Spectroscopy

Course number: BIP-31306

Principles and Applications of Spectroscopic Techniques in Molecular Sciences

Programme BMW - Molecular Life Sciences

Laboratory of Biophysics
Wageningen University

September 2007

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Contents

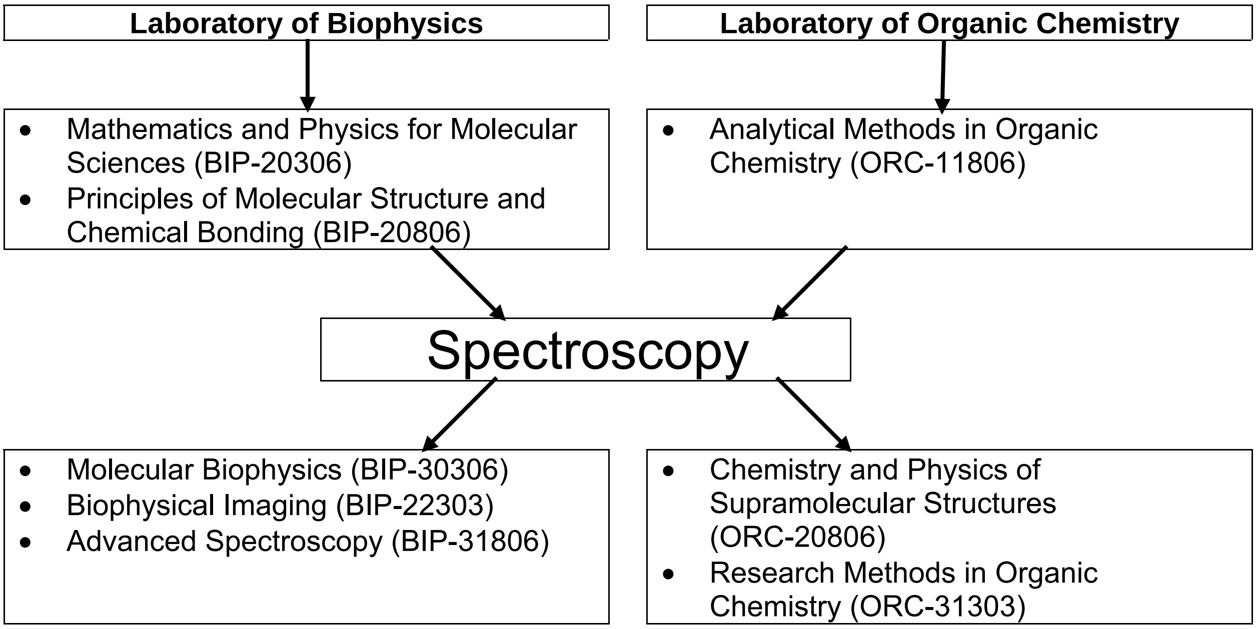
1. General

Introduction

This course deals with the physical background of various spectroscopic techniques and the use of these techniques in the analysis of the structure and dynamics of organic and biological compounds. It is an extension of the courses Analytical Methods in Organic Chemistry and Principles of Molecular Structure and Chemical Bonding. The course will focus on optical spectroscopy (UV/VIS, fluorescence and phosphorescence) and magnetic resonance spectroscopy (NMR and ESR). The practical part of the course will give an illustration of the subjects treated in the lectures and provides training in the use of spectroscopic equipment and the interpretation of spectra. At the end of the course the students will work in small groups to learn to select and apply spectroscopic methods in practice.
The laboratory of Biophysics is involved in the main part of the course and organization of the written exam. The projects are supervised by both staff of the laboratory of Organic Chemistry and the laboratory of Biophysics.

Setting

The setting of the course Spectroscopy within the course programme Molecular Sciences is shown below. Please note that a successful completion of the courses Principles of Molecular Structure and Chemical Bonding (BIP-20806) and Analytical Methods in Organic Chemistry (ORC-11806) is a strong advantage in successful participating in the course Spectroscopy.



Aims

The first aim of the course Spectroscopy is to introduce the participants into elementary spectroscopic techniques and to apply those techniques to:

- The determination of a number of physical properties of molecules
- The structure determination of organic compounds

The second aim is to learn how to integrate the knowledge of spectroscopy in applied research projects.

Subjects

The following spectroscopic methods will be treated:

- Optical spectroscopy (UV/VIS, fluorescence and phosphorescence)
- Magnetic resonance spectroscopy (NMR and ESR)

Of each spectroscopic technique an explanation will be given of the basic principles. Furthermore, areas will be indicated where these methods can be applied to problems in Molecular Sciences. Subsequently the course material is completed with

- Exercises and problems
- Laboratory experiments
- Applied projects

The laboratory experiments are meant as a direct illustration of the course material and an introduction into the spectroscopic equipment. The aim of the applied projects is to integrate the spectroscopic knowledge in applying the methods to a practical problem from the field of molecular sciences.

Course elements

The course consists of lectures, tutorials, laboratory experiments and projects.

Lectures

- Presentation of the subject matter
- Sketching the broad outlines
- Preparation for the tutorials, exercises, laboratory experiments

Tutorials

- Further explanation of the subject matter
- Testing the knowledge by practising with exercises and problems
- Preparation for the laboratory experiments and final written exam
- Feedback with lectures and laboratory experiments

Laboratory experiments

- Performing spectroscopic experiments
- Interpretation of the spectroscopic results
- Learn spectroscopy through working with spectroscopic instruments
- Practicing the knowledge and understanding of the subject matter by working on experimental problems
- Feedback with lectures and tutorials

Projects

- Integration of the spectroscopic knowledge, including the knowledge obtained in the courses Principles of Molecular Structure and Chemical Bonding (BIP-20806) and Analytical Methods in Organic Chemistry (ORC-11806)
- Application of the course material in applied research projects

- As good as possible solving of an applied scientific problem
- Working out of experimental results
- Processing the results and conclusions in a written report and oral presentation

Course programme

Assignment of the groups
The total number of participants is subdivided into at most four groups (A to D) for the laboratory experiments and projects. The Group Assignment is handed over at the start of the course.

Lectures and tutorials
The lectures and tutorials will be presented to all groups together in lecture room C26 (previously C89, room 3029), Transitorium 3rd floor.

Laboratory experiments
The planning of the laboratory experiments is indicated in the course schedule. Refer to chapter 7 “Laboratory Experiments” for the codes of the experiments.

Projects
The aim of the projects is to get trained in the use of spectroscopic techniques in solving scientific problems in Biophysics and Organic Chemistry. Based on a project description, spectroscopic experiments should be proposed to work on a particular problem. Then the measurements should be done and interpreted, leading to a written report and oral presentation.
In the projects, the staff of the laboratories involved will give feedback to the students, but in this part of the course the emphasis will be on one's own initiatives and insight in the spectral analysis. As a result, it is real life research.
A plenary meeting of all groups will end this part, where the results and conclusions of the research projects will be presented,

Course material

- Course book: Atkins, P. and De Paula, J., Physical Chemistry, 8th edition, 2006 (Oxford University Press, ISBN 0-19-870072-5).
- Course Syllabus and manual (will be handed over at the beginning of the course).
- Handouts and background material are available on the Spectroscopy Blackboard site.
- Group Assignment (will be hand over at the beginning of the course).

