

Pianificazione, Gestione ed esecuzione di un Esperimento Scientifico in un centro di ricerca internazionale



La spettroscopia vibrazionale di assorbimento Infrarosso e scattering Raman

Due facce della stessa medaglia

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Infrared Spectroscopy Basic Concepts on Theory and Instrumentation

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Electromagnetic Spectrum



Speed of light, c = 299.792.458 m/s ~300.000 Km/s

- Planck Constant, h = 6.62606957 × 10⁻³⁴ m² kg / s
- > The wavelength, λ ([μm])
- The frequency, v ([Hz], $v=c/\lambda$)
- ➤The energy, E ([eV], E=hv)
- > The wavenumber, $\widetilde{\mathcal{V}}$ ([cm⁻¹], $\widetilde{\mathcal{V}}$ =1/ λ)



Electromagnetic Spectrum

A closer view into the IR spectral range





	NIR	MIR		FIR
λ (μm)	0.74	3	30	300
v (THz)	400	100	10	1
v (cm ⁻¹)	~13000	~3333	~333	~33
E (eV)	1.65	0.413	0.041	0.004
E (Kcal/mol)	37	10	1	0.1

Molecular Vibrations



Molecular Rotations

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The Born-Oppenheimer Approximation

1- Electronic motion and nuclear motion in molecules can be separated and independently considered

 Ψ molecule $\uparrow(r\downarrow i , R\downarrow j) = \Psi$ electrons $\uparrow(r\downarrow i ,$

The electronic wavefunction depends upon the nuclear positions but not on nuclei velocities \rightarrow The nuclear motion is so much slower than electron motion that nuclei can be considered to be fixed.

Electronic transitions (10⁻¹⁵ s) are at least 10² times faster than nuclear transitions and involve energies 10 to 50 times greater

Degree of freedom

Degree of freedom is the number of variables required to completely describe the motion of a particle/ molecule. For a molecule made by N atoms moving in 3-dimensional space, the degree of freedom becomes 3N. For non-linear molecules, all translational/rotational motions can be described in terms of translation/ rotations along/around 3 axes. The remaining 3N-6 degrees of freedom constitute vibrational motion. For a linear molecule however there are only 2 rotational degrees of freedom for any linear molecule leaving 3N-5 degrees of freedom for vibration.

2- Vibrational and rotational motion can also be considered independently

The energies involved in rotational transitions (10⁻¹⁰ s) are about 10³ times smaller than the ones involved in vibrational transitions (10⁻¹³ s). Pure vibrational transitions falls in the MIR-FIR regime, while pure rotational transition in the FIR-THz regime

The classical description of vibrational motion

The simplest example: a diatomic heteronuclear molecule AB

 $\mu \downarrow AB = m \downarrow A \ m \downarrow B /_{\text{Reduced Mass of AB molecule}}$ $m\downarrow A + m\downarrow B$

The equilibrium internuclear distance is denoted by r_{eq} . However as a result of molecular vibrations, the internuclear distance is continuously changing; let this distance be called r(t).

Let $x(t)=r(t)-r_{eq}$

When x is non-zero, a restoring force F exists which tries to bring the molecule back to x=0, that is equilibrium. For small displacements this force can be taken to be proportional to x.

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F(restoring force) = -k x
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k = Force constant [Nm<sup>-1</sup>]
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 $x(t) = Asin(2\pi v t)$ $v = 1/2\pi \sqrt{k/\mu}AB$ E = K + U = 1/2 kAT2

[The Hooke's law]

 $K, U, E (/kA^2)$





 $-h^{2}/8\pi^{2}\mu^{4}AB d\psi/dx^{2} + 1/2 kx^{2}\psi_{\overline{Q}}$ thum mechanical Model of harmonic oscillator $E\downarrow vib = hv(n+1/2)$

n: Vibrational quantum number (0,1,2,3,...)

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A series of equally spaced never ending vibrational levels







Normal modes of vibration

- A normal mode is a molecular vibration where some or all atoms vibrate together at the same frequency.
- The number of "normal modes" is equal to the vibrational degree of freedom available
- Each mode has a definite frequency of vibration. Sometimes 2 or 3 modes may have the same frequency but that does not change the fact that they are distinct modes; these modes are called degenerate.

