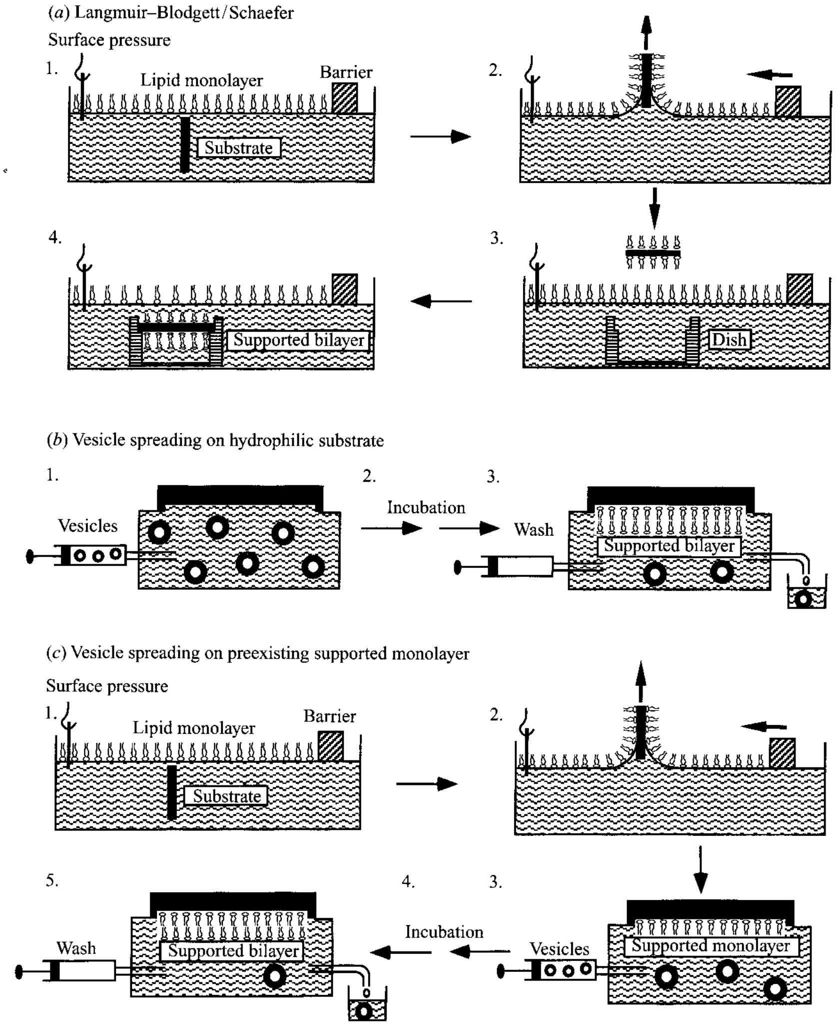
be accomplished by using the Langmuir/Blodgett/Schaefer technique for the generation of the so-called supported phospholipid bilayers (Figure 2.20). A lipid mono-layer is first transferred from the air-water interface of a Langmuir trough to the hydrophilic support at a constant surface pressure of 32-36 mN/m, i.e. the bilayer equivalence pressure. A second monolayer is deposited onto this surface by horizontal apposition of the substrate to a monolayer at the same pressure. The substrate with the bilayer is then collected in a dish or measuring cell under water, avoiding any subsequent exposure to air. Fully hydrated high quality bilayers are obtained in this way. However, this method is inadequate for reconstituting membranes proteins. A second method to prepare supported bilayers is by spreading vesicles on a hydrophilic substrate. A dispersion of small unilamellar lipid vesicles, which may also include reconstituted membrane proteins is brought into contact with the clean surface of the internal reflection

Preparation of fully hydrated supported bilayers



Methods for preparation of fully hydrated supported lipid bilayers. (*a*) Langmuir-Blodgett/Schaefer technique. (*b*) Spreading of small unilamellar vesicles on a hydrophilic substrate. (*c*) Spreading of small unilamellar vesicles on a preexisting supported monolayer.

Figure 2.20 Techniques to prepare supported bilayers for the use in ATR spectroscopy.

element assembled in a liquid holding cell. These vesicles will spontaneously spread on the substrate and form a continuous planar bilayer in about an hour at room temperature. Again, these bilayers must not be exposed to air after formation, but excess vesicles can be easily flushed out of the cell by a large volume of buffer. Most of the studies performed today have been performed with third method, in which vesicles are spread on a preexisting monolayer. In this method, which is also often referred to as monolayer fusion, a lipid mono-layer is first transferred from the air-water interface of a Langmuir trough to the hydrophilic internal reflection element at the bilayer equivalence pressure. The monolayer-coated plate is then assembled in the ATR holding cell and a dispersion of lipid vesicles with or without protein is then injected. As in the direct spreading method, a bilayer forms by spontaneous self-assembly and excess vesicles are removed by flushing the cell with buffer. A D_2O containing buffer may be introduced either at this or at a later stage. This monolayer fusion method allows the reconstitution of integral membrane proteins.

Samples like the supported bilayers on a total internal reflection element are different from samples that are prepared in normal cuvette. In a cuvette, the samples are isotropically oriented an the radiation interacts with the molecules from any possible direction. By contrast, on a supported bilayer the samples are oriented an additional information can be obtained.

As an example we consider the UV-absorption spectrum of Poly-L-glutamine. The absorption of the UV light is monitored parallel and perpendicular to the long axis of the molecules. The absorption is different, because changes in the transition dipole moment of the molecules have different components along the two axis. The different absorption in the two directions is called *Linear Dichroism*. For each substance there is a specific ratio of the extinction coefficients

$$d = \frac{\varepsilon_{||} - \varepsilon_{\perp}}{\varepsilon_{||} + \varepsilon_{\perp}}$$

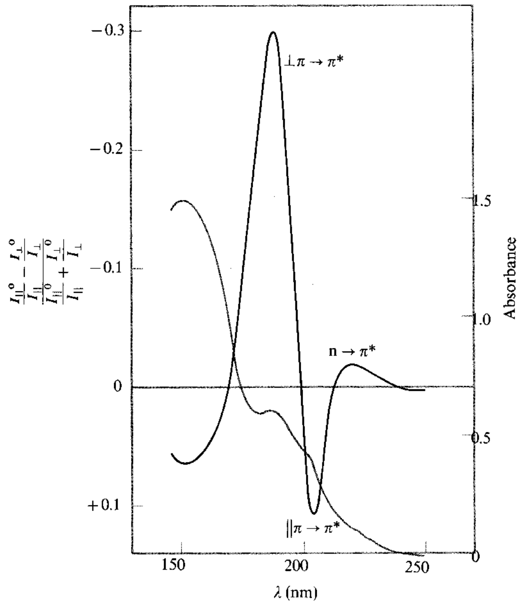
with the Beer Lambert Law

$$A = \log\left(\frac{I_0}{I}\right) = -\log(T) = \varepsilon \cdot c_M \cdot l$$