Reports

- Both reports of the laboratory experiments and projects should conform to the guidelines as given in the brochure “Recording, reporting and presenting your work” by the former School of Technology and Nutrition (see “Guidelines for reports” on the Spectroscopy Blackboard site)
- Concerning the reports of the laboratory experiments, make clear with the instructor and your fellow students what the requirements are and what your contribution should be. All reports must be submitted to the instructors for the experiments, in principle within one working day. The latest date reports of the laboratory experiments will be accepted is **Monday October 1st 2007**.
- The project reports must be submitted **directly after** the project, i.e. on **Friday October 5th 2007** (project in week 5) and **Thursday October 11th 2007** (project in week 6; before the plenary presentation of the projects). On these dates also the

reflection reports for respectively project 1 and 2 have to be submitted (see chapter 8).
- Without giving one's reasons late submission of the reports will lead to exclusion at the written exam ("*Zonder opgave van redenen leidt te late inlevering van de verslagen tot uitsluiting van het schriftelijke examen*").

Presence

- Presence at the lectures, tutorials, laboratory experiments and projects is **obligatory**.

Evaluation

The evaluation of the course elements will be based on the following points:
- Active presence
- Willingness to acquire knowledge
- Execution of problems and experiments
- Execution of the problems and assignments in the experiments
- Execution of the reports
- Oral presentation of the results and conclusions of the projects

Indication of marks

- 1-4: (unacceptable)
- 5 (Just sufficient)
- 6 (Sufficient)
- 7 (Satisfactory)
- 8 (Good)
- 9 (Very good)
- 10 (Excellent)

If a student tends to a mark ≤ 5 in laboratory experiments or projects, he will be addressed immediately by the instructor. To let the student improve the instructor may decide to provide the student with additional tasks and assignments.

Key words for evaluation of practical work

Category	*Key words for evaluation of laboratory experiments and projects*
Character	Social – self-assured – independent – division of tasks – contribution to group and project – leader – dominant
Scientific knowledge	Grasp on spectroscopy – understanding – insight – interest
Attitude	Will to learn – catching up – planning – organization – enthusiasm – active presence – interest
Motivation	Contribution – effort – dedication
Initiatives	Creative – independent
Communication	(Active) English speaking – quality of writing – clarity
Presentation	Speaking – clarity – use of PowerPoint slides

Final mark

The final mark for the course is calculated using the following weight factors:

Written exam	5
Laboratory experiments	1
Project Biophysics	2
Project Organic Chemistry	2
Total	10

For all parts of the course the **bottom mark is 5.0** (i.e. a course part should be done again if the mark is below 5.0). Only after all parts of the course are finished, the final mark can be passed on to the Central Student Administration.

Open-book written exam

The open-book written exam is intended to monitor your understanding and knowledge of the subject matter treated in the course. The **subject matter (NL: examenstof)** is the course material treated in all lectures, tutorials, laboratory experiments and projects. An **open-book exam** means that all books, notes and course materials may be used at the exam.
The emphasis of this course is on the acquisition of insight and understanding and not on cramming the subject matter. For this reason, the written exam consists of a number of open problems. Therefore problem solving is an important issue in this course as well as using the knowledge in applications of the spectroscopic techniques. An active participation in the lectures, tutorials and experiments, and actively training in the solution of exercises and problems is therefore essential.

Lecturers and instructors

Course coordinator and lecturer for the lectures and tutorials

- Frank Vergeldt, laboratory of biophysics, room 1024, Transitorium 1st floor
  E-mail: Frank.Vergeldt@wur.nl

Instructors for the laboratory experiments

- Optical spectroscopy
  Ruud Spruijt, laboratory of biophysics, room 1052, Transitorium 1st floor
  E-mail: Ruud.Spruijt@wur.nl
- ESR
  Cor Wolfs, laboratory of biophysics, room 1052, Transitorium 1st floor
  E-mail: Cor.Wolfs@wur.nl
- NMR
  Pieter de Waard, laboratory of biophysics, room 1068, Transitorium 1st floor
  E-mail: Pieter.deWaard@wur.nl

Instructors for the projects

Laboratory of Biophysics

- Rob Koehorst, laboratory of biophysics, room 1059, Transitorium 1st floor
  E-mail: Rob.Koehorst@wur.nl

Laboratory of Organic Chemistry

- Barend van Lagen, laboratory of organic chemistry, room K171, basement Chemistry building
  E-mail: Barend.vanLagen@wur.nl

Locations

Lectures and tutorials	Lecture room C26 (old C89), Transitorium 3rd floor
Experiments OS	Biophysics, room 1071[1], Transitorium 1st floor
Experiments ESR	Biophysics, room 1029, Transitorium 1st floor
Experiments NMR	Biophysics, room 1071[1], Transitorium 1ste floor
Projects Biophysics	Biophysics, room 1071[1], Transitorium 1st floor
Projects Organic Chemistry	Organic chemistry, room 169, basement Chemistry building
Plenary presentation	Lecture room C26 (old C89), Transitorium 3rd floor
Written exam	Lecture room C17 (old C94), Agrotechnion
Written re-exam	Lecture room C25 (old C85), Transitorium
Total re-exam	Lecture room C25 (old C85), Transitorium

[1] This is the coffee room Biophysics in the northeast corner of the Transitorium.

General study hints

- Prepare yourself thoroughly for the lectures, tutorials and laboratory experiments
- Work out properly the lectures and tutorials
- Work out as many problems and exercises as possible: learn by doing
- Work out the laboratory experiments as soon as possible and do not wait for the final deadline
- See the brochure “Recording, reporting and presenting your work” by the former School of Technology and Nutrition (see http://www.technologyandnutrition.www), for instructions how to present your work
- Make good agreements with your fellow students in the laboratory experiments and projects and allocate the tasks in a clear and honest way
- Do not create backlogs and catch up with the work all the time

2. Course Schedule

Lectures and tutorials 3/9 up to 27/9 2007

Week 1 and 3

Monday	08:30 - 10:15, lecture room C26
Tuesday	08:30 - 10:15, lecture room C26
Wednesday	08:30 - 10:15, lecture room C26

Week 2 and 4

Monday	08:30 - 10:15, lecture room C26
Wednesday	08:30 - 10:15, lecture room C26
Thursday	08:30 - 10:15, lecture room C26

The subject matter (NL: examenstof) is from chapters 8 to 15, plus section 23.7 from the course book "Physical Chemistry" of Atkins and De Paula, 8th edition. In addition all material from chapters 8 to 12 as treated during the course Principles of Molecular Structure and Chemical Bonding (BIP-20806) is presumed to be familiar.