The 3 normal modes of vibratine of a triatomic molecule , defined by 3 normal coordinates (Q_1, Q_2, Q_3) may be defined in terms of internal coordinates

$$Q_{1} = l_{11}\Delta r_{1} + l_{21}\Delta r_{2} + l_{31}\Delta \alpha \qquad v_{1}$$

$$Q_{2} = l_{12}\Delta r_{1} + l_{22}\Delta r_{2} + l_{32}\Delta \alpha \qquad v_{2}$$

$$Q_{3} = l_{13}\Delta r_{1} + l_{23}\Delta r_{2} + l_{33}\Delta \alpha \qquad v_{3}$$

$$Evib = \sum_{i=1}^{3N-6} \left(ni + \frac{1}{2}\right)hv_{i}$$

 $E_0 = \frac{1}{2} \sum_{i=1}^{3N-6} h v_i$

3 quantum numbers: n_1 , n_2 , n_3

3 fundamental vibrations :

$$E(0,0,0) \rightarrow E(1,0,0) \quad v_1$$

 $E(0,0,0) \rightarrow E(0,1,0) \quad v_2$

 $E(0,0,0) \rightarrow E(0,0,1) \quad v_3$

Overtones and combinations bands

 $\begin{array}{ll} (000) \rightarrow (020) & 2v_2 \\ (000) \rightarrow (110) \end{array}$



Infrared Spectroscopy

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Basic concepts on Theory

Infrared Spectroscopy

Basic concepts on Theory

FROM PEAK POSITION, INTENSITY AND WIDTH

CONCENTRATION OF THE SPECIES (BEER-LAMBER LAW)

 $A = \varepsilon \downarrow absrptivity \ l \downarrow path \ lenght \ c \downarrow concnetration$

NATURE OF ATOMS INVOLVED IN THE SPECIFIC VIBRATION PARAMETERS OF THE ATOMIC BOND : BOND STRENGTH AND LENGHT BOND CONFORMATION: DOUBLE BOND CIS/TRANS, CHEMICAL ENVIRONMENT (THROUGH MODULATION OF THE DIPOLE MOMENT) ROTATIONAL MODES IN THE FIR REGION

FROM WHOLE SPECTRUM

NATURE OF THE MOLECULE: SPECTRAL FINGERPRINT=> MOLECULAR IDENTIFICATION SAMPLE INTERACTIONS: FREE/BOUND WATER ...

SAMPLE EVOLUTION: REACTION KINETIC, AGING, PHYSICO CHEMICAL TREATMENT, CONSTRAINTS (PRESSURE, TEMPERATURE, pH) ...

QUANTITATIVE or SEMI-QUANTITATIVE ANALYSIS

SIMPLE MIXTURES: BEER LAMBERT BOUGUER LAW

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 $\nu = 1/2\pi \sqrt{k/\mu}AB$

When dealing with molecular species (normal modes of vibration 3N-6), the absorption profile at a single frequency (or limited spectral range) is scarcely useful.
Only a multi-frequency profile can account for the system complexity and its interaction with the environment

An FTIR spectrum needs to be <u>energy resolved</u> over a <u>large spectral range</u>

The past instrumentation: Dispersive Interferometers

http://www.chemicool.com/definition/fourier_transform_infrared_spectrometer_ftir.htm

This slow acquisition time limited the wide spreading of infrared spectroscopy until 1960s', when Fourier Transform Interferometer have been first proposed. www.ceric-eric.eu/project/pages

The present instrumentation: Fourier Transform InfraRed Interferometers

Conventional sources
 NIR: Tungsten lamp
 MIR: Glow bar (SiC)
 FIR: Hg-Arc
 FIR: Hg-Arc
 Beamsplitters
 NIR: CaF₂
 MIR: KBr
 FIR: Mylar, Silicon
 Detectors
 NIR – InGaAs, InSb, Ge, Si room

NIR – InGaAS, INSD, Ge, SI room temperature detectors MIR: Room temperature DLaTGS Nitrogen cooled MCT FIR – He Cooled Silicon Bolometer Room temperature DLaTGS

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<image>

For a single wavelength

 $I(\mathbf{x}) = I(\widetilde{\mathbf{v}})[1 + \cos(2\pi \mathbf{x}\widetilde{\mathbf{v}})]$

