbidity, the effectively collected Raman light for a scattering length L is found

$$I_R^T = I_0 \mu_R L (\delta/\gamma_R) (1 - I_T/I_0) C. \tag{2.5}$$

It comes out from the equation that the intensity of stray-light and Raman bands from both water and bacteria in the suspension depends on C_B, at least through I_T.

For a given μ_T, always exists the C_B value to optimize I/I_T [4] and it comes from the simple relation

$$\mu_T LC_B \simeq 1 \tag{2.6}$$

i.e. from an experimental point of view we get this value diluting the bacteria suspension till the light transmitted over a scattering length L becomes $1/e$ of the incoming beam intensity.

3. Experimental results

In our experiment, the sample was a suspension of *Escherichia Coli* bacteria. The bacterial suspension was contained in a glass cylinder fitted with optical flat ends of diameter 1''. We were thus able to focus the 1 watt laser beam (from C.R.L. 53 A Argon laser) in the centre of the suspension, so that spontaneous convection prevented local overheating. The turbidity coefficient μ_T of this kind of bacteria makes the condition (2.6) experimentally verified for C_B value of about 1 %, with L = 1 cm. From these experimental data the Tyndall scattering coefficient is $\mu_T \simeq 100\ cm^{-1}$ and the light scattered elastically $I_T/I_0 \simeq 0.65$: in these conditions the " attenuation coefficient " will be

$$(1 - I_T/I_0) \simeq 0.35.$$

The detecting condition (a) in our experimental situation is fulfilled, providing that

$$\mu_B \geq 2 \times 10^2 \, \Gamma \, (\nu_B/\gamma_B) \tag{3.1}$$

The equation (3.1) gives the minimum detectable μ–value for a bacteria Raman band of width γ_B shifted ν_B from the exciting line. In fig. 4 the unshaded zone represents all the μ_B values which can be detected. The dotted straight line shows an assumed μ_B, as it comes from the following considerations.