the dichroic ratio is also given as

$$d = \frac{A_{||} - A_{\perp}}{A_{||} + A_{\perp}}$$

Linear dichroism of a poly-L-glutamic acid film

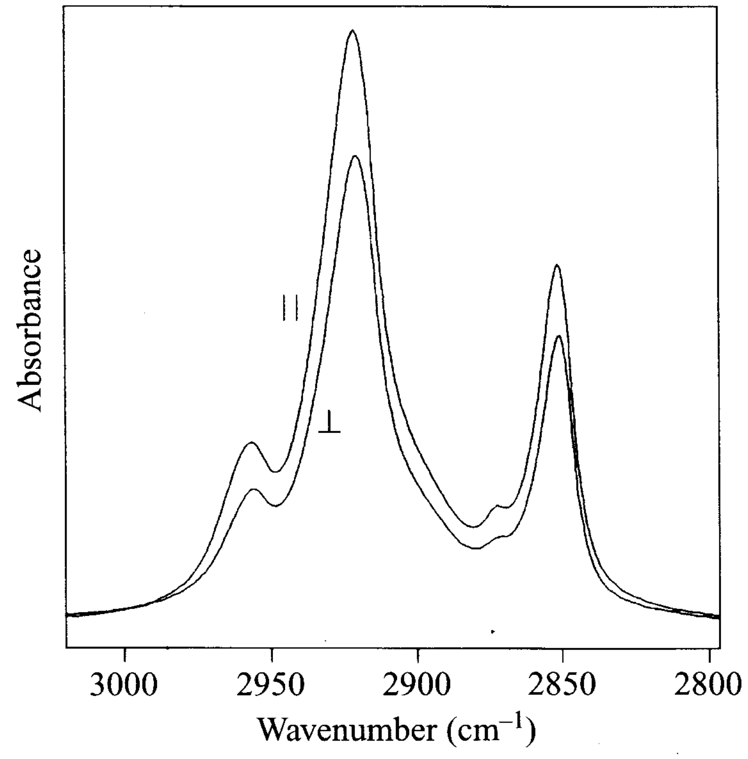


Linear dichroism (black curve) *and absorbance* (gray curve) of a poly-L-glutamic acid film. The polypeptide has an α-helical structure. Bands assigned to the n → π* and to the parallel and perpendicular polarized π → π* transitions are indicated on the dichroism spectrum. Note that this spectrum is plotted in terms of raw light intensities rather than absorbance. [After J. Brahms, J. Pilet, H. Damang, and V. Chandrasekharan, *Proc. Natl. Acad. Sci. USA* 60:1130 (1968).]

Figure 2.21 Linear dichroism of poly-L-glutamic acid

To determine the dichroic ratio, it is necessary to use linearly polarized light (i.e. the electrical field vector of the light oscillates only in one direction). If the transition dipole moment is known for a particular absorbance, one can determine the direction of the molecule by measuring the dichroic ratio.

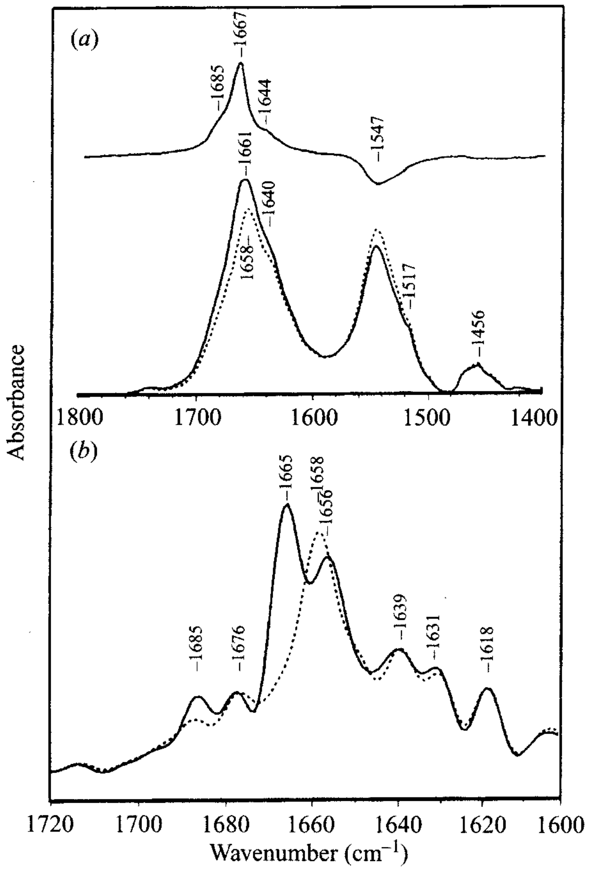
Linear dichroism of lipid molecules in supported planar bilayers
by ATR-FTIR spectroscopy



ATR–FTIR spectra in the methylene stretching region of a fully hydrated single supported bilayer of DMPC, prepared by the monolayer fusion technique, at parallel and perpendicular polarizations of the IR beam. The measuring temperature was approximately 21 °C. The dichroic ratio and the derived order parameter are 1·25 ± 0·02 and 0·45 ± 0·02, respectively. (Reproduced from Tatulian *et al.* 1995*b*.)

Figure 2.22 Linear dichroism of phospholipid molecules obtained by ATR-FTIR spectroscopy.

Polarized transmission FTIR spectra in the amide I and amide II region of bacteriorhodopsin



Polarized transmission FTIR spectra in the amide I and II region of bacteriorhodopsin in hydrated oriented purple membranes. (*a*) Spectra recorded with a parallel (solid line) and perpendicular (dashed line) polarized IR beam. The spectrum on the top is the linear dichroism spectrum, $A_{\|}-A_{\perp}$. (*b*) Expanded amide I region of spectra recorded with a parallel (solid line) and perpendicular (dashed line) polarized IR beam at 80 K after Fourier self-deconvolution. The main amide I peak is split into two components (~ 1665 and ~ 1656 cm^{-1}) of which the higher frequency component exhibits stronger dichroism. (

Figure 2.23 Polarized FTIR spectra of bacteriorhodopsin in the amide I and amide II regions.

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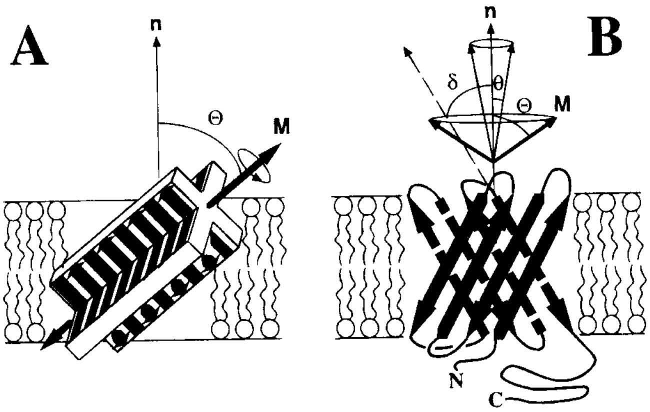


FIGURE 8: Models illustrating symmetry relations between β-sheets, their amide I transition dipole moments, and a planar lipid bilayer. *Panel A:* The orientation of planar antiparallel β-sheets is not uniquely determined by the angle Θ between the transition dipole moment M and the membrane normal n. The β-sheets can assume many orientations that are rotationally symmetric around M. Two orthogonal orientations are shown. When $\Theta = 0°$, the strands run parallel to the plane of the membrane, but adjacent strands of the sheet penetrate the membrane. When $\Theta = 90°$, the strands may run parallel to the plane of the membrane with alternating residues up and down, respectively, or they may be oriented parallel to the membrane normal. *Panel B:* The orientation of a β-barrel relative to the bilayer normal (angle θ) is determined by the angle Θ and the angle δ of the strands relative to the bilayer normal.

