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Light Scattering Spectroscopy.

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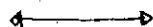


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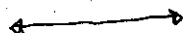
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## INTRODUCTORY NOTES FOR THE SPECTROSCOPIC INVESTIGATIONS



### PREFACE

The aim of these lessons is to give a rather general introduction to the use of spectroscopic techniques. Although a short review of many technique is performed (mainly to discuss spectroscopic techniques from a unified point of view), the course is essentially devoted to optical techniques, in particular Raman scattering and correlation spectroscopy.

In chapter I we discuss some fundamental concept, related to the "spectroscopic language". Chapter 2 is devoted to the corresponding description of a physical system. A review of various spectroscopic techniques is given in chapter 3, while in chapter 4 we discuss in some details Raman scattering and its application to biological macromolecules.

Special results in selected system are not discussed in the present paper, because they are available in many text books.

## BASIC CONCEPT IN SPECTROSCOPY

### 1.1] General Considerations

Spectroscopy is a formidable tool for the experimental observation of the structure of the matter, as well as for the investigation of the dynamical properties of various systems. Especially in the last years, spectroscopic technique has been greatly improved and, correspondingly, the theoretical treatment of itself more and more assuming a "spectroscopic" language, describing physical systems in terms of collective excitations. Such a tendency, among other things, results in an increase of the experimental techniques that, at present, are considered as spectroscopies, apart from the classical ones, like light scattering, X-ray measurements and neutron diffraction.

Although the subject of these lessons are mainly concerned with optical spectroscopies, it is worthwhile to note that also NMR, dielectric measurements, EPR, ESCA, Auger, acoustic measurements and so on, can be treated as special spectroscopic techniques.

From a very general point of view, in a spectroscopic experiment one is concerned with two systems: the first one is the system under investigation (main system), whose properties are, at least partially, unknown. The other one (the "probe" system), on the contrary, is perfectly known and its actual state can be determined with enough accuracy. The two systems are allowed to interact, then the state assumed by the probe system after the interaction is recognized. The goal of the experiment is to deduce the properties of the system under investigation from the change of state induced in the probe system.

Obviously the change of the probe system must fulfill some conditions:

- i) The strength of the interaction with the main system must be strong enough to give appreciable changes in the state of the probe, but not so strong to drastically change the state of the main system.
- ii) The expected change in the state of the probe must be easily detectable with the requested accuracy.
- iii) It is desirable that the interaction of the probe with the main system turns out to be specific enough, in order to ensure that only the properties

under investigation are involved in the interaction process.

- iv) The space-time structure of the probe (its "graininess") must be adequate to the space-time domain to which the properties under investigation belong.

In a spectroscopic experiment some information can be obtained from fundamental physical laws, without detailed considerations concerning the actual interaction process. Although in many cases such kind of information are not sufficient for the purposes of the experiment, they are of paramount importance, because their validity is fully granted apart from any model-the hypothesis. On the contrary a detailed investigation of the interaction process requires more or less drastic approximations that, in turn, could give rise to some ambiguity and/or uncertainty in the interpretation of the results.

The implied fundamental physical laws are, of course, energy and momentum conservation laws: a change of energy  $\Delta E$  and momentum  $\Delta \vec{p}$  in the probe system, formally implies that an equal and opposite change occurred in the main system. The latter therefore is able to sustain some "elementary excitation" characterized by the energy  $\Delta E$  and momentum  $\Delta \vec{p}$ .

In principle, therefore, performing enough spectroscopic experiments, one can reconstruct the whole distribution of the elementary excitations of the system under investigation, i.e. a (generalized) structure factor  $S(\vec{k}, \omega)$ . In turn such a knowledge would correspond to a complete knowledge of the system itself.

Actually, however, the straightforward application of the conservation laws, although can give informations about the existence of an elementary excitation, cannot give quantitative informations about the density of states; i.e. one can argue that  $S(\vec{k}, \omega)$  is non zero for some values of its arguments, but its actual value is beyond the results obtainable through the simple application of conservation laws. Therefore a deeper insight into the specific scattering process is needed, mainly in order to get the "transition probabilities", ~~section~~ (or the "cross section" of the scattering process), so that the probability that an allowed exchange of energy and momentum actually occurs, can be calculated.

The description of the scattering process and its theoretical treatment can be carried out from two different point of view.

In the first one (the "ondulatory" point of view), the probe system is

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treated as a wave field, characterized by a given angular frequency  $\omega$  and wave-vector  $\vec{k}$ . In many cases also a polarization (spin) sector is requested. Correspondingly the main system is described in terms of a (generalized) susceptibility  $\chi(\vec{r}, t)$  that represent the response function of the system to the wave field. The experimental results are then obtained as informations about the Fourier transform  $\chi(\vec{k}, \omega)$  of the susceptibility in some regions of the  $\vec{k}, \omega$  space.

In the second point of view both the probe system and the main system are treated as an ensemble of "quasi-particles" (photons, phonons, electrons and so on), and a fully quantific treatment is carried out. In such a case the elementary excitations of the <sup>main</sup> system are also treated as quasi-particles (phonons, magnons, excitations and so on), and information about their existence and the corresponding density of states are obtained as the result of the experiment.

### 1.2] The ondulatory point of view.

As mentioned before, in such a case the system under observation is characterized by some response function  $\chi(\vec{r}, t)$  that connect, at each space-time point, the scattered incident field and the scattered one. Obviously the response function is determined by the structural and dynamical properties of the system in a way that, in turn, depend on the physical nature of the ~~pro~~ wave field. A detailed investigation of such an argument will be performed in the further sections, in connection with specific spectroscopic techniques. At present we assume a phenomenological point of view, so that the response function is to be considered ~~as~~ known. It is to be noted that in general  $\chi(\vec{r}, t)$  has a tensorial character, due to the vectorial character of the wave field that represent the probe system. However, for the sake of simplicity, in the present section we consider a scalar field, characterized by a "disturbance"  $V$  that behaves like a plane monochromatic wave:

$$V_i = V_0 e^{i(\vec{k} \cdot \vec{r} - \omega t)} \quad [1.2.1]$$

where  $\vec{k}$  is the ~~vector~~ wave vector, and  $\omega$  the frequency. Here  $\vec{r}$  characterizes the position of each volume element  $dV$  of the main system that because of the interaction with the incident wave irradiate a scattered wave, whose magnitude is proportional to  $V_i$  and to the response function  $\chi(\vec{r}, t)$ . ~~Looking at all different directions~~ Looking at a given direction  $\hat{e}$ , we

can write for the ~~scattered~~ wave scattered in such a direction by the whole system:

$$V_s(k_s, t) = \int V_0 e^{-i\omega_s t} \chi(z, t) e^{i k_s z} e^{i k_s \cdot (R-z)} dz \quad [1.2.2]$$

$$= \int V_0 e^{-i\omega_s t} e^{i k_s \cdot R} e^{i q \cdot z} dz$$

$$= V_0 e^{-i\omega_s t} e^{i k_s \cdot R} \int \chi(z, t) e^{i q \cdot z} dz$$

where  $k_s$ , the scattered wave vector, points in the  $z$  direction, and  $q = k_s - k_i$  is the so called "scattering wave-vector". The spatial coordinate  $z$ ,  $R$  and  $d$  are sketched in fig. 1, from an arbitrary origin  $O$  located into the medium system.



Actually eq. 1.2.2 gives the ~~scattered field~~ <sup>disturbance</sup> into a wave front  $d$ , in a decomposition of the scattered field in plane wave.

Let us now consider a frame of reference with the  $z$ -axis pointing in the  $q$  direction. Then we have

$$\int \chi(z, t) e^{i q z} dz = \iiint \chi(x, y, z, t) e^{i q z} dx dy dz = \int e^{i q z} dz \iint \chi(x, y, z, t) dx dy = \int \tilde{\chi}(z, t) e^{i q z} dz \quad [1.2.3]$$

where

$$\tilde{\chi}(z, t) = \iint \chi(x, y, z, t) dx dy$$

behave like a mean value of the response function onto the  $xy$  plane, perpendicular to the  $q$  direction.

It is to be noted that in the case of light scattering (with which we are mainly concerned),  $k_i \approx k_s$ , so that  $q = 2k_i \sin \frac{\theta}{2} = \frac{4\pi}{\lambda} \sin \frac{\theta}{2}$ , being  $\lambda$  the wavelength of the incident field and  $\theta$  the angle of scattering.

(see fig. 2). As a consequence the  $xy$  plane is parallel to the bisectrix of the scattering angle. The physical meaning of such a construction is quite clear: all the volume elements located in the  $xy$  plane give rise to scattered waves (in the  $z$  direction) that have exactly ~~in~~ the same phase. With reference to the fig. 3

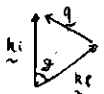


Fig. 2

one can see that the longer optical path characterizing an element  $dz'$ , ~~in~~ in comparison with another volume element  $dz$ , as far as the incident wave is concerned, is exactly balanced by a shorter optical path as far as the scattered wave in the  $z$  direction is concerned.



Fig. 3

Looking now to the intensity of scattered field (in the  $z$  direction), ~~it~~ it is proportional to the square modulus of the disturbance  $V$ , i.e.

$$I(k_s, t) \propto \iint \hat{\chi}(z, t) \hat{\chi}^*(z', t) e^{i q(z-z')} dz dz' \quad [1.2.4]$$

By putting  $S = z - z'$  one can write:

$$I(k_s, t) \propto \int dS e^{i q S} \int \hat{\chi}(z') \hat{\chi}(z'+S) dz' = \int C(S) e^{i q S} dS \quad [1.2.5]$$

where

$$C(S) = \int \hat{\chi}(z') \hat{\chi}(z'+S) dz'$$

is the <sup>spatial</sup> autocorrelation function of the "plane-averaged" response function  $\hat{\chi}(z, t)$ .

Actually the above treatment requires that scattered waves can interfere, i.e. that in the scattering process no phase shift occurs. If however a phase shift, takes place randomly distributed <sup>in the various</sup> volume elements, scattered waves are no longer able to interfere, and ~~the intensity~~ <sup>the scattered intensity</sup> becomes independent from the scattering angle, being simply proportional to the square modulus of the response function ~~integrated~~ <sup>integrated</sup> over the scattering volume:  $I(t) \propto \int \chi^2(z, t) dz \quad [1.2.6]$

From such a point of view one can therefore distinguish between a "coherent" scattering, given by [1.2.5] and an "incoherent" one, given by [1.2.6]. ~~In~~ the case of light in many text books the scattering given by [1.2.5] is also called "elastic" or "quasi elastic". From a physical point of view it correspond to the Rayleigh and Brillouin scattering. In contrast, scattering given by [1.2.6] is also called "anelastic" and correspond mainly to Raman scattering (provided that a suitable definition of the response function is given).

Let us now go back to equation [1.2.5], ~~which~~ <sup>which</sup> gives the scattered intensity as the Fourier transform of the spatial autocorrelation function  $C(S)$ . From the well known Wiener-Kintchine theorem this means that  $I(k_s, t)$  just reproduce the (spatial) power spectrum of  $\hat{\chi}(z, t)$ , being proportional to the  $q$ -component of such a spectrum.

Another comments can be made, concerning the range of the autocorrelation function  $C(S)$ . Let us suppose, for the sake of simplicity, that  $C(S)$  behave like an exponential:  $C(S) = C e^{-\gamma S}$  (for example, this happens if some fluctuation of concentration or entropy gives rise to the spatial variation of  $\chi(z, t)$ ). In such a case  $\gamma$  will be related to the <sup>approx.</sup> diffusion coefficient. It is easy to show that the integral [1.2.5] furnishes a value  $I$  greatly different from zero only if  $\gamma$  is neither too smaller nor too larger than  $q$ , i.e. the range of the autocorrelation function must be of the order of  $q^{-1}$  in order to have a  $I$  ~~greatly~~ scattered intensity.

Let us now consider the temporal behaviour of the scattered intensity. Generally

speaking it correspond to the temporal behaviour of the response function, that modulate the amplitude of the scattered waves. As a consequence the (temporal) power spectrum of the scattered intensity is no longer monochromatic, like the incident wave, but reproduce the temporal power spectrum of the response function. In other words, the space-time <sup>variations</sup> fluctuations of the response function  $\chi(\mathbf{r}, t)$  can be Fourier-transformed giving rise to a  $\chi(\mathbf{q}, \omega)$ . Selecting a scattering angle one select a spatial component  $\chi_q(\omega)$  that contribute to behave like a diffraction grating that scatters the incident wave at the appropriate Bragg's angle. The "contrast" of such a grating, however, changes in time, ~~so that the~~ according to its own ~~own~~ temporal power spectrum that, therefore, is "imprinted" in the scattered intensity. Obviously, because of the phenomenological point of view we have assumed, is not surprising that a single ~~one~~ spatial component of the response function  $\chi_q(\omega)$  could have a rather complicated <sup>temporal</sup> power spectrum: actually many different physical phenomena can contribute to build up the ~~response~~ phenomenological response function, each of one being characterized by a precise temporal behaviour. As an example let us show the temporal power spectrum of light scattered by a pure liquid, ~~is~~ qualitatively sketched in fig. 4. In such a case the response function correspond to the refractive index of the medium, i.e. to its dielectric constant,  $\epsilon(\mathbf{r}, t)$ . Changes in the dielectric constant can occur <sup>both</sup> because of adiabatic fluctuations in the pressure, ~~that~~ that propagate in the medium like damped acoustic waves, and because of isobaric fluctuations in the entropy, that decays exponentially in time. As a consequence we shows in the spectrum of scattered light the "Brillouin" doublet, in which the incident frequency is shifted by  $\omega = \pm c q$ , being  $c$  the sound velocity, and a "Rayleigh" central line, whose width  $\Gamma$  correspond to the decay-constant of the entropy fluctuations, and is related to the thermal diffusion coefficient of the liquid.

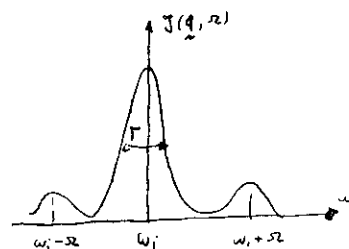


Fig. 4  
Scattered light intensity at a fixed  $q$ .

In the case of the so-called inelastic scattering, the same considerations holds, in principle. However, because of the leakage of interference effects, no selection on the spatial components of the response function occurs. As a consequence, at any scattering angle we have the superposition of the temporal spectra of any component; so that in many cases, the interpretation of the frequency spectrum requires some care and can give rise

to some ambiguity, especially as far as the low-frequency part of the spectrum is concerned. For the true Raman spectrum, however, the physical origin of the phenomenon itself originate a  $q$ -independent temporal behaviour in the response function, so that no ambiguity arise in the interpretation of the spectra, at least in connection with the present discussion.

