Analytical Methods

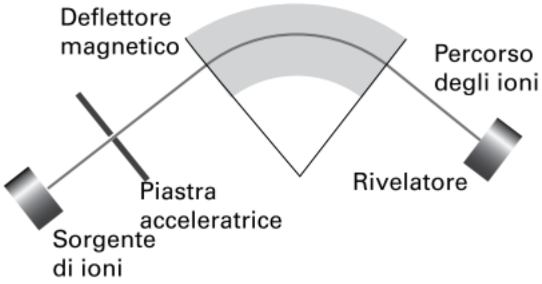
How can we characterize a supramolecular structure?

- qualitative data : structure of the edifice, mode of interactions
- quantitative data (thermo and kinetics) : stoichiometry, stability, selectivity.

- X-Ray Diffraction
- NMR (1D, 2D)
- UV-vis   Fluorimetry
- Mass Spectrometry
- Circular Dichroism
- Electrochemistry, Potentiometry (pH)
- Microcalorimetry (Isothermal Titration Calorimetry)
- Computational Methods

- DLS dynamic light scattering: size distributions
- SEM   scanning electron microscopy
- TEM   trasmission electron microscopy
- STM   scanning tunneling microscopy
- AFM atomic force microscopy

Mass Spectrometry/Cyclic Voltammetry



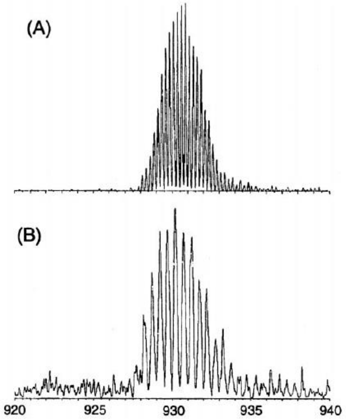


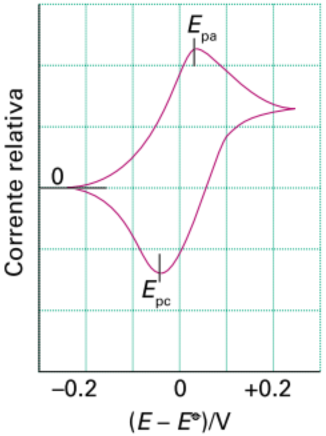
Figure 13. The isotopic patterns of 930 (*m/z*): (A) dimer (4+) and (B) monomer (2+).

ESI-MS

$[U]^{2+}$ - monomer

$[U{-}{-}U]^{4+}$ - dimer

Equal m/z, different line spacing in the isotopic distribution.

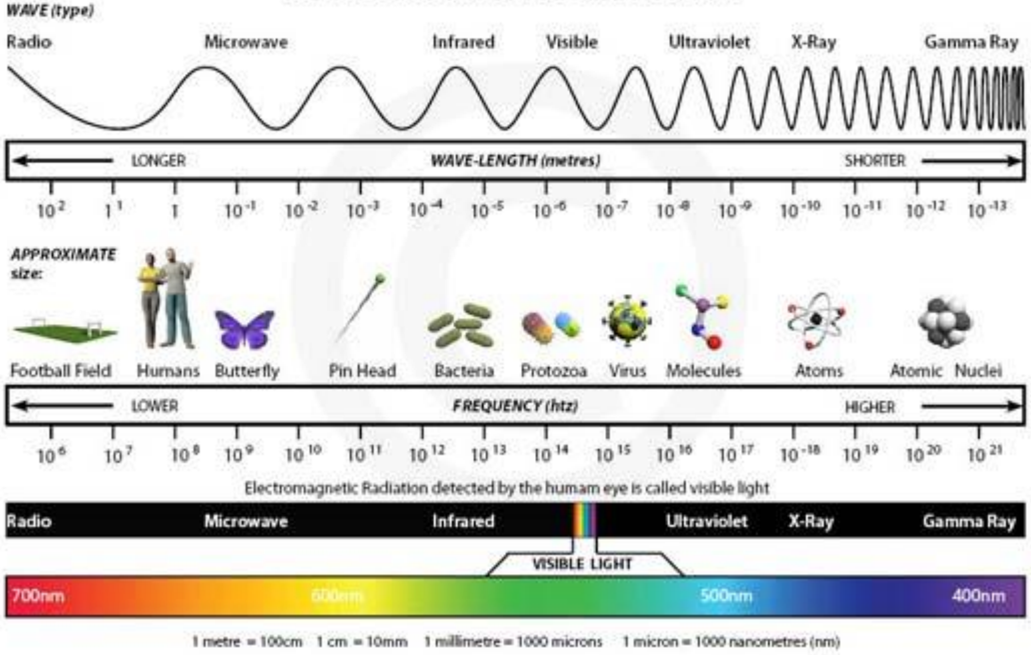


CV

Mesurement of current flow by cycling the voltage – linear ramp in one direction and linear ramp in the opposite direction of the working electrode; reference electrode (fixed voltage) and counter electrode.

Redox Active species in Solution;
e^- transfer process must be reversible;
Solvent redox inactive in the experimental window;
Conc $\leq$ mM;
Supporting electolyte for conducticity;
E_{redox} (V) = (E_{ox} – E_{rid})/2.

THE ELECTRO MAGNETIC SPECTRUM



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Different Analytical Techniques for compound characterization

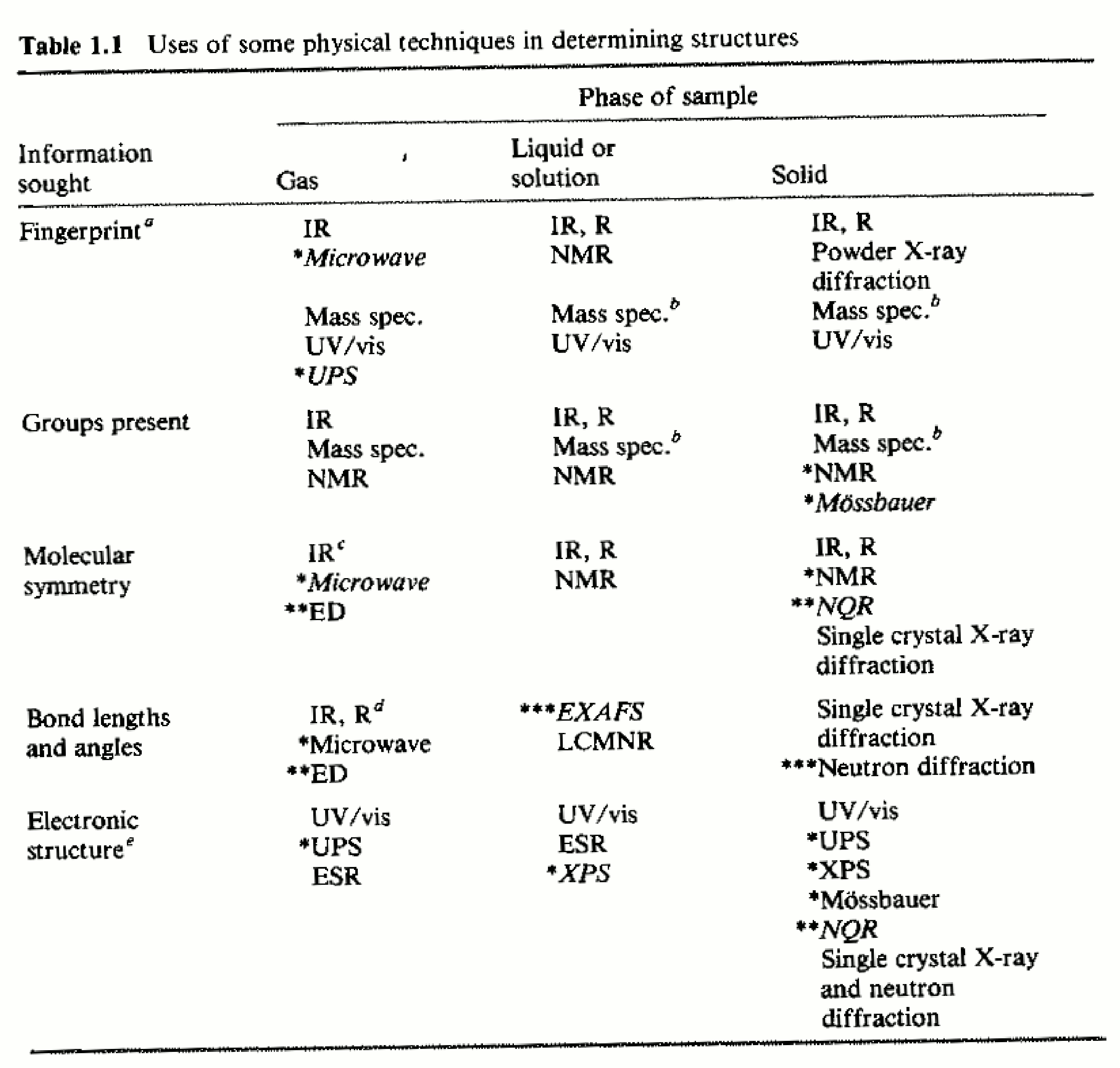


Table 1.1 Uses of some physical techniques in determining structures

Information sought	Phase of sample		
	Gas	Liquid or solution	Solid
Fingerprint[a]	IR *_Microwave_ Mass spec. UV/vis *_UPS_	IR, R NMR Mass spec.[b] UV/vis	IR, R Powder X-ray diffraction Mass spec.[b] UV/vis
Groups present	IR Mass spec. NMR	IR, R Mass spec.[b] NMR	IR, R Mass spec.[b] *NMR *_Mössbauer_
Molecular symmetry	IR[c] *_Microwave_ **ED	IR, R NMR	IR, R *NMR **_NQR_ Single crystal X-ray diffraction
Bond lengths and angles	IR, R[d] *Microwave **ED	***_EXAFS_ LCMNR	Single crystal X-ray diffraction ***Neutron diffraction
Electronic structure[e]	UV/vis *UPS ESR	UV/vis ESR *_XPS_	UV/vis *UPS *XPS *Mössbauer **_NQR_ Single crystal X-ray and neutron diffraction

UV-vis Absorption Spectroscopy

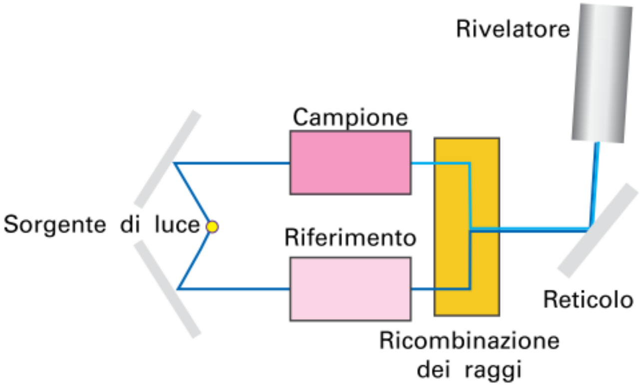
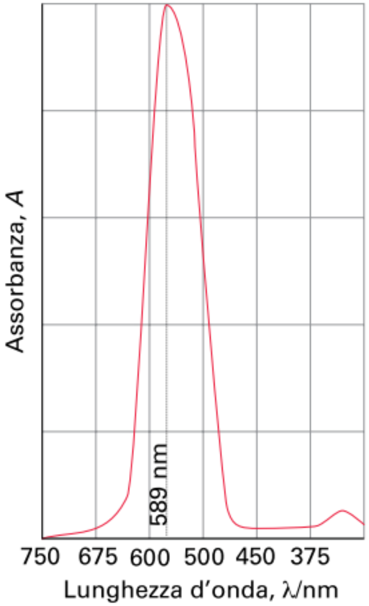


Figure 8.9 Lo schema di un tipico spettrometro per assorbimento UV-visibile.



UV-vis Absorption Spectroscopy

Selection rules

Electronic transitions obey the following selection rules.