Week	Block #	Subject (global)
Week 1	1	• Principles of optical spectroscopy (section 13.1, 13.2 and 13.3)
	2	• The theory of spectroscopic transitions (section 9.10, 10.3, 10.7, 12.6, and 13.2(b))
	3	• Electronic spectra (section 9.1, 9.6, 9.7, 10.1, 11.6, 14.1(c) and (d), and 14.2)
Week 2	4	• The fate of electronically excited states (section 14.3 and 14.4)
	5	• Kinetics of photophysical and photochemical processes (section 23.7)
	6	• Lasers and their application in spectroscopy (section 13.2(a), 14.5, and 14.6(a) and (e))
Week 3	7	• Energies of electrons and nuclei in magnetic fields (section 9.7, 9.8, 15.1, 15.2 and 15.3)
	8	• Nuclear magnetic resonance spectroscopy (section 15.4, 15.5 and 15.6)
	9	• CW and FT NMR spectroscopy (section 15.8)
Week 4	10	• Time domain NMR spectroscopy (section 15.8 and 15.9)
	11	• Two-dimensional NMR spectroscopy (section 15.10, 15.11 and 15.12)
	12	• Electron spin resonance spectroscopy (section 15.14, 15.15 and 15.16)

Laboratory experiments 6/9 up to 28/9 2007

Week 1 and 3

Thursday	08:30 - 12:30, Laboratory of Biophysics
Friday	08:30 - 12:30, Laboratory of Biophysics

Week 2 and 4

Tuesday	08:30 - 12:30, Laboratory of Biophysics
Friday	08:30 - 12:30, Laboratory of Biophysics

Date	**Day**	**Group A**	**Group B**	**Group C**	**Group D**
Week 1 **6/9 and 7/9**	Thursday	OS			
	Friday		OS		
Week 2 **11/9 and 14/9**	Tuesday			OS	
	Friday				OS
Week 3 **20/9 and 21/9**	Thursday	ESR	NMR		
	Friday			ESR	NMR
Week 4 **25/9 and 28/9**	Tuesday	NMR	ESR		
	Friday			NMR	ESR

Instructors

Experiment Code	**Instructor**
OS	Ruud Spruijt
ESR	Cor Wolfs
NMR	Pieter de Waard

Projects 1/10 up to 12/10 2006

Monday to Friday 08:30 - 12:30

Datum	**Group A**	**Group B**	**Group C**	**Group D**
Week 5				
Monday 1/10	Projects Biophysics		Projects Organic chemistry	
Tuesday 2/10	Projects Biophysics		Projects Organic chemistry	
Wednesday 3/10	Projects Biophysics		Projects Organic chemistry	
Thursday 4/10	Work out the data Start writing of report			
Friday 5/10	Finalizing report Preparation oral presentation **Submission of report** **Submission of reflection report week 5 (see chapter 7)**			
Week 6				
Monday 8/10	Projects Organic chemistry		Projects Biophysics	
Tuesday 9/10	Projects Organic chemistry		Project Biophysics	
Wednesday 10/10	Projects Organic chemistry Work out the data		Project Biophysics Work out the data	
Thursday 11/10	Writing of report **Submission of report** **Submission of reflection report week 6 (see chapter 7)**			
Friday 12/10	Plenary presentation projects			

Supervision Laboratory of Biophysics

Project 1:	Rob Koehorst
Project 2:	Frank Vergeldt

Supervision Laboratory of Organic Chemistry

Barend van Lagen

Written exam

Exam
Thursday 25-10-2007, 14:00 - 17:00, lecture room C17 (old C94, Agrotechnion)

Re-exam
Friday 21-12-2007, 9:00 - 12:00, lecture room C25 (old C85, Transitorium, 3rd floor)

Total re-exam
Thursday 21-8-2008, 9:00 - 12:00, lecture room C25 (old C85, Transitorium, 3rd floor)

3. Excersices tutorials

Block 1. Principles of optical spectroscopy

Question 1.1
Calculate the energy of the photons involved in following transitions:

a) Absorption at 523 nm
b) Emission at 15342 cm^{-1}
c) A NMR transition at 300 MHz
d) An ESR transition at 3 cm

Question 1.2
Suppose we have blue green light (500 nm) with an intensity of 1 micro Einstein per second per square meter ($1*10^{-6}$ Einstein s^{-1} m^{-2}), where 1 Einstein represents the energy of 1 mole of photons at a given wavelength. Convert this intensity to watt per square meter (W m^{-2}).

Question 1.3
We want to mimic the emission spectrum of the sun, which has a maximum at approximately 520 nm, in an experimental setup. Assume that both the sun and our light source behave as “black bodies” (see Atkins section 8.1(a)). At what temperature should the radiation source operate?

Question 1.4
What is the essential difference between detection based on a monochromator, a polychromator, and a Fourier transform technique?

Question 1.5
Consider the design of a monochromator. What tradeoff (NL: afweging) is made in the choice of distance from grating (NL: rooster) to slit and the slit width?

Question 1.6
Design a spectrometer to measure the absorption spectra of fluorescent solutions by detecting the fluorescence maximum of that specific solution, the so-called fluorescence excitation spectrum. Make a schematic drawing and label all essential parts. Which type of detector as discussed in question 1.4 is best suited for this device?

Question 1.7 (discussion question 13.1 Atkins on page 476)
Describe the physical origins of linewidths in the absorption and emission spectra of gases, liquids, and solids.

Question 1.8 (exercise 13.2b in Atkins on page 476)
At what speed of approach would a red (660 nm) traffic light appear green (520 nm)?

Question 1.9 (exercise 13.3b in Atkins on page 476)
Estimate the lifetime of a state that gives rise to a line of width (a) 100 MHZ, (b) 2.14 cm^{-1}.

Block 2. The theory of spectroscopic transitions

Question 2.1 (exercise 10.14b on page 359 in Atkins)
Which of the following transitions are allowed in the normal electronic emission spectrum of an atom: (a) 5*d* → 2*s*, (b) 5*p* → 3*s*, (c) 6*p* → 4*f*?

Question 2.2 (exercise 12.14a on page 427 in Atkins)
What states of (a) benzene, (b) naphthalene may be reached by electric dipole transitions from their (totally symmetrical) ground states?

Question 2.3
Calculate the population differences between the states involved in the following transitions:
a) An electronic transition at 523 nm
b) A vibration transition at 565 cm^{-1}
c) A rotational transition at 20.9 cm^{-1}
d) An ESR transition at 3 cm
e) An NMR transition at 300 MHz
What can you conclude from these results?
Hint: $e^x \approx 1 + x$ when $x << 1.0$ (see justification 15.1 on page 518 in Atkins)

Question 2.4 (exercise 14.2b on page 509 in Atkins)
The molar absorption coefficient of a substance dissolved in hexane is known to be 327 $dm^3\ mol^{-1}\ cm^{-1}$ at 300 nm. Calculate the percentage reduction in intensity when light of that wavelength passes through 1.50 mm of a solution of concentration 2.22 mmol dm^{-3}.

Question 2.5 (exercise 14.9a on page 509 in Atkins)
A swimmer enters a gloomier world (in one sense) on diving to greater depths. Given that the mean molar absorption coefficient of sea water in the visible region is 6.2×10^{-3} $dm^3\ mol^{-1}\ cm^{-1}$, calculate the depth at which a diver will experience (a) half the surface intensity of light, (b) one tenth the surface intensity.