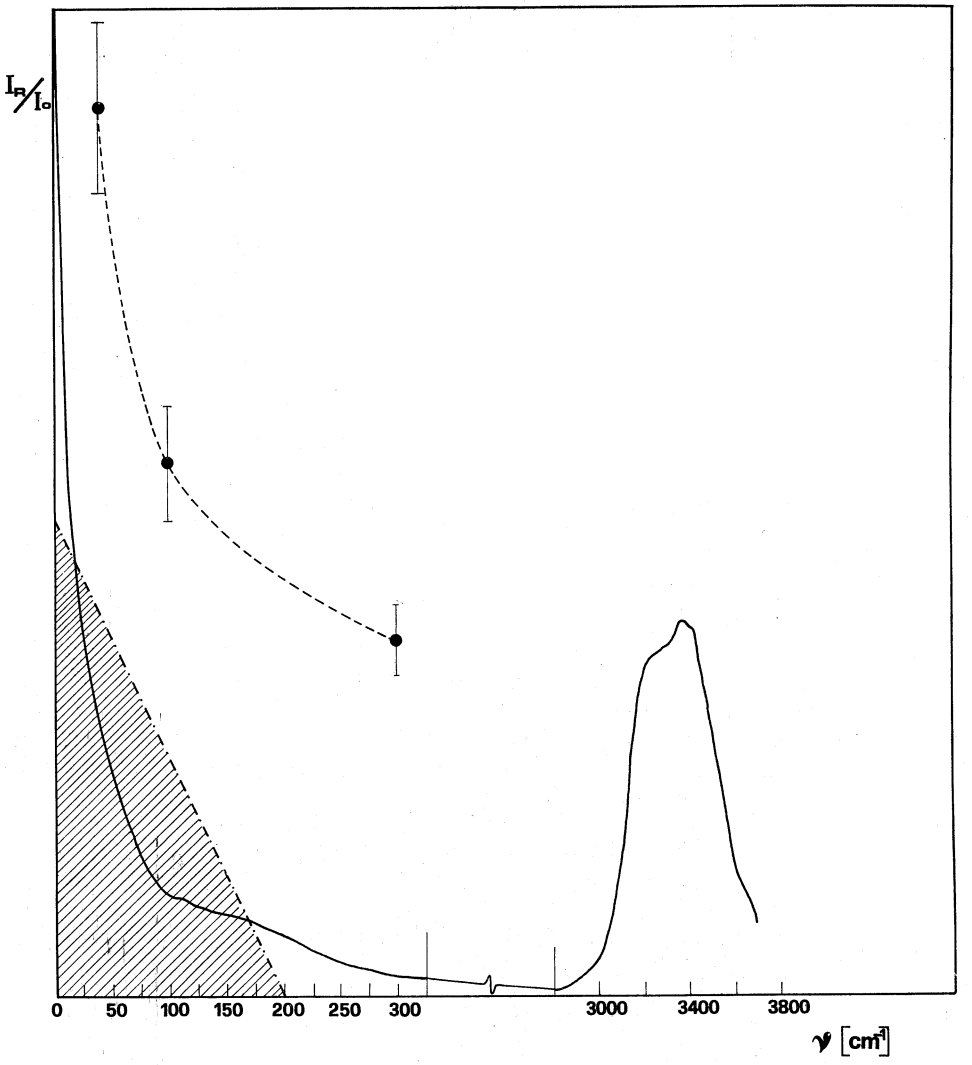

Fig. 2. – The solid line is the pure water Raman spectrum in the $0 \div 300$ cm^{-1} region (translational modes) and 2800–3800 m^{-1} region (OH–stretching band). The resolving power is about R.P. $=$ 10 cm^{-1}. The shaded triangle area is about equivalent to the integrated Raman intensity of the whole translational mode band (0–200 cm^{-1}) i.e. one-half of the OH-stretching integrated intensity. The dotted curve is the stray-light intensity from our bacteria solution, in the same experimental conditions.

Bacteria are mainly composed of water, so that we may suppose to be of the same order of magnitude both the integrated scattering coefficient of the predicted mode (μ_B) and that of all the pure water translation motions. Latter's intensity is about equivalent to the dotted triangle area in fig. 2, that is to say one half of OH–stretching mode integrated intensity. The band-width γ_B may be assumed about 1 cm^{-1}.

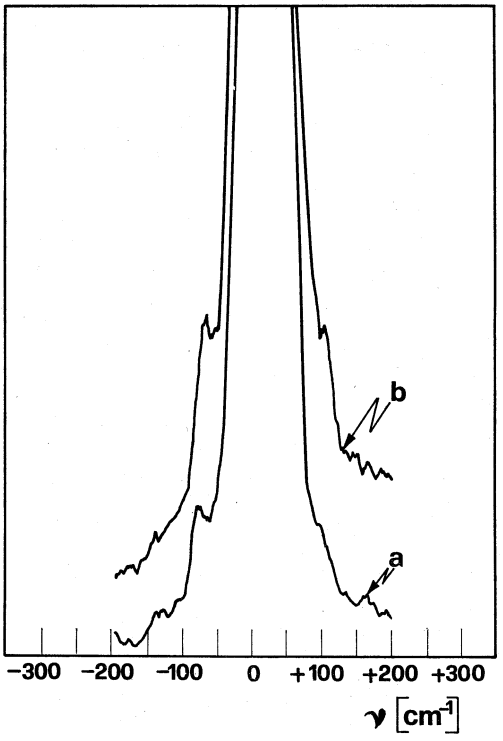

Fig. 3. – Two Raman spectra from our bacteria suspension, taken with different incident light power ($\lambda_{exc} = 4880$ Å, $P_a = 300$ mW, $P_b = 600$ mW) and R.P. = 1 cm^{-1}. The shape of these spectra shows the percent magnitude of the stray-light fluctuations ($\sim 20\%$) which make these spectra practically uncorrelated to each other unless for the monochromator's ghost at about 80 cm^{-1}.

The experimental value of OH–stretching integrated scattering coefficient μ_{OH} was evaluated on the basis of the Raman spectra of our bacteria suspension in the 3.000 cm^{-1} region, taken with 10 cm^{-1} resolving power.