Figure 2.24

2.9. Approximate frequencies of amino acid side chain absorptions in the 1400-1800 cm^{-1}-region.

Vibration			in H_2O ν_0 (cm^{-1})	in D_2O ν_0 (cm^{-1})
Phe	ring		1494	
Tyr	ring—0$^-$		1498	1500
terminal	—NH^{3+}	(δ_s)	1515	
Tyr	ring-OH		1518	1615,1515
Lys	—NH^{3+}	(δ_s)	1526	
terminal	-NH$_2$	(δ)	1560	
Glu	—COO$^-$	(ν_{as})	1560	1567
Asp	—COO$^-$	(ν_{as})	1574	1584
His	ring		1596	
terminal	—COO$^-$	(ν_{as})	1598	1592
Tyr	ring--0-		1602	1603
Gln	—NH$_2$	(δ)	1610	
Asn	—NH$_2$	(δ)	1622	
Lys	—NH^{3+}	(δ_{as})	1629	
terminal	—NH^{3+}	(δ_{as})	1631	
Arg	—CN$_3$H$_5^+$	(ν_s)	1633	1586
Gln	—C=O	(ν)	1670	1635
Arg	—CN$_3$H$_5^+$	(ν_{as})	1673	1608
Asn	—C=O	(ν)	1678	1648
Glu	—COOH	(ν)	1712	1706
Asp	—COOH	(ν)	1716	1713
terminal	—COOH	(ν)	1740	1720

'Adapted from Chirgadze et al., 1975 and Venyaminov & Kalnin, 1990a,b

δ_s: symmetric deformation vibration (bending vibration)
δ_{as}: asymmetric deformation vibration
ν_s: symmetric stretching vibration
ν_{as}: asymmetric stretching vibration

Table 2.3 IR absorption frequencies of amino acid sidechains.

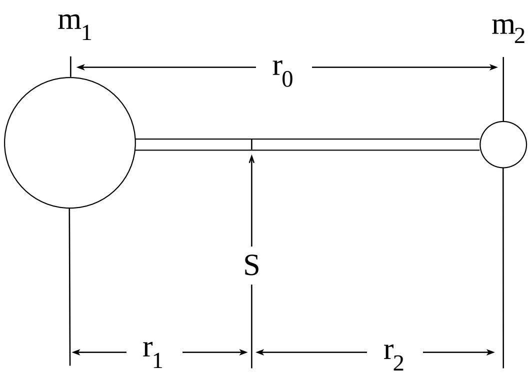
In nucleic acids the melting of the double helix can be monitored by IR spectroscopy.

2.10. Fine Structure of Infrared Spectra

Rotational spectra and the rotating diatomic molecule.

Rotational energy, like other forms of molecular energy is quantized, which means that a molecule cannot have any arbitrary amount of rotational energy (and thus an arbitrary value of its angular momentum). The energy is limited to certain definite values, depending on the shape and the size of the molecule concerned. The permitted energy values — the so-called rotational energy levels — can in principle be calculated by the Schrödinger equation. But even for simple molecules the mathematical analysis is complex and for bigger molecules, it is necessary to work with approximations. Here we will just describe some very basic principles on the example of a simple diatomic molecule.

The rigid diatomic molecule



A rigid diatomic molecule treated as two masses
connected at a rigid distance. S: center of gravity.

Figure 2.25 The rigid diatomic molecule

Two atoms (or masses) are joined by a rigid bar (the bond) whose length is

$$r_0 = r_1 + r_2$$

The molecule rotates end-over-end about a point S, the center of gravity, which is defined by the moment of balancing equation:

$$m_1 r_1 = m_2 r_2$$

The moment of inertia about C is defined by:

$$I = m_1 r_1^2 + m_2 r_2^2$$

or

$$I = m_2 r_2 r_1 + m_1 r_1 r_2 = r_1 r_2 (m_1 + m_2)$$

Since $m_1 r_1 = m_2 r_2 = m_2 (r_0 - r_1)$ the distances r_1 and r_2 from the center of gravity can be substituted by the distance r_0 between the two atoms (masses):

$$r_1 = \frac{m_2 r_0}{m_1 + m_2} \text{ and } r_2 = \frac{m_1 r_0}{m_1 + m_2}$$

The moment of inertia can now be written as

$$I = \frac{m_1 \cdot m_2}{m_1 + m_2} r_0^2 = \mu r_0^2$$

The quotient

$$\mu = \frac{m_1 \cdot m_2}{m_1 + m_2}$$

is called the reduced mass of the system. The moment of inertia for the diatomic molecule is therefore described by the atomic masses and the bond length.

Applying the Schroedinger equation to the rotating diatomic molecule, it can be shown that the rotational energy levels are given by the expression:

$$E_j = \frac{h^2}{8\pi^2 I} \cdot J \cdot (J+1), \text{ with J = 0, 1, 2, 3, ...}$$

I is the moment of inertia, h is Planck’s constant, and J is the rotational quantum number. As a direct result of solving the Schroedinger equation, J can only be zero or a positive integer number. Therefore the rotational energy can have only discrete values, the energy levels of rotation of the molecule. The corresponding frequencies, $\nu = \frac{\Delta E}{h}$, or wavenumbers of rotation,

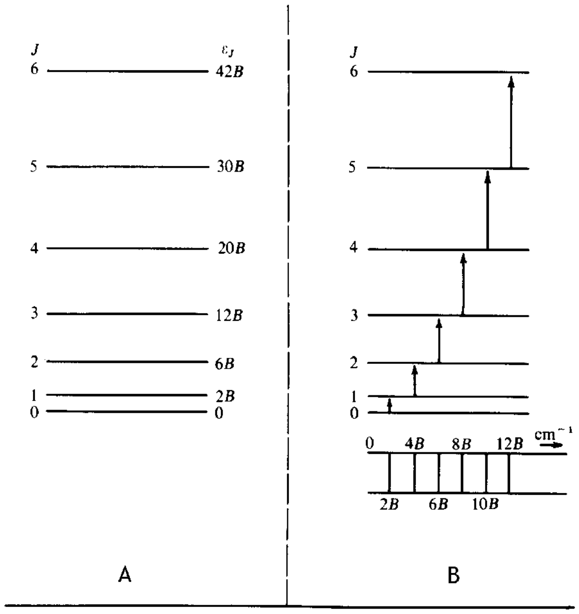
$\tilde{\nu} = \frac{\Delta E}{hc}$ can also be used to define the possible energy levels. For rotational and vibrational spectroscopy it is common to describe the energy levels in wavenumbers (cm^{-1}):

$$\varepsilon_j = \frac{E_J}{hc} = \frac{h}{8\pi^2 Ic} J(J+1) \qquad (\mathrm{cm}^{-1})$$

The equation is usually simply written as:

$$\varepsilon_J = BJ(J+1) \quad (\mathrm{cm}^{-1}) \qquad \text{with} \quad B = \frac{h}{8\pi^2 I_B c} \qquad (\mathrm{cm}^{-1})$$

The relation between the rotational quantum number J and the energy levels ε_j allow us to draw the energy diagram. For J=0 the energy is also 0 and the molecule does not rotate. For J=1 the energy is 2B and the molecule has its lowest angular momentum. These and higher energy levels are given in Figure 2.26 below:



A: Rotational energy levels of a rigid diatomic molecule
B: Allowed transitions between the energy levels of a rigid diatomic molecule

Figure 2.26 Rotational energy levels of the rigid diatomic molecule

In principle, there is no limit how rapidly a molecule may rotate. However, the centrifugal force on the atoms may be greater then the strength of the bond and the molecule is then disrupted, but this point is not reached at normal temperatures.

As indicated in the Figure above, the energy difference between different rotational states depends on the levels between a transition takes place. If we imagine the molecule to be in the ground state (J=0) the incident radiation can be absorbed to raise it to the J=1 state. The energy that is necessary is given by

$$\varepsilon_{J=1} - \varepsilon_{J=0} = 2B - 0 = 2B \ [\mathrm{cm}^{-1}]$$

In other words, an absorption line will appear at 2B cm^{-1}.