Question 2.6 (application 14.26 on page 512 in Atkins)
The abundance of ozone is typically inferred from measurements of UV absorption and is often expressed in terms of *Dobson units* (DU): 1 DU is equivalent to a layer of pure ozone 10^{-3} cm thick at 1 atm and 0°C. Compute the absorbance of UV radiation at 300 nm expected for an ozone abundance of 300 DU (a typical value) and 100 DU (a value reached during seasonal Antarctic ozone depletions) given a molar absorption coefficient of 476 $dm^3\ mol^{-1}\ cm^{-1}$.

Question 2.7 (examen 23/12/2005)
Van een imaginair eiwit is bekend dat het één tryptofaan (Trp) bevat. Met een absorptiespectrometer (absorption spectrometer) zijn bij drie verschillende golflengtes de optische dichtheden (optical densities) gemeten (tabel 1). Daarnaast zijn voor de aminozuren tryptofaan, tyrosine en fenylalanine de molaire absorptiecoëfficiënt ε (molar absorption coefficient) als functie van een aantal golflengtes gemeten (tabel 2).
a. Waarom wordt bij 200 nm een cuvet met een optische weglengte van 1 mm gebruikt?
b. Bepaal de concentratie van het eiwit.

c. Bepaal het aantal tyrosines en fenylalanines in het eiwit.
d. Welke aannames zijn er gemaakt bij onderdeel b en c?

Tabel 1 Optische dichtheid van de eiwit oplossing bij verschillende golflengtes.

Golflengte (nm)	Cuvetdikte (cm)	OD
200	0.1	0.866
250	1	0.344
300	1	0.061

Tabel 2 Molaire absorptiecoëfficiënt ε (in liter mol^{-1} cm^{-1}) bij verschillende golflengtes voor enkele aminozuren in oplossing.

Golflengte (nm)	Tryptofaan	Tyrosine	Fenylalanine
200	23000	15000	9300
250	2500	220	140
300	610	0	0

Block 3. Electronic spectra

Question 3.1 (discussion question 9.1 on page 316 in Atkins)
Discuss the physical origin of quantization energy for a particle confined to moving inside a one-dimensional box or on a ring.

Question 3.2
Explain what the expression HOMO and LUMO means, what their energy level determine and why they are so important in spectroscopy?

Question 3.3 (application 9.31 on page 318 in Atkins)
When β-carotene is oxidized *in vivo*, it breaks in half and forms two molecules of retinal (vitamin A), which is a precursor to the pigment in the retina responsible for vision (*Impact I14.1*). The conjugated system of retinal consists of 11 C atoms and one O atom. In the ground state of retinal, each level up to $n = 6$ is occupied by two electrons. Assuming an average internuclear distance of 140 pm, calculate (a) the separation in energy between the ground state and the first excited state in which one electron occupies the state with $n = 7$, and (b) the frequency of the radiation required to produce a transition between these two states. (c) Using your results and *Illustration 9.1*, choose among the words in parentheses to generate a rule for the prediction of frequency shifts in the absorption spectra of linear polyenes:
The absorption spectrum of a linear polyene shifts to (higher/lower) frequency as the number of conjugated atoms (increases/decreases).

Question 3.4 (discussion question 14.4 on page 508 in Atkins)
Explain how colour can arise from molecules. Consider only the effect of absorption. Why is grass green and blood red?

Question 3.5 (exercise 14.3a on page 509 in Atkins)
A solution of an unknown component of a biological sample when placed in an absorption cell of path length 1.00 cm transmits 20.1 per cent of light of 340 nm incident upon it. If the concentration of the component is 0.111 mmol dm^{-3}, what is the molar absorption coefficient?

Question 3.6 (numerical problem 14.5 on page 510 in Atkins)
A lot of information about the energy levels and wavefunctions of small inorganic molecules can be obtained from their ultraviolet spectra. An example of a spectrum

with considerable vibrational structure, that of gaseous SO2 at 25°C, is shown in Fig. 14.6 on page 484 in Atkins. Estimate the integrated absorption coefficient for the transition. transition. What electronic states are accessible from the A1 ground state of this Cv molecule by electric dipole transitions?. The problem can be solved by assuming that the peak forms a triangle.

Block 4. The fate of electronically excited states

Question 4.1
Reconsider discussion question 14.4 on page 508 in Atkins, now taking into account the role of light absorption, emission and scattering. Why does grass change color when you pour boiling water on it? How to explain the light effects of the peacock feather (NL: pauwenveer) or the wing of a butterfly?

Question 4.2
Refer to figure 14.9 on page 486 and the spectra in figure 14.22 on page 493 in Atkins. Sketch both the absorption and fluorescence spectra for the hypothetical situations reflected by positions 0, 1 and 2 on the x-axis of figure 14.9.

Question 4.3
Consider figure 14.22 on page 493 in Atkins. Sketch both the absorption and fluorescence spectra when the effect of solvent relaxation as shown in figure 14.23 on page 493 in Atkins is weak and strong.

Question 4.4
Explain why spectroscopists in general and biophysicists in particular prefer fluorescence spectroscopy above absorption spectroscopy.

Block 5. Kinetics of photophysical and photochemical processes

Question 5.1
Derive equation 23.35 on page 849 in Atkins.

Question 5.2 (exercise 23.7b on page 864 in Atkins)
In a photochemical reaction A → B + C, the quantum efficiency with 500 nm light is 1.2×10^2 mol einstein^{-1}. After exposure of 200 mmol A to the light, 1.77 mmol B is formed. How many photons were absorbed by A?

Question 5.3 (exercise 23.8b on page 864 in Atkins)
In an experiment to measure the quantum efficiency of a photochemical reaction, the absorbing substance was exposed to 320 nm radiation from a 87.5 W source for 28.0 min. The intensity of the transmitted light was 0.257 that of the incident light. As a result of irradiation, 0.324 mol of the absorbing substance decomposed. Determine the quantum efficiency.

Question 5.4
When benzophenone is illuminated with ultraviolet light it is excited into a singlet state. Triethylamine acts as a quencher for the singlet state. In an experiment in methanol as a solvent, the fluorescence intensity varied with amine concentration as shown below. A time-resolved laser spectroscopy experiment had also shown that the half-life of the fluorescence in absence of quencher is 29 us. What is the value of k_q?

[Q]/(mol L^{-1})	0.0010	0.0050	0.0100
I_f/(arbitrary units)	0.41	0.25	0.16

Question 5.5 (numerical problem 23.10 on page 865 in Atkins)

Describe an approach to solve numerical question 23.10 on page 865 in Atkins. So you don't need to answer the question.