A detailed analysis of the Raman spectroscopy will be done in chapter 3.

### 1.3] Concept of quasi-particles. The quantum point of view.

It is well known that one of the fundamental aspects of quantum mechanics, consists in the possibility of a dualistic description of natural phenomena. Electromagnetic waves can be treated in terms of photons, and correspondingly electrons, neutrons and so on, that classically are to be considered as particles, can be described by appropriate wave fields. For the connection between the two alternative descriptions, of crucial importance are the so-called De Broglie equations:

$$\vec{p} = \hbar \vec{k} \quad E = \hbar \omega \quad 1.3.1$$

where  $\vec{p}$  and  $E$  refers to the momentum and energy of a particle, while  $\vec{k}$  and  $\omega$  are the wave vector and the angular frequency of the corresponding oscillatory description. The dynamical connection between energy and momentum, in a particular description translate itself in a relation between wave vector (i.e. wavelength) and frequency:

$$E = E(p) \quad \omega = \omega(k) \quad 1.3.2$$

Equations like 1.3.2 are called "dispersion laws", and are characteristic of the particular system.

In the course of the evolution of the physics of collective systems, the possibility of a "particular" description of the collective behaviour has been extensively applied. Such a generalization, among other things, allowed a better understanding of the meaning that is to be attributed to the "particles", that, in fact, are more properly called "quasi-particles" in order to stress their peculiar behaviour in comparison with the usual particles of the classical physics.

As an example let us consider briefly elastic waves in a solid. In a first rough approximation a solid can be considered as an ensemble of point-like masses, each of one having a fixed equilibrium position. Small displacements from such equilibrium positions give rise to restoring forces that can be considered proportional to the displacement (harmonic approximation). In such a conditions, classical mechanics provides the

existence of "eigenmodes"  $\omega_i$ , whose number is equal to the number of degrees of freedom of the system ( $3N$ , if  $N$  is the number of point-masses), each of ours being a collective ~~property~~ of the entire system. The motion of each point mass, in turn, can be obtained as a linear superposition of harmonic oscillations of frequency  $\omega_i$ . Moreover the Hamiltonian of the system can be rewritten in terms of the eigenfrequencies as the sum of  $3N$  independent ~~Hamiltonians, each of one referring to an~~ harmonic oscillator characterized by the frequency  $\omega_i$ .

$$H = \sum_{i=1}^{3N} H_i = \sum_{i=1}^{3N} C_i^2 \omega_i^2 \quad 1.3.3$$

Obviously such harmonic oscillators are not the point-masses. Each "oscillator" shares a collective property involving all the point-masses of the system. According to eq. 1.3.3 a status of the system is fully determined by the knowledge of the amplitudes  $C_i$ .

The fundamental contribution of the quantum mechanics amounts to the conclusion that the energy  $H_i$  ~~of each oscillator~~ stored in each eigenmode of the system cannot assume arbitrary values, being quantized according to the rule:

$$H_i = (n_i + \frac{1}{2}) \hbar \omega_i \quad 1.3.4$$

where  $n$  is an integer. In such a condition one can speak of the  $i$ th mode as being excited with  $n_i$  quanta of energy  $\hbar \omega_i$  ( $\frac{1}{2} \hbar \omega_i$  is the zero-point energy). Then a status of the system is fully determined by the "occupation numbers"  $n_i$ . Moreover the phase with which each eigenfrequency enters to determine the motion of each point-mass is such that a ~~wavevector~~ wavevector  $k_i$  ~~can be defined~~ can be defined. The wavevector  $k_i$  and frequency  $\omega_i$  are related by the dispersion relation  $\omega_i = c k_i$ , being  $c$  the "sound velocity". Then one can attribute the meaning of "quasi-particles" to the energy quanta  $\hbar \omega_i$ , each of one travelling through the system with the velocity  $c$ . Then the "momentum"  $p$  will be given by  $\hbar k$ , i.e.  $p = \hbar k$ . The term "phonon" is used for such a "quasi-particles".

Like the phonons, that describe essentially density fluctuations, one can speak of "magnons" to describe magnetization fluctuations, or "plasmons" to describe charge fluctuations, and so on. In such a way a physical system can be characterized by an ensemble of "elementary excitations" belonging to the various physical properties of the system. Although such a description usually is used to describe propagating excitation, it ~~can~~ <sup>could</sup> be extended to comprehend ~~the~~ diffusive processes, with suitable definition of the dispersion relation. (See e.g. the concept of "Brownian", to describe the brownian motion of a particle suspended in a fluid).

As far as the spectroscopic processes are concerned, from such a point of view ~~the probe system~~ the process becomes similar to a collision between a "quasi-particles"

belonging to the probe system (e.g. a photon in the case of light scattering) and a quasi-particles belonging to the ~~main~~ <sup>system</sup> (e.g. a ~~phonon~~ <sup>acoustic phonon</sup>, in the case of the Brillouin scattering). Obviously the number of quasi-particles is not a conserved quantity: they can be created and destroyed in the collision process.

As an example let us think again to Brillouin scattering. The hypersound waves are now represented by a population of phonons, characterized by the energy  $E_p = \hbar \omega_p$  and momentum  $p_p = \hbar k_p$ . Also the incident light is represented by a population of photons, all with the same energy  $E_i = \hbar \omega_i$  and momentum  $p_i = \hbar k_i$ . The interaction of light with system is shown as a photon-phonon interaction, in the course of which the incoming particles are destroyed and new particles of the same kind are created. In fig. (5, a) a process of such a kind ~~is~~ <sup>is</sup> shown: The outgoing photon (straight line) possesses an energy that is the sum of the energy of the destroyed incoming photon and phonon, and the same happens for the momentum:

$$\left. \begin{aligned} E_f &= E_i + E_p = \hbar(\omega_i + \omega_p) \\ p_f &= p_i + p_p = \hbar(k_i + k_p) \end{aligned} \right\} \quad 1.3.5$$

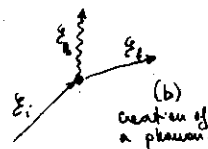
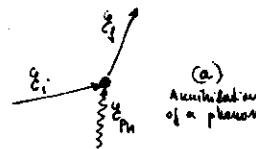


fig. 5

The opposite process is shown in fig. 5.b. Now a phonon is created, and the outgoing photon is characterized by a lower energy and momentum:

$$\left. \begin{aligned} E_f &= E_i - E_p = \hbar(\omega_i - \omega_p) \\ p_f &= p_i - p_p = \hbar(k_i - k_p) \end{aligned} \right\} \quad 1.3.6$$

It is quite obvious that such two processes give rise to the Brillouin doublet, i.e. scattered light shows a frequency shift  $\pm \omega_p$ . The process of kind (b) is also called a Stokes process: The frequency of scattered light is lower than that of the incident beam. The opposite (a) process is called an ~~Anti-Stokes~~ <sup>Anti-Stokes</sup> one: ~~scattered~~ <sup>scattered</sup> light has an higher frequency. Although the qualitative predictions of the classical (oscillatory) and quantum descriptions ~~are~~ <sup>mutually</sup> agree, there is an important difference as far as the intensities of the Stokes and Anti-Stokes lines are concerned. In the oscillatory point of view the ~~fluctuations~~ <sup>fluctuations</sup> of the ~~disturbance~~ <sup>disturbance</sup> pressure when decomposed in plane acoustic waves that act as moving diffraction gratings. The Stokes and Anti-Stokes lines are originated by the couple of waves that move

in the  $q$  and  $-q$  directions. A statistical analysis shows that the average amplitude of such waves is the same, due to the isotropic character of the fluid. Therefore the Stokes and Anti-Stokes lines will have the same intensity.

In the quantum picture of view, however, processes of kind (b) are more probable than those of kind (a), the second being proportional to the probability of phonons in the excited state of energy  $\hbar\omega_p$ . In the first case, in fact, the status of the  $p$  system passes from  $|n\rangle$  to  $|n+1\rangle$  <sup>(being in the phonon occupation number)</sup> while in the second case the opposite transition, from  $|n+1\rangle$  to  $|n\rangle$ , takes place. Now the state  $|n\rangle$  and  $|n+1\rangle$  differs for an energy content given by  $\hbar\omega_p$ , so that the probability for the system to be in the status  $|n\rangle$  is larger than that of being in the status  $|n+1\rangle$ . The ratio between the two probabilities is given by the usual Boltzmann factor  $\exp(-\hbar\omega_p/kT)$ . As a consequence the same ratio will characterize the intensities of Stokes and Anti-Stokes lines. Such a difference becomes relevant when  $\hbar\omega_p \gg kT$ , so that an experimental observation of high frequency Anti-Stokes lines cannot be performed. In the usual Brillouin scattering  $\omega_p \approx 10^{12}$  sec<sup>-1</sup> or less, so that  $\hbar\omega_p/kT$  is of the order of  $10^{-3}$  and the Boltzmann factor becomes practically equal to one.

#### 1.4.3 Conservation Laws.

As discussed in the preceding section, the scattering process in a quantum description is described as the annihilation or creation of an elementary excitation. In the course of the process both energy<sup>2</sup> and momentum<sup>2</sup> must be conserved. Actually for each type of quasi-particles there is a <sup>characteristic</sup> relationship between  $\mathcal{E}$  and  $p$ , i.e. between the angular frequency  $\omega$  and wavevector  $k$  (Dispersion laws). As a consequence for a given incident wavevector  $k_i$  and a fixed scattering angle there are a couple of equations that must be fulfilled by the scattered wavevector  $k_f$  and the wavevector of the created (or annihilated) elementary excitation,  $K$ :

$$\begin{cases} k_i^2 + k_f^2 - 2k_i k_f \cos \theta = K^2 \\ f(k_i) = f(k_f) + \varphi(K) \end{cases} \quad 1.4.1$$

where  $\theta$  is the scattering angle and  $f(k)$ ,  $\varphi(K)$  are the dispersion laws for the probe and the elementary excitation respectively:

$$\begin{aligned} \omega &= f(k) \quad (\text{probe}) \\ \Omega &= \varphi(K) \quad (\text{elementary excitation}) \end{aligned}$$

The system of equations (1.4.1) imposes some constraints, giving rise to allowed and forbidden processes.

As an example, let us consider the creation or annihilation of a phonon in a

neutron scattering experiment. For the sake of simplicity we consider the scattering along a straight line (zero scattering angle). The dispersion laws are  $\mathcal{E} = p^2/2m$  for neutrons and  $\mathcal{E} = cP$  for phonons,  $c$  being  $c$  the sound velocity. In terms of incident and scattered wave vector  $k_i$  and  $k_f$  of the neutron, and of the wavevector of the phonon  $K$ , eqs. (1.3.7) furnish:

$$k_f = \frac{2m c}{h} - k_i$$

$$K = 2k_i - \frac{2m c}{h}$$

Obviously  $k_f$  and  $k_i$  must be positive, while  $K$  can be either positive (creation) or negative (annihilation). As a consequence  $k_i$  cannot be larger than  $2m c/h$ . In a creation process we get the condition  $\frac{m c}{h} < k_i < \frac{2m c}{h}$ , while for an annihilation process the condition becomes  $0 < k_i < \frac{m c}{h}$ . If, in addition, we suppose that  $K$  is much smaller than  $k_i$ , the conditions above imply that the process can take place only if the velocity of the neutron practically coincide with sound velocity.

In a transparent medium, however, the momentum conservation law can be relaxed in the sense that momentum can be supplied by the center-of-mass motion of the system, in an arbitrary quantity.

Applying the same considerations to the scattering of electromagnetic radiation (photons), due to the enormous value of the velocity of light, it turns out  $k_i = k_f$  and, as a consequence one gets the well known relationship

$$K = 2k_i \sin \frac{\theta}{2} = \frac{4\pi n}{\lambda_0} \sin \frac{\theta}{2} \quad 1.4.2$$

where  $n$  is the refractive index of the medium,  $\lambda_0$  the wavelength (in vacuum) of the incident radiation.



## II

### "SPECTROSCOPIC" DESCRIPTION OF COLLECTIVE SYSTEMS

#### 2.1) Distributions ~~probabilities~~ and densities. Correlation functions.

From the spectroscopic point of view, a physical system is to be considered as a finite region of space in which an interaction takes place between the system itself and the probe field. Thus, depending on the specific kind of the interaction, some property of the system will be implied, according to a rather general scheme consisting in writing an Hamiltonian of the kind

$$H = H_s + H_p + H_i \quad 2.1.1$$

where  $H_s$  and  $H_p$  are the Hamiltonians of the system and the probe, respectively, and  $H_i$  describe the interaction. In terms the latter can be written as:

$$H_i(t) = \int A(x,t) \rho(x,t) dx \quad 2.1.2$$

and  $A$  represent a generalized field strength, carried by the probe, that couple with a generalized charge distribution  $\rho(x,t)$  of the system. For example in a infrared absorption spectroscopy,  $A$  is just the electric field of the incoming wave, while  $\rho$  describe the ~~polarization~~ permanent electric dipole moment distribution. In eq. 2.1.2  $x$  represent the scattering volume, i.e. the part of the system actually interested by the interaction with the probe field.

Obviously, in a more general case, high power of the field strength can be involved; however the ~~general structure of~~ general structure of eq. 2.1.2 can be assumed. ~~At the present considerations~~ as far as some general considerations are implied.

Actually two rather general comments can be made:

- i) Different kind of probes (i.e. different spectroscopic techniques) couples with different properties of the system. Depending on the technique, therefore, different distribution  $\rho(x,t)$  can be involved. As a consequence, different spectroscopic experiment can give different results, although always the same system is investigated. For example in a crystal whose molecules

presents a anisotropic plangibility, and in addition there are orientational disorder, polarized light scattering will reveal the crystal order (Bragg peaks), while depolarized scattering behave like in a disordered system. We will show in chapter 3 that such a consideration is fundamental in order to appreciate the difference between the so called coherent and incoherent neutron scattering.

- ii) Generally speaking, it is a common feature of spectroscopic investigation, to detect a (generalized) intensity, proportional to the square of the (generalized) field strength. As a consequence, taking into account eq. 2.1.2, it turns out that the experimental result furnishes the correlation function of the ~~the~~ implied distribution  $\rho(x,t)$ , rather than the ~~the~~ distribution itself. Now, while a given distribution gives rise to a well defined correlation function, the vice-versa is not true: many different distributions could give rise to the same correlation function, so that the experimental results cannot determine unambiguously the implied distribution  $\rho(x,t)$ .