Fourier Transform (FT) \rightarrow

$$I(x) = \int I(\tilde{v}) d\tilde{v} + \int I(\tilde{v}) \cos(2\pi x \tilde{v}) d\tilde{v}$$

$$I(ZPD) = 2 \int I(\tilde{v}) d\tilde{v} = I_0$$

$$I(x) = \frac{1}{2} I_0 + \int I(\tilde{v}) \cos(2\pi x \tilde{v}) d\tilde{v}$$

$$I(x) - \frac{1}{2} I_0 = I'(x) = \int I(\tilde{v}) \cos(2\pi x \tilde{v}) d\tilde{v}$$

$$I(\tilde{v}) \propto \int_{-\infty}^{+\infty} I'(x) \cos(2\pi x \tilde{v}) dx$$

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Optical Scheme of a FTIR spectrometer

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Acquisition of an infrared spectrum

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Infrared Spectroscopy

Basic concepts on Instrumentation

TRANSMISSION

Lambert-Beer Law

 $-\log_{10} (I/I_0) = \varepsilon dc$ $L \cdot mol^{-1} \cdot cm^{-1} [c] = mol \cdot L^{-1} [d] = cm$

• SPECULAR

Typical angle of incidence = 10-30° The refraction behavior of the bulk sample is investigated

Attenuated Total Reflection: The surface properties of the

material are investigated for penetration depth of

• DIFFUSE

The diffusive-reflection spectrum is defined by the absorption-scattering behavior of the sample

• TOTAL

• GRAZING INCIDENCE

hundreds of nanometers

Typical angle of incidence = 50-85° The surface properties of the sample are investigated

Infrared bio-spectroscopy From macro to nanoscale on the molecules of Life

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Biospectroscopy

Biospectroscopy is the spectroscopy of the Molecule of Life

Organic molecules are the Molecules of Life. They are built on chains of carbon atoms, usually very long (bio-macromolecules)

There are four main groups of bio-macromolecules to build sub-cellular structure, cells, tissue, organs up to living beings:

Proteins; Lipids; Nucleic Acids; Carbohydrates

Samples conventionally studied by FTIR Spectroscopy

Samples conventionally studied by FTIR Microscopy

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Proteins

Functions and Structure

Proteins perform a vast array of functions within organisms, exhibiting activity strictly related to their structure (<u>Structure-Activity relationship</u>)

FTIR spectroscopy for protein conformational studies

Extended beta-Aggregates

Lipids Functions and Structure

FTIR spectroscopy of lipid

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=C-H: Unsaturated fatty acids increase membrane fluidity

Shifts and broadening of the methyl and methylene bands are indicative of increased lipid disorder/fluidity

Nucleic acids Structure and Function

DNA stores information

Image credit https://www.wonderwhizkids.com/gene-expression

RNA transfer information

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Image credit

https://biology.tutorvista.com/biomolecules/nucleic-acids.html

FTIR spectroscopy for Nucleic acids conformational

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FTIR spectroscopy of Carbohydrates

Mammalian cells

<u>At a first glance</u>, the FTIR spectrum of a mammalian cell can be viewed as the over imposition of the diverse spectral contribution of each individual components

Typical mammalian dried cell chemical composition (component percent of total cell weight)

Mammalian cells

Band intensity, position, width and shape (band components) are sensitive to subtle biochemical changes of bio-specimens.

Letterature

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- (4) Kuhn, L. P. Infrared Spectra of Carbohydrates. Anal. Chem. 1950, 22 (2), 276–283.
- (5) Tipson, R. S. Infrared Spectroscopy of Carbohydrates, National Burea of Standards Monograph 110, June 968
- (6) Movasaghi, Z.; Rehman, S.; ur Rehman, D. I. Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues. Appl. Spectrosc. Rev. 2008, 43 (2), 134–179.