The Förster theory of resonance energy transfer and the basis for the FRET technique can be tested by performing fluorescence measurements on a series of compounds in which an energy donor and an energy acceptor are covalently linked by a rigid molecular linker of variable and known length. L. Stryer and R.P. Haugland (*Proc. Natl. Acad. Sci. USA* **58**, 719 (1967)) collected the following data on a family of compounds with the general composition dansyl-(L-prolyl)$_n$-naphthyl, in which the distance *R* between the naphthyl donor and the dansyl acceptor was varied from 1.2 nm to 4.6 nm by increasing the number of prolyl units in the linker:

R/nm	1.2	1.5	1.8	2.8	3.1	3.4	3.7	4.0	4.3	4.6
$1 - E_T$	0.99	0.94	0.97	0.82	0.74	0.65	0.40	0.28	0.24	0.16

Are the data described adequately by eqn 23.38? If so, what is the value of R_0 for the naphthyl-dansyl pair?

Block 6. Lasers and their application in spectroscopy

Question 6.1

We want to study the absorption spectra of the first excited singlet state of chlorophyll, which has a lifetime of 10 * 10^{-12} seconds (10 ps), and the triplet state, which has a lifetime of 1.3 * 10^{-3} seconds (1.3 ms). Design one or two schematic spectrometers, eventually using one or more pulsed laser, to measure these states.

Question 6.2

Discuss the fundamental principles of laser action.

Question 6.3 (numerical problem 14.10 on page 510 in Atkins)

A certain molecule fluoresces at a wavelength of 400 nm with a half-life of 1.0 ns. It phosphoresces at 500 nm. If the ratio of the transition probabilities for stimulated emission for the $S^* \rightarrow S$ to the $T \rightarrow S$ transitions is 1.0×10^5, what is the half-life of the phosphorescent state?

Block 7. Energies of electrons and nuclei in magnetic fields

Question 7.1 (exercise 15.1b on page 556 in Atkins)

What is the resonance frequency of a ^{19}F nucleus in a magnetic field of 16.2 T?

Question 7.2 (exercise 15.2a on page 556 in Atkins)

^{33}S has a nuclear spin of $^3/_2$ and a nuclear *g*-factor of 0.4289. Calculate the energies of the nuclear spin states in a magnetic field of 7.500 T.

Question 7.3 (exercise 15.4b on page 556 in Atkins)

In which of the following systems is the energy level separation the largest? (a) A ^{14}N nucleus in (for protons) a 600 MHz NMR spectrometer, (b) an electron in a radical in a field of 0.300 T.

Question 7.4

Which technique is more sensitive, ESR or NMR? Calculate the sensitivity ratio of the two techniques? Why is this a very bad question?

Question 7.5 (exercise 15.6b on page 556 in Atkins)

Use Table 15.2 to predict the magnetic fields at which (a) ^{14}N, (b) ^{19}F, and (c) ^{31}P come into resonance at (i) 300 MHz, (ii) 750 MHz.

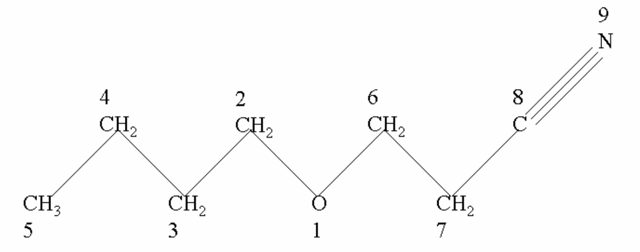
Question 7.6 (exercise 15.8a on page 556 in Atkins)

The first generally available NMR spectrometers operated at a frequency of 60 MHz; today it is not uncommon to use a spectrometer that operates at 800 MHz. What are the relative population differences of ^{13}C spin states in these two spectrometers at 25°C?

Block 8. Nuclear magnetic resonance spectroscopy

Question 8.1

Try to assign the ^1H-spectrum of 3-butoxypropionitril in $CDCL_3$ (spectrum A in the appendix of this chapter). Discuss all details. Do you have enough data to make a full assignment?



3-butoxypropionitril

Question 8.2

The peak at 77 in spectrum B and C (see the appendix of this chapter) is assigned to $CDCl_3$ (deuterated chloroform, meaning the ^1H proton is replaced by a ^2D proton). Explain why the fine structure (a triplet!) still can be seen in the uncoupled ^{13}C-spectrum (spectrum C), why is the pattern 1:1:1 instead of 1:2:1?

Question 8.3

Try to assign the coupled and uncoupled ^{13}C-spectra of 3-butoxypropionitril in $CDCl_3$ (spectrum B coupled, spectrum C uncoupled). Discuss all details.

Question 8.4 (exercise 15.9b on page 556 in Atkins)

The chemical shift of the CH_3 protons in acetaldehyde (ethanal) is $\delta = 2.20$ and that of the CHO proton is 9.80. What is the difference in local magnetic field between the two regions of the molecule when the applied field is (a) 1.5 T, (b) 15 T?

Block 9. CW and FT NMR spectroscopy

Question 9.1

Two groups of protons have δ=4.0 and δ=5.2 and can chemically exchange. In a 60 MHz spectrometer the two peaks turn into one peak at 280 K. In a 300 MHz spectrometer the temperature has to be raised to 300 K before to two peaks collapse into one peak. Give the exchange rate at both temperatures.

Question 9.2

Refer to question 8.2. Make a diagram of the nuclear spin energy levels and show all allowed transitions to explain the 1:1:1 triplet ^{13}C-resonance of $CDCl_3$ (deuterated chloroform) at 77 ppm in spectrum B and C (see “Questions for block 8”, and the appendix of this chapter).

Question 9.3

Refer to spectrum A in the appendix of this chapter. Explain why one speaks of “going from high field to low field”, when going from 0.0 ppm towards 3.5 ppm.
Given a fixed magnetic field strength; which nuclear magnetic resonance transition takes place at highest frequency, transitions at 0.0 ppm or at 3.5 ppm?

Block 10 Time domain NMR spectroscopy

Question 10.1

Describe in your own words the processes characterized by the time constants T_1, T_2 and T_2^*. Which experiments can be used to measure these parameters independently?

Question 10.2 (application 15.22 on page 559 in Atkins)

Suggest a reason why the spin–lattice relaxation time of benzene (a small molecule) in a mobile, deuterated hydrocarbon solvent increases with temperature whereas that of an oligonucleotide (a large molecule) decreases.

Question 10.3 (excercise 15.15a on page 557 in Atkins)

The duration of a 90° or 180° pulse depends on the strength of the $\mathcal{B}_1$ field. If a 90° pulse requires 10 μs, what is the strength of the $\mathcal{B}_1$ field? How long would the corresponding 180° pulse require?

Block 11. Two-dimensional NMR spectroscopy

Question 11.1

Try to assign the ^{1}H COSY spectrum (spectrum D in the appendix of this chapter) of 3-butoxypropionitril in $CDCL_3$ (see question 8.1). Compare this spectrum to the spectra A-C in question 8.1 to 8.3. Does this spectrum give the extra information necessary to resolve all uncertainties?

Question 11.2

Try to assign the ^{1}H ^{13}C hetero nuclear correlated (HETCOR) spectrum (spectrum E in the appendix of this chapter) of 3-butoxypropionitril in $CDCL_3$. The HETCOR experiment is similar to the COSY experiment with the exception that it concerns two different nuclei. The simplest pulse sequence that can be proposed for such 2D involves simply two 90° pulse, the last one being applied simultaneously to both nuclei. Compare this spectrum to the spectra A-C in question 8.1 to 8.3 and to spectrum D in question 11.1. Does this spectrum give the extra information necessary to resolve all uncertainties?