If the molecule is then raised from the J=1 to the J=2 level by absorption of more energy, the corresponding energy difference is

$$\begin{aligned} \tilde{v}_{J=1\to J=2} &= \varepsilon_{J=2} - \varepsilon_{J=1} \\ &= 6\ \mathrm{B} - 2\ \mathrm{B} = 4\ \mathrm{B}\ (\mathrm{cm}^{-1}) \end{aligned}$$

In general to raise the molecule from the state J to state J+1, we would have:

$$\begin{aligned} \tilde{v}_{J\to J+1} &= B(J+1)(J+2) - BJ(J+1) \\ &= B(J^2 + 3J + 2 - J^2 - J) \\ &= B(2J+2) \\ &= 2B(J+1) \end{aligned}$$

Thus, a incremental raise of the energy of rotation leads to absorption lines at 2B, 4 B, 6B, 8B, ..., cm^{-1}. Until now, we have assumed that transitions between energy levels occur only between immediate neighbors on the energy scale. We have not considered that transitions may occur, for example between J=0 and J =2. Quantum mechanical consideration show that such transitions are indeed “forbidden”. The selection rule for transitions between rotational energy levels is $\Delta J = \pm 1$.

The relation

$$\tilde{v}_{J\to J+1} = 2B(J+1)$$

gives the *whole* spectrum of the molecule, provided that the molecule has a dipole moment (it has to be asymmetric, heteronuclear). HCl, NO, CO will show a rotational spectrum, while H_2, CO_2, N_2 and O_2 will not.

2.10.1 Effect of isotopic substitution.

When one atom in the molecule is replaced by its isotope, the same element but with a different number of neutrons in the nucleus and therefore with a different mass, the resulting molecule is chemically identical with the original molecule. In particular, there is no appreciable change in internuclear distance on isotopic substitution. However, there is a change in the reduced mass of the molecule, which results in change of the moment of inertia

$$I = \frac{m_1 \cdot m_2}{m_1 + m_2} r_0^2 = \mu r_0^2 .$$

The moment of inertia is directly related to the rotational constant B

$$B = \frac{h}{8\pi^2 I_B c}$$

Gilliam et al. found the first rotational absorption line of $^{12}C^{16}O$ to be at 3. 84235 cm^{-1}, while that of $^{13}C^{16}O$ was at 3.67337 cm^{-1}. The values of B can be calculated from these wavenumbers: B($^{12}C^{16}O$)=1.92118 cm^{-1}, B($^{13}C^{16}O$)=1.83669 cm^{-1}. The ratio B($^{12}C^{16}O$) / B($^{13}C^{16}O$) is then:

$$\frac{\mathrm{B}(^{12}\mathrm{C}^{16}\mathrm{O})}{\mathrm{B}(^{13}\mathrm{C}^{16}\mathrm{O})} = \frac{h}{8\pi^2 I(^{12}\mathrm{C}^{16}\mathrm{O})c} \cdot \frac{8\pi^2 I(^{13}\mathrm{C}^{16}\mathrm{O})c}{h} = \frac{I(^{13}\mathrm{C}^{16}\mathrm{O})}{I(^{12}\mathrm{C}^{16}\mathrm{O})}$$

and finally

$$\frac{\mathrm{B}(^{12}\mathrm{C}^{16}\mathrm{O})}{\mathrm{B}(^{13}\mathrm{C}^{16}\mathrm{O})} = \frac{\mu(^{13}\mathrm{C}^{16}\mathrm{O})}{\mu(^{12}\mathrm{C}^{16}\mathrm{O})}$$

which is 1.046. The introduction of the isotope ^{13}C into carbon monoxide thus leads to smaller wavenumbers of the absorbed radiation (and therefore longer wavelength and smaller frequencies).

The composition of isotopes in a given substance can be determined using rotational absorption spectroscopy.

Until now, it was assumed that the bond is rigid. However, we know that there are vibrations between the connected atoms. These vibrations result in a change of the distance between the atoms. Furthermore, increased rotational energy and an increase in the rotational frequency results in an increase of the centrifugal force that works against the force that connects the two atoms. Thus, the bond length will increase with the rotational frequency.

$$F = -k(r - r_0) = 4\pi^2 \nu_{rot}^2 r\mu$$
$$r = \frac{kr_0}{\left(k - 4\pi^2 \nu_{rot}^2 \mu\right)}$$

From vibrational spectroscopy we know that in case of a harmonic oscillation, the oscillation frequency is given by

$$\nu_{vib} = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

which can be rearranged to give the force constant k:

$$k = 4\pi^2\nu_{vib}^2\mu = 4\pi^2\tilde{\nu}_{vib}^2c^2\mu$$

where $\tilde{\nu}$ is the wavenumber (in cm^{-1}), c the speed of light, μ the reduced mass and k the force constant of the vibration.

The second consequence of elasticity is that the quantities B and r vary during a vibration.

From the definition of B

$$B = \frac{h}{8\pi^2 I_B c} = \frac{h}{8\pi^2 c\mu r^2}$$

it follows that B is proportional to the reciprocal square of the distance between the two atoms. However, in a simple harmonic motion a molecular bond is compressed and extended an equal amount on each side of the equilibrium distance (r_e). The average value of this distance is therefore unchanged. The average value of $1/r^2$ is not equal to the average value of $1/r_e^2$. To give an example, we consider a bond of an equilibrium length, 0.1 nm that vibrates between the limits 0.09 and 0.11 nm. We have:

$$\langle r\rangle_{av} = \frac{0.09+0.11}{2} = 0.1 = r_e$$

and

$$\left\langle\frac{1}{r^2}\right\rangle_{av} = \frac{\left(1/0.09\right)^2+\left(1/0.11\right)^2}{2} = 103.05\ \text{nm}^2$$

which results in a radius of $\sqrt{103.05\ \text{nm}^2} = 0.0985\ \text{nm}$. The difference is not negligible compared with the precision with which B can be measured spectroscopically.

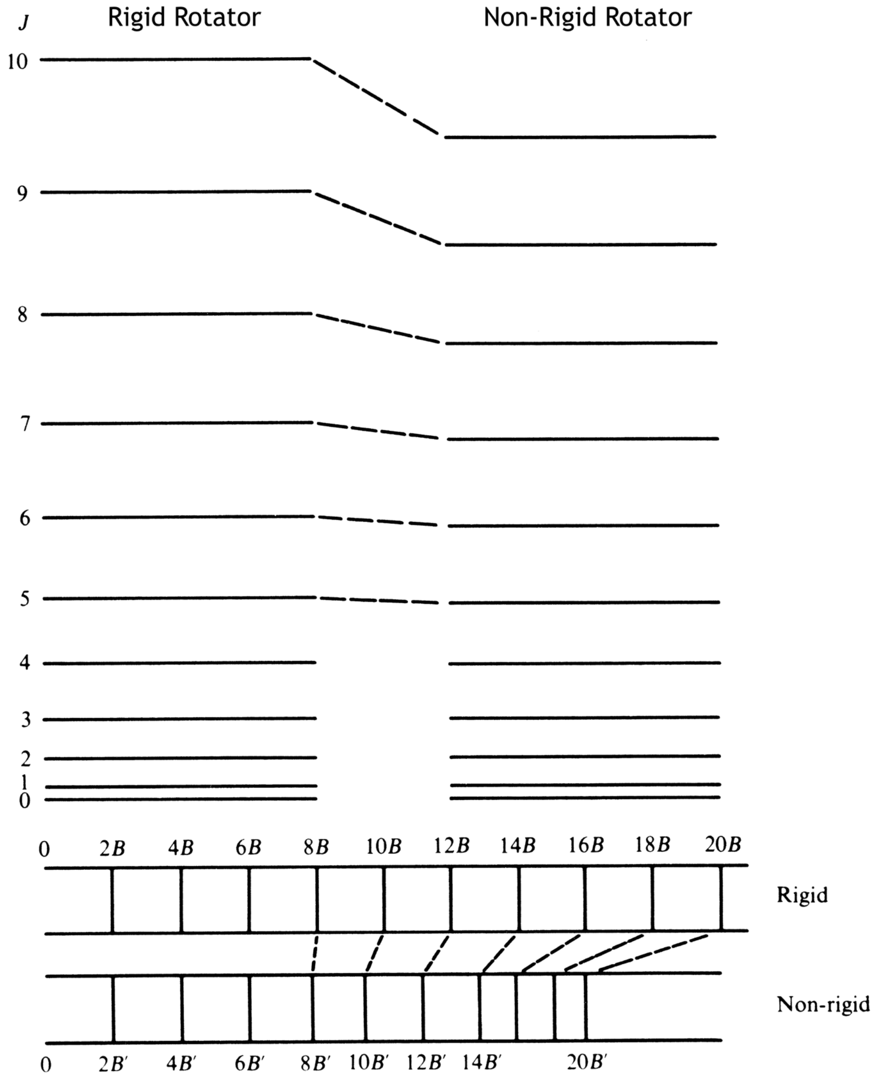
The mathematical treatment of the non-rigid rotator in quantum mechanics gives the rotational energy levels as