Spin selection rule: $\Delta S = 0$

Transitions may occur from singlet to singlet, or triplet to triplet states and so on, but a change in spin multiplicity is *forbidden*.

Laporte selection rule: There must be a change in parity:

allowed transitions: $g \leftrightarrow u$

forbidden transitions: $g \leftrightarrow g$ $u \leftrightarrow u$

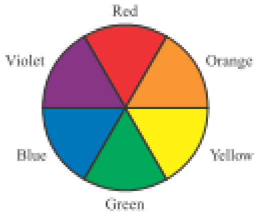
This leads to the selection rule:

$\Delta l = \pm 1$

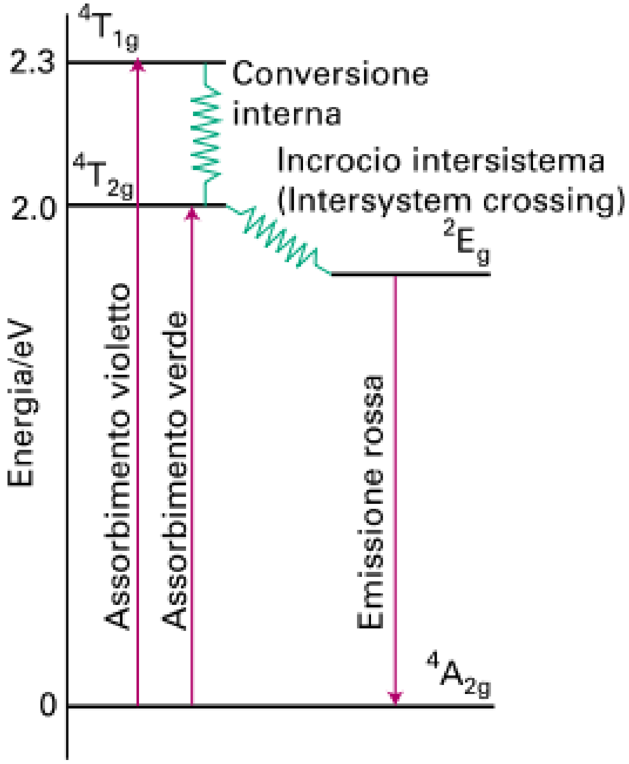
and, thus, *allowed* transitions are $s \longrightarrow p$, $p \longrightarrow d$, $d \longrightarrow f$; *forbidden* transitions are $s \longrightarrow s$, $p \longrightarrow p$, $d \longrightarrow d$, $f \longrightarrow f$, $s \longrightarrow d$, $p \longrightarrow f$ etc.

Type of transition	**Typical ε_{max} / dm^3 mol^{-1} cm^{-1}**
Spin-forbidden '*d*–*d*'	<1
Laporte-forbidden, spin-allowed '*d*–*d*'	1–10
	10–1000
Charge transfer (fully allowed)	1000–50 000

Table 19.2 The visible part of the electromagnetic spectrum.

Colour of light *absorbed*	Approximate wavelength ranges / nm	Corresponding wavenumbers (approximate values) / cm^{-1}	Colour of light *transmitted*, i.e. complementary colour of the absorbed light	In a 'colour wheel' representation, complementary colours are in opposite sectors
Red	700–620	14 300–16 100	Green	
Orange	620–580	16 100–17 200	Blue	
Yellow	580–560	17 200–17 900	Violet	
Green	560–490	17 900–20 400	Red	
Blue	490–430	20 400–23 250	Orange	
Violet	430–380	23 250–26 300	Yellow	

Absorption and Emission Spectroscopy



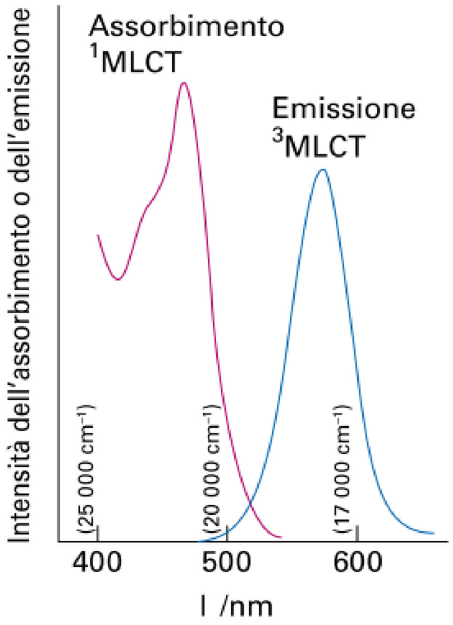


Figura 20.36 Gli spettri di assorbimento e di fosforescenza di $[Ru(bpy)_3]^{2+}$.

Absorption Spectroscopy – kinetic studies

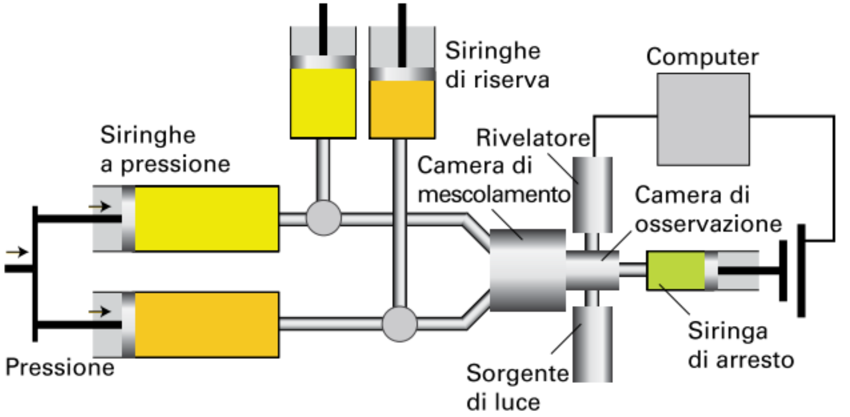
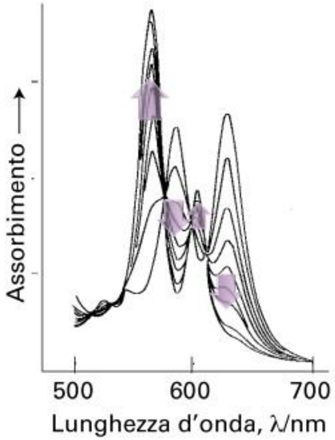


Figure 8.14 La struttura di uno strumento *stopped-flow* per studiare reazioni veloci in soluzione.



NMR spectroscopy : basic reminders

A particle in motion possess a magnetic moment m and an angular moment p, these are described as quantized vectors.

For a nucleus the total magnetic moment moment of nuclear spin and the total angular momentum of nuclear spin are defined; the quantum number associated with these is the nuclear spin quantum number I.

For each isotope of each element there is a fundamental state with $I = n/2$ (n = integer).

The value of I depends from the atomic number Z and from the mass number A:

Nuclei with even Z + even A possess $I = 0$, NMR silent ($^{12}\mathrm{C}$, $^{16}\mathrm{O}$);

Nuclei with even or odd Z + even A possess odd n (I = ½, 3/2, 5/2), NMR active nuclei ($^{1}\mathrm{H}$, $^{13}\mathrm{C}$, $^{15}\mathrm{N}$, $^{31}\mathrm{P}$);

Nuclei with odd Z + even A possess even n (I = integer) ($^{2}\mathrm{H}$, $^{14}\mathrm{N}$), NMR active nuclei.

NMR spectroscopy : basic reminders

If $I \neq 0$, the nuclei possess a total magnetic moment moment of nuclear spin μ expressed by:

$$\mu = \gamma \hbar [I\,(I+1)]^{1/2}$$

γ is the gyromagnetic ratio, differing in value for each isotope

In the presence of an external magnetic field B_0 the nuclear spin axes can assume a set of orientations, in dependence of the magnetic quantum number *m*

$$m = I, I-1, I-2, \ldots -I$$

the total number of possible orientations is $2I+1$

NMR spectroscopy : basic reminders

Possible orientations for nuclei with I = 1/2

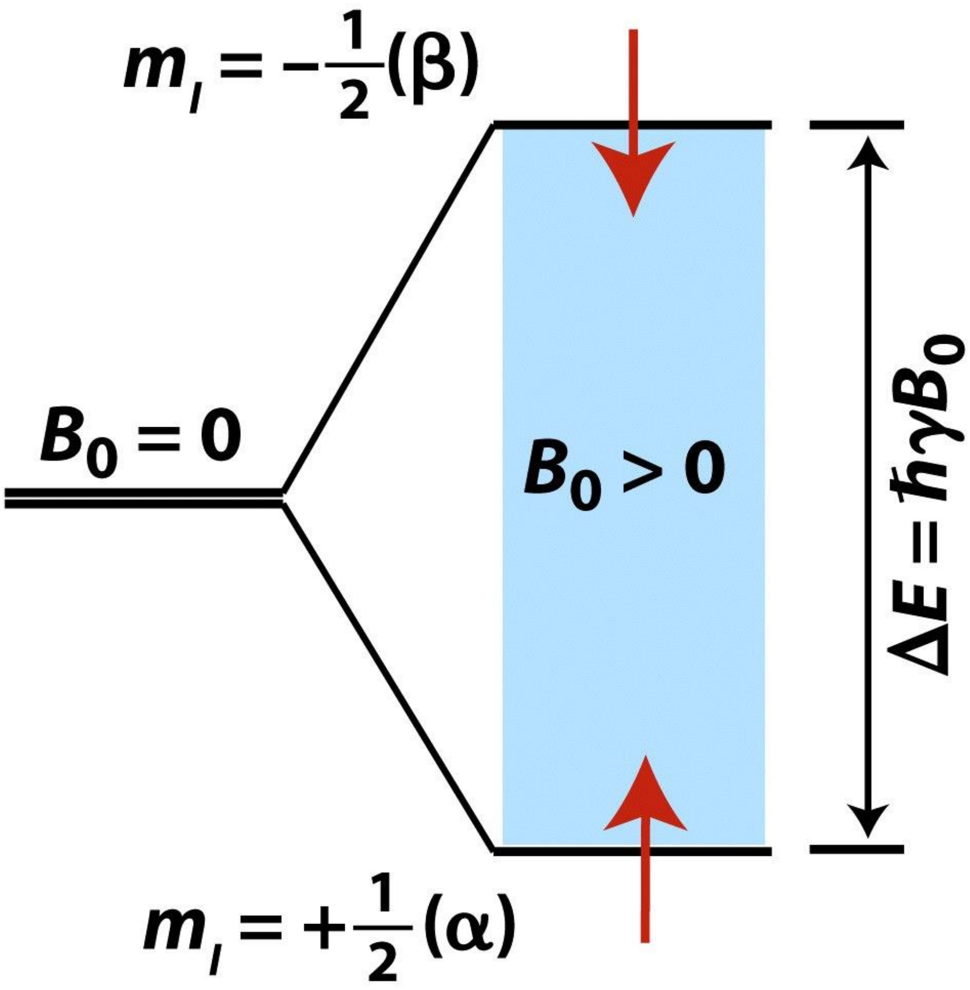


Figure 6-7
Shriver & Atkins Inorganic Chemistry, Fourth Edition

The energetic levels build up onlu in the presence of the external B_0.

The difference between the energetic levels is not fixed, but depends from B_0.

NMR spectroscopy : basic reminders

When a sample is irradiated with a radiation with a specific frequency (and an energy), a transition betweenthe Quando si irradia il campione con una radiazione elettromagnetica che ha una frequenza, e quindi un’energia pari a ΔE, avviene la transizione tra i livelli energetici secondo la regola di selezione $\Delta m = \pm 1$.

LARMOR FREQUENCY - is the precession of the magnetic moment of the nuclei about the external magnetic field.

$$\nu_0 = \gamma / 2\pi \; B_0$$

The sensitivity of NMR spectroscopy depends on the difference in population between the two levels, and therefore depends on B_0 and γ.