It is well known, e.g., that the inverse problem in optics (i.e. the knowledge of the properties of a source, starting from the properties of the irradiated field) ~~cannot~~ cannot be resolved in a unique way.

Therefore a rather common procedure request the use of a "model" for the system under investigation, from which the implied distribution  $\rho(x,t)$  can be ~~calculated~~ deduced and the scattered field intensity can be calculated and compared with experimental results.

~~2.2) Correlation function~~

~~Define a function  $F(x)$ , the autocorrelation function  $F(x)$  is defined as~~

$$F(x) = \int \rho(x,t) \rho(x,t) dx \quad 2.2.1$$

~~The variable  $x$  can be a space or a time variable. Autocorrelation function~~

The relevance of the correlation function can be ~~explained~~ explained as seen by considering, as an example, the particular case in which  $f(x)$  represent the mass density of an isotropic, amorphous fluid.

In an ~~the~~ equilibrium state the (mean) density  $\rho_0$  ~~has a~~ precise value; however point by point there are small fluctuations around the equilibrium value. Let us put (in a one-dimensional case):

$$f(x) = \rho(x) - \rho_0$$

The fluctuating part of the density  $f(x)$  and its dynamical behaviour is a well-defined physical object, that is of interest mainly because it is directly connected with the results of a spectroscopic experiment. ~~the case~~ In the case of light scattering, in fact, the refractive index is (at least as a first approximation) proportional to the density. In a x-rays experiment the change density is obviously proportional to the density. Also in the case of neutron scattering, the concentration of scatterers (nuclei) is proportional to the density.

Taking into account eq. 2.1.2, it follows that the scattered intensity  $I(k)$  will be proportional to:

$$I(k) \propto \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} f(x)f(x') e^{ik(x-x')} dx dx' \quad 2.1.3$$

where  $k$  is the scattering wave vector (Compare also with eq. 1.2.5). Because of the translational invariance of the system, ~~the only~~ only the difference  $\xi = x - x'$  plays a role, so that one obtains

$$I(k) \propto \int_{-\infty}^{\infty} f(x)f(x+\xi) e^{ik\xi} dx d\xi \quad 2.1.4$$

that is the Fourier Transform of the <sup>(not of)</sup> autocorrelation function. Actually, from the well known Wiener-Kintchine theorem, such a quantity just correspond to the power spectrum of the  $f(x)$ :

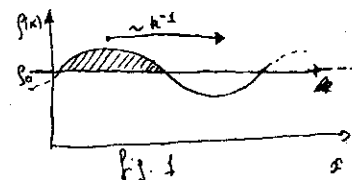
$$I(k) \propto \text{F.T.} |f(x)|^2 \quad 2.1.5$$

It can be noted that ~~such a Fourier~~ the existence of such a Fourier Transform can be argued on a physical ground: one expects, in fact, that for large  $\xi$  the fluctuations become uncorrelated, so that the autocorrelation function implied in eq. 2.1.4 converges strongly enough to permit a Fourier Transform.

From a physical point of view, ~~exceeding~~ the preceding considerations can be summarized as follows: the spatial fluctuation  $f(x)$  of some physical quantity <sup>(that is, a function of  $x$ )</sup> can be considered as the superposition of simple sinusoidal functions of the kind  $A(k) \sin(kx + \phi(k))$ . The spectroscopic observation select one of ~~such~~ this components, the selected wave vector depending both on the wavelength of the probe field and on the scattering angle.

As far as the dynamical behaviour is concerned, the time-dependence of the scattered intensity will be ~~proportional~~ given by the temporal dependence of the amplitude  $A(k)$  of the selected component.

Let us consider, as an example, the case in which  $f(x)$  represent the fluctuations of some conserved quantity, like the mass density. Now, in order that the spatial component of wavevector  $k$  disappear, some quantity of matter must travel through a distance of order of  $k^{-1}$  (see fig. 1).



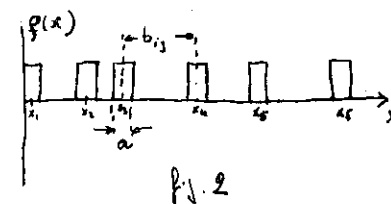
process such a process requires a time proportional to the square of the travelled distance, so that one gets ~~the~~ the dynamical behaviour of the given component (and therefore of the scattered intensity) as ~~being~~ characterized by a time-constant of the kind  $\tau = (Dk^2)^{-1}$ ,  $D$  being the

diffusion coefficient.

## 2.2) State and dynamic Structure Factor.

In the preceding section we considered the system under investigation as a continuous system. In many cases, however, the system is to be treated as an ensemble of elementary object (atoms, molecules, and so on). Actually ~~the discreteness of the system~~ <sup>discreteness of the system</sup> ~~is not negligible~~ <sup>is not negligible</sup> when the wavelength of the probe field becomes comparable with the dimension of the elementary objects, or lots. As an example one can think to x-rays scattering from an atomic or molecular system, or to light scattering from a macromolecular or particulate solution.

In such a case the ~~spatial distribution of the generalized charges  $\rho(x)$~~  <sup>spatial distribution of the generalized charges  $\rho(x)$</sup>  will behave like ~~the~~ the function schematically drawn in fig. 2.



Here "a" indicate the size of the elementary object, ~~and~~ that is always the same (at least for a one-component system), while  $b_i$  is the variable distance between neighbouring objects.

One can write:

$$\rho(x) = \sum_n \text{rect}(a, x_n)$$

where the "rectangle" function of width "a" are centered at the points  $x_n$

In terms:

$$x_n = nL + \sum_{i=0}^n \epsilon_i = nL + \Delta_n \quad 2.2.2$$

where  $L$  is the mean distance between two neighbouring objects, while  $\epsilon_i$  are stochastic variables, both  $\epsilon_i$  and the sum  $\Delta_n = \sum_{i=0}^n \epsilon_i$  are stochastic variables. In order to perform an explicit calculation, let us assume that  $\epsilon_i$  ~~always~~ ~~is~~ Gaussian is distributed according to a gaussian law, with ~~with mean~~ <sup>(r.m.s.)</sup> around a zero mean value, with a root mean square  $\sigma$ . Then also  $\Delta_n$  is gaussian, with rms  $\sigma\sqrt{n}$ .

It can be noted that the limit  $\sigma \rightarrow 0$  correspond to a perfectly ordered array of object, i.e. to a lattice characterized by the spacing  $L$ . On the contrary as  $\sigma$  increases becomes larger and larger, we are dealing with an increasingly disordered system.

Now, in order to calculate the angular distribution of scattered light,  $I(k)$ , we take the square modulus of the Fourier Transform of the distribution 2.2.1 (or, equivalently the Fourier transform of its autocorrelation function). In such a way we obtain the power spectrum of the distribution.

In terms, because of the finite time requested by ~~any~~ a any physical observation, a mean is to be performed, according to the postulated gaussian distribution.

The straightforward but tedious calculus, furnishes (apart from a proportionality constant):

$$I(k) = \frac{\sin \frac{k\sigma}{2}}{\frac{k\sigma}{2}} \left( 1 - e^{-\frac{k^2 L^2}{4}} \cos(kL) \right)$$

$$I(k) = \left( \frac{\sin \frac{k\sigma}{2}}{\frac{k\sigma}{2}} \right)^2 \left( \frac{1 - e^{-\frac{k^2 L^2}{4}} \cos(kL)}{1 - 2e^{-\frac{k^2 L^2}{4}} \cos(kL) + e^{-\frac{k^2 L^2}{2}}} \right)^2 \quad 2.2.3$$

~~The~~ <sup>The</sup> results shows ~~the typical feature~~ a typical feature: it is the product of two terms, the first one being related solely to the shape of the single object, while the second one depends on the way in which the objects are spatially distributed.

~~Therefore~~ Such a results is quite general, although the kind of the implied functions <sup>can</sup> changes, according to the shape and to the kind of distribution of the objects.

The first factor is called FORM FACTOR, while the second one correspond to the so called STRUCTURE FACTOR.

The physical meaning of such a result is quite clear. The scattered intensity from a single object behave in a way described by the form factor. In terms such an intensity is modulated by interference effects because of the superposition of the waves scattered by different objects, and such an interference pattern depend on the spatial arrangement of the scatterers and is described by the structure factor.

In fig. 2 we plot the function given by the eq. 2.2.3, in the following case:

- i)  $\sigma = 0$ , i.e. a perfect ordered array
- ii)  $\sigma = 0.2a$
- iii)  $\sigma = a$
- iv)  $\sigma = 2a$
- v)  $\sigma = \infty$ , i.e. a completely disordered system.

It can be seen that in the  $\sigma=0$  case the scattered intensity behave like a ~~succession~~ succession of peaks (Bragg's peaks), whose height is modulated by the form factor. On the contrary in the  $\sigma=\infty$  case, only the form factor survive, the structure factor being now a constant equal to unity.

It is worthwhile to note that the width of the form factor depend on the product  $k\sigma$ . ~~Therefore~~ In terms the "dimension" of the scatterers, " $a$ " depends on the kind of interaction i.e. on the kind of probe field. For example, considering an atomic system ~~investigated~~ investigated by means of X-Rays scattering, the "dimension" " $a$ " will refers to the electronic cloud of the atom ~~that~~ that is of the order of  $\approx$  Angstrom. The same system, investigated ~~is~~ is a neutron scattering experiment, will show a dimension " $a$ " corresponding to <sup>the</sup> nuclear length, i.e. practically zero. Therefore, although both X-rays and neutron can have the same wavelength, the form factor will be completely different in the two cases.

As a final remark we want to stress that the above mentioned considerations held only in the case of "coherent" scattering. If changes of phase occurs in the scattering process, interference effects no longer occur.

Up to now we considered an "integrated" scattered intensity, i.e. regardless to its time behaviour. Actually the distribution implied in the scattering process ~~also~~ depend on time, as well as on the position:

$$\rho = \rho(\mathbf{r}, t)$$

2.3.4

so that ~~its~~ partial Fourier transform is a time-dependent quantity:

$$\hat{\rho}(\mathbf{k}, t) = \int \rho(\mathbf{r}, t) e^{i\mathbf{k} \cdot \mathbf{r}} d\mathbf{r} \quad 2.2.5$$

Such a function is called "intermediate scattering function" (Van Hove). In the first part of the present section we just considered ~~the~~ <sup>the</sup> time integral (integrated intensity) of its square modulus (integrated intensity).

However the time behaviour is of relevant interest, in order to investigate the dynamical properties of the system. In principle such an investigation can be performed ~~either~~ in the time or in the frequency domain. For example one can consider the time autocorrelation function:

$$F(\mathbf{k}, \tau) = \int \hat{\rho}(\mathbf{k}, t) \hat{\rho}(\mathbf{k}, t + \tau) dt \quad 2.2.6$$

whose Fourier transform is the <sup>(temporal)</sup> power spectrum of the ~~static~~ <sup>dynamic</sup> eq. function  $\rho(\mathbf{k}, t)$ :

$$S(\mathbf{k}, \omega) = \int F(\mathbf{k}, \tau) e^{i\omega\tau} d\tau \quad 2.2.7$$

The function  $S(\mathbf{k}, \omega)$  is the dynamical structure factor. Its  $\omega=0$  value corresponds to the static structure factor discussed above (more precisely it is the product of the form factor <sup>time</sup> the structure factor).

The experimental investigation of  $S(\mathbf{k}, \omega)$  can be performed by various methods, depending on the implied time-scale, ~~or on~~ <sup>or equivalently,</sup> ~~on~~ i.e. on the frequency range). As far as small frequencies are implied (up to some MHz), the function  $F(\mathbf{k}, \tau)$  (eq. 2.3.6) can be experimentally measured by means of an autocorrelation technique (optical beating spectroscopy). At larger frequencies (up to some GHz), the function  $S(\mathbf{k}, \omega)$  can be obtained through interferometric techniques (Fabry-Pérot interferometer). Higher frequencies request the use of ~~grating~~ diffraction gratings (monochromator).

### III

## SURVEY OF SPECTROSCOPIC TECHNIQUES

### 3.1] Electromagnetic Scattering: X-rays and light scattering

An electromagnetic wave carries an electric field variable in space and time. For a ~~monochromatic~~ <sup>linearly polarized</sup> plane wave such a field can be described as

$$\mathbf{E}(\mathbf{r}, t) = \mathbf{E}_0 e^{i(\mathbf{k} \cdot \mathbf{r} - \omega t)} \quad 3.1.1$$

The wavevector  $\mathbf{k}$  determines the direction of propagation, while the direction of the vector  $\mathbf{E}_0$ , orthogonal to  $\mathbf{k}$ , determines the direction of the polarization.

The interaction Hamiltonian ~~with~~ of such a field with the scatterer, can be expanded in power of  $E_0$ . Up to the second order, a simple physical meaning can be attributed to the various contributions. One can write (cf. eq. 2.1.1):

$$H_i = - \int d\mathbf{r} \left( \mathbf{E} \cdot \mathbf{M} + \frac{1}{2} \mathbf{E} \cdot \underline{\alpha} \cdot \mathbf{E} + \dots \right) \quad 3.1.2$$

In eq. 3.1.2  $\mathbf{M}$  is the permanent dipole moment density, and  $\underline{\alpha}$  is the polarizability tensor, so that  $\underline{\alpha} \cdot \mathbf{E}$  is the induced moment dipole density.

It is to be noted that eq. 3.1.2 implies that we are concerned with a neutral medium. Such an approximation loses its validity if the wavelength of the incident field becomes small enough. In such a case, in fact, the incident wave "sees" the elementary charges ~~not~~ contained in an atom or molecule. Elementary charges are forced in motion by the electric field of the wave and in turn radiate electromagnetic waves. Because of the mass ratio between electrons and nucleus, the dominant contribution comes from the former. ~~Of course~~ In a quantitative description, one can speak of scattering of photons by electrons, so that the scattered field will reflect the distribution of electrons in the matter: we are concerned with X-Rays scattering.

Because we are mainly concerned with light scattering, do not enter in detail on this argument, and limit myself to a few comments:

- i) The available experimental techniques ~~do not~~ <sup>do not</sup> allow an easy frequency analysis of scattered X-Rays. As a consequence X-Ray scattering is mainly used for the investigation of static structure factors.

ii) As mentioned in the previous section, the scattered intensity is the product of a form factor, time a structure factor. The first one refers to the distribution of electronic charges in an atom, while the second reflect the spatial arrangement of atoms in a molecule, or in a ~~fluid~~ (macroscopic) fluid or crystal, and so on. X-Rays, in fact, has been extensively used to determine the spatial structure of molecules and macromolecules, and also crystal structures.