Question 11.3

The actual pulse sequence used in question 11.2 is more complicated than described, this to achieve ^{1}H-decoupling at the same time. How can the effect of this decoupling be seen in spectrum E?

Block 12. Electron spin resonance spectroscopy

Question 12.1 (exercise 15.16b on page 557 in Atkins)
Some commercial EPR spectrometers use 8 mm microwave radiation (the Q band). What magnetic field is needed to satisfy the resonance condition?

Question 12.2 (exercise 15.18b on page 557 in Atkins)
A radical containing three equivalent protons shows a four–line spectrum with an intensity distribution 1:3:3:1. The lines occur at 331.4 mT, 333.6 mT, 335.8 mT, and 338.0 mT. What is the hyperfine coupling constant for each proton? What is the g-value of the radical given that the spectrometer is operating at 9.332 GHz?

Question 12.3 (exercise 15.19b on page 557 in Atkins)
A radical containing three inequivalent protons with hyperfine constants 2.11 mT, 2.87 mT, and 2.89 mT gives a spectrum centred on 332.8 mT. At what fields do the hyperfine lines occur and what are their relative intensities?

Question 12.4 (exercise 15.21b on page 557 in Atkins)
The naphthalene radical anion has $g = 2.0024$. At what field should you search for resonance in a spectrometer operating at (a) 9.312 GHz, (b) 33.88 GHz?

Question 12.5 (application 15.26 on page 559 in Atkins)
You are designing an MRI spectrometer. What field gradient (in microtesla per metre, $\mu T\ m^{-1}$) is required to produce a separation of 100 Hz between two protons separated by the long diameter of a human kidney (taken as 8 cm) given that they are in environments with $\delta = 3.4$? The radiofrequency field of the spectrometer is at 400 MHz and the applied field is 9.4 T.

Extra questions.

Exercises in Adkins that reflect the level of knowledge assumed for the exam.

8.4a and 8.7a on page 274, and 8.26 on page 276.

9.1a on page 316, 9.31 on page 318.

10.2, 10.3, 10.8 on page 358, 10.14b, 10.1 and 10.3 on page 359, and 10.27(a) on page 361.

11.1b on page 399.
Given the eigenfunctions and eigenvalues of a conjugated molecule from a Huckel calculation, calculate HOMO and LUMO energy levels, and electron density distribution.

12.7 and 12.4a on page 426, and 12.13a on page 427.

13.4a on page 476.

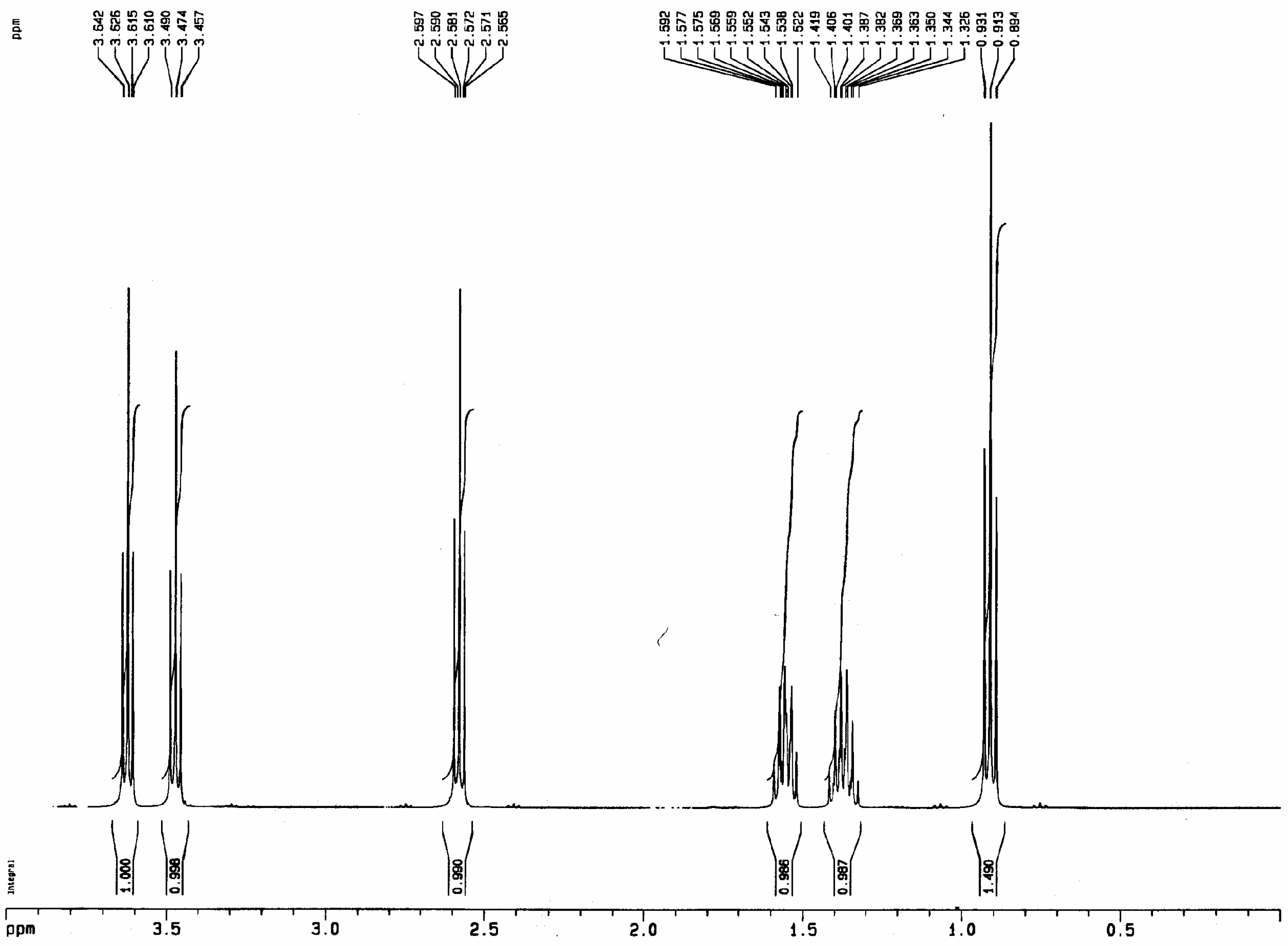
14.2, 14.4, 14.5, 14.7 on page 508, 14.2a, 14.3a, 14.5a and 14.8a on page 509.

15.2, 15.5, 15.2a, 15.7b, 15.11b, 15.12b on page 556, 15.15b and 13.23b on page 557, 15.10 and 15.12 on page 558.

23.8a on page 864, 23.9 on page 865, and 23.32 on page 867.