Proposed Experiments at SISSI

- (1) The students will have the opportunity to look "inside" the interferometer
- (2) The spectra will be acquired starting from hydrated molecules
 - \rightarrow Water features will be highlighted as well the dominant effect on IR spectra
- (3) They will start to become confident with IR spectral interpretation They will collect spectra of:
 - Proteins (with different conformations) -> IR sensitivity to protein secondary structure
 - Raman spectra of the same molecules will be commented for highlighting technique complementary
 - Nucleic acids (DNA) -> IR sensitivity to DNA folding
 - Raman spectra of the same molecules will be commented for highlighting technique complementary
 - Sugars (Trehalose) -> RAMAN SPECTRA WILL BE AQUIRED AT DIFFERENT CONCENTRATIONS FOR HIGHLIGTHING THE DIFFERENT CONTRIBUTION OF WATER TO IR AND Raman spectra

UV Resonant Raman Spectroscopy Basic Concepts on Theory and Instrumentation

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Raman scattering

When the electromagnetic radiation interacts with matter and its energy is not in resonance with any energy level difference, absorption does not take place but **scattering processes** can occur at second order in perturbation theory

Inelastic scattering experiments

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Raman scattering

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Raman effect

Raman bands arise from changes in the molecular polarizability during the vibrations

derived polarizability tensor

Resonant Raman scattering

Raman cross section increased

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UV Resonant Raman scattering

200-300 nm Aromatic compounds

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225-300 nm DNA nitrogenous bases

Further advantages on the use of UVRR

- **Absence** of *fluorescence background* on the spectra
- Measurements in water and/or buffer solutions at low solute concentrations
- Higher Raman cross section with respect to the Raman scattering performed exploting visible near/IR laser sources

Exciting wavelength selectivity

- Laser: fixed wavelength sources
- SR: tunable CW wavelength source → better selectivity

Exciting wavelength selectivity

- Laser: fixed wavelength sources
- SR: tunable CW wavelength source → better selectivity

UVRR set-up, IUVS at Elettra

UVRR set-up, IUVS at Elettra

UV micro Raman system

Hydration of biomolecules studied by Raman spectroscopy

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Spettro Raman dell'acqua

di un Esperimento Scientifico in un Centro di Ricerca Internazionale

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C. Bottari et al. , JRS 2018

Caso scientifico:"memoria dell'acqua" ?

NATURE VOL. 333 30 JUNE 1988

Human basophil degranulation triggered by very dilute antiserum against IgE

SCIENTIFIC PAPER

E. Dayenas, F. Beauvais, J. Amara*, M. Oberbaum*, B. Robinzon t, A. Miadonna t, A. Tedeschit, B. Pomeranz§,

transmission of the biological information could be related to the molecular organization of water.

S Departments of Zoology and Physiology, Ramsay wright Zoological Laboratories, University of Foronto. 23 Harbord Street, Toronto. Optatio M5S 1A1 Canada

To whom co

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Editorial reservation

When, huma READERS of this article may share the incredulity of the many referees surface, are e The latter ca who have commented on several versions of it during the past several degranulatio months. The essence of the result is that an aqueous solution of an dilutions nee related to the antibody retains its ability to evoke a biological response even when diluted to such an extent that there is a negligible chance of there being a single molecule in any sample. There is no physical basis for such an activity. With the kind collaboration of Professor Benveniste, Nature has therefore arranged for independent investigators to observe repetitions of the experiments. A report of this investigation will appear shortly.

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Caso scientifico:"memoria dell'acqua" ?

NATURE VOL. 334 28 JULY 1988

NEWS AND VIEWS

"High-dilution" experiments a delusion

The now celebrated report by Dr J. Benveniste and colleagues elsewhere is found, by a visiting Nature team, to be an insubstantial basis for the claims made for them.

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Caso scientifico:"memoria dell'acqua" ?

Esperimento proposto:

Verificare la presenza di un "effetto memoria" dell'acqua in soluzioni omeopatiche per mezzo dell'analisi di spettri vibrazionali raccolti tramite spettroscopia Raman.

L'esperimento consiste nel preparare set di soluzioni acquose di trealosio e eventualmente glicina a diverse concentrazioni, diluendole prograssivamente fino a raggiungere diluizioni di tipo omeopatico (meno di una molecola di trealosio per litro di solvente).

Si mostrareranno le modifiche alla banda OH-stretching dell'acqua in presenza di soluzione di trealosio (presenza della "hydration shell") passando da concentrazioni alte a diluizione omeopatiche. Si verificherà se vi sono differenze tra la forma della banda OH stretching in soluzioni omeopatiche rispettto alla banda del solvente puro (acqua).

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Thank you

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