At larger wavelength, however, correlation between molecules, or molecules aggregate, can be detected. In practice, with usual X-Rays diffraction techniques, structural arrangement involving distances not larger than 100 Å about, can be investigated. Larger distances would imply the use of longer wavelength and ultimately results in light scattering experiments.

iii) Usual sources of X-ray, ~~and~~ (X-ray tube) furnishes ~~a~~ nearly monochromatic radiation, whose wavelength is of the order of some Å, depending on the kind of the anode. The continuous part of the spectrum (Bremsstrahlung), is, in fact, ~~the~~ <sup>generally</sup> not enough intense to allow a selection of any required wavelength (monochromatization). Such a circumstance ~~is~~ can be overcome by the use of diffraction radiation. We will return shortly on this subject ~~in~~ in the last section of this chapter.

iv) The circumstance mentioned sub i) is a consequence of the trivial fact that ~~an~~ X-Rays <sup>photons</sup> having a large wavevector  $k$ , possess an ~~energy~~ <sup>energy</sup>  $\hbar\omega = \hbar ck$  that is very high compared to the energies (frequencies) characteristic of atomic or molecular motion. As a consequence the possible variations of the frequency of scattered radiation is behind the resolution of available instrumentation. Such a difficulty can be overcome by using neutron scattering. The wavelength of neutrons beam can be easily of the same order of magnitude of ~~that of~~ X-Rays, but their energy is noticeably lower, ~~and~~ ~~and~~

### 3.2) Elastic, quasi-elastic, and inelastic light scattering

It is usual to distinguish, in light scattering experiments, the kind of scattered radiation according to its frequency change and coherence properties.

### 3.2) Dielectric and infrared absorption.

As far as the first terms in eq. 3.1.2 is concerned, one can see that it gives rise to the absorption of electromagnetic waves. ~~As for~~ The permanent dipole density  $\mu$  changes in time both because: ~~the orientation of molecules~~

- The number density of molecules, each of ones carries its own moment, changes in time, at a given space-point;
- The ~~mag~~ direction of any molecular dipole moment changes, according to rotational motion of molecules;
- The magnitude of the dipole moment of any given molecule, changes in time, because of internal motions of the molecule (vibrations).

In terms of autocorrelation function, one is concerned with the quantity:

$$G(t) = \left\langle \sum_{i,j} (\mu_i(t) \cdot \mu_j(0)) e^{ik(z_i(t) - z_j(0))} \right\rangle \quad 3.2.1$$

where  $i, j$  refers to various molecules, whose dipole moment is  $\mu_i$  and  $\mu_j$  respectively, while  $z_i$  and  $z_j$  are the coordinates of the molecules, and  $k$  is the wavevector of incident radiation. ~~where polarization is described by the unit vector  $e$ .~~

Actually rotational and vibrational motions are very fast compared to the translational motion described by  $z(t)$ . It follows that correlation between magnitude and/or orientation of dipoles decays much faster than the exponential term in eq. 3.2.1 ~~that therefore~~ (due also to the smallness of  $k$ ), so that in eq. 3.2.1 the exponential can be neglected.

Now the internal degree of freedom of a molecule can be expressed in terms of normal modes,  $q^\nu$ , so that the instantaneous value of the dipole moment can be written as:

$$\mu(t) = \mu^0 + \sum_{\nu} \mu^{\nu} q^{\nu}(t) \quad 3.2.2$$

where

$$\mu^{\nu} = \left( \frac{\partial \mu}{\partial q^{\nu}} \right)_{q^{\nu}=0} \quad 3.2.3$$

In eq. 3.2.2  $\mu^0$  is the permanent dipole moment, i.e. the value of corresponding to the equilibrium configuration of the molecule, while  $q^{\nu}$  are the ~~normal modes of the molecule~~ <sup>normal modes</sup>. The sum in eq. 3.2.2 range over the number of normal modes, characterized by the frequencies  $\nu$ .

~~But neglected~~ In eq. 3.2.2 the change in time of  $\mu(t)$  reflect the rotational motion, while the sum takes into account the vibrations of the molecule. Because ~~rotational~~ rotational and vibrational degree of freedom can be considered as uncorrelated, the correlation function, eq. 3.2.1, reduces to the sum of two terms, namely:

$$C(t) = C_0(t) + C_v(t) \quad 3.2.4$$

where

$$C_0(t) \propto \langle \sum_{i,j} \mu_i(t) \mu_j(0) \rangle$$

and

$$C_v(t) \propto \langle \sum_{i,j} \sum_{v,v'} (\mu_i^v(0) \mu_j^{v'}(t)) (q_i^v(0) q_j^{v'}(t)) \rangle \quad 3.2.5$$

Again the two correlations decays on very different time scale, so that they originate two entirely different physical effect. The first one is usually characterized by time constants of the order of micro or at most nano-seconds, so that gives rise to absorption of electromagnetic waves (or microwaves), and is known as dielectric relaxation. The second terms in eq 3.2.5 decays much faster, and gives rise to absorption in the infrared part of the electromagnetic spectrum.

### 3.3) Elastic, quasi elastic and anelastic light scattering.

In the preceding section, the physical process involved is mainly the absorption of a photon. Experimental observation reduces to the measurement of the ratio between incident and transmitted intensity of an electromagnetic wave of given frequency.

On the contrary, the second term in eq. 3.2.2 gives rise to scattering process, that can be described as the annihilation of a photon of frequency  $\omega$ , and ~~and wavevector  $k$~~  <sup>and wavevector  $k$</sup>  and creation of a photon of frequency  $\omega'$  and wavevector  $k'$ .

As far as scattering processes are concerned, interference effects between scattered waves become of paramount importance, and one can distinguish between the three following cases:

- i) Scattered waves are able to interfere with the incident beam, irrespective of their wavevector  $k'$ .
- ii) Scattered waves ~~are~~ characterized by the same wavevector  $k'$  are able to <sup>mutually</sup> interfere, ~~but~~ However waves with different wavevector ~~do~~ no longer can give interference effects.

- ii) ~~These~~ Scattered waves cannot <sup>even</sup> interfere, ~~also~~ if they are characterized by the same wavevector  $k'$ .

Accordingly we call elastic, quasi-elastic and anelastic, the scattering phenomena characterized respectively by the <sup>above used</sup> properties.

It is to be noted that such a classification is quite arbitrary. ~~Scattered waves often in the scattering~~ Moreover there is no a clear-cut distinction between the three cases.

In order to understand the ~~scattering~~ physical meaning of the above classification, we can think to the diffraction of light from a diffraction grating. We know that scattered radiation is characterized by precise value of the wavevector  $k$  (i.e. of the scattering angle  $\theta$ ), corresponding to the first, second and so on diffraction orders.

If the grating is at rest, diffracted light is characterized by the same frequency  $\omega_0$  of the incident light. Provided that the latter is a ~~plane~~ coherent, monochromatic wave, each diffracted beam is also coherent and can therefore interfere with the incident beam. We are faced with elastic scattering; no transfer of energy occurs between the incident wave and the grating.

Let us now suppose that the grating moves, sliding on its own plane, in a direction perpendicular to the ~~diffraction lines~~ <sup>its</sup> rows. In such a case, scattered light is Doppler shifted, the frequency shift being related to the velocity  $v$  of the grating and to the scattering angle, i.e. to the <sup>exchanged</sup> wavevector  $k = k_0 - k' = \frac{2\pi}{\lambda} \sin \frac{\theta}{2}$ :

$$\Delta\omega = v \cdot k$$

3.3.1

It is obvious that light scattered at different angles cannot give interference, because of the difference in frequency. For the same reason scattered light cannot interfere with the incoming beam. However waves scattered at the same angle ~~can~~ interfere: actually the interference pattern turns out to be the same as in the case of grating at rest.

Finally we can think to a grating, which rows moves in a random way, around their equilibrium position. In such a case, ~~scattered~~ scattered waves, even if characterized by the same exchanged wave vector  $k$ , suffer a ~~small~~ frequency shift whose amplitude is randomly distributed, so that interference effects no longer occurs. Such a situation ~~can~~ can be assumed as characterizing anelastic scattering.

### 3.4) Rayleigh-Brillouin scattering

In the prev section we are concerned with quasi-elastic light scattering, i.e. with the second term in the integral of eq. 3.1.2. The underlying physical process can be schematized as follows: the incident field induces a dipole moment, which, in turn, interacts. In such a case the relevant quantity is the polarizability tensor  $\alpha$ . In addition, unlike the case treated in the preceding section, such a phenomenon ~~turns out~~ turns out to be of the second order in the electric field. In a quantum language this implies that we are looking to two-photon process. Therefore, ~~apart from the possibility of absorption of two photons that two photons are absorbed~~ The possible processes are schematically drawn in fig. 1; here (a) and (b) represent the

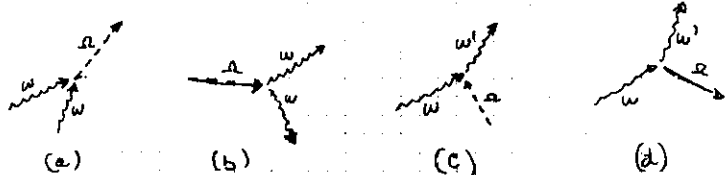


Fig. 1

absorption of two or emission of two photons. Obviously the frequency  $\omega$  (as well as the wavevector  $\mathbf{k}$ ) of the involved elementary excitation of the system, obeys the conservation rules:  $\omega = 2\omega'$ . The experimental observation of such an absorption process would allow the investigation of rotational, vibrational and translational degree of freedom of molecules, like in the infrared absorption technique, the only difference being that now the polarizability tensor  $\alpha$  is implied, rather than the permanent dipole moment  $\mu$ . However the absorption due to such a process hardly can be separated from other absorption processes.

The cases schematized in fig. 1, (c) and (d), correspond to scattering. In (c) a photon of frequency  $\omega$  is absorbed, together with an elementary excitation of frequency  $\omega'$ , and a photon of different frequency  $\omega'' = \omega + \omega'$  is emitted. In (d) the elementary excitation is created, and the frequency of the emitted photon is now  $\omega'' = \omega - \omega'$ . Usually processes of the kind (c) are called "Anti-Stokes", while processes of the kind (d) are called "Stokes".

The probability of occurrence of the two kind of processes are not equal because of the population factor implied in the distribution of elementary excitations; the ratio between the probabilities of Stokes and Anti-Stokes processes

being ~~proportional~~ proportional to  $\exp[-h\omega'/k_B T]$ , where  $k_B$  is the Boltzmann constant and  $T$  the temperature. We will see that, in the case of high frequency mode ~~(Rayleigh scattering)~~ such a circumstance prevents the experimental detection of anti-Stokes processes. On the contrary, at low frequency (Rayleigh and Brillouin scattering) the Stokes-Anti-Stokes ratio turns out to be nearly unity. Like in eq. 3.2.1, we are now concerned with a correlation function of the kind:

$$C_p^{(2)}(t) = \left\langle \sum_{ij} (\mathbf{E}_i \cdot \alpha_i(0) \mathbf{E}_j) (\mathbf{E}_i \cdot \alpha_j(t) \mathbf{E}_j) e^{i\mathbf{k} \cdot (\mathbf{r}_i(t) - \mathbf{r}_j(0))} \right\rangle \quad 3.4.1$$

where, as before,  $i$  and  $j$  refer to single molecules,  $\alpha$  is the tensor of molecular polarizability,  $\mathbf{k}$  is the exchanged wave vector and  $\mathbf{r}_i$  the coordinate of a molecule. In eq. 3.4.1 attention is paid to the polarization of light, through the unit vector  $\mathbf{E}_i$  (incident beam) and  $\mathbf{E}_j$  (scattered beam).

The polarizability tensor  $\alpha$  can change in time because of rotational and vibrational degree of freedom of the molecules. Like the case discussed in the preceding section, we can write (at the first order in the vibrational coordinates  $q^\nu$ ):

$$\alpha = \alpha^0 + \sum_{\nu} \alpha^{\nu} q^{\nu} \quad 3.4.2$$

where

$$\alpha^{\nu} = \left( \frac{\partial \alpha}{\partial q^{\nu}} \right)_{q^{\nu}=0} \quad 3.4.3$$

In eq. 3.4.2  $\alpha^0$  is the <sup>"permanent"</sup> polarizability tensor, i.e. for a non-vibrating molecule. Correspondingly the correlation function can be split in two terms:

$$C_p^{(2)}(t) = C_p^{(2)}(t) + C_p^{(2)}(t) \quad 3.4.4$$

where

$$C_p^{(2)}(t) = \left\langle \sum_{ij} (\mathbf{E}_i \cdot \alpha_i^0(0) \mathbf{E}_j) (\mathbf{E}_i \cdot \alpha_j^0(t) \mathbf{E}_j) e^{i\mathbf{k} \cdot (\mathbf{r}_i(t) - \mathbf{r}_j(0))} \right\rangle \quad 3.4.5$$

and

$$C_{vp}^{(2)}(t) = \left\langle \sum_{ij} \sum_{\nu\nu'} (\mathbf{E}_i \cdot \alpha_i^{\nu}(0) \mathbf{E}_j) (\mathbf{E}_i \cdot \alpha_j^{\nu'}(t) \mathbf{E}_j) q_i^{\nu}(0) q_j^{\nu'}(t) e^{i\mathbf{k} \cdot (\mathbf{r}_i(t) - \mathbf{r}_j(0))} \right\rangle \quad 3.4.6$$

The correlation function 3.4.5 is related both to rotational degrees of freedom of the molecules and to translational motion through the exponential factor. The corresponding part of the scattering is usually known as Rayleigh-Brillouin scattering. The correlation function 3.4.6 takes into account the vibrations of a molecule, and represent the so-called Raman scattering. We will discuss Raman scattering in the next section. Here we would to stress the circumstance that the presence of the normal-mode coordinate  $q^\nu$  of different molecules gives rise to phase shift in the scattered waves that, as a consequence, no longer can

interfere, so that, in principle, Raman scattering (inelastic scattering). In addition in eq. 3.4.5 the orientational factor can be put equal to unity, because correlation between vibration modes decays much faster than translational motion. As a consequence Raman scattering turns out to be independent from the exchanged wavevector (i.e. from the scattering angle) apart for a trivial geometrical factor.

Let us now return to  $C_0^{(0)}(t)$ , eq. 3.4.4. The permanent polarizability tensor can be written as the sum of an isotropic ~~tensor~~ and an anisotropic tensor:

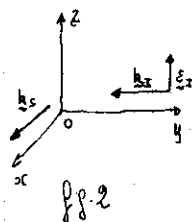
$$\underline{\alpha}^0 = \bar{\alpha}^0 \underline{I} + \underline{\beta}^0 \quad 3.4.6$$

where  $\underline{I}$  is the diagonal unit tensor and:

$$\bar{\alpha}^0 = \frac{1}{3} \text{Tr } \underline{\alpha}^0 \quad \underline{\beta}^0 = \underline{\alpha}^0 - \bar{\alpha}^0 \underline{I} \quad 3.4.7$$

If one is concerned with a single-component system,  $\bar{\alpha}^0$  turns out to be a constant, while  $\underline{\beta}^0$  can change both from molecule to molecule and in time, because of rotational motion.