Appendix

Spectrum A



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NAME             BIP04H
EXPNO                 1
PROCNO                1

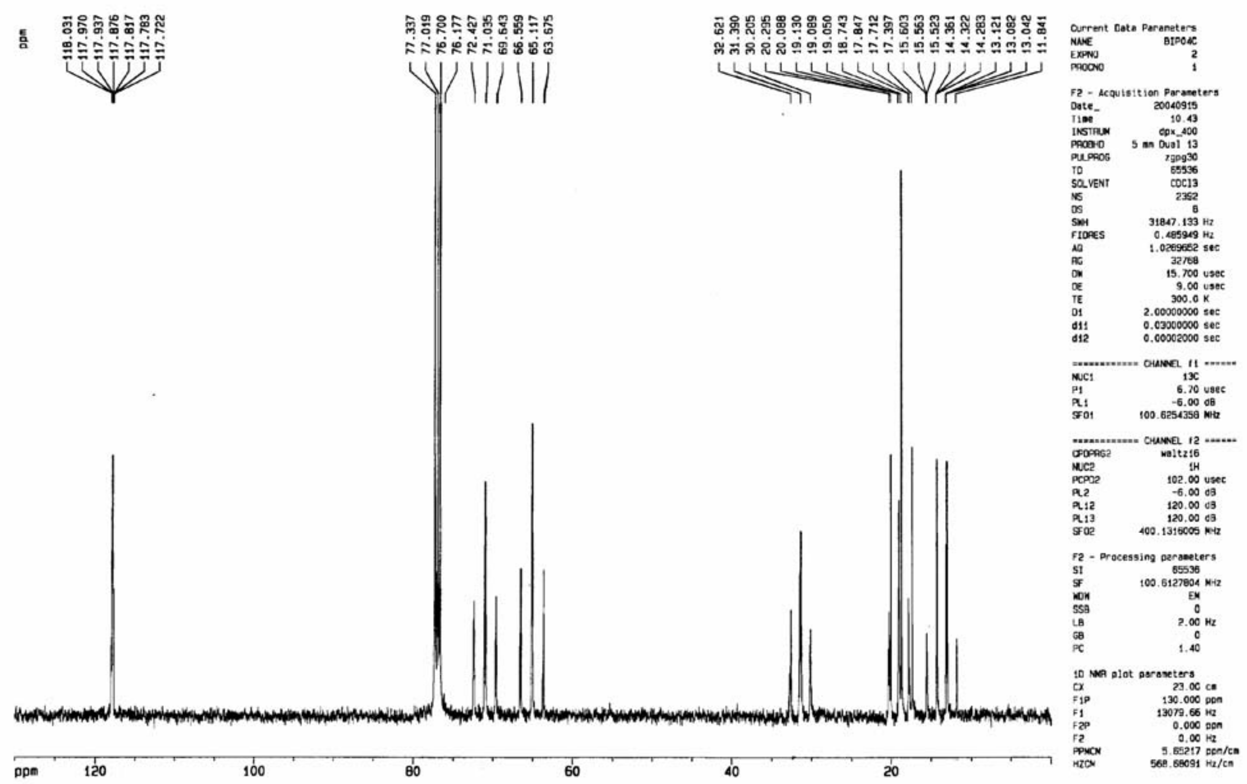
F2 - Acquisition Parameters
Date_          20040915
Time               9.22
INSTRUM         dpx_400
PROBHD      5 mm Dual 13
PULPROG            zg30
TD                32768
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            8223.685 Hz
FIDRES         0.250967 Hz
AQ            1.9923444 sec
RG                   64
DW               60.800 usec
DE                 9.00 usec
TE                300.0 K
D1           1.00000000 sec

============ CHANNEL f1 =========
NUC1                 1H
P1                 9.50 usec
PL1               -6.00 dB
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                32768
SF          400.1300000 MHz
WDW                  GM
SSB                   0
LB                -0.30 Hz
GB                  0.3
PC                 1.00

1D NMR plot parameters
CX                23.00 cm
F1P               4.000 ppm
F1              1600.52 Hz
F2P               0.000 ppm
F2                 0.00 Hz
PPMCM           0.17391 ppm/cm
HZCM           69.58783 Hz/cm
```

Spectrum B



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EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
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Time               10.43
INSTRUM          dpx_400
PROBHD      5 mm Dual 13
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  2362
DS                     8
SWH            31847.133 Hz
FIDRES          0.485949 Hz
AQ             1.0289652 sec
RG                 32768
DW                15.700 usec
DE                  9.00 usec
TE                 300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

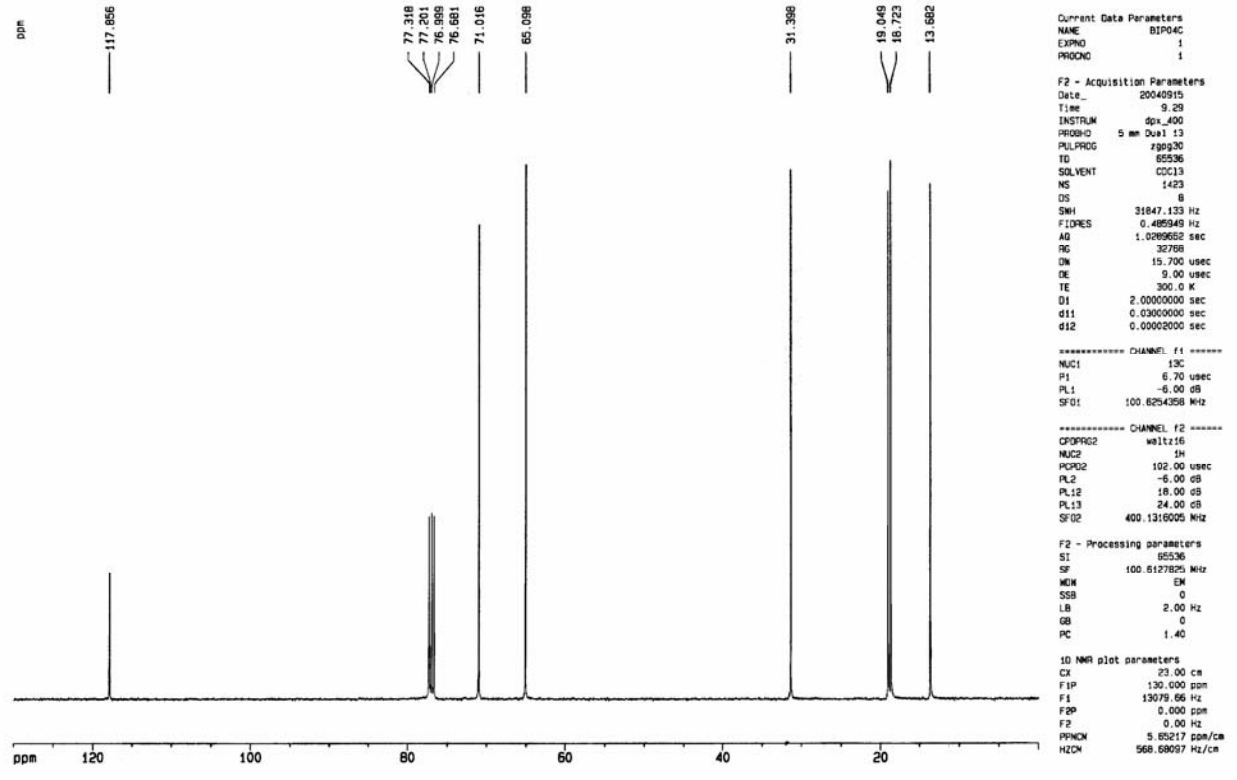
============ CHANNEL f1 ======
NUC1                 13C
P1                  6.70 usec
PL1                -6.00 dB
SFO1         100.6254358 MHz

============ CHANNEL f2 ======
CPDPRG2          waltz16
NUC2                  1H
PCPD2             102.00 usec
PL2                -6.00 dB
PL12              120.00 dB
PL13              120.00 dB
SFO2         400.1316005 MHz

F2 - Processing parameters
SI                 65536
SF           100.6127804 MHz
WDW                   EM
SSB                    0
LB                  2.00 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX                 23.00 cm
F1P              130.000 ppm
F1              13079.66 Hz
F2P                0.000 ppm
F2                  0.00 Hz
PPMCM            5.65217 ppm/cm
HZCM           568.68091 Hz/cm
```