NMR spectroscopy : basic reminders

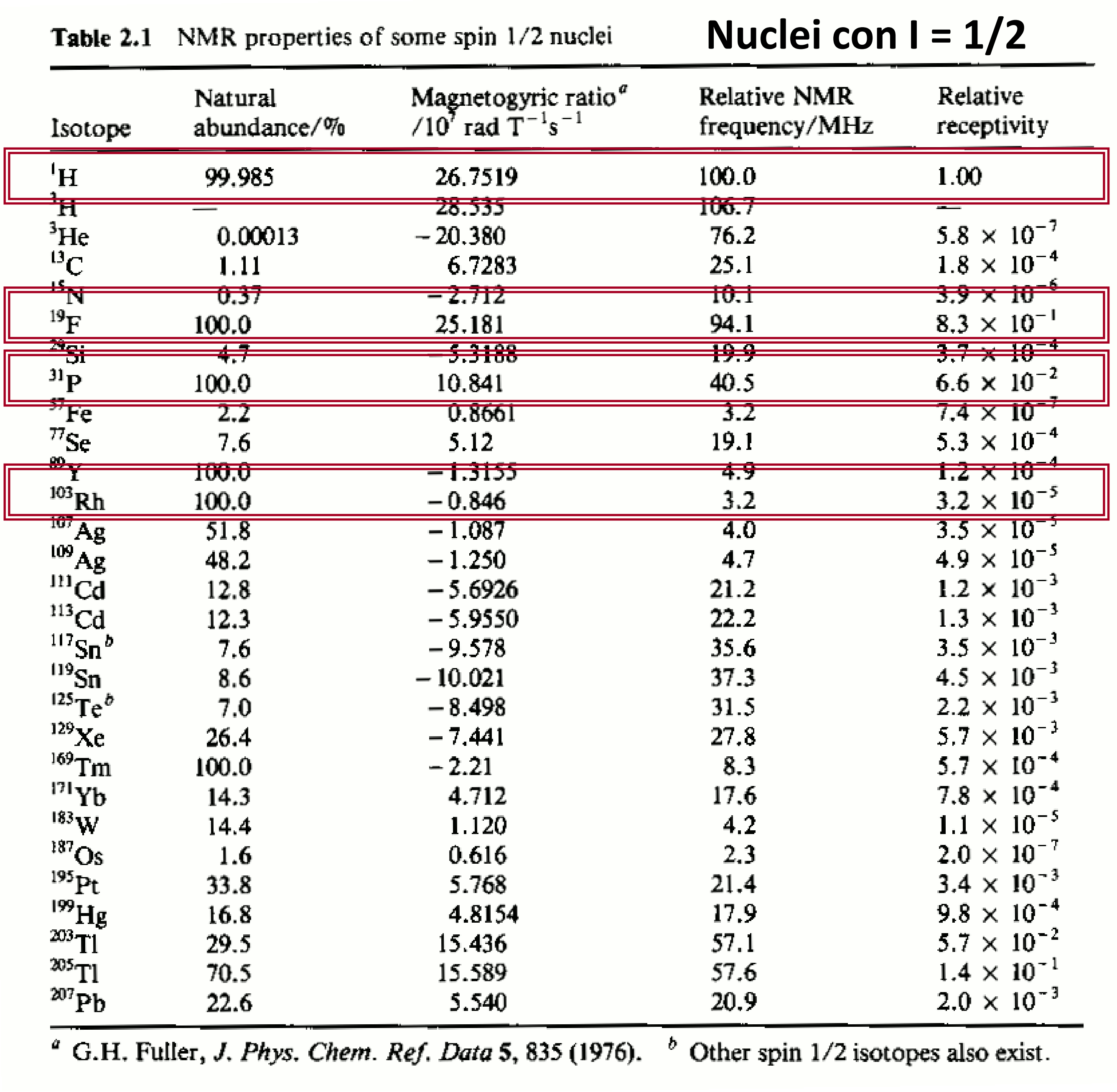


Table 2.1 NMR properties of some spin 1/2 nuclei

Nuclei con I = 1/2

Isotope	Natural abundance/%	Magnetogyric ratio[a] /10^7 rad $T^{-1}s^{-1}$	Relative NMR frequency/MHz	Relative receptivity
^{1}H	99.985	26.7519	100.0	1.00
^{3}H	—	28.535	106.7	—
^{3}He	0.00013	−20.380	76.2	5.8×10^{-7}
^{13}C	1.11	6.7283	25.1	1.8×10^{-4}
^{15}N	0.37	−2.712	10.1	3.9×10^{-6}
^{19}F	100.0	25.181	94.1	8.3×10^{-1}
^{29}Si	4.7	−5.3188	19.9	3.7×10^{-4}
^{31}P	100.0	10.841	40.5	6.6×10^{-2}
^{57}Fe	2.2	0.8661	3.2	7.4×10^{-7}
^{77}Se	7.6	5.12	19.1	5.3×10^{-4}
^{89}Y	100.0	−1.3155	4.9	1.2×10^{-4}
^{103}Rh	100.0	−0.846	3.2	3.2×10^{-5}
^{107}Ag	51.8	−1.087	4.0	3.5×10^{-5}
^{109}Ag	48.2	−1.250	4.7	4.9×10^{-5}
^{111}Cd	12.8	−5.6926	21.2	1.2×10^{-3}
^{113}Cd	12.3	−5.9550	22.2	1.3×10^{-3}
^{117}Sn[b]	7.6	−9.578	35.6	3.5×10^{-3}
^{119}Sn	8.6	−10.021	37.3	4.5×10^{-3}
^{125}Te[b]	7.0	−8.498	31.5	2.2×10^{-3}
^{129}Xe	26.4	−7.441	27.8	5.7×10^{-3}
^{169}Tm	100.0	−2.21	8.3	5.7×10^{-4}
^{171}Yb	14.3	4.712	17.6	7.8×10^{-4}
^{183}W	14.4	1.120	4.2	1.1×10^{-5}
^{187}Os	1.6	0.616	2.3	2.0×10^{-7}
^{195}Pt	33.8	5.768	21.4	3.4×10^{-3}
^{199}Hg	16.8	4.8154	17.9	9.8×10^{-4}
^{203}Tl	29.5	15.436	57.1	5.7×10^{-2}
^{205}Tl	70.5	15.589	57.6	1.4×10^{-1}
^{207}Pb	22.6	5.540	20.9	2.0×10^{-3}

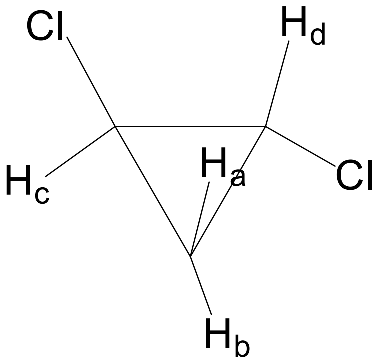
[a] G.H. Fuller, *J. Phys. Chem. Ref. Data* **5**, 835 (1976). [b] Other spin 1/2 isotopes also exist.

NMR spectroscopy : basic reminders

Chemical and/or Magnetic equivalence:

Chemically equivalent nuclei: the ones that can interconvert by a symmetry operation on the molecule or that are equivalent in time by a very fast intramolecular dynamic process. These have the same chemical shift

Magnetically equivalent nuclei: the ones that are chemically equivalent and that couples in the same way with the other nuclei of the molecules. These have the same coupling constant. J.



H_a e H_b are chemically equivalent but not magnetically equivalent.

NMR spectroscopy : basic reminders

The resonances multiplicity (or splitting) is a consequence of coupling bewteen nuclei:

^nJ: coupling constant, with n = numeber of bonds between the coupling nuclei:

^1J direct

^2J geminal

^3J vicinal

^4J, ^5J long range

Homonuclear coupling ^{1}H-^{1}H J $\leq$ 18 Hz;

Heteronuclear coupling ^{1}H-X J hundreds of Hz;

Homo or Heteronuclear coupling X-X o Y-X J kHz.

NMR spectroscopy : basic reminders

The number of lines for first order spin systems is = (2 n I + 1);
n = number of coupling nuclei with the one giving rise to the resonance,
I = spin quantic number of the coupling nuclei;
For nuclei with I = ½ the relative intensity of the lines is given by the Pascal Triangle

1
1 1
1 2 1
1 3 3 1
1 4 6 4 1
1 5 10 10 5 1

1 Pascal's triangle

Structure 6-1
Shriver & Atkins Inorganic Chemistry, Fourth Edition

NMR spectroscopy : basic reminders

2D spectra are of the type:

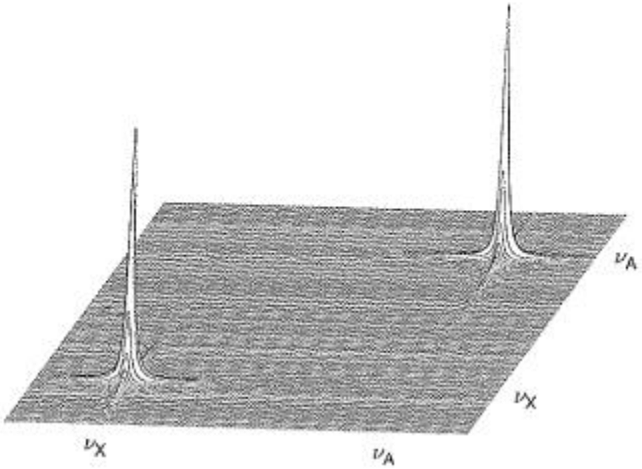
Homonuclear: same isotope frequency on the two dimensions;

Heteronuclear: frequncies of two different isotopes in the twodimensions.

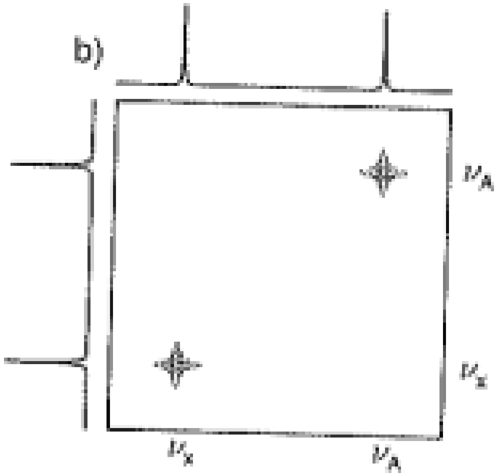
2D spectra giving informations on nuclei that couple through bonds are called J-correlated – COSY experiments (COrrelated SpectroscopY).