Taking into account the polarization of incident and scattered light, one can also distinguish between "polarized" and "depolarized" spectrum. For the sake of simplicity let us consider the scattering geometry sketched in Fig. 2.



The incident wave comes along the y-direction, and one detects the wave scattered at  $90^\circ$ , along the x-direction. The polarization of incident wave ~~is parallel~~,  $E_i$ , is parallel to z-direction, i.e. normal to the scattering plane x,y.

If one looks at scattered waves with polarization  $E_s$  again parallel to z-axis, ~~the~~ the scattering is called Vertical-Vertical (V-V) or "polarized". While if the waves are polarized along the y-axis are detected, one speaks of Vertical-Horizontal (V-H) or "depolarized" scattering.

In the first case the  $\alpha_{zz}$  component of the polarizability tensor is involved in the process. In the second one the relevant component is the  $\alpha_{yz}$  one.

Let us consider firstly the polarized spectrum. According to eq. 3.4.6 one has

$$\alpha'_{zz} = \bar{\alpha}^0 + \beta^0_{zz} \quad 3.4.8$$

Accordingly, two correlation functions are involved:

$$C_{0,zz}^{(2)}(t) = \left\langle \sum_{ij} \alpha_i^0 \alpha_j^0 e^{iK(z_i(t) - z_j(0))} \right\rangle + \left\langle \sum_{ij} \beta_{zz}^0 \beta_{zz}^0 e^{iK(z_i(t) - z_j(0))} \right\rangle \quad 3.4.9$$

The first addendum corresponds to the "isotropic" part of the polarized spectrum, while the second one corresponds to the "anisotropic" part. It is quite evident that the isotropic part is concerned solely with translational degree of freedom. Actually, because of the small value of the implied wavevector  $K$ , due to the long wavelength of light, the expression of the first addendum in eq. 3.4.9 is slightly misleading: light cannot "see" a single molecule. We can define a volume element  $d\tau$ , whose size is much smaller than  $K^{-1}$  ~~but still contains a large number of molecules~~ but again containing a large number of molecules. ~~The mean polarizability  $\bar{\alpha}^0$  of such an element depends only~~ Then one can define a density of polarizability  $\bar{\alpha}^0(z)$

$$\bar{\alpha}^0(z) d\tau = \sum_{i \in d\tau} \alpha_i^0 \quad 3.4.10$$

Accordingly the isotropic part of the <sup>polarized</sup> spectrum can be written as:

$$C_{0,zz}^{(2)} = \left\langle \iint \bar{\alpha}^0(z,0) \bar{\alpha}^0(z,t) e^{iK(z(t) - z(0))} d\tau d\tau' \right\rangle \quad 3.4.11$$

where  $\bar{\alpha}^0$  changes from point to point and in time because of the fluctuations of the number density of molecules. Hydrodynamic and thermodynamic analysis shows that fluctuations of density can be split in:

- isobaric ~~pressure~~ fluctuations, that obeys to a diffusion equation, governed by the heat diffusion coefficient  $D$ . The corresponding part of the spectrum is a line centered at the incident frequency  $\omega_0$ , whose width, in a simple case, is given by  $D K^2$ , ~~by the heat diffusion coefficient~~ (Rayleigh scattering).
- adiabatic pressure fluctuations, that obey to a wave-equation, and can be seen as acoustic waves travelling the medium. The corresponding part of the spectrum consists in two lines centered about  $\omega_0 + \omega$  and  $\omega_0 - \omega$ , where  $\omega = vK$ ,  $v$  being the sound velocity, and whose width is proportional to the acoustic absorption coefficient. (Brillouin scattering). (See Fig. 4 of Ch. I).

Actually such a simple picture is ~~more or less~~ blurred in a real case, because of interactions ~~between different modes~~ that give rise to a mode-mode coupling. For example a coupling between external



(rotational and translational) and internal (vibrational) degrees of freedom can exist, being moreover frequency dependent (relaxation processes). In such a case adiabatic pressure fluctuations can give contributions to the line centered at  $\omega_0$ , and a "transfer" of intensity between Brillouin and Rayleigh lines ~~occurs~~ occurs (Mountain modes).

A second contribution to the planar spectrum comes from the anisotropic part of the polarizability tensor  $\bar{\alpha}_{ij}$ . Such an anisotropic part, in turn, is the only that gives contribution to the depolarized spectrum. We have, in fact:

$$C_{0,UV}^{(2)ANIS} = \left\langle \iint \bar{\alpha}_{ij}(z,0) \bar{\alpha}_{ij}(z,t) e^{ik(z(t)-z(0))} dz dz' \right\rangle \quad 3.4.12$$

and

$$C_{0,UV}^{(2)ROT} = \left\langle \iint \bar{\alpha}_{ij}(z,0) \bar{\alpha}_{ij}(z,t) e^{ik(z(t)-z(0))} dz dz' \right\rangle \quad 3.4.13$$

Where  $\bar{\alpha}_{ij}(z,t)$  is defined as  $\bar{\alpha}_{ij}^{(0)}(z,t)$ .

In the above equation the main point is the possibility of separation between rotational degree of freedom that contribute to the variations of  $\bar{\alpha}_{ij}$  (and  $\bar{\alpha}_{ij}$ ) and translational degree of freedom that are implied in the exponential factor. If the two kind of motion are uncorrelated, the correlation functions can be factorized. In addition ~~correlation between rotation~~ the orientational correlation decays much faster than the translational one, so that the exponential factor can be put equal to unity.

The corresponding part of ~~scattered~~ the spectrum of scattered line turns out to be a broad band, centered at the incident frequency  $\omega_0$  whose width is related to the "rotational" diffusion coefficient. Such a line is the only present in the VV spectrum, while it is superimposed to the Rayleigh-Brillouin lines in the VV spectrum.

### 3-5) Correlation and interference techniques.

Let us now discuss the experimental techniques that allows the detection of the Rayleigh-Brillouin part of the spectrum of scattered light.

First of all we notice that, in any case, the use of a laser source is practically the only possibility, because of the monochromaticity and coherence characterizing the light emitted by such a source.

As far as the detection and analysis of the scattered field ~~are~~ concerned, the main point is the magnitude of the frequency shift, i.e. the distance, in terms of frequency, from the ~~value~~ <sup>characteristic</sup> ~~frequency~~  $\omega_0$  of the incident light.

Up to some megahertz of frequency shift, the only possible technique is the so called "light beating spectroscopy" together with the detection of the time autocorrelation of the scattered light intensity. Such a technique allows the detection of frequency shift as small as a few ~~hertz~~ <sup>of</sup> hertz, i.e. correlation times as large as ~~micro~~ <sup>micro</sup> seconds.

The main point of such a technique is to ~~at~~ <sup>subtract</sup> "subtract" the frequency  $\omega_0$  to the spectrum of scattered line, i.e. to shift around the zero frequency the spectrum centered at  $\omega_0$  frequency.

Such a result can be obtained by means of a technique well known in the radio-communication field: a "local oscillator" furnishes a constant signal at frequency  $\omega_0$ , and such a signal is "mixed" with the incoming signal at frequency e.g.  $\omega_0 + \Omega$ . The superposition of the two signal is then detected through a square-law detector, so that the output signal contains the "frequency difference", i.e.  $\Omega$ . Obviously in the case of light scattering the local oscillator consists in a ~~part~~ part of the same light beam sent into the sample, the square-law detector being a photomultiplier (see Fig. 3). Such kind of experimental disposition is called "heterodyne". It is also possible ~~to~~ <sup>in many cases</sup> avoid the use of the local oscillator. In fact, if the ~~power spectrum of the~~ <sup>power spectrum of the</sup> scattered beam consists in a more or less large band centered at  $\omega_0$ , each frequency component "beats" with each other. As a result the central frequency  $\omega_0$  is subtracted, the power spectrum of the detected signal being now centered at zero frequency. In such a case one speaks of "homodyne".

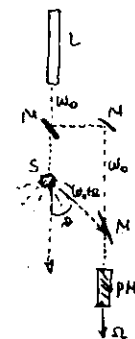


Fig. 3

L: laser  
M: mirror  
S: sample  
PM: photomultiplier  
 $\theta$ : scattering angle

Let us suppose that the scattered field  $E(t)$  can be written in terms of its frequency spectrum  $\hat{E}(\omega)$ :

$$E(t) = \int \hat{E}(\omega) e^{-i\omega t} d\omega \quad 3.5.1$$

Then the intensity will be given by:

$$I(t) = \iint \hat{E}(\omega) \hat{E}^*(\omega') e^{-i(\omega - \omega')t} d\omega d\omega' \quad 3.5.2$$

By putting  $\omega = \omega_0 + \Omega$ ,  $\omega_0$  being the frequency of the incident wave, one obtains:

$$I(t) = \iint \hat{E}(x) \hat{E}^*(x') e^{-i(\omega - \omega')t} d\omega d\omega' \quad 3.5.3$$

that shows that the temporal behaviour of the scattered intensity will depend only on the frequency shift  $\omega$ , the central frequency  $\omega_0$  being neglected. ~~Moreover~~ such an intensity is related to the square modulus of the scattered field.

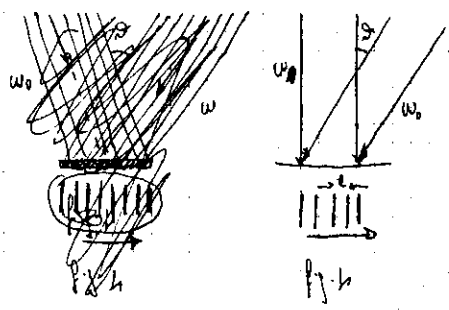
In the heterodyne case, the field onto the detector is the sum of the scattered field  $\hat{E}_s$  and the local oscillator field  $E_0 = A e^{i\omega_0 t}$ . In such a case we have

$$I(t) = \iint (\hat{E}_s(\omega) + A \delta(\omega - \omega_0)) (\hat{E}_s^*(\omega') + A \delta(\omega' - \omega_0)) e^{-i(\omega - \omega')t} d\omega d\omega' \quad 3.5.4$$

$$= \iint \hat{E}_s(\omega) \hat{E}_s^*(\omega') e^{-i(\omega - \omega')t} d\omega d\omega' + 2A \operatorname{Re} \int \hat{E}_s(\omega) e^{-i(\omega - \omega_0)t} d\omega + |A|^2$$

Now, if ~~also~~ the local field amplitude  $A$  is <sup>much</sup> larger than the amplitude of the scattered field, the first integral can be neglected; the last term is a constant so that the time behaviour of the scattered intensity turns out to be related to the amplitude of the scattered field rather than to its square modulus as in the ~~ordinary~~ case.

It is to be noted that, ~~in a real case, the~~ ~~heterodyne~~ experiment request very stringent geometrical condition. In particular, in order to ~~have~~ ~~actually~~ ~~have~~  $E$  that the beating between scattered and local field actually takes place, the two waves must have the same wavevector  $k$ . Let us think, for the sake of simplicity to two plane waves, of frequency  $\omega$  and  $\omega_0$  ~~with different directions of propagation~~ ~~from~~ with different direction of propagation, that come onto the photodiode surface (see fig. 4). As a consequence interference fringes appear, whose spacing is given by  $l = \lambda / \sin \theta$ , being  $\theta$  the angle between the two direction of propagation. Moreover, because of the (small) frequency difference, the fringe pattern moves in such a way that the distance  $l$  is covered in a time  $\tau = 2\pi / |\omega - \omega_0|$ . Now, if the dimension of the photodiode is smaller



than  $l$ , the consequence is a periodic variation of ~~the~~ light intensity onto the photodiode, so that the photocurrent oscillate at a frequency  $\Omega = |\omega - \omega_0|$  (Beating). If, however, the dimension of the photodiode becomes larger and larger than  $l$ , such an oscillation become smaller and smaller around an increasing mean value. Such a circumstance requires that  $D$  must be small enough: in practice a collimation between the two beams better than  $10^{-3}$  rad is required. In other words the heterodyne technique request <sup>an</sup> optical alignment with a ~~comparable~~ ~~with~~ ~~of the same order of precision~~ as good as that required in an interferometric experiment. For such a reason usually the heterodyne technique is preferred.

It is to be noted, however, that with the heterodyne technique, a constant shift of frequency cannot be detected, so that such a technique cannot be used to detect, e.g., the Doppler shift due to a ~~common~~ ~~motion~~ motion of the scatterers.

The actual analysis of the time behaviour of the photocurrent  $I(t)$  could be performed through a spectrum analyzer. However it is <sup>a</sup> common practice to use a "correlator", in which a multichannel stores the quantities

$$C_j = \sum_{i=1}^N n(t_i, 0) n(t_i + \tau_j) \quad 3.5.5$$

where  $n(t_i, 0)$  is the number of photocount detected in a small time interval  $\Delta t$ , at  $t$  the instant  $t_i$ , while  $\tau_j$  is a fixed delay,  $\tau_j = j \cdot \Delta t$ . Obviously  $\Delta t \leq \Delta t'$ . The total time of integration,  $T = N \Delta t'$ , must be long enough to ensure a good ~~ratio~~ <sup>ratio</sup> signal to noise. Such a procedure furnishes a good sampling of the ~~intensity~~ <sup>auto</sup> autocorrelation function, provided that the ~~phenomenon~~ <sup>phenomenon</sup> the phenomenon under investigation is a stationary one.

In order to increase the working speed, so that smallest time intervals  $\Delta t'$  can be <sup>explored</sup> ~~used~~, a "clipped" correlation ~~function~~ <sup>technique</sup> can be used. In such a technique  $n(t_i + \tau_j)$  ~~assume the value 1 or 0~~ <sup>is</sup> substituted by  $n_c(t_i + \tau_j)$ , where  $n_c$  can be 1 ~~or 0~~ (if  $N > K$ ) or zero (if  $N < K$ ),  $K$  being a fixed number, usually equal to one. It is easy to show that if the intensity is small enough, in order that the probability of detecting one photon in the time interval  $\Delta t$  turns out to be ~~to~~ <sup>be</sup> noticeably less than one, then the clipped autocorrelation function do not differ appreciably from the true autocorrelation function.