$$E_J = \frac{h^2}{8\pi^2 I}J(J+1) - \frac{h^4}{32\pi^4 I^2 r^2 kc}J^2(\text{J}+1)^2 \quad (\text{in cm}^{-1})$$

or

$$\varepsilon_J = \frac{E_J}{hc} = BJ(J+1) - DJ^2(J+1)^2 \qquad (\text{in cm}^{-1})$$

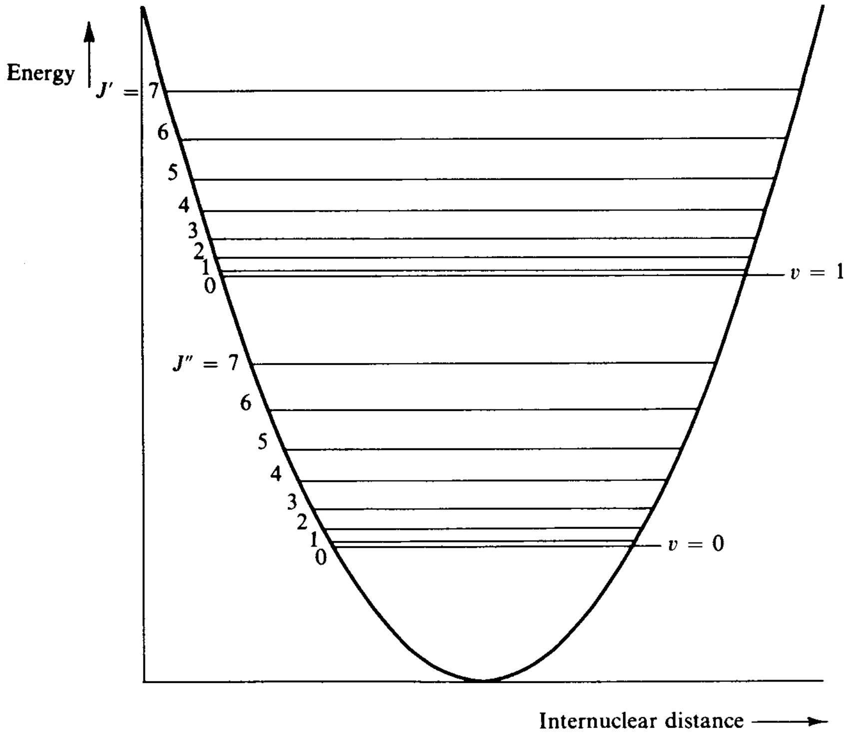
A comparison of the energy levels of a rigid and non-rigid rotator is given in Figure 2.27 below.



Differences in the energy levels when changing from a rigid to a non-rigid model of the diatomic molecule. (after Banwell, Fundamentals of Molecular Spectroscopy, McGraw-Hill, London 1983)

Figure 2.27 Energy levels of the rigid and non-rigid rotator

A typical diatomic molecule has rotational energy separations of 1-10 cm^{-1}. In contrast we saw that vibrational transitions have energy separations in the region of 400 to 4000 cm^{-1}. Since the energy levels are separated by very different margins, we may in approximation consider that a diatomic molecule can execute rotations and vibrations independently of each other. This assumption is called the Born-Oppenheimer approximation (although this includes the separation of different energy levels of electrons in electron transitions).



The rotational energy levels for two different vibrational states of a diatomic molecule.

Figure 2.28 Rotational energy levels of two vibrational modes.

This approximation includes that the energy of a given system is simply the sum of the separate energies:

$$E_{total} = E_{rot} + E_{vib}$$

in Joule, or

$$\varepsilon_{total} = \varepsilon_{vib} + \varepsilon_{rot}$$

Taking the separate expressions for ε_{vib} and ε_{rot} the total energy is given by:

$$\varepsilon_J = BJ(J+1) - DJ^2(J+1)^2 + HJ^3(J+1)^3$$
$$+\left(v+\frac{1}{2}\right)c\tilde{\nu}_{vib} - x\left(v+\frac{1}{2}\right)^2 c\tilde{\nu}_{vib}$$

If the centrifugal distortion constants are neglected the equation can be written:

$$\varepsilon_{total} = \varepsilon_{j,v} = BJ(J+1) + \left(v+\frac{1}{2}\right)c\tilde{\nu}_{vib} - x\left(v+\frac{1}{2}\right)^2 c\tilde{\nu}_{vib}$$

(It should be noted that it is not logical to ignore D, since the molecule is treated as vibrating, but not rigid. However if D is taken into account, the effect on the spectrum would still be minor because of the large separation in the energy levels of a vibration compared to a rotation).

In the figure above (previous page), the energy levels for rotation and vibration are sketched for the lowest levels of vibration, v=0 and v=1. The relative proportions of energy differences in vibration and rotation are neglected in this graph, since separations in rotational energy levels are only 1/1000 of the separations of vibrational energy levels.

Since the rotational constant B is assumed to be unaffected by differences in the values of J and v (a consequence of the Born-Oppenheimer Approximation), the separations between the rotational energy levels at the vibrational level v=0 is the same as that of the rotational energy levels at the vibrational level v=1.

The selection rules for the combined motions are the same as the selection rules for each separately:

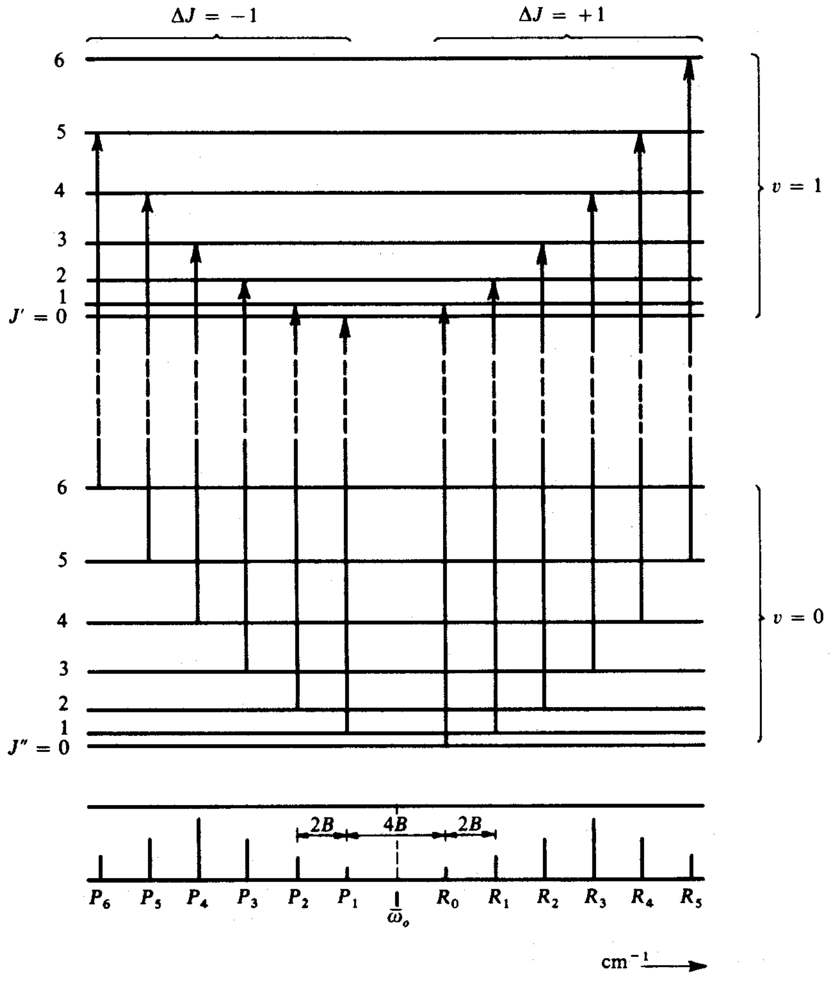
$$\Delta v = 0,\ \pm 1,\ \pm 2,\ \pm 3\ \ ....\ \ ;\quad \Delta J = \pm 1$$

The case Δv=0 is the simple case of a transition between rotational energies only. Quantum mechanical considerations show that a diatomic molecule, with very rare and special exceptions must not undergo vibrational transitions with no change in the rotational energy (ΔJ must not be 0).

The vibration-rotation spectrum for the transition from v=0 to v=1 can be obtained with the selection rules:

$$\Delta\varepsilon_{v,J} = \varepsilon_{J',v=1} - \varepsilon_{J'',v=0}$$

$$\begin{aligned}\Delta\varepsilon_{v,J} &= BJ'(J'+1) + 1\frac{1}{2}\tilde{v}_e - 2\frac{1}{4}x_e\tilde{v}_e - \left\{BJ''(J''+1) + \frac{1}{2}\tilde{v}_e - \frac{1}{4}x_e\tilde{v}_e\right\} \\ &= \tilde{v}_0 + B(J'-J'')(J'+J''+1) \quad (\text{in cm}^{-1}), \quad \text{with } \tilde{v}_0 = \tilde{v}_e(1-2x_e)\end{aligned}$$



Some transitions between rotational-vibrational energy levels of a diatomic molecule together with a spectrum arising from these transitions.

Figure 2.29 Transitions between two vibrational modes and rotational fine structure.

B is taken identical in the upper and lower vibrational states, which is a direct consequence of the Born-Oppenheimer approximation – rotation is unaffected by vibrational changes.

If we have:

1. $\Delta J = +1,\ i.e.\ J' = J'' + 1 \quad \text{or} \quad J' - J'' = +1$

the energy difference is

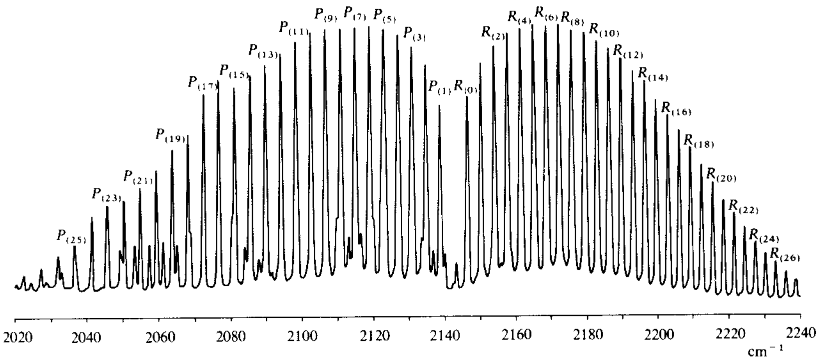
$$\Delta\varepsilon_{v,J} = \tilde{v}_0 + 2B(J'' + 1) \quad \text{cm}^{-1}, \qquad J'' = 0, 1, 2.....$$

If we have:

2. $\Delta J = -1,\ i.e.\ J'' = J' + 1 \quad \text{or} \quad J' - J'' = -1$

the energy difference is

$$\Delta\varepsilon_{v,J} = \tilde{v}_0 - 2B(J' + 1) \quad \text{cm}^{-1}, \qquad J' = 0, 1, 2.....$$



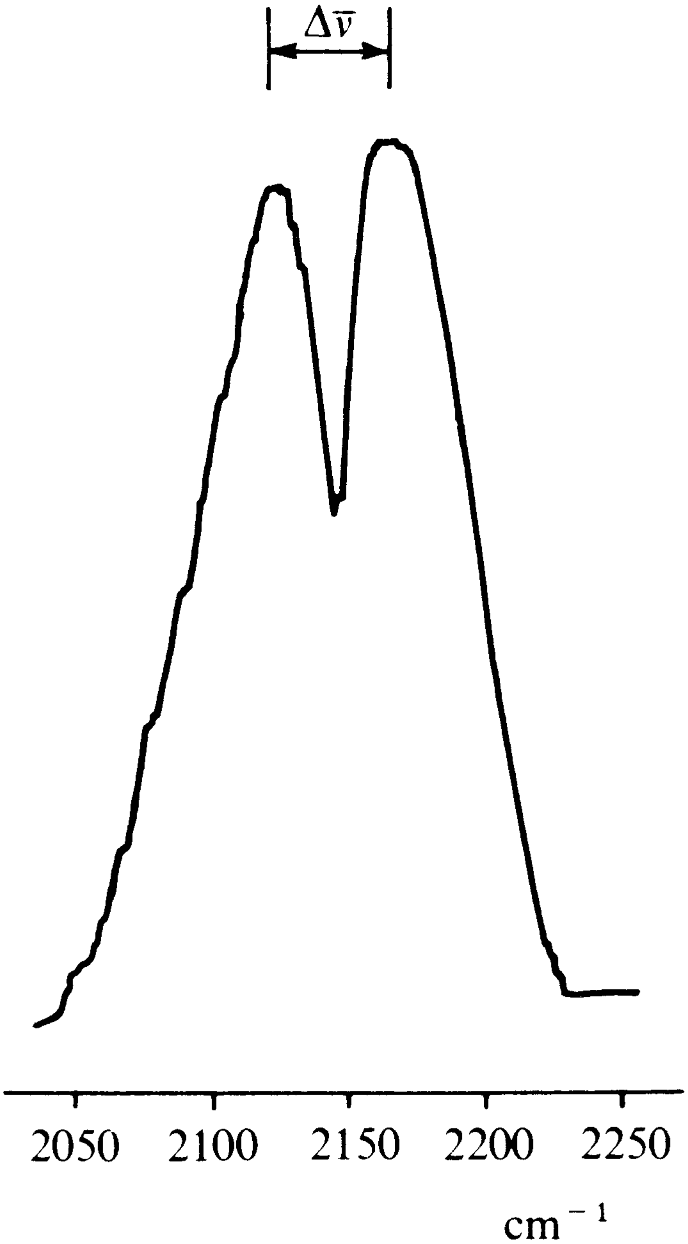
Rotation-vibration spectrum of carbon monoxide (at a gas pressure of 120 mbar). The lines are labeled according to their J values. The P-branch is complicated by the presence of a band centered at about 2100 cm^{-1} due to one per cent of ^{13}CO in the sample; Some of the rotational lines from this band appear between P branch lines, others are overlapped by a P-branch line and give it an enhanced intensity.