NMR spectroscopy : basic reminders

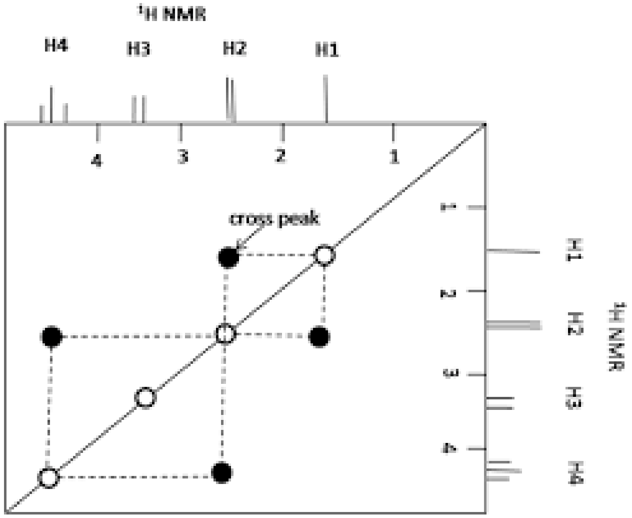
2D COSY of non-coupled nuclei



2D map



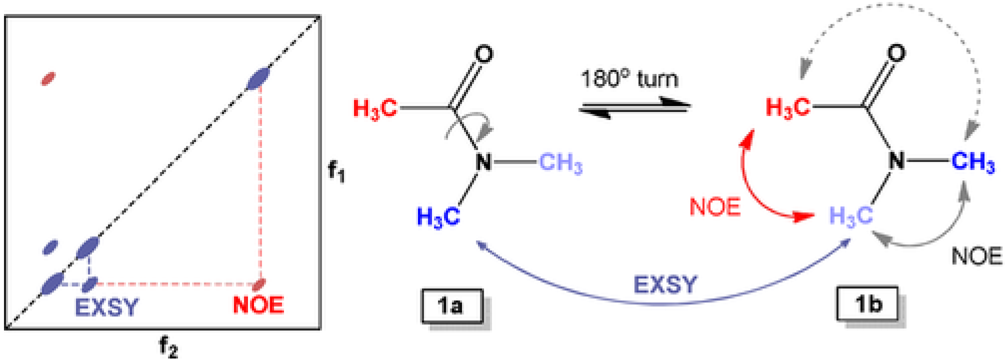
2D COSY of coupling nuclei



Nuclear Overhouser Effect NMR spectroscopy (NOESY)
Exchange spectroscopy (EXSY)

Useful for determining which signals arise from NMR-active nucleus that are close to each other in space even if they are not bonded;

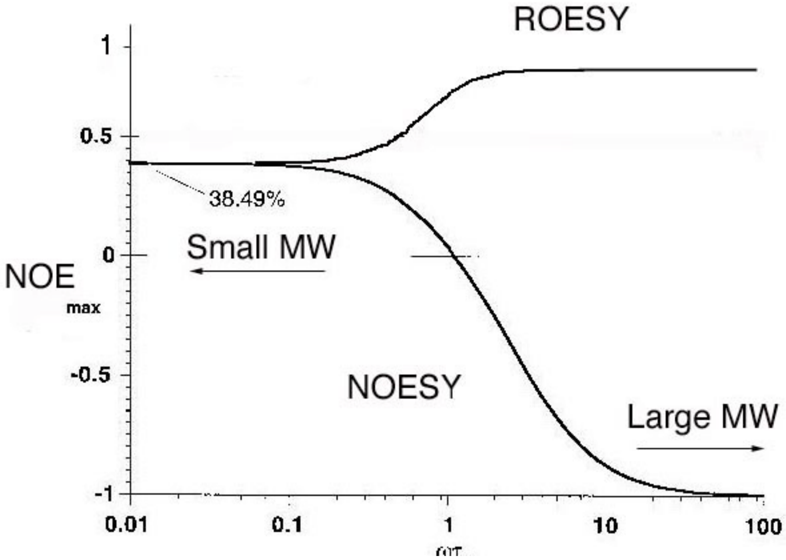
NOESY also detects chemical and conformational exchange (termed often EXSY-exchange spectroscopy-in this case); nOe may be observed between protons that are up to **4Å** apart, while the upper limit for large molecules is about **5Å**.



Rotating frame Overhouser Effect NMR spectroscopy (ROESY)

Molecular Weight and Maximum NOE

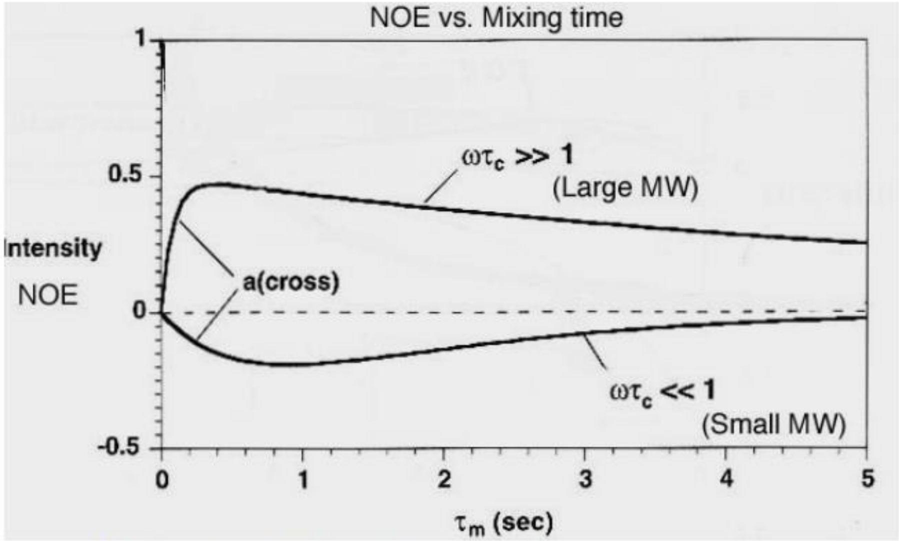
The maximum possible NOE depends on the correlation time (or approximately the inverse of the rate of molecular tumbling), which is in large part determined by the molecular weight and solvent viscosity. Larger molecular weights and higher viscosities lead to larger correlation times. The NOE is positive for small molecules (MW< 600), goes through zero for medium-sized molecules (MW range 700 – 1200), and becomes negative for large molecules (MW>1200). (These MW ranges are approximate only.) For medium sized molecules, the NOE may be theoretically zero. See the figure below that is adapted from Newhaus and Williamson text. The ROESY experiment (rotating frame NOE) is preferred for medium-sized molecules since the ROE is always positive.



Time Dependence of NOE - Mixing Times

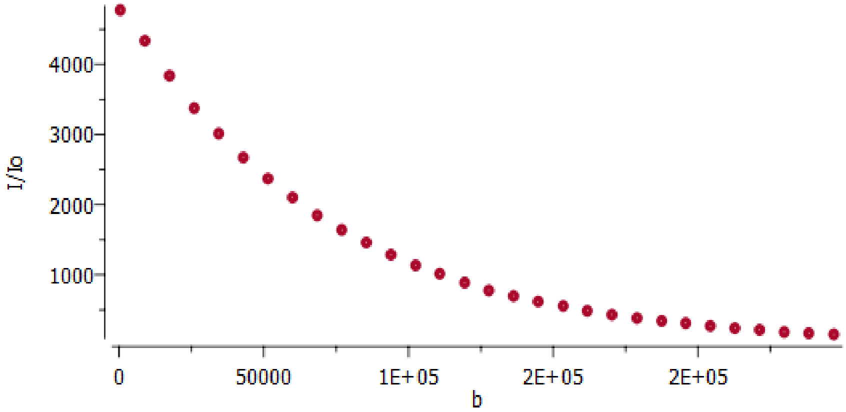
In transient experiments, such as NOESY and ROESY, the NOE dynamically builds up and then decays due to relaxation during the mixing time, as shown below in the plot of NOE versus mixing time. The NOE, thus, goes through a maximum as function of mixing time. The location of the maximum NOE and rate of build-up depend on the correlation time, or its proxy, the molecular weight, and the distance between protons for a particular NOE. In general, large molecules build-up NOE quickly while small molecules build-up NOE more slowly. That is, for large molecules the point of maximum NOE is shifted to shorter mixing times. A shorter distance between protons will also lead to faster build-up of NOE and a shift of the maximum to shorter mixing times.

There is only one mixing time specified per NOE experiment, and it is the most important parameter for NOE experiments. For small molecules, a mixing time that maximizes the NOE is desirable, unless you intend to calculate an actual distance (see analysis section). Generally, one is interested in a range of distances so the choice depends on molecular weight rather than a particular distance. For large molecules, the mixing time must be kept small so that the build-up obeys the linear approximation and spin diffusion is avoided (see analysis section). The following are guidelines:



1) small molecules 0.5 -1 sec. Start with 0.5 sec.
2) medium size molecules 0.1 -0.5 sec. Start with 0.25 sec.
3) large molecules 0.05 - 0.2 sec. Start with 0.1 sec.

Diffusion-Ordered NMR spectroscopy (DOSY)

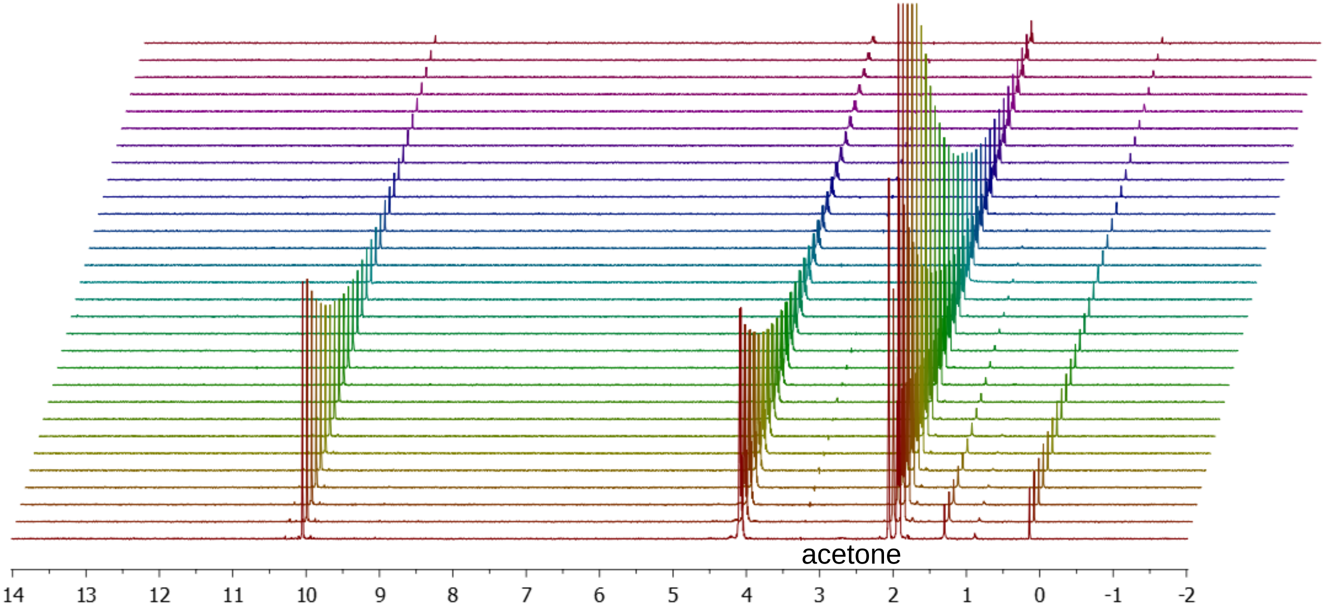


$$\ln\frac{I}{I_o} = -b\boldsymbol{D}$$

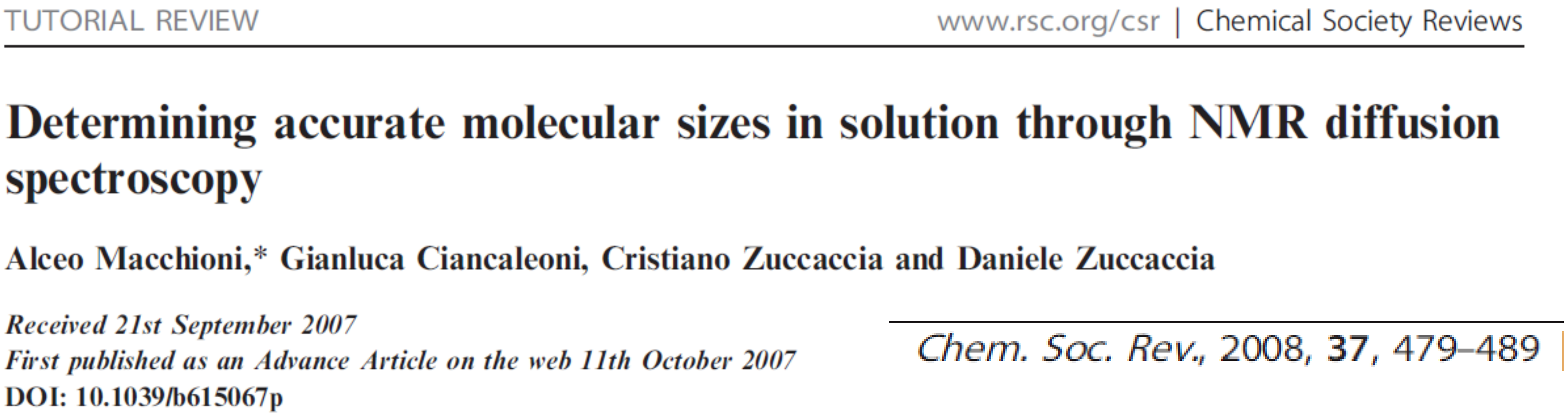
Stejskal-Tanner equation

$$r_H = \frac{k_B T}{6\pi\eta\boldsymbol{D}}$$

Stokes-Einstein equation



Diffusion-Ordered NMR spectroscopy (DOSY)