According to the autocorrelation function of the photocurrent is related to the autocorrelation of the scattered field, in the heterodyne case:

$$I_{\text{het}}(t) \equiv I_2(t) = \langle E_s^*(0) E_s(t) \rangle \quad 3.5.6$$

while in the homodyne case the same function is related to the autocorrelation of the square modulus, i.e. of the intensity of the scattered field:

$$I_{\text{hom}}(t) \equiv I_2(t) = \langle |E_s(0)|^2 |E_s(t)|^2 \rangle \quad 3.5.7$$

Such a circumstance is to be taken into proper account in the interpretation of experimental results. For example, if the scatterers perform a random Brownian motion, so that the fluctuations of concentration decay exponentially with a time constant  $\tau^{-1}$ , then  $I_{\text{het}}(t)$  will decay with the same time constant, while  $I_{\text{hom}}(t)$  will decay with a time constant  $(2\tau)^{-1}$ .

In fig. 5 we show a simplified scheme of a clipped correlator. Notice that because  $n_i$  can be only zero or one, the product  $n_i n_c$  can be performed quickly by a simple "and" circuit.

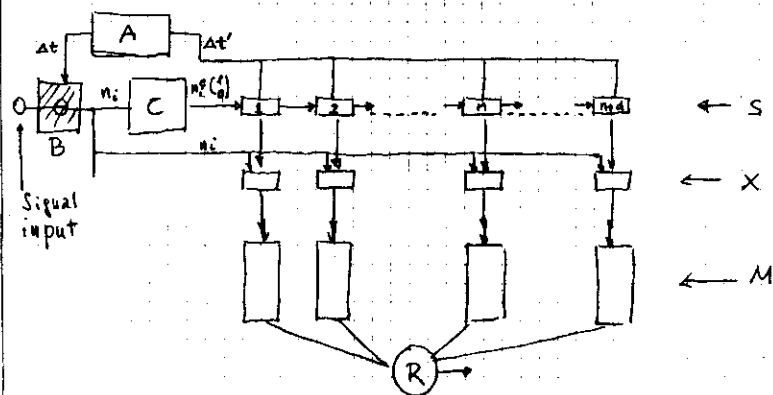


Fig. 5

A is the main clock, that fixes the intervals  $\Delta t, \Delta t'$ . B is a gate, while C is the clipper. S is a series of shift registers, whose content (1 or 0) is transferred to the next one at each time interval  $\Delta t'$ . X are "and" circuits, and M is the multi-channel; each channel accumulates the value  $n_i$  only if the content of the corresponding shift register is one. R is some circuitry that allows to show the content information stored in the multi-channel.

### 3.6) Interferometric techniques

The correlation technique described in the preceding section, allows the experimental detection of the (clipped) autocorrelation function until the required time interval is not much less than a microsecond. This means that the autocorrelation function is "sampled" with such a <sup>temporal</sup> graininess. According to the Wiener-Kinchine theorem, it follows that the spectrum of the scattered light can be reconstructed in the frequency range from zero to some MHz. Various experimental arrangements have been proposed in order to extend such a range toward higher frequency. For example the photocurrent can be recorded at high speed in a ~~mag~~ magnetic tape, and analyzed ~~later~~ with a reduced speed  $f$ .

However in order to analyze frequency shift of the order of several megacycle up to the order of GHz, the most commonly used technique is the interferometric one.

Essentially with such a technique, collective motions of the system can be investigated, namely sound absorption and velocity, and rotational diffusion coefficient. <sup>However</sup> apart from some informations concerning the latter phenomena that are connected with the shape and type of scatterers, as well as with their mutual interaction, interferometric techniques are up to now scarcely used for biophysical purposes. For such a reason we limit ourselves to a short description of interferometric techniques. <sup>More precisely</sup> A Fabry-Perot interferometer consists ~~essentially~~ in two plane parallel mirrors, whose reflectivity is larger than 90% (see fig. 6). Incident light  $I$ ,

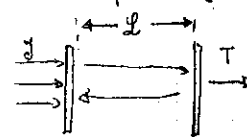


Fig. 6

usually as a plane wave, enters into the optical cavity  $L$ , being successively reflected ~~back and forth~~ between the two mirrors. A part of the optical energy stored into the cavity passes through the exit mirror as a transmitted wave,  $T$ . However different spectral components of the incident beam are damped in a different way into the cavity. In particular the component whose

wavelength  $\lambda$  satisfies the condition:

$$L = \frac{n\lambda}{2}$$

3.6.1

where  $n$  is an integer, is practically undamped, so that the transmitted wave  $T$  contains only such a component. Because the length  $L$  of

the cavity can be continuously changed, it is clear that a spectral analysis of the incident light can be performed.

In ~~the case of~~ a Fabry-Pérot interferometer, two parameters are of great importance:

i) The "Free Spectral range".

Let us suppose that eq. 3.6.1 is satisfied for a given value of  $L$ ,  $\lambda_0 = \lambda_0$  and  $n = n_0$ . A change in  $L$  results in a change of the value of  $\lambda$  for which eq. 3.6.1 is fulfilled, until the same equation is again fulfilled with  $\lambda = \lambda_0$  and  $n = n_0 + 1$ . As a consequence the range of frequency that can be explored is limited. An evaluation of such "free spectral range"  $\Delta \omega$  can be easily performed by putting  $(n+1)\lambda_0 = n\lambda_0 + 2L$ . Then  $\Delta \lambda$ , ~~can be~~ and therefore  $\Delta \omega$ , can be calculated. It results  $\Delta \omega = 2\pi c / L$ , being  $c$  the speed of light. The free spectral range, therefore, increases when the length of cavity is shortened. For example, with a cavity length of one centimeter, a free spectral range  $\Delta \omega \approx 10^{14}$  (rad/sec) is available.

ii) The "Finesse".

Measures essentially the resolving power of the instrument, and is related to the damping of light into the cavity, to the reflectivity of the mirrors, to their parallelism and planarity and so on.

### 3.7) Raman Scattering.

As mentioned in Section 4.1, the ~~responsible~~ interaction responsible for Raman scattering are the  $\alpha''$ 's, i.e. the derivative of the polarizability tensor with respect to the internal degree of freedom described by the normal-mode coordinate  $q''$ . In other words we are now concerned with the correlation function:

$$C_{\nu\nu}^{(2)}(t) = \left\langle \sum_{i,j} \sum_{\nu,\nu'} (\epsilon_i \alpha''_{\nu}(0) \epsilon_j) (\epsilon_i \alpha''_{\nu'}(t) \epsilon_j) q''_{\nu}(0) q''_{\nu'}(t) e^{i\omega_{\nu}(t) - i\omega_{\nu'}(0)} \right\rangle \quad 3.7.1$$

Usually the following simplifying assumption can be made:

- Vibrational or rotational motions of different molecules are totally uncorrelated, so that only terms with  $i=j$ , in eq. 3.7.1 ~~are~~ give a non zero contribution.
- Also considering a single molecule, the various degree of freedom do not interact each others, (harmonic approximation), so that only terms with  $\nu=\nu'$  give non zero contribution.

- The translational degree of freedom has a correlation time very long compared with the correlation time associated with internal degree of freedom, so that the exponential factor in eq. 3.7.1 can be put equal to one.

With these assumptions eq. 3.7.1 becomes:

$$C_{\nu}^{(2)}(t) = \left\langle \sum_{\nu} (\epsilon_i \alpha''_{\nu}(0) \epsilon_i) (\epsilon_i \alpha''_{\nu}(t) \epsilon_i) q''_{\nu}(0) q''_{\nu}(t) \right\rangle \quad 3.7.2$$

where the summation over the various ~~molecules~~ molecule has been dropped because all the molecules are considered identical.

Notice that in eq. 3.7.2 rotational degree of freedom are again present because  $\alpha''_{\nu}$  depends on the instantaneous orientation of the molecule.

According to the i) assumption above, the mean splits in the product:

$$C_{\nu}^{(2)}(t) = \sum_{\nu} \langle (\epsilon_i \alpha''_{\nu}(0) \epsilon_i) (\epsilon_i \alpha''_{\nu}(t) \epsilon_i) \rangle \langle q''_{\nu}(0) q''_{\nu}(t) \rangle \quad 3.7.3$$

Let us now suppose that each vibrational mode is harmonic in character, so that each  $q''$  can be defined as ~~a~~ a damped harmonic oscillator with frequency  $\omega_{\nu}$ , and amplitude  $q''_0$ :

$$q''_{\nu}(t) = q''_0 e^{-i\omega_{\nu}t} G_{\nu}(t) \quad 3.7.4$$

where the ~~fast~~ decreasing function  $G_{\nu}(t)$  describe the damping. It follows:

$$C_{\nu}^{(2)}(t) = \sum_{\nu} q''_0^2 \langle (\epsilon_i \alpha''_{\nu}(0) \epsilon_i) (\epsilon_i \alpha''_{\nu}(t) \epsilon_i) \rangle G_{\nu}(t) e^{-i\omega_{\nu}t} \quad 3.7.5$$

Eq. 3.7.5 shows that the spectrum of the scattered radiation is given by a series of lines, centered at the ~~vibrational~~ frequencies  $\omega_{\nu}$ , characteristic of the scatterer. Each line, in turn, is the convolution of the ~~proper~~ "intrinsic" line-shape, given by the damping function  $G_{\nu}(t)$  with the ~~rotational~~ spectrum of the scatterer, given by

$$R_{\nu}^{(2)}(t) = \langle (\epsilon_i \alpha''_{\nu}(0) \epsilon_i) (\epsilon_i \alpha''_{\nu}(t) \epsilon_i) \rangle \quad 3.7.6$$

It is to be noticed that the assumption i) above, implies that light scattered from different molecules or ~~even~~ even from different modes of the same molecule cannot interfere anyway: actually eq. 3.7.5 represent the scattering from a single point-scatterer, and in the scattered intensity neither form factor nor structure factor ~~can~~ <sup>can be</sup> be recognized. In such a sense Raman scattering is often ~~called~~ <sup>described</sup> as an incoherent process.

Let us now, as before, split the derivative of the polarizability tensor  $\alpha^{\nu}$  in a ~~isotropic~~ isotropic ~~part~~ and anisotropic part (compare with eq. 3.4.4):

$$\alpha^{\nu} = \bar{\alpha}^{\nu} \frac{1}{3} + \beta^{\nu} \quad 3.7.7$$

Following the same procedure sketched in sec. 4, we can distinguish again between a "polarized" or V-V spectrum, and a "depolarized", or V-H spectrum. In the first case we have

$$C_{V,V}^{(2)0} = C_{V,V}^{(2)IS} + C_{V,V}^{(2)ANIS}$$

where

$$C_{V,V}^{(2)IS} = \sum_{\nu} \langle \bar{\alpha}^{\nu}(0) \bar{\alpha}^{\nu}(t) \rangle q_0^2 G_{\nu}(t) e^{-i\Omega_{\nu} t} \quad 3.7.8$$

$$C_{V,V}^{(2)ANIS} = \sum_{\nu} \langle \beta_{zz}^{\nu}(0) \beta_{zz}^{\nu}(t) \rangle q_0^2 G_{\nu}(t) e^{-i\Omega_{\nu} t} \quad 3.7.9$$

While for the V-H configuration only the anisotropic part of the spectrum gives contribution:

$$C_{V,H}^{(2)} = \sum_{\nu} \langle \beta_{zz}^{\nu}(0) \beta_{zz}^{\nu}(t) \rangle q_0^2 G_{\nu}(t) e^{-i\Omega_{\nu} t} \quad 3.7.10$$

Like the results obtained in sec. 4, the VV spectrum consists in a vibrational term, involving  $\bar{\alpha}^{\nu}$  and in a term that is the convolution of both vibrational and rotational contributions. The latter, as before, is the only contribution to the V-H spectrum.

It is easily understood that a comparison between VV and V-H (or infrared) spectra allows an evaluation of both vibrational and rotational spectra, ~~and~~ ~~can~~ that, e.g., can be useful in order to test if the two kind of degree of freedom are, or not, more or less coupled.

### 3.8) Neutron scattering

The physical property of matter involved in the process of ~~the~~ neutron scattering is the interaction of a neutron with an atomic nucleus. Such an interaction, in turn, is the sum of a contribution due to nuclear forces and one due to spin-spin interaction. The latter is practically absent in diamagnetic materials and can be usually neglected.

It is however to be noticed that the mutual orientation of the <sup>spins of</sup> neutron and nucleus modify the strength of the nuclear interaction.

Such an interaction can be written as:

$$V(\mathbf{r}) = \frac{2\pi\hbar}{m} b_i \delta(\mathbf{r} - \mathbf{r}_i) \quad 3.8.1$$

where  $\mathbf{r}_i$  specify the position of the  $i$ th atomic nucleus. Such a potential, known as Fermi potential, is characterized by an infinitely short range: In a neutron scattering experiment, the scatterers are, in fact, atomic nuclei, that can be considered point-like.

The strength of the interaction is characterized by the length  $b_i$ , (scattering length). The value of  $b_i$  depends:

- on the kind of nucleus, so that also for the same element, can change from isotope to isotope
- on the mutual orientation of spins (parallel or antiparallel), as mentioned before.

The  $b_i$  can be positive or negative according to ~~the~~ the kind (attractive or repulsive) of the interaction. Such a circumstance plays a noticeable role in practical experiments. It is in fact possible, for some selected substances, to use a mixture of isotopes of the same element in order to obtain  $\sum b_i = 0$  for such an element. As a consequence, in a scattering experiment the contribution of that element is cancelled, and a <sup>partial</sup> structure factor can be <sup>exactly</sup> obtained.

Another relevant consequence of the variability of <sup>the</sup> scattering length is the distinction between "coherent" and "incoherent" neutron scattering. It is to be noted that such a terminology is rather improper. Actually any ~~of~~ scattering process is, in itself, coherent in the sense that no phase shift (or time delay) is involved. The possibility of interference effect, that characterise coherent radiations, is however conditioned by the physical arrangement of the scatterers, or by their dynamical states. For example the "incoherence" of Raman scattering comes from the fact (if true) that rotational and vibrational motion of different molecules are uncorrelated.

Let us now consider, as an example, a perfectly ordered array of neutron scatterers.

It is also to be noted, in comparison with light scattering, that in a neutron scattering experiment the incoming beam "see" the triple atomic nuclei, with which <sup>in a way that can be different from scattering to neutrons</sup> interact. In other words a substitution of

a volume integral in place of a summation, like in eq. 3.4.11, cannot be performed. Therefore, we are concerned with the correlation function (apart from proportionality factor):

$$C(t) = \sum_{i,j} \langle b_i b_j e^{iK(z_i(t) - z_j(0))} \rangle \quad 3.8.2$$

In such a case, however, the mean has a twofold meaning: firstly, for each scatterer, a mean is to be performed over the various isotopic and spin states; successively the usual thermodynamic mean can be performed. Next, in addition, that the mean of the scattering lengths can be performed independently of the exponential factor, ~~because~~ the former ~~is~~ do not depend on the position.