Figure 2.30 Rotational vibrational spectrum of carbon monoxide.

In combination it can be written:

$$\Delta\varepsilon_{v,J} = \tilde{\nu}_0 + 2Bm \quad (\text{cm}^{-1}), \qquad m = \pm 1, \pm 2.....$$

The last equation represents the combined rotation-vibration spectrum. It will consist of



The fundamental band of carbon monoxide under very low resolution. All rotational fine structure is lost and a PR contour is observed.

Figure 2.31 Fundamental band of carbon monoxide under very low resolution.

equally spaced bands with a spacing of 2B on each side of the band origin $\tilde{\nu}_0$. Since m cannot be zero, the band origin itself will not appear. Lines to the low frequency side of $\tilde{\nu}_0$ are referred to as the P-branch, while those to the high frequency side are called the R-branch.

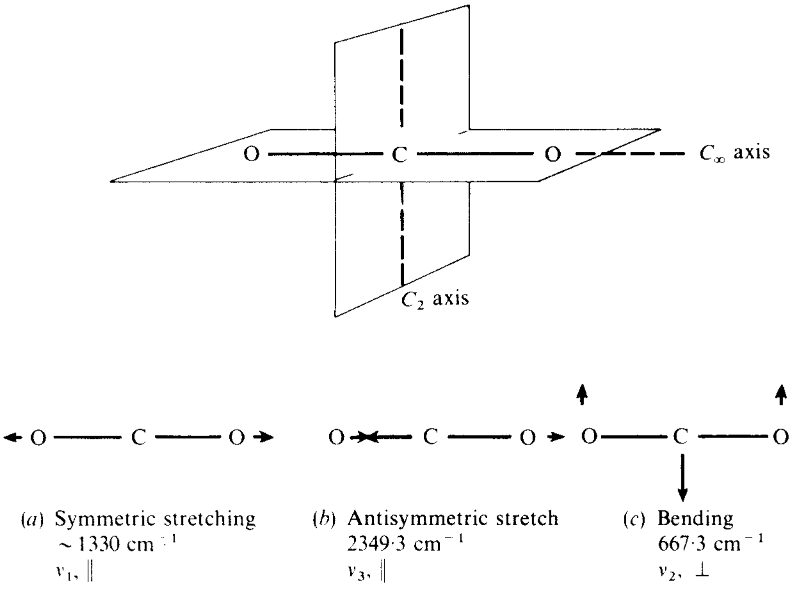
The influence of rotation on the spectra of polyatomic molecules.

We described that the selection rules of diatomic molecules for a simultaneous rotation and vibration were given by

$$\Delta v = 0, \pm 1, \pm 2, \pm 3 \; .... \; ; \quad \Delta J = \pm 1, \; \Delta J \neq 0$$

and that this gave rise to a spectrum consisting of approximately equally spaced line series on each side of a central minimum, designated as the band center.

We now consider molecules with more than two atoms. We can divide the possible vibrations of complex molecules into those causing a dipole change either (1) parallel or (2) perpendicular to the major axis of rotational symmetry. The purpose of this distinction is that the selection rules depend on the type of vibration, parallel or perpendicular, which the molecule is undergoing (see Figure 2.32 below, a diatomic molecule can only have parallel vibrations):



(*a*) Symmetric stretching $\sim 1330 \text{ cm}^{-1}$ ν_1, $\parallel$

(*b*) Antisymmetric stretch $2349{\cdot}3 \text{ cm}^{-1}$ ν_3, $\parallel$

(*c*) Bending $667{\cdot}3 \text{ cm}^{-1}$ ν_2, $\perp$

The symmetry and fundamental vibrations of the carbon dioxide molecule.

Figure 2.32 Symmetry and fundamental vibrations of the carbon dioxide molecule.

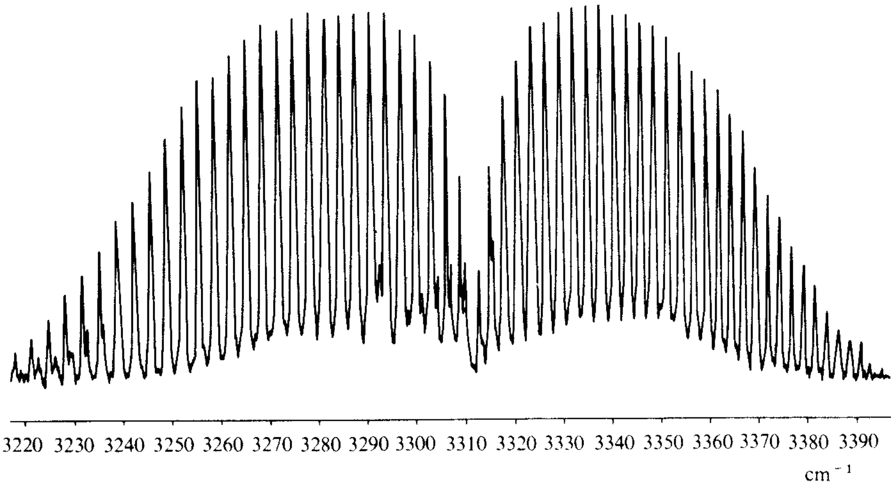
As a simple case, we consider a linear molecule of three atoms. Parallel vibrations have selection rules that are identical with those for diatomic molecules.

$$\Delta J = \pm 1, \;\; \Delta v = \pm 1 \text{ for harmonic vibrations}$$

and

$$\Delta J = \pm 1, \;\; \Delta v = \;\pm 1, \;\pm 2, \;\pm 3, \ldots \text{ for anharmonic vibrations}$$

The spectrum of a parallel vibration will thus be similar in appearance to that of a diatomic molecule (see the parallel stretching vibration of the HCN molecule in Figure 2.33).



Spectrum of the symmetric stretching vibration of the HCN molecule showing the P and R branch lines

Figure 2.33 Spectrum of the symmetric stretching vibration of the HCN molecule.

Perpendicular vibrations. For these the selection rule is found to be

$$\Delta v = \pm 1, \;\; \Delta J = \;0, \;\pm 1 \text{ for simple harmonic motion}$$

This implies for the first time, that a vibrational change can take place with no simultaneous rotational transition. The result is depicted in Figure 2.34 below, which shows the same energy levels and transitions as a linear diatomic molecule, with the addition of ΔJ=0 transitions. If the oscillation is taken as simple harmonic, the energy levels are identical with those previously given:

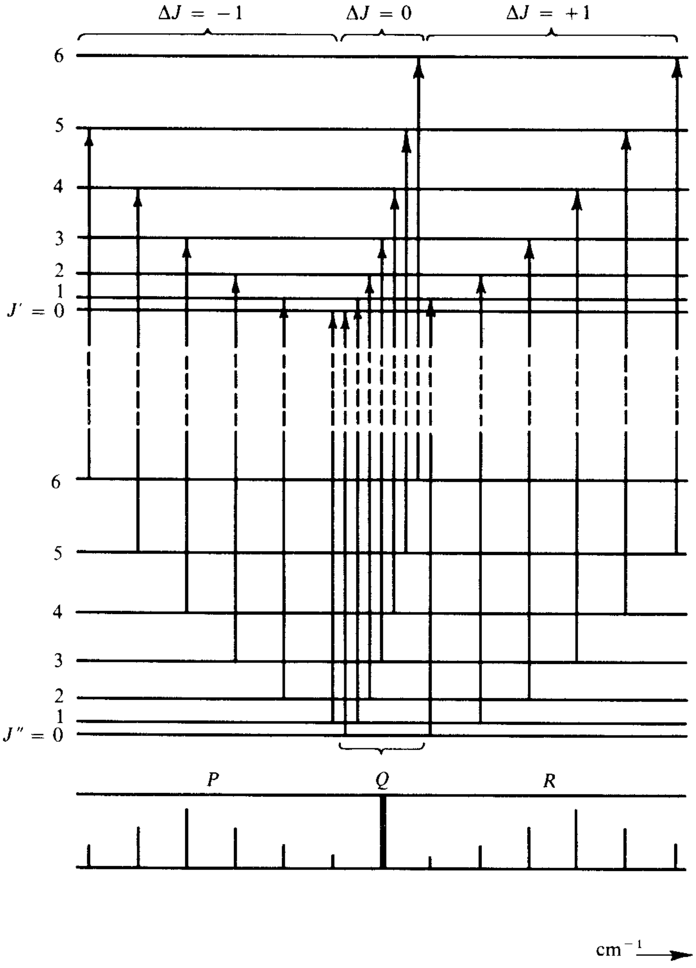
$$\varepsilon_{total} = \varepsilon_{j,v} = BJ(J+1) + \left(v + \frac{1}{2}\right) c\tilde{v}_{vib} - x\left(v + \frac{1}{2}\right)^2 c\tilde{v}_{vib}$$

with the corresponding P and R branch lines.

Transitions with a ΔJ=0, however, correspond to a Q-branch whose lines are derived from the equation:

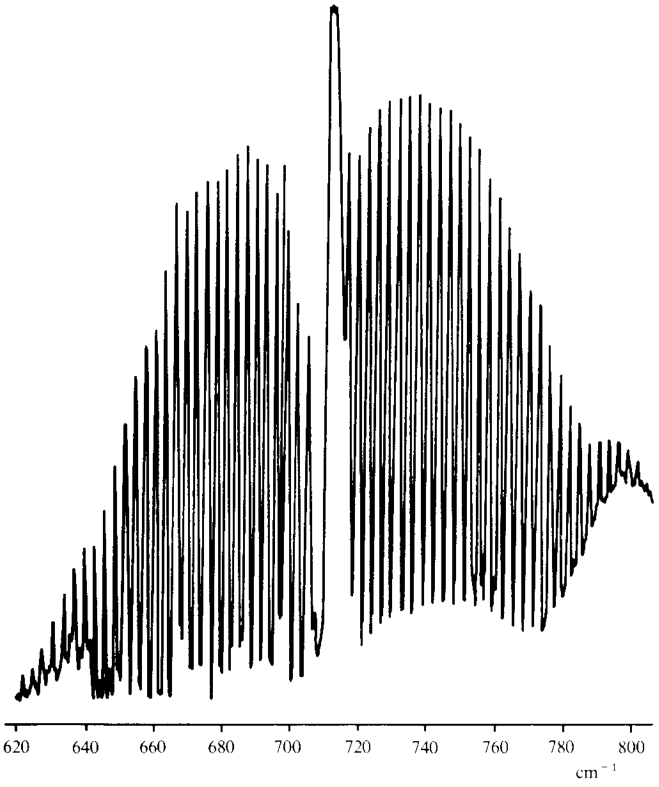
$$
\begin{aligned}
\Delta\varepsilon_{v,J} &= \varepsilon_{J+1,v+1} - \varepsilon_{J,v} \\
&= 1\frac{1}{2}\tilde{\nu}_e - 2\frac{1}{4}x_e\tilde{\nu}_e + BJ(J+1) - \left\{\frac{1}{2}\tilde{\nu}_e - \frac{1}{4}x_e\tilde{\nu}_e + BJ(J+1)\right\} \\
&= \tilde{\nu}_0 \quad (\text{in cm}^{-1}), \qquad \text{for all } J
\end{aligned}
$$

Thus the Q branch consists of lines superimposed upon each other at the band center $\tilde{\nu}_0$. One contribution arising from each of the populated J values gives usually a very intense line. See diagram (Figure 2.34) and spectra (Figure 2.35) of the H-CN bending vibration below.

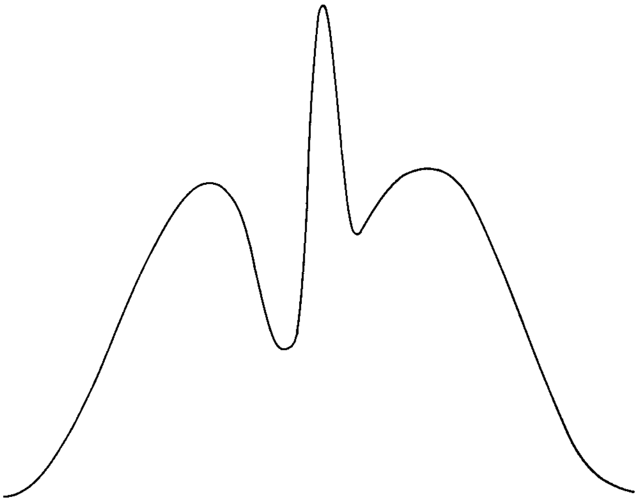


The rotational energy levels for two vibrational states showing the effect on the spectrum of transitions for which ΔJ=0.

Figure 2.34 Appearance of the Q branch in rotation-vibration spectra of the H-CN bending vibration.

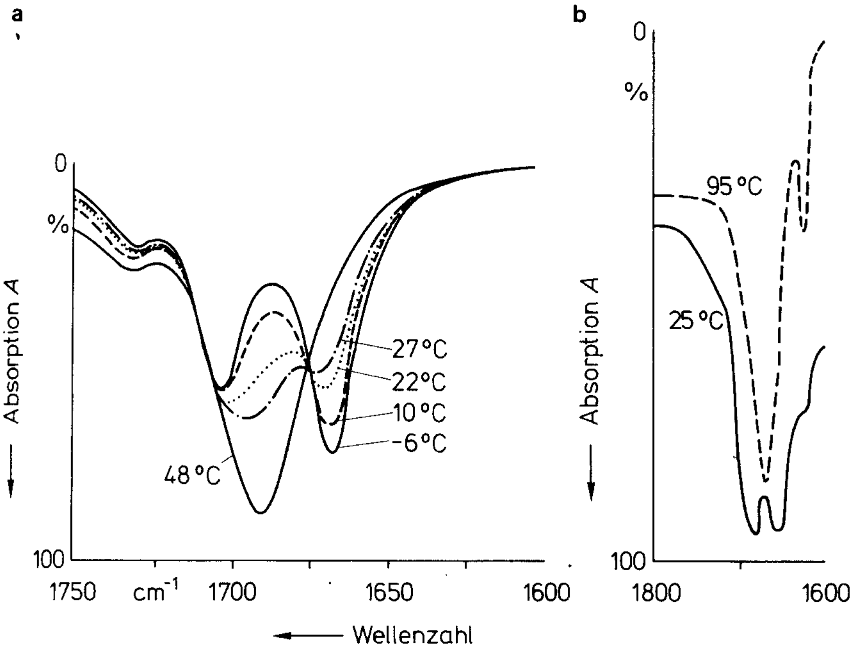


Spectrum of the bending mode vibration of the HCN molecule. The PQR structure of the spectrum becomes evident.



The contour of a PQR band under low resolution.

Figure 2.35 Rotation vibration spectrum of the bending vibration in an HCN molecule.



IR-spectra of a selected region of a, poly-cytidine/5-guanosine monophoshate and b, DNA as a function of temperature.
(After Howard, F.B. et al. (1969), Proc. Nat. Acad. Sci., USA 64, 451 and after Kolkenbeck, K., Zundel, G. (1975), Biophysics Struct. Mech., 1, 203.)

Figure 2.36 IR spectra of polynucleotides.