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Determining accurate molecular sizes in solution through NMR diffusion spectroscopy

Alceo Macchioni,* Gianluca Ciancaleoni, Cristiano Zuccaccia and Daniele Zuccaccia

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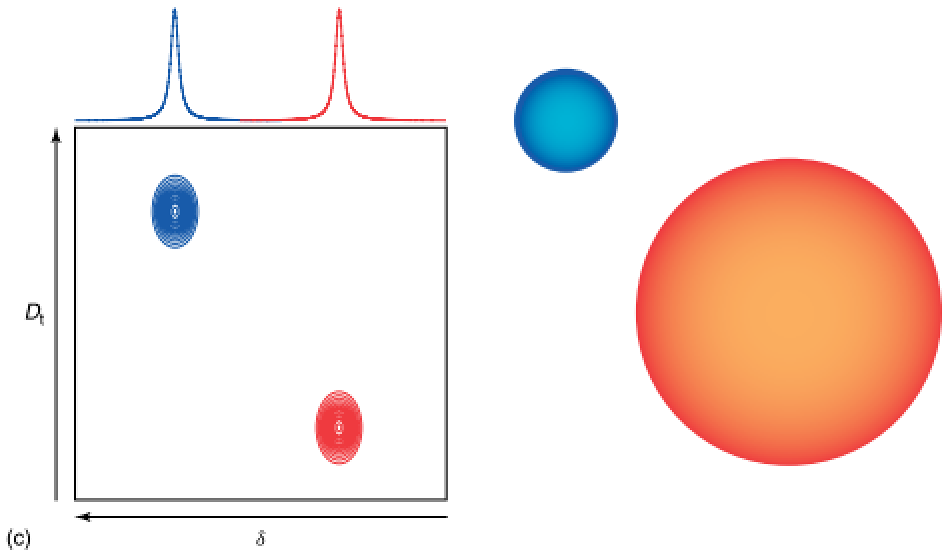
Chem. Soc. Rev., 2008, **37**, 479–489

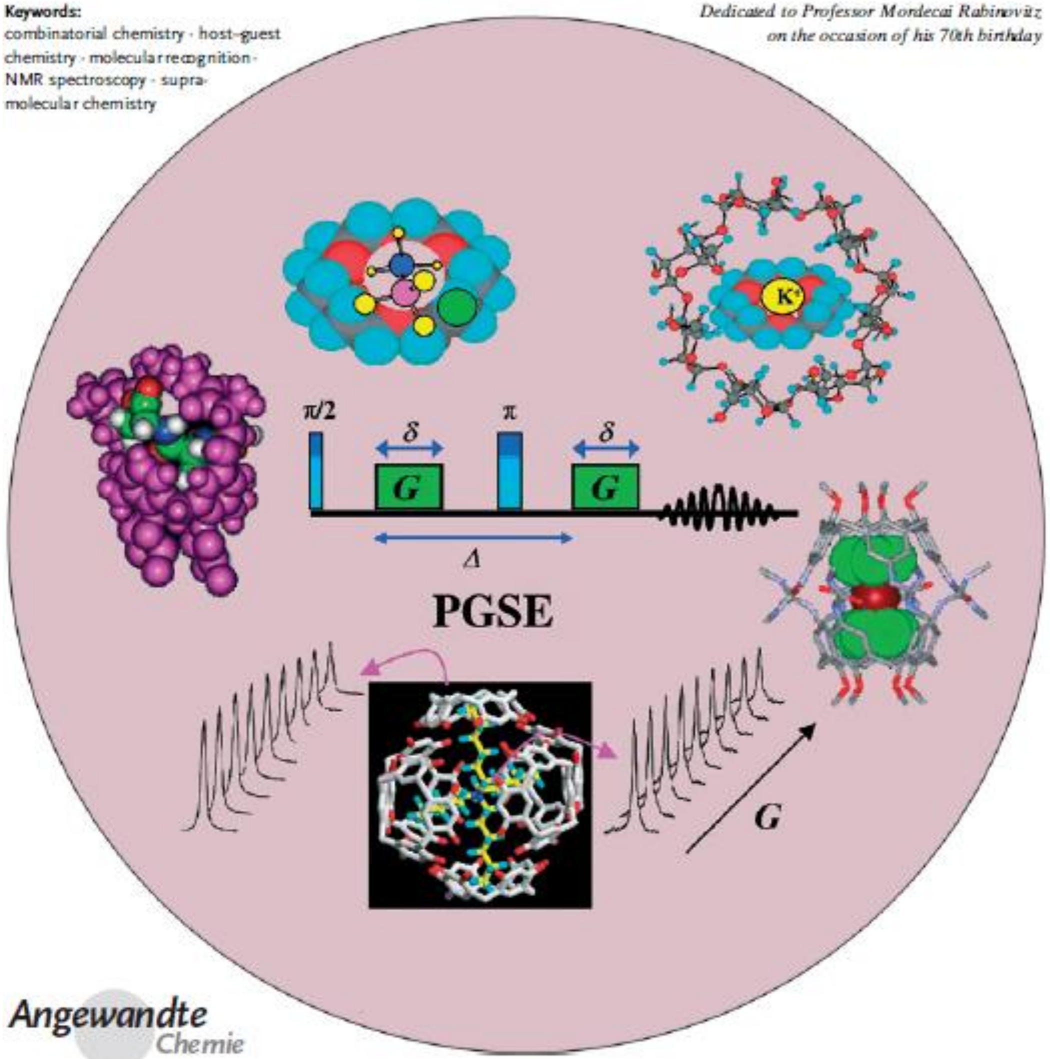


Diffusion NMR Spectroscopy in Supramolecular and Combinatorial Chemistry: An Old Parameter—New Insights

Yoram Cohen, Liat Avram, and Limor Frish*

Keywords:
combinatorial chemistry · host–guest chemistry · molecular recognition · NMR spectroscopy · supramolecular chemistry

Dedicated to Professor Mordecai Rabinovitz on the occasion of his 70th birthday



Angewandte Chemie

DOI: 10.1002/anie.200300637

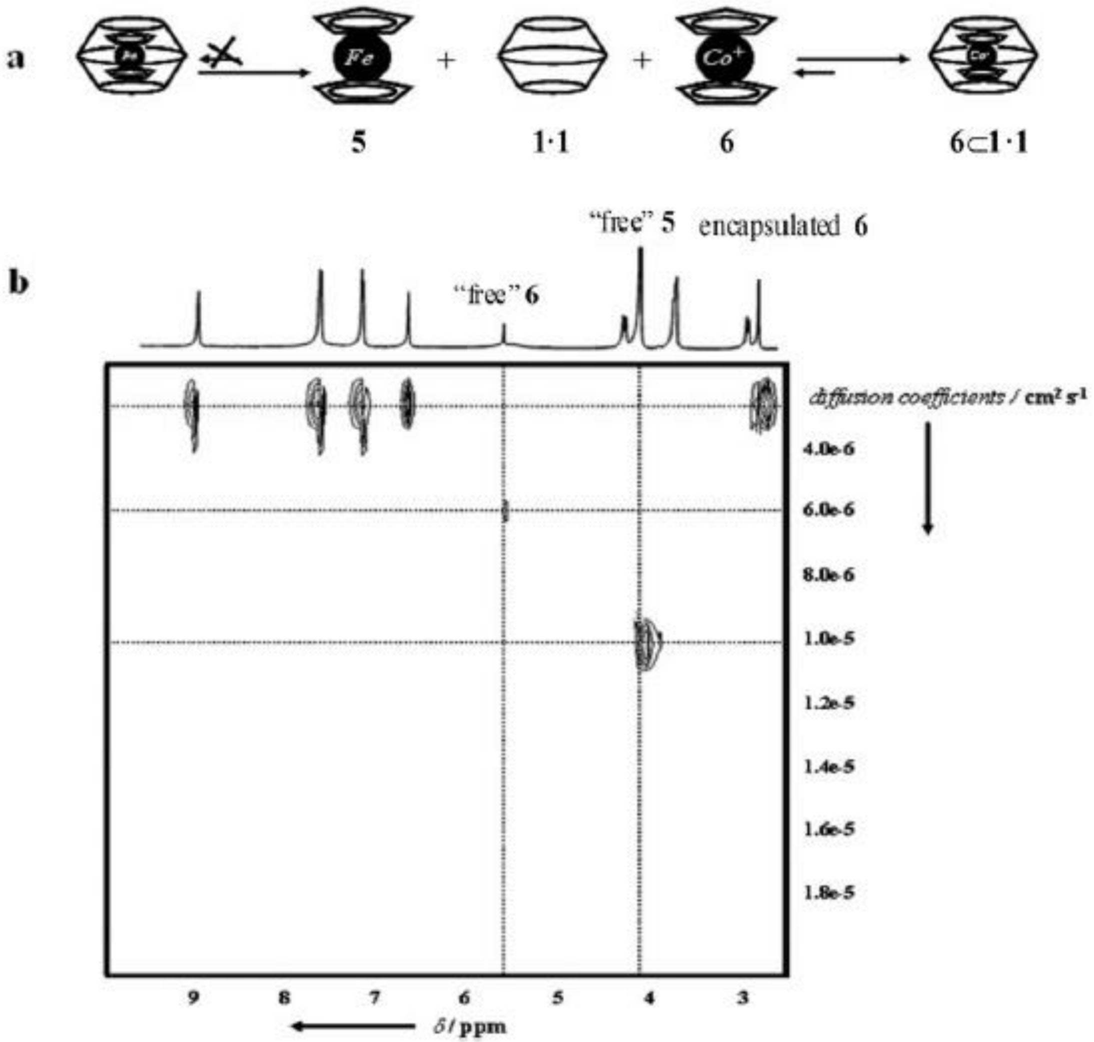
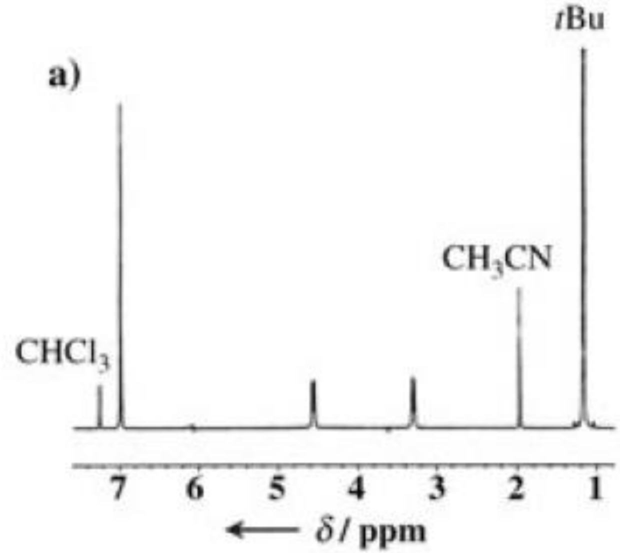
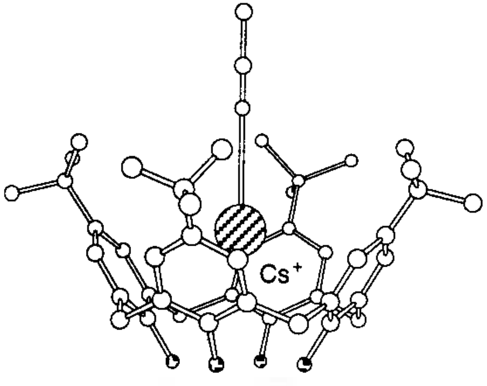
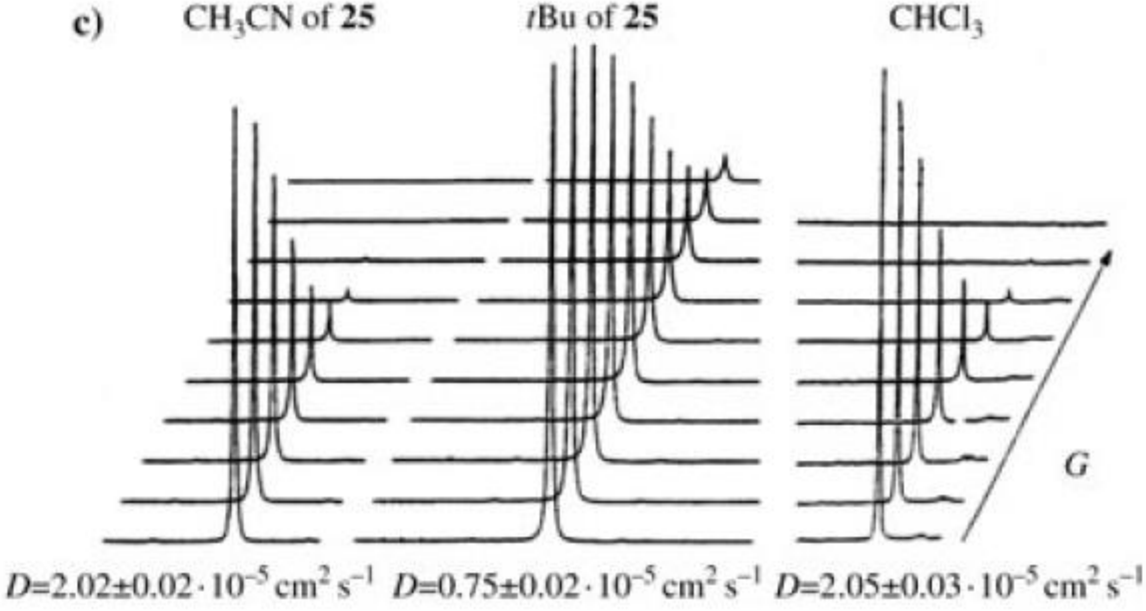