If we assume that isotopic and spin states are ~~mutually~~ independent random variables, for the first mean one gets:

$$\overline{b_i b_j} = \begin{cases} \overline{b_i^2} = \overline{b^2} & \text{for } i=j \\ \overline{b_i} \overline{b_j} = (\overline{b})^2 & \text{for } i \neq j \end{cases} \quad 3.8.3$$

where the bar indicates such kind of ~~mean~~ mean. Eq. 3.8.3 can be also written:

$$\overline{b_i b_j} = (\overline{b^2} - (\overline{b})^2) \delta_{ij} + (\overline{b})^2 \quad 3.8.4$$

so that eq. 3.8.2 becomes

$$C(t) = \sum_i (\overline{b} - (\overline{b})^2) \langle e^{iK(z_i(t) - z_i(0))} \rangle + \sum_{i \neq j} (\overline{b})^2 \langle e^{iK(z_i(t) - z_j(0))} \rangle \quad 3.8.5$$

It can be seen that we can distinguish between two contributions: the first one reflects the motion of an individual scatterer, <sup>(self correlation)</sup> while the second is concerned with the pair correlation between different scatterers, and therefore reflect collective motions. It is usual to call "coherent" scattering length the ~~quantity~~ mean:

$$(b^{\text{coh}})^2 = (\overline{b})^2$$

and "incoherent" scattering length the mean square deviation:

$$(b^{\text{inc}})^2 = \overline{b^2} - (\overline{b})^2 \quad 3.8.6$$

The above equations refers to the case in which the system under investigation consists in a simple atomic specie. For compound substances, the index  $i, j$  again labels the scattering lengths, being now referred to the various chemical component, so that  $C(t)$  turns out to be the sum of various "partial structure factor".

From an intuitive point of view one can think to the distribution of the  $b_i$  as the superposition of a mean value  $\overline{b}$ , that gives rise to coherent scattering, i.e. to interference effect, plus a fluctuating quantity that takes into account the difference, point by point, from the actual  $b_i$  value and the mean value  $\overline{b}$ . Because of the complete randomness of the latter distribution ~~the~~ interference effect no longer takes place, and one is concerned with incoherent scattering.

It is however to be noticed that an experimental separation of the two contribution is not easy, unless  $b^{\text{coh}} \gg b^{\text{inc}}$  or vice-versa, so that in general various experimental tricks are needed, like isotopic substitution of one or more component, and so on.

### 9) Special Techniques - EXAFS, MFXRD.

Although behind the purpose of our lessons, it can be useful to mention shortly some recently developed experimental techniques, that can improve the ~~investigation~~ spectroscopic investigations.

Let us consider the absorption of X-rays. When the energy of the incoming photons,  $h\nu$ , becomes equal to ~~the~~ some ionization energy  $E$  (e.g. K-Edge), the absorption increases abruptly, while electrons are ejected from the absorbing atom. The ejected electron possesses a kinetic energy given by  $(h\nu - E)$ .

Now if the ~~process~~ ~~absorbing~~ atoms ~~can~~ be considered as isolated (e.g. in a gas), the ejected electrons escape diffusely, while in the case of condensed matter there are a probability that the electron will be <sup>back-scattered</sup> ~~reflected~~ by some neighbouring atom and thus re-absorbed.

~~As a consequence~~ ~~for~~ In such a case a photon is emitted and, as a consequence, the absorption is lowered. ~~side-effect~~ The probability of such an event depends on the mutual distance between the emitting and back-scattering atoms, being sizeably different from zero when  $\pm$  such a distance corresponds to an integer multiple of half the wavelength associated with the electron. Because the latter ~~is proportional~~ depends on the kinetic energy, the absorption coefficient, just after the K-edge will show an oscillating behaviour. In fig. 7 we report a qualitative sketch of such kind of phenomenon. (a) represents the absorption of isolated atoms, (b) the absorption in condensed matter. The difference (c) contains therefore informations about the number and distances of nearest neighbours atoms, and is called EXAFS spectrum (Extended X-rays Absorption Fine Structure).

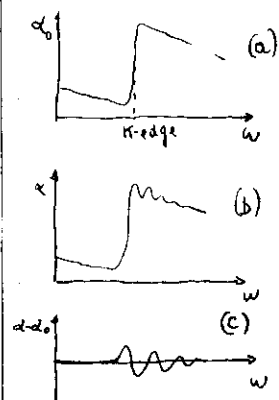


Fig. 7

The great advantage of such a technique consists in the possibility of fixing a selected chemical element, through the choice of the K-edge value (X-rays energy), and thus one can investigate the structural arrangement around the selected one.

Obviously such a technique requires the availability of X-Rays whose energy can be continuously changed. The bremsstrahlung radiation of usual X-Ray tubes could be used, although in such a case the intensity is very small so that very long experimental times are requested. The main source for such kind of experiment is given by the synchrotron radiation, and therefore requires

the use of big machines, i.e. the storage ring.

It is also to be noted that the actual interpretation of experimental data is not easy, because many parameters are involved in the phenomenon (e.g. thermal motion that blurred the ~~the~~ interference pattern (the so-called Debye-Waller factor), presence of various atomic species, value of the phase-shift of the emitted and back-scattered electrons, and so on).

Another recent technique, at present not fully developed, is the so-called MFXRD (Modulated Frequency X-Rays Diffraction). Shortly such a technique consists in ~~the~~ an usual X-Ray diffraction experiment, in which however, the frequency  $\omega$  of the X-Rays is modulated (with a low frequency  $\Omega$ ) around the K-edge of some selected atomic species A. It can be shown that in such conditions the ~~interf~~ diffraction pattern is the superposition of

- i) a pattern, constant in time, corresponding to the total structure factor
- ii) a pattern that ~~oscillate~~ oscillate in time with frequency  $\Omega$  corresponding to correlation between A and other atomic species.
- iii) a pattern that oscillate in time with frequency  $2\Omega$ , corresponding to the ~~correlation~~ correlation between A and A.

It is easily understood that a phase-lock detection of the scattered intensity, allows the separation of the ~~three~~ three conditions above.

## APPLICATION OF <sup>RAMAN</sup> IV TO BIOLOGICAL MOLECULES

### 4.1) Introductory remarks.

The use of spectroscopic techniques for the investigation of biological systems is very powerful mainly because of its flexibility. Elastic scattering gives informations about structural properties, and changing the wavelength of the incident radiation from some Å to micrometers, the structure can be investigated on a wide scale of distances so that, starting from intramolecular configuration, we can arrive to collective arrangement, local order and so on.

However it is the quasi-elastic and inelastic scattering that carries the large number of informations, because of its intimate relationship with the dynamical properties of the system. Also in this field we can explore a wide range of characteristic times: ~~starting~~ starting from relative motion of single atoms in a molecule, we can explore the dynamic of intramolecular group, as well as the dynamic of the entire molecule up to collective motions that involve a large number of molecules.

There is a rather general rule, whose origin is easily understood: the bigger is the ~~mass~~ mass implied in some kind of ~~motion~~ motion, the lower is the corresponding frequency, so that exploring a Raman (and or an IR spectrum) from frequency shift as low as  $1 \text{ cm}^{-1}$  <sup>or less</sup> to high frequency, a rather complete analysis of the various degree of freedom can be made, as well as useful informations can be drawn about interactions, collective properties and so on.

In the present and in the next chapters we will be concerned mainly with Raman Scattering. It is to be noticed that Raman scattering and IR spectroscopy would give, in principle, the same informations about the normal mode of the system  $q^j$ . However there is an important difference: in the IR spectroscopy it is the permanent dipole moment (and its derivatives with respect to the  $q^j$ ) that is involved, while for the Raman effect the same role is played by the polarizability tensor (and 1st derivatives).

For such a reason not all the  $q^j$ 's contribute to both the Raman and IR spectrum.

In the next section we give a short survey of some fundamental ~~eigenmodes~~ eigenmodes of selected, simple molecules. We will show, in fact, in

each case, if the mode is Raman and/or IR active.

## 2) Normal modes of simple molecules and spectroscopic Activity.

The simplest molecule is a diatomic one (linear molecule) with identical atoms, like e.g.  $N_2$ . Such a molecule possesses a single vibrational degree of freedom: the stretching one. The molecule does not have any dipole moment, so that the stretching is IR inactive. Its polarizability tensor (ellipsoidal) however changes with the distance between the two atoms, so that the stretching is Raman active.

On the contrary — a molecule like HCl possesses a well defined dipole moment so that in such a case the stretching is both Raman and IR active.

~~More complicated~~ A situation a bit more complicated arises in a linear triatomic molecule, like  $CO_2$  (see fig. 1). Here we have two kinds of stretching (symmetric and antisymmetric) and a bending mode. The molecule in itself does not possess a permanent dipole moment, but a moment appears in the consequence of the antisymmetrical stretching and of the bending mode. In fig. 2 we report the behaviour of the derivative of both the dipole moment and of the polarizability. Taking into account the values of such derivatives at  $q=0$ , it follows that:

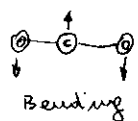
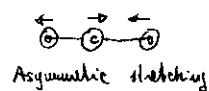
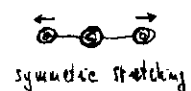
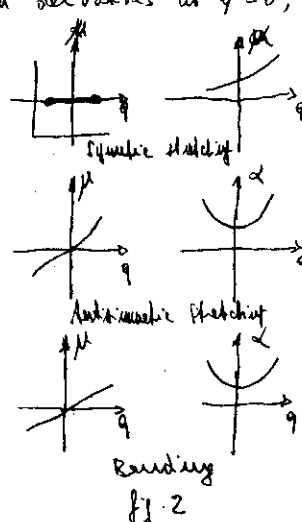


fig. 1

- i) Symmetrical stretching is Raman active, but IR inactive;
- ii) Antisymmetrical stretching is Raman inactive, but IR active;

iii) Bending is Raman inactive and IR active.

It can be seen that in such a case the Raman activity excludes the IR activity and vice-versa. The origin of such a circumstance stays in the fact that the molecule possesses a "centre of symmetry". Every molecule possessing a



a center of symmetry (e.g. benzene, ethylene etc) obeys to such a principle of mutual exclusion.

As the complexity of the molecules increases, also increases the number of vibrational degree of freedom, and an exact assignment of the peaks showed in the experimental spectra is not easy. In such a case a theoretical analysis (e.g. group theory) as well as a comparison between IR and Raman spectra can be very useful.

It is also to be noticed that the frequency of a mode can change if the involved group of atoms are bonded as a part of different molecule. For example the  $C=O$  ~~stretching~~ ~~group~~ appears in many functional groups, and its stretching frequency changes accordingly.

## 3) Localized and collective modes

When Raman scattering from condensed matter, or also from atomic groups belonging to a macromolecule are involved, there are reasons to distinguish between localized vibrational modes and collective behaviour.

In principle we speak of localized modes if the phases of vibrations, belonging to the same normal mode, ~~for different~~ but interesting different molecules (or atomic groups in the same macromolecule) are uncorrelated and randomly distributed. In such a case the radiation scattered by different scattering centers is incoherent, and the ~~the~~ angular distribution of intensity (at the frequency of the involved eigenmode) does not show any peculiarity, apart from a geometrical factor: neither form factor, nor structure factor are showed.

In some case, however, there is a precise relationship between the phases of vibrations of neighbouring molecules or atomic groups. In such a case one cannot longer speak of eigenmodes of a single molecule, but, rather, one is concerned with a collective excitation (a phonon). ~~For~~ In such a case a strong angular dependence of scattered light takes place, that gives information about the spatial arrangement of the scatterers. This is the case, for example, in molecular crystals, where the vibrations of single molecules are coupled, and behave like an elastic wave travelling the system (optical phonon).

Up to now, however, no extensive investigations have been performed about angular dependence of Raman scattering ~~in~~ in macromolecules, although



such kind of measurement could be very interesting.

Apart from Raman scattering, low frequency modes likely are connected with collective behaviour, involving translational and rotational degrees of freedom. We will see in the last chapter, for example, that diffusion coefficient turns out to be a non-local quantity in the sense that the characteristic time of concentration fluctuations depends on the wavelength of the fluctuation itself.

#### 4) ~~See~~ Special Raman techniques.

In the recent years many experimental techniques have been ~~employed~~ used to improve Raman scattering observation. Such new techniques are of relevant importance for the identification of biological matter, because in such a case, usual Raman techniques suffers of some trouble. First of all a Raman spectrum from macromolecules shows a rather complicated pattern, because of the large number of vibrational degrees of freedom, involving simple atoms, ~~and~~ functional groups and large sub-units. As a consequence it is rather difficult to separate a wanted contribution. In addition solutions of biological molecules shows in almost all cases a more or less pronounced fluorescence, whose origin up to now is not clearly understood, that ~~makes the Raman~~ blurred the true Raman spectrum. In the present section, we notice shortly on some of such implemented Raman techniques.

##### 4.a) Resonant Raman scattering

From a quantitative point of view, the physical process that originate the Raman scattering can be seen as follows (see Fig. 3): an electronic state is splitted because of vibrational degree of freedom, the spacing between splitted levels corresponding to the vibrational eigenfrequency  $\Omega$ . Incoming photons of frequency  $\omega_i$  raise the electron to a virtual state, from which the electron decays again to a ground level corresponding to the vibrationally excited ground state. The emitted photon has a frequency  $\omega_s = \omega_i - \Omega$ .

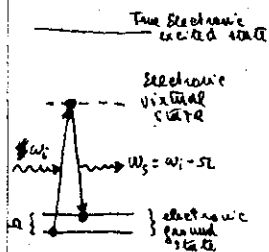


Fig. 3

The virtual state to which the scatterer is excited after the absorption of the incident photon, is an unstable one, ~~falling~~ in general, to a forbidden energy value.

From an intuitive point of view, such a state can be reached because of the indeterminacy principle.

It is possible to make a calculation from which one can see that the transition probability for a process of the kind depicted in Fig. 3 is inversely proportional to the energy difference between the ~~two~~ allowed excited states and the virtual one. As a consequence such a probability becomes larger and larger when the energy of the incident photon becomes equal to the energy of an allowed electronic state. ~~(i.e. when the frequency of incident light corresponds to an absorption band)~~ In such a case one speaks of "Resonant Raman Scattering" (see Fig. 4), whose magnitude can be order of magnitude larger than the usual Raman scattering.