We found

$$\mu_{OH} \cong 4 \times 10^{-5} \text{ cm}^{-1}$$

which gives

$$\mu_B \cong 1/2\, \mu_{OH} = 2 \times 10^{-5} \text{ cm}^{-1}.$$

This number is represented by the dotted line in fig. 4. Then we deduced that, as a consequence of the assumptions on the intensity and width of the requested mode, we should not be allowed to detect it, unless $\nu_B > 30\ cm^{-1}$.

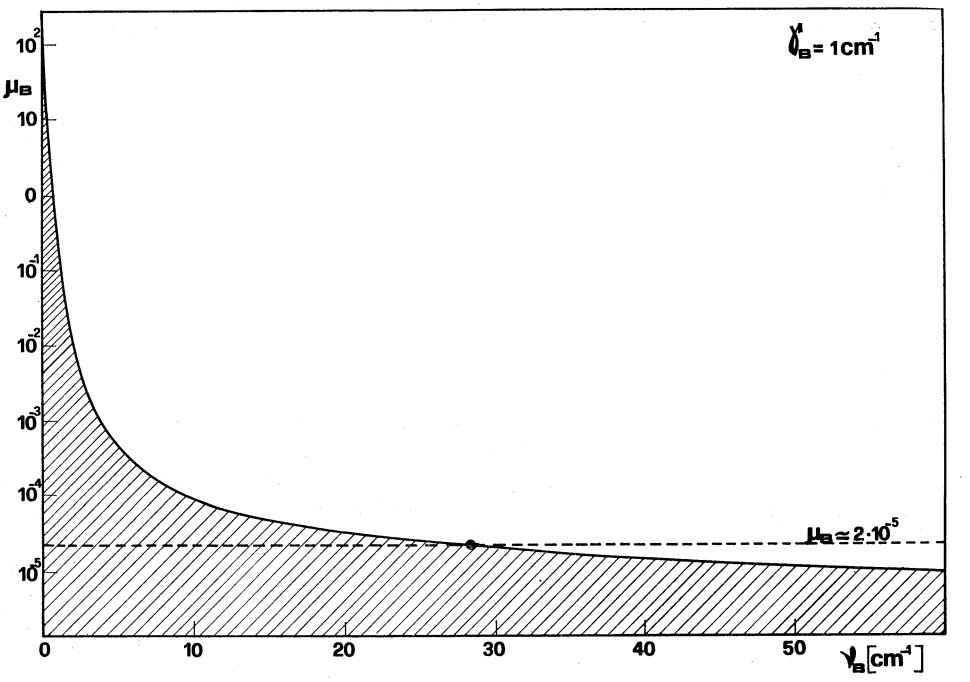

Fig. 4. – The solid curve represents the equation $\mu_B = 200\,\Gamma_B\,(\nu_B/\gamma_B)$ i.e. the detecting limit as a function of the ν_B increment from the exciting line in γ_B units. The working area is unshaded in fig. Dotted straight line is the upper limit of the estimated μ_B value as mentioned in par. 3 of the paper.

We want to point out a further consideration, deduceable from fig. 4; if the μ_B value is only a factor of ten less than the estimated one, at any frequency shift we would never be able to detect this collective mode.

4. CONCLUSION

The results of our experiment are that a band of the proposed intensity and bandwidth does not exist beyond $30\ cm^{-1}$; nevertheless we could not say anything on the existence of a band either weaker or wider or closer to the central line than the one above.

Furthermore the idea of averaging over a great number of " identical " spectra, in order to increase the signal to noise ratio, does not improve the situation very much, because the number of spectra we should have to get would be the square of the factor we want to gain: i.e. in order to improve one order of magnitude the S/N ratio, we ought to take 100 spectra; as the typical time needed for one spectrum is about half an hour, it is clear that

it is almost impossible to keep constant both the experimental apparatus and the living sample for such a long time.

Therefore the Raman technique seems to be quite useless in order to study opalescent suspensions, unless bringing down the stray-light a few orders of magnitude. Further studies on this line are in progress in our laboratories [4].

We want to thank Prof. G. Careri for suggesting this experiment and guiding the discussion of our result. We thank also M. Sampoli and M. Cerdonio for discussing their F.I.R. data and Prof. A. Tonolo for samples preparation.

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