Fig. 3 (a) Schematic representation of the reaction of **5** and **6** with $\mathbf{1}_2$, and (b) the DOSY spectrum (400 MHz, 298 K) of the $C_2D_4Cl_2$ solution of $\mathbf{1}_2$ in the presence of **5** and **6**.[38] Adapted with permission from ref. 19*b*, copyright © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.


a)
tBu
CH3CN
CHCl3
7
6
5
4
3
2
1
← δ / ppm


Cs+


c)
CH3CN of 25
tBu of 25
CHCl3
G
D=2.02±0.02·10−5 cm2 s−1
D=0.75±0.02·10−5 cm2 s−1
D=2.05±0.03·10−5 cm2 s−1

The Time-scales

Time-lapse required for interaction between radaition and sample

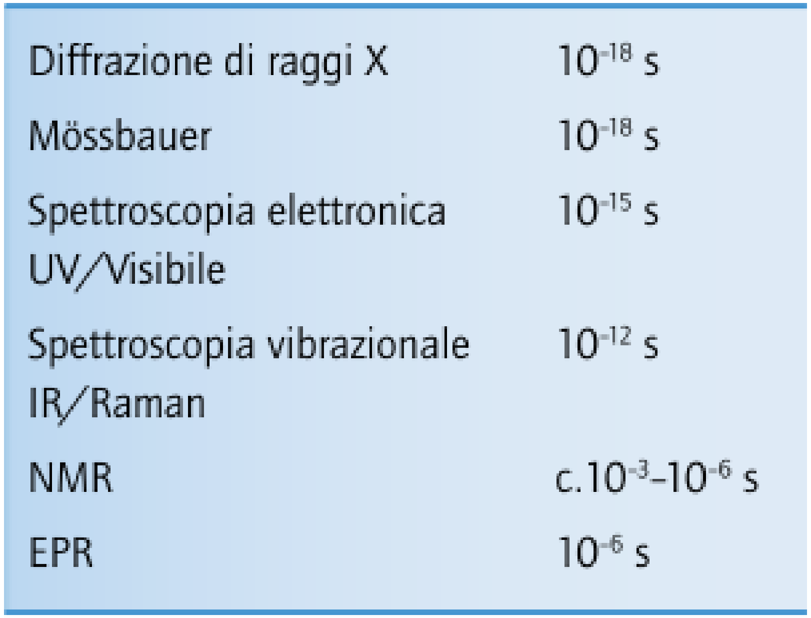
For X-ray, the photon interacts with the sample in a time-lapse 10^{-16} - 10^{-19} s, shorter than molecular vibrations (10^{-13} – 10^{-14} s), and rotations (10^{-10} – 10^{-12} s).

↓

The photon captures the molecules in a fixed vibrational and rotational situation, like a snapshot.

The Time-scales

The interaction between a radiation and a sample pomotes processes that occur in given time-scales.



Diffrazione di raggi X	10^{-18} s
Mössbauer	10^{-18} s
Spettroscopia elettronica UV/Visibile	10^{-15} s
Spettroscopia vibrazionale IR/Raman	10^{-12} s
NMR	c.10^{-3}–10^{-6} s
EPR	10^{-6} s

The Time-scales

Lifetime of the excited states

Some spectroscopic techniques relay on the population of excited states: UV-Visibile, IR, NMR, ...

The excited state lifetime is the time required for an excited state to go back to the fundamental state: relaxation time.

Heisenberg:

$$\tau \Delta E = h$$

τ is the lifetime of the excited state; ΔE is the uncertainty on the energy of the excited state

$h \approx 10^{-34}$ J s

$\Delta\nu = \Delta E/h = h/2\pi\tau h \approx 1/\tau$ uncertainty on the frequency

Es: $\tau = 10^{-15}$ s for electronic spectroscopy $\Delta\nu = 10^{15}$ Hz same range of UV-Vis:

BROAD BANDS!

The Time-scales

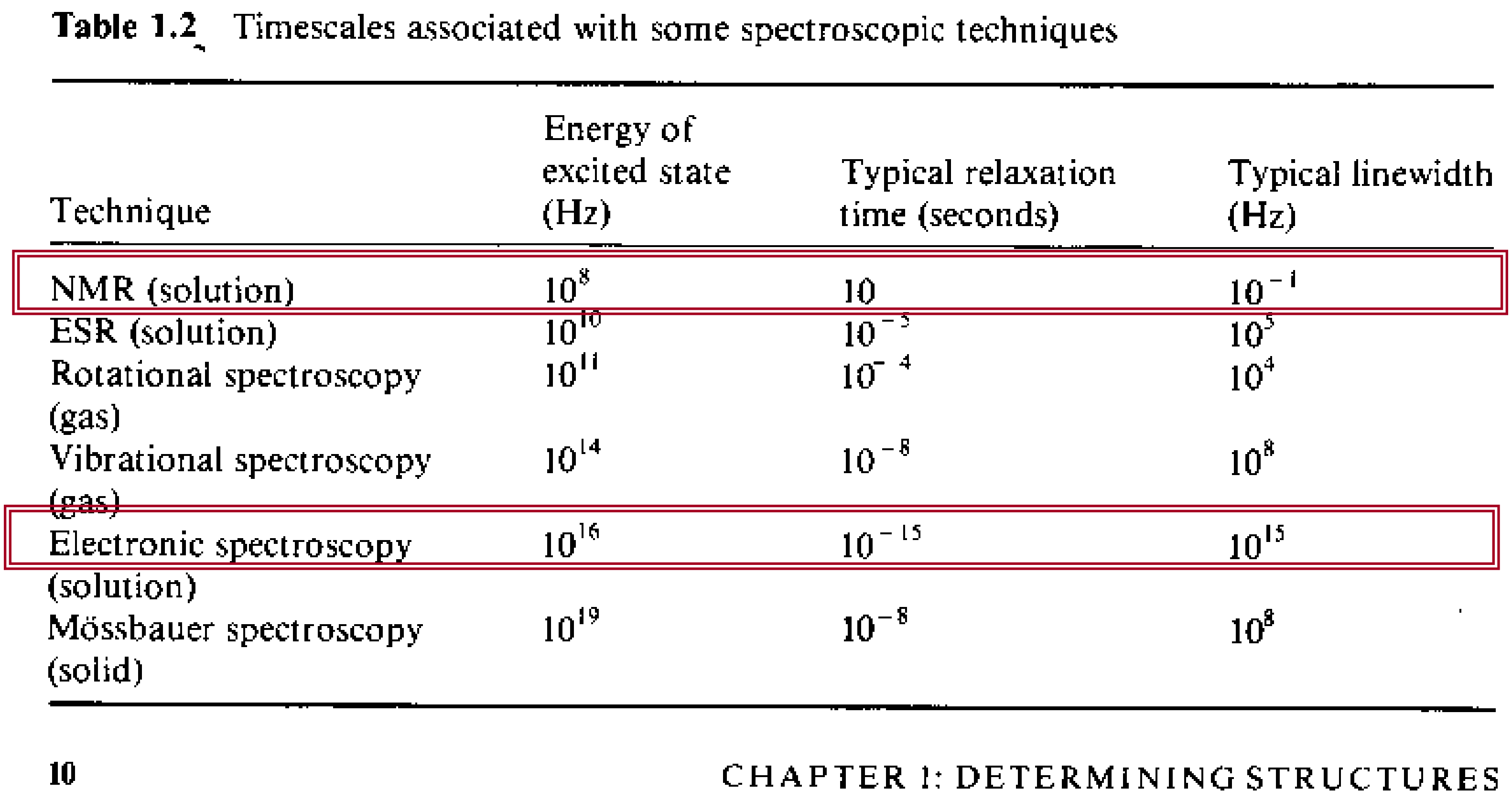
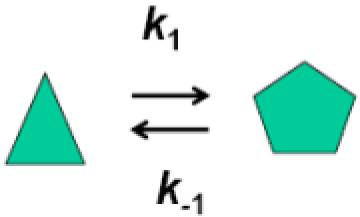
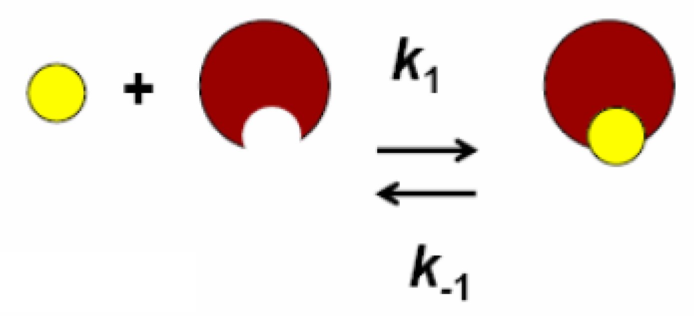


Table 1.2 Timescales associated with some spectroscopic techniques

Technique	Energy of excited state (Hz)	Typical relaxation time (seconds)	Typical linewidth (Hz)
NMR (solution)	10^8	10	10^{-1}
ESR (solution)	10^{10}	10^{-5}	10^5
Rotational spectroscopy (gas)	10^{11}	10^{-4}	10^4
Vibrational spectroscopy (gas)	10^{14}	10^{-8}	10^8
Electronic spectroscopy (solution)	10^{16}	10^{-15}	10^{15}
Mössbauer spectroscopy (solid)	10^{19}	10^{-8}	10^8