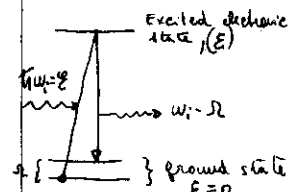


Fig. 4

Resonant Raman scattering is a formidable tool for the study of macromolecules, because it allows the enhancement of those vibrational lines involving the atomic group to which the excited electronic state is referred (chromophore).

Such a relevance can be easily understood if one thinks that usually, in a macromolecule, the site of biological activity coincide or is close to a chromophore group: for example in haemoglobin the oxygen atom attaches to the iron atom that is at the center of the heme group, the latter being a chromophore.

Obviously the use of Resonant Raman effect require the availability of a continuously tunable light source (e.g. a dye laser), in order to match the frequency of the incident beam with the frequency of ~~the~~ absorption band of the group under investigation.

##### 4.b) Coherent Anti-Stokes Raman Spectroscopy (CARS)

Usually Raman lines (i.e. corresponding to a frequency higher than the incident one) are of very small intensity and become practically undetectable for vibrational frequencies larger than some tenth of  $\text{cm}^{-1}$ . This is because such a process correspond to the annihilation of a vibrational quantum of energy and at normal temperature vibrational excited state are ~~practically~~ practically empty. In the CARS effect such excited state are out of equilibrium by means of a combination of the usual Raman effect and the stimulated emission process.

In the CARS effect the sample is illuminated by two light beams, one (the pump) with a fixed frequency  $\omega_1$ , the other ~~at a~~ with a continuously variable frequency  $\omega_2$ . If  $\omega_2$  becomes equal to  $\omega_1 - \Omega$ , being  $\Omega$  the vibrational frequency under investigation, to the absorption of two quanta  $\hbar\omega_1$  and  $\hbar\omega_2$  correspond the emission of two quanta  $\hbar\omega_1$  and  $\hbar\omega_2$ , the system being leaved in a vibrational excited status (i.e. an excitation  $\hbar\Omega$  is created). As a consequence to a successive absorption of a quantum  $\hbar\omega_1$ , correspond the emission of a quantum  $\hbar(\omega_1 + \Omega)$  (i.e. Anti-Stokes line), the vibrational excitation being annihilated (see Fig. 5). In a sense the process is like to that of a so called parametric amplifier or in a MASER, where  $\omega_1$  correspond to the pump frequency,  $\Omega$  is the signal frequency and  $\omega_2$  the idler frequency.

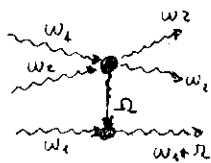


Fig. 5

A CARS spectrum is obtained by scanning the frequency  $\omega_2$ , and recording the emitted intensity as a function of  $\omega_1$ . The advantage of the CARS technique is to avoid the trouble

of the fluorescence: the latter, in fact, has always a frequency lower than the exciting one, so that is superimposed to the usual Stokes spectrum of ordinary Raman effect ( $\omega_1 - \Omega$ ). On the contrary the frequency of the Anti-Stokes lines is larger than the incident one and is not disturbed by the fluorescence. However, such a technique present some ~~technical~~ <sup>major</sup> technical difficulties, like the collimation of the two incident beams, the continuous scan of  $\omega_2$  and so on.

#### 4.c) Time Resolved Raman Spectroscopy

Another method, developed to avoid the fluorescence trouble, takes advantage of the very different times characteristic of the Raman and fluorescence effect, respectively. In fact the lifetime of the virtual status implied in the Raman effect is of the order of  $10^{-13}$  to  $10^{-14}$  sec, while fluorescence takes place with a delay of the order of  $10^{-9}$  to  $10^{-7}$  sec.

Therefore, by using a very short pulse of laser light (of the order of some picosecond), it is possible, through a suitable electronic gating, to detect the scattered light before that fluorescence can takes place.

~~Back at the~~ Notwithstanding its conceptual simplicity, such a technique is indeed a very complicated one.

First of all it requires the generation of very short light pulse, that in turns must be intense enough to give a feasible signal in a very short time. In addition the spectrum of scattered light cannot be obtained by usual scanning monochromator, but it is to be recorded simultaneously by means of suitable array of detector (up to  $10^{-9}$  sec) or, for faster detection, by spreading the signal using electronic beam (streak camera, up to  $10^{-12}$  sec).

#### 5] The Raman Spectrometer

We conclude this chapter by describing shortly the spectrometer that is usually ~~employed~~ is adopted for Raman spectroscopic measurement.

In principle the working instrument is a diffraction grating that disperses the collected scattered light, according to its spectral distribution. However, because the Raman signal is ~~very~~ very faint (in some cases the ratio between incident and Raman-scattered light exceeds  $10^9$ ), not only a great dispersion power is required, but also a high reception power. Usually the latter requirement is ~~not~~ fulfilled by using double (or else triple) monochromators: light dispersed by the first is re-focused and then re-dispersed by the second one.

In Fig. 6 a schematic view of the instrument is shown. The most commonly used geometrical arrangement correspond to a  $90^\circ$  scattering. In Fig. 6 L is the laser, M the mirrors, and X is the sample, usually contained in thin cylindrical tube optically polished. The light scattered at  $90^\circ$  is collected by the collecting optics O. A polarization analyzer P allows the detection of both the VV or the VH spectrum. Through the entrance slit  $S_1$ , light reaches the first ~~monochromator~~ diffraction grating  $G_1$ , and is therefore dispersed. A ~~small~~ small spectral interval is then selected by the intermediate slit  $S_2$ , and again dispersed by the second diffraction grating  $G_2$ . Ultimately the spectral interval is selected

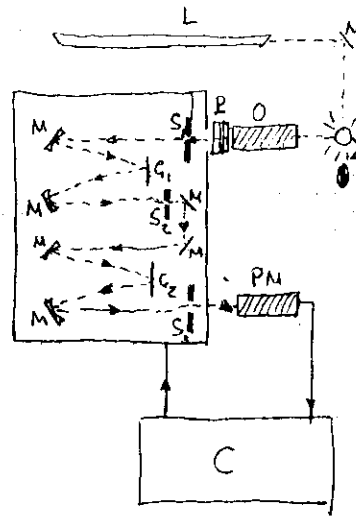


Fig. 6

by the exit slit  $S_2$  and revealed by a photomultiplier PM. A computer C collects and processes the signal, as well as controls the ~~double monochromator~~ <sup>double monochromator</sup>.

The analysis of the spectrum is performed by rotating the diffraction gratings, so that different part of the spectrum fall onto the ~~intermediate~~ <sup>intermediate</sup> and the exit slits. It is quite clear that the rotations of the two gratings are to be strictly correlated: this is obtained through a suitable mechanical connection (cosecant bar). In order to have a good optical purity and a high enough dispersion and rejection power, the gratings are very large (up to 10 cm), and ~~they~~ <sup>they</sup> possess up to 1800-2000 lines per millimeter. Gratings of such a type are obtained by means of holographic techniques. Sometimes the gratings are ~~blazed~~ <sup>blazed</sup> in such a way that the second diffraction order can be revealed, in order to increase the dispersion power. The selectivity of the instrument can be regulated by the slits. ~~p~~ <sup>p</sup>.

In a normal operation mode, the collection of a spectrum (from 0 to 3000  $\text{cm}^{-1}$ , say) requires a long time, because of the weakness of the signal that implies long acquisition times. Sometimes it could be useful to ~~accumulate~~ <sup>accumulate</sup> several spectra, each of one registered in a short time (and therefore of poor quality) and then to add the spectra, in order to minimize unwanted spurious signal.

Finally, because the long exposure to laser light can damage the sample (e.g. by heating), especially in the case of coloured substances, the sample itself can be continuously rotated, in order to expose to light different volume element. In some cases ~~the sample~~ <sup>the sample</sup> a liquid sample can be shaped as a Laminar jet, so that it is continuously renewed during the measurement.

## 6) Raman spectroscopy and conformation of macromolecules.

As previously discussed, each line in a Raman spectrum correspond to a same vibrational mode of the molecule, the frequency of the mode being related to the masses and force constant that are involved in the vibration. For example the stretching of a diatomic molecule is characterized by the frequency

$$\omega = \sqrt{\frac{k}{\mu}} \quad 4.6.1$$

where

$$\mu = \frac{m_A m_B}{m_A + m_B} \quad 4.6.2$$

is the "reduced mass", being  $m_A$  and  $m_B$  the masses of the two atoms and  $k$  is the force constant.

In an actual Raman spectrum, however, the various lines are more or less broadened, so that one is concerned with bands, having a characteristic shape and width. The ~~largest~~ <sup>largest</sup> of the broadening is originated by many circumstance. First of all the vibrational mode itself is more or less damped. For a simple exponential damping of the mode, the corresponding line is broadened in a Lorentzian band, whose width correspond to the damping constant. Such kind of ~~the~~ <sup>the</sup> broadening will be the same for each molecule, and is often called "homogeneous broadening". On the other hand the frequency of the mode can be ~~more~~ <sup>more</sup> slightly modified, according to the structural arrangement involving the vibrating masses and their nearest neighbours. As an

example, we show in fig. 7 the Raman band ~~of the~~ <sup>of the</sup> corresponding to the C=O stretching in acetic acid, respectively in the monomer and in dimer state: the occurrence of the hydrogen bond modify the stretching frequency.

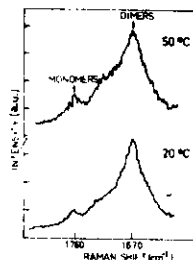
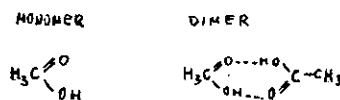


Fig. 7. Raman spectrum of acetic acid showing the band of the C=O stretching.

Fig. 7

In an actual physical system, ~~as~~ <sup>in</sup> general, different molecules can be in different conformational state, so that the same vibrational mode takes place at slightly different frequencies in different molecules. As a consequence one reveals a superposition of lines, each ~~more~~ <sup>more</sup> broadened, that gives rise to a larger band, whose shape can be ~~more~~ <sup>more</sup> less regular and depends on the distribution of the various conformational arrangement. Such a broadening

is usually called "inhomogeneous".

A relevant consequence of such a situation, is that in general the shape ~~not~~ <sup>not</sup> of a Raman band turns out to be ~~more~~ <sup>more</sup> dependent from the temperature, as well as from other thermodynamic variables like ~~the~~ <sup>the</sup> pressure, concentration and so on. Actually the eigenfrequencies of a molecule are not modified by temperature changes. It is the distribution among various structural and conformational arrangements that changes according to the thermodynamic status of the system.

Let us, finally, mention, among the causes of inhomogeneous broadening, the

possible existence of different isotopic species of the same atom.

From the above discussion it is quite clear that Raman spectroscopy can be fruitfully used to obtain informations about the structure of a macromolecule.

In order to give a practical example, we discuss in some details the vibrational behaviour - and therefore the corresponding Raman spectrum - of a peptide bond.

A peptide bond <sup>consists in a amide group, that</sup> connects different amino-acids, and is schematically indicated in fig. 8. The chemical bonds of the amide-group -CONH- maintain

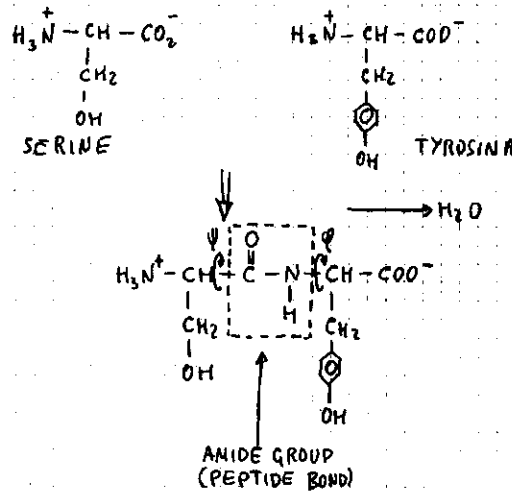


fig. 8

the latter in an essentially planar configuration. However rotations ( $\psi$  and  $\phi$ , called Ramachandran angle) of the neighbouring amino-acid are allowed. Depending on such angle, a polypeptide can obviously assume different configuration (secondary structure). On the other hand the vibrational modes of the amide-group in itself are more or less affected by the actual values of the angle  $\psi$  or  $\phi$ , so that an analysis of the Raman bands corresponding to the amide-group modes, results in a structural analysis

concerning the secondary structure of the polypeptide chain.

In the Raman spectrum of the amide-group, up to seven bands can be recognized; in addition there are "combination" bands as we shall see later.

The most important vibrational modes, that gives rise to pronounced Raman bands are the I and III. The amide II band is weak in Raman spectrum, but is strong in the IR one. Its origin is shown in fig. 9. Amide I, II and III bands are associated with out-of-plane vibration, and give a <sup>characteristic</sup> contribution to Raman spectra.

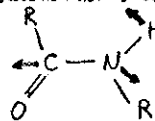


fig. 9

The amide-III band is a bending of the CONH group. Similar kind of bending appears also in other groups so that its identification is often questionable.

The physical origin of the important I & II amide bands ~~is~~ shown in fig. 10. The Amide-I band is essentially related to the carbonyl stretching, or small contribution coming from the NH bending. Its frequency is usually between 1600 and 1700  $\text{cm}^{-1}$ , depending on the kind of polypeptide and on its conformation.

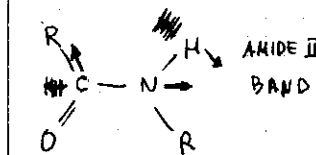


fig. 10

The amide-III band is strongly affected by the conformation, essentially through the value of the  $\psi$  angle. Such a band can be recognized easily by means of the isotopic substitution of hydrogen with deuterium (the band shifts to the lower frequency of about 1000  $\text{cm}^{-1}$ ). ~~These are also two bands~~

In addition there are also two bands, called A and B bands, that arise as combination bands, from a phenomenon called "Fermi resonance" (see fig. 11).

The second harmonic (overtone) of the amide-II band falls very near another vibrational frequency, namely the NH stretching. In such a condition there are a mode-mode coupling that results in a splitting of the two quasi-coincident frequencies in two bands well separated: in a sense the overtone is intensified - but its frequency is lowered - ~~from~~ by energy exchange from the fundamental (NH stretching), whose frequency, in turns, is raised.

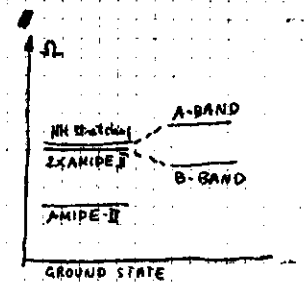


fig. 11