Chemical Exchange

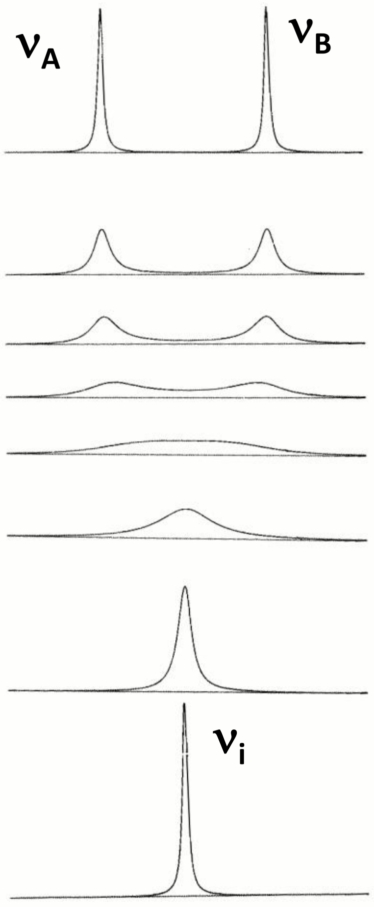




Chemical Exchange

Exchange between equally populated sites

$$A \underset{k_B}{\overset{k_A}{\rightleftharpoons}} B$$



Temp. 1

Slow

$p_a = p_b = 0.50$

$\nu_i = p_a \nu_a + p_b \nu_b$

Coalescence Temperature

Temp. 1 < Temp. 2

Temp. 2

Fast

Chemical Exchange

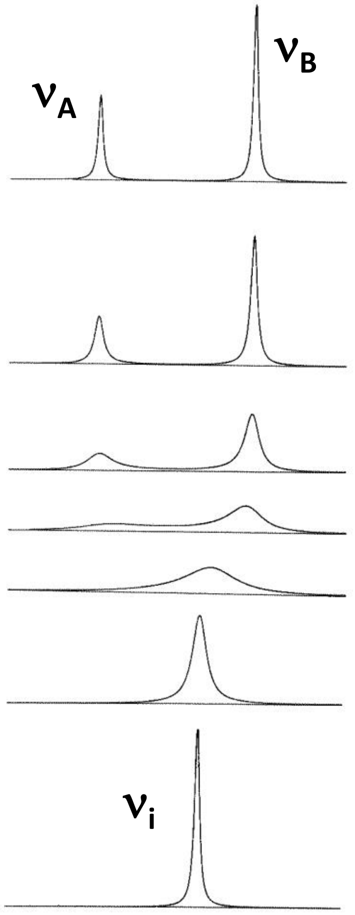
Exchange between non-equally populated sites

$$A \underset{k_B}{\overset{k_A}{\rightleftharpoons}} B$$

$p_a = 0.33$ $p_b = 0.66$

$\nu_i = p_a \nu_a + p_b \nu_b$

Temp. 1



Slow

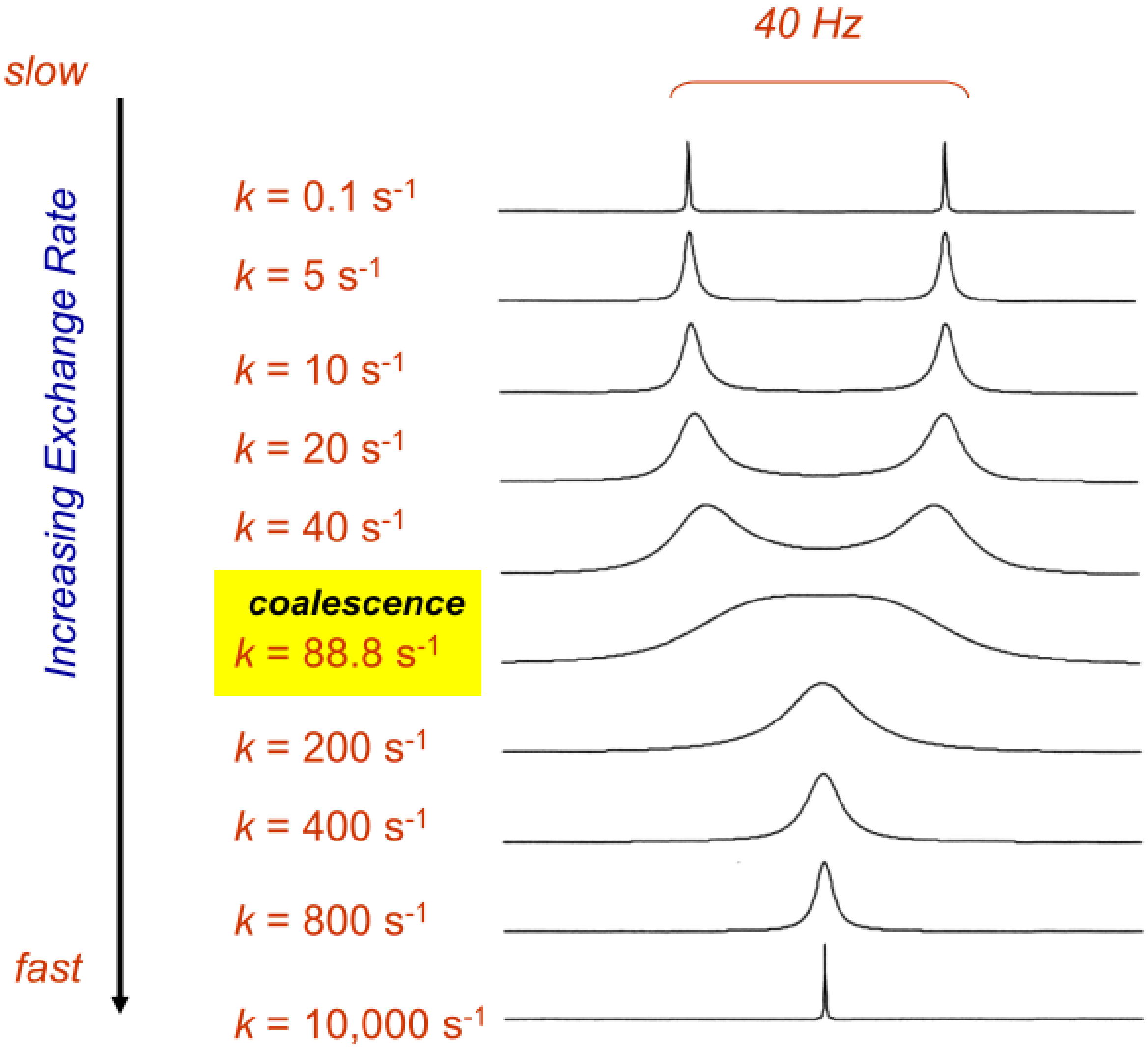
Coalescence Temperature

Temp. 1 < Temp. 2

Temp. 2

Fast

Chemical Exchange



40 Hz

slow

Increasing Exchange Rate

$k = 0.1\ s^{-1}$

$k = 5\ s^{-1}$

$k = 10\ s^{-1}$

$k = 20\ s^{-1}$

$k = 40\ s^{-1}$

coalescence

$k = 88.8\ s^{-1}$

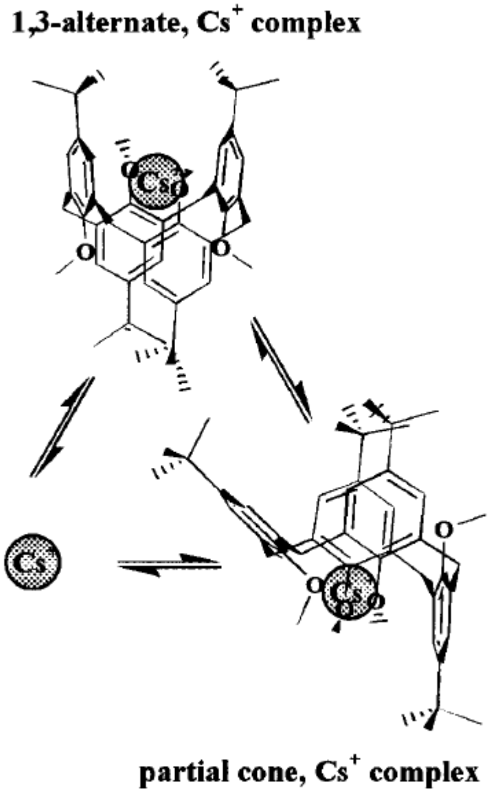
$k = 200\ s^{-1}$

$k = 400\ s^{-1}$

$k = 800\ s^{-1}$

fast

$k = 10{,}000\ s^{-1}$



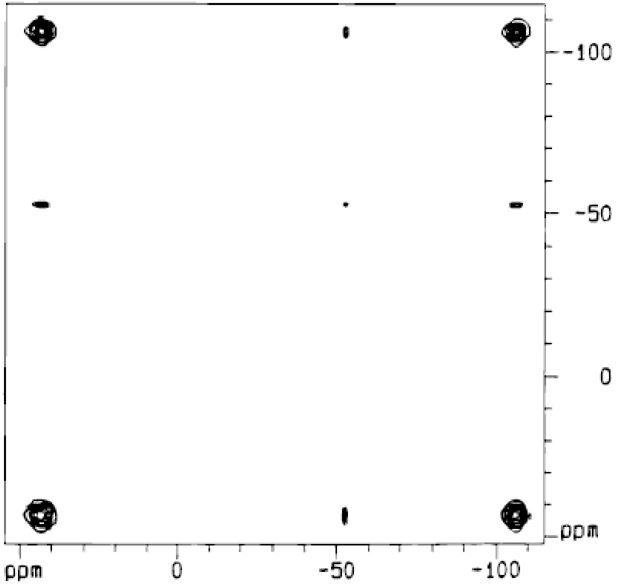


Figure 5. ^{133}Cs 2D-EXSY spectrum of [**1**] = 43.3 mM, [CsI_3] = 80.4 mM at 283 K: mixing time (τ_m) = 0.1 s.

Lineshape analysis – determinatin of kinetic constants at different Temperatures

(suitable for the study of exchange processes on timescales from 10 µs to 100 ms)

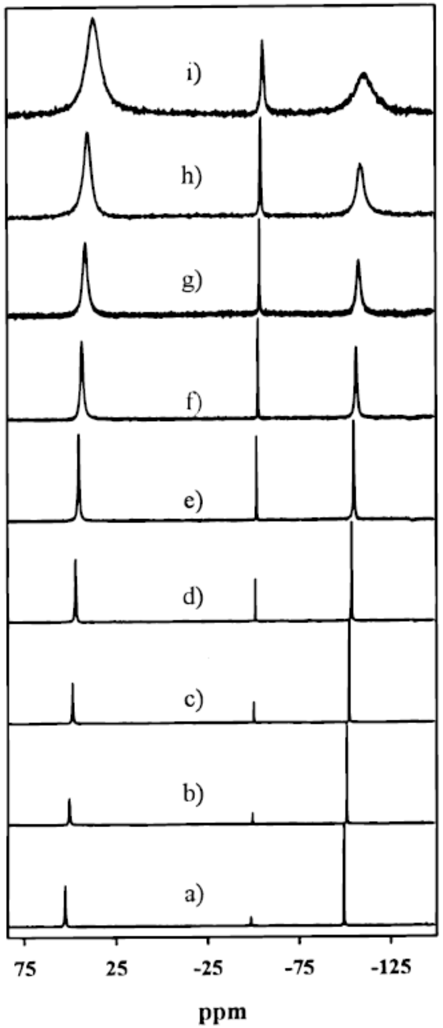


Figure 3. ^{133}Cs NMR spectra of CsI_3 80.4 mM, in the presence of 43.3 mM tetramethoxycalix[4]arene (**1**). The spectra were recorded at various temperatures: 236 (a), 246 (b), 254 (c), 264 (d), 274 (e), 283 (f), 292 (g), 300 (h), and 314 K (i).

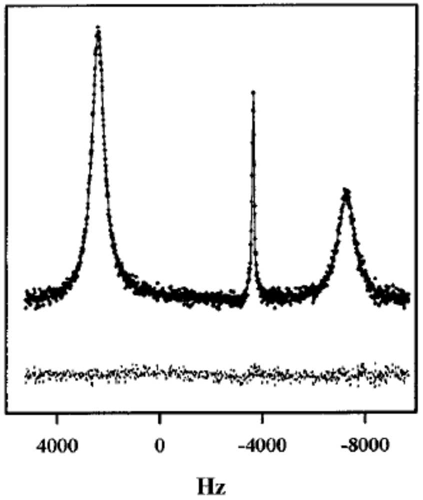
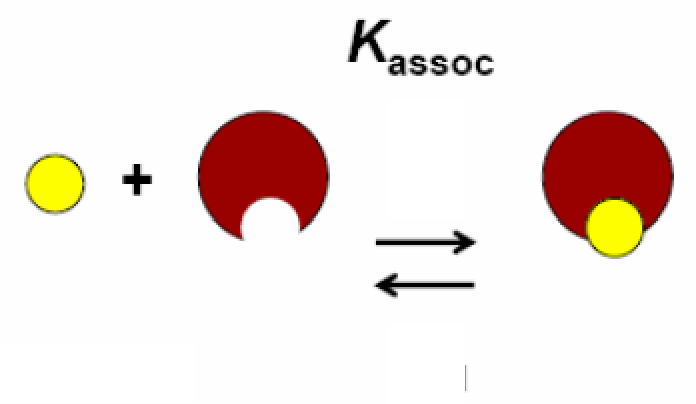


Figure 4. ^{133}Cs NMR spectra of [**1**] = 43.3 mM, [CsI_3] = 80.4 mM at 314 K showing the measured spectrum, the fit (solid line) from DNMR5, and the difference.

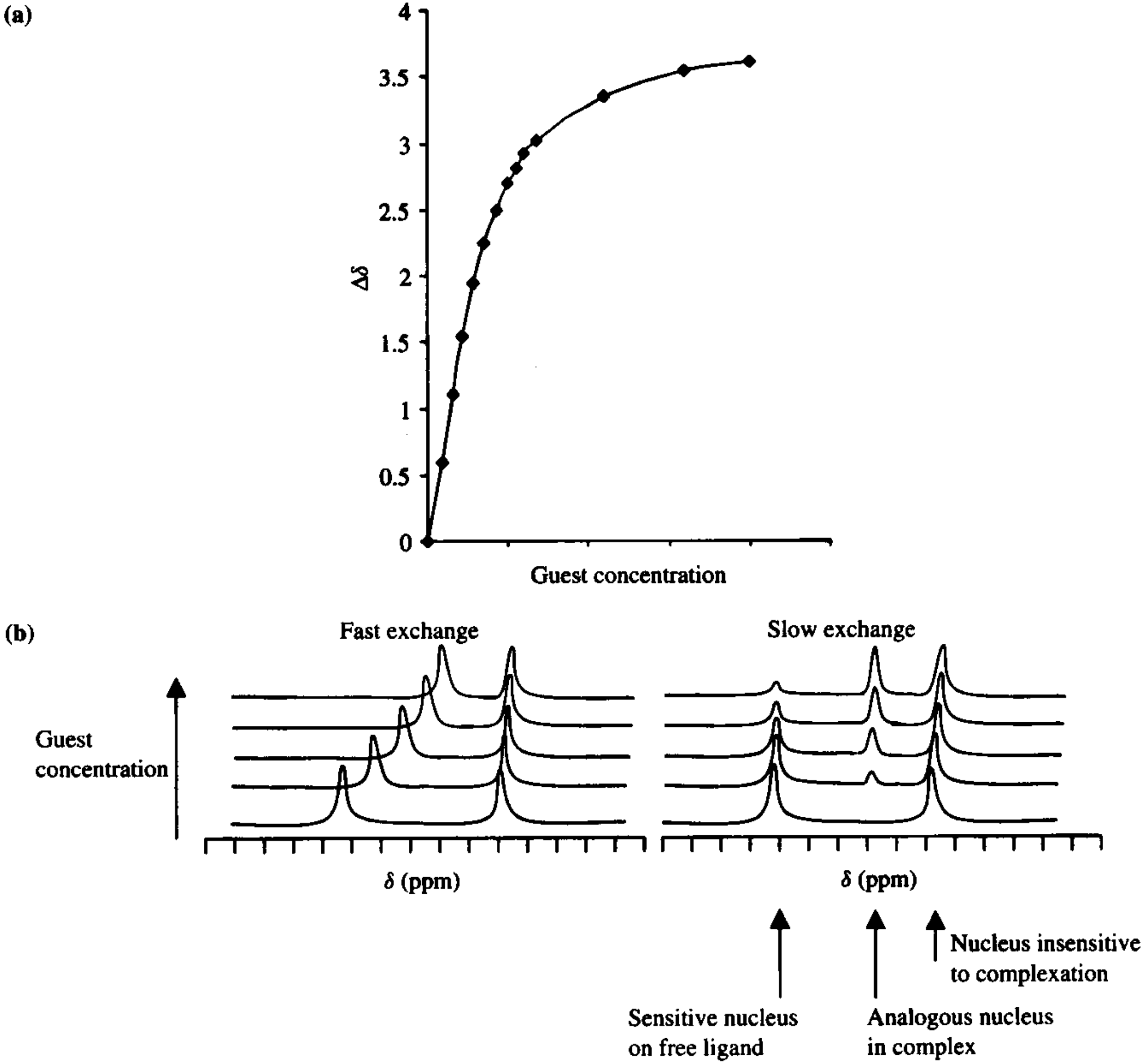


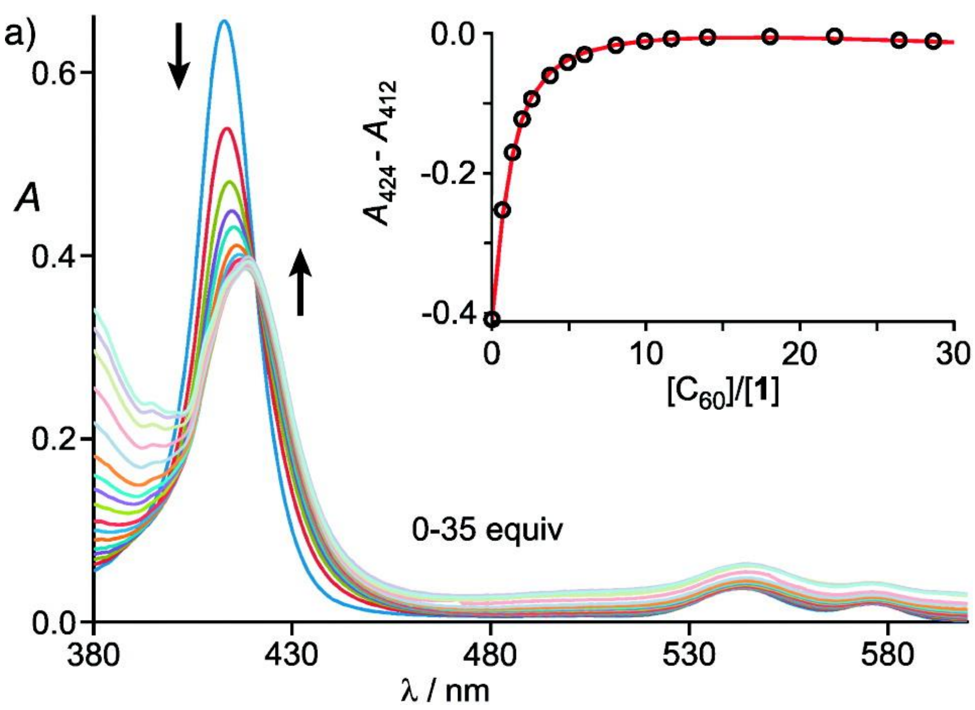
- NMR : [i] ≈ mM, K_{max} ≈ 10^4

- Microcalorimetry : [i] ≈ mM, K_{max} ≈ 10^7

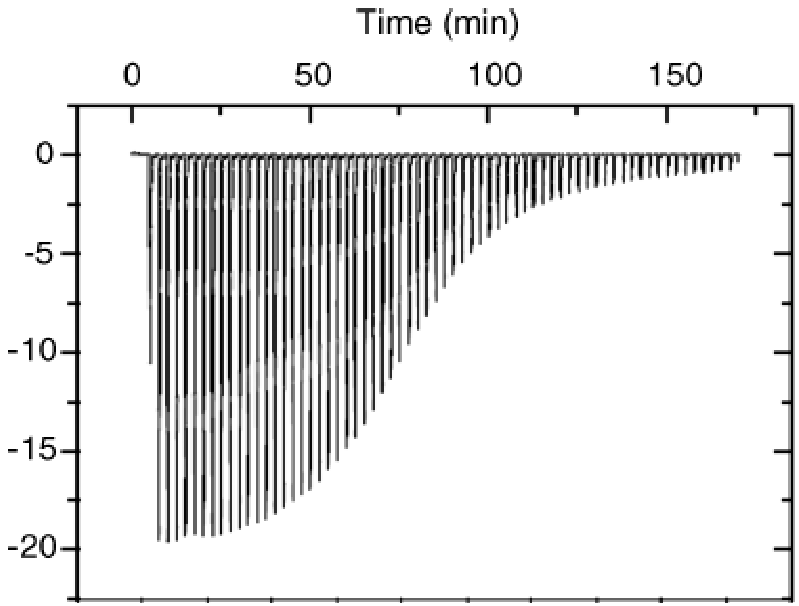
- UV-vis : 0.01 mM < [i] < mM, K_{max} ≈ 10^6

- Fluorimetry : [i] ≈ µM, K_{max} ≈ 10^8

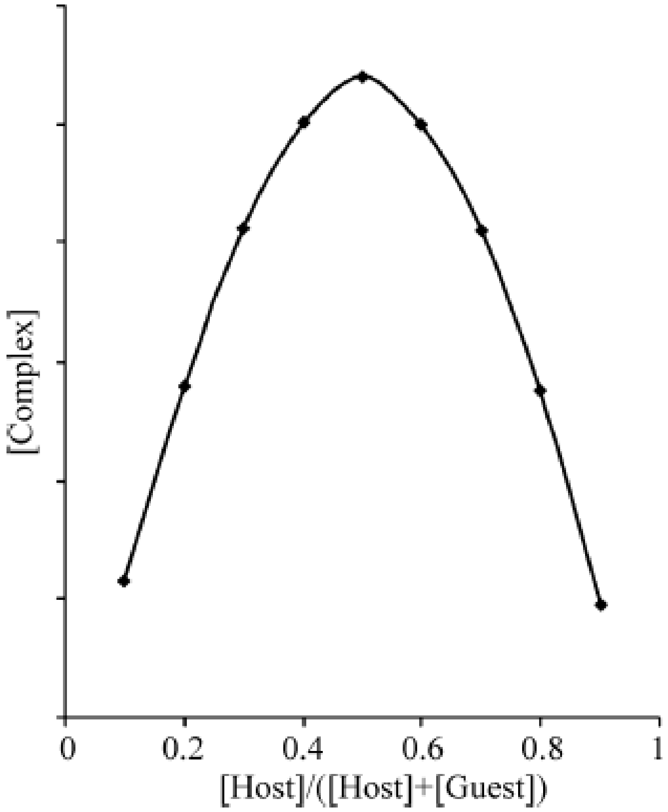

(a)
4
3.5
3
2.5
2
1.5
1
0.5
0
Δδ
Guest concentration
(b)
Fast exchange
Slow exchange
Guest concentration
δ (ppm)
δ (ppm)
Nucleus insensitive to complexation
Sensitive nucleus on free ligand
Analogous nucleus in complex



IsoThermal Calorimetry (ITC)



The job-plot



The total molar concentration of the two binding partners are held constant, but their mole fractions are varied.

An observable that is proportional to complex formation is plotted against the mole fractions of these two components.

The maximum on the plot corresponds to the stoichiometry of the two species.