Spectrum C



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Current Data Parameters
NAME              BIP04C
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20040915
Time                9.29
INSTRUM          dpx_400
PROBHD      5 mm Dual 13
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1423
DS                     8
SWH            31847.133 Hz
FIDRES          0.485949 Hz
AQ             1.0289652 sec
RG                 32768
DW                15.700 usec
DE                  9.00 usec
TE                 300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

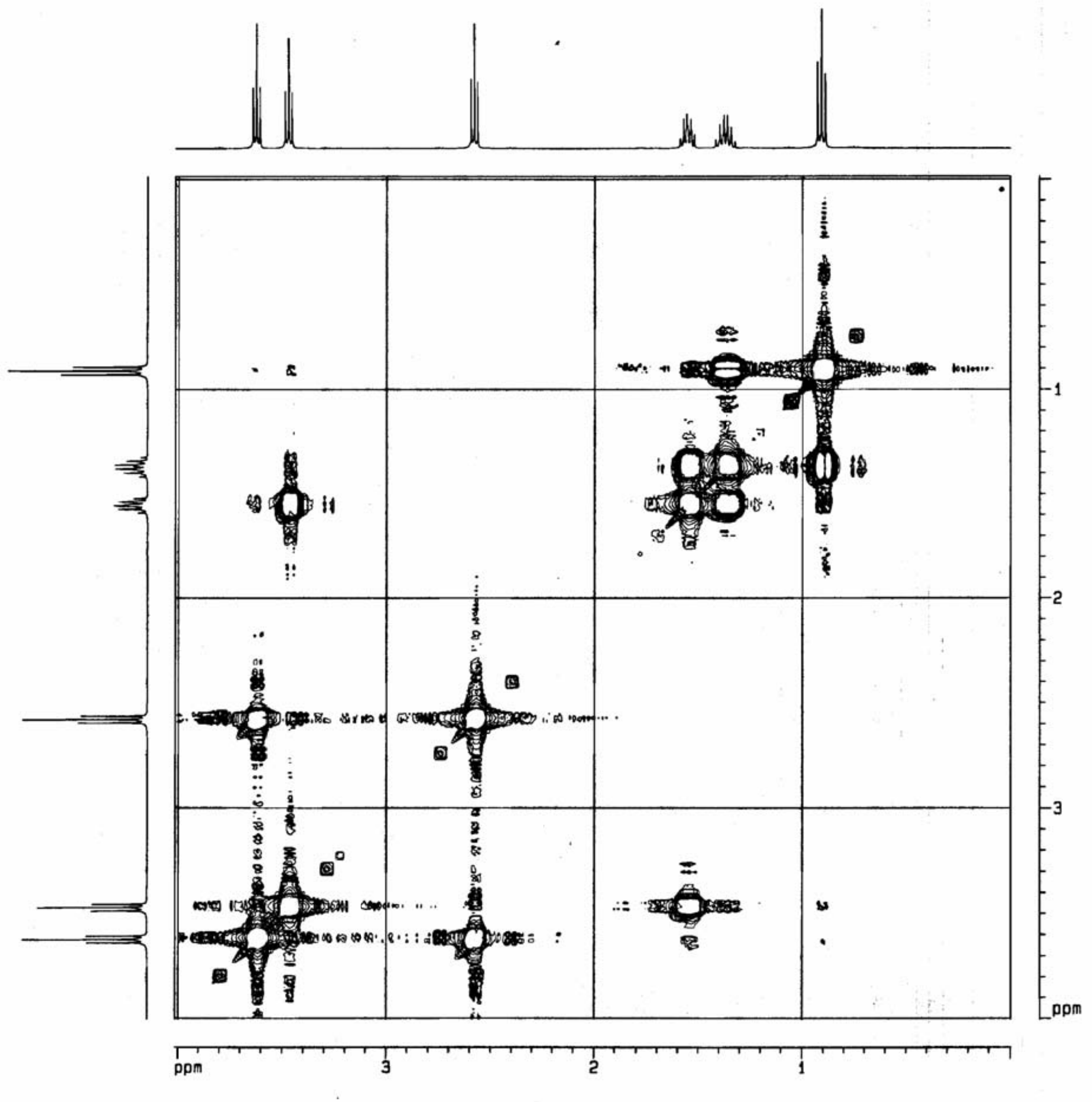
============ CHANNEL f1 ======
NUC1                 13C
P1                  6.70 usec
PL1                -6.00 dB
SFO1         100.6254358 MHz

============ CHANNEL f2 ======
CPDPRG2          waltz16
NUC2                  1H
PCPD2             102.00 usec
PL2                -6.00 dB
PL12               18.00 dB
PL13               24.00 dB
SFO2         400.1316005 MHz

F2 - Processing parameters
SI                 65536
SF           100.6127825 MHz
WDW                   EM
SSB                    0
LB                  2.00 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX                 23.00 cm
F1P              130.000 ppm
F1              13079.66 Hz
F2P                0.000 ppm
F2                  0.00 Hz
PPMCM            5.65217 ppm/cm
HZCM           568.68097 Hz/cm
```

Spectrum D



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NAME               BIP04co1
EXPNO                     1
PROCNO                    1

        F2 - Acquisition Parameters
Date_              20040915
Time                  16.56
INSTRUM             dpx_400
PROBHD         5 mm Dual 13
PULPROG              cosy90
TD                     1024
SOLVENT                MeOH
NS                       16
DS                       16
SWH                4006.410 Hz
FIDRES             3.912510 Hz
AQ                0.1278452 sec
RG                       64
DW                  124.800 usec
DE                     9.00 usec
TE                    300.0 K
d0               0.00000300 sec
D1               3.00000000 sec
IN0              0.00024991 sec

============ CHANNEL f1 =============
NUC1                     1H
P1                     9.50 usec
PL1                   -6.00 dB
SFO1            400.1315479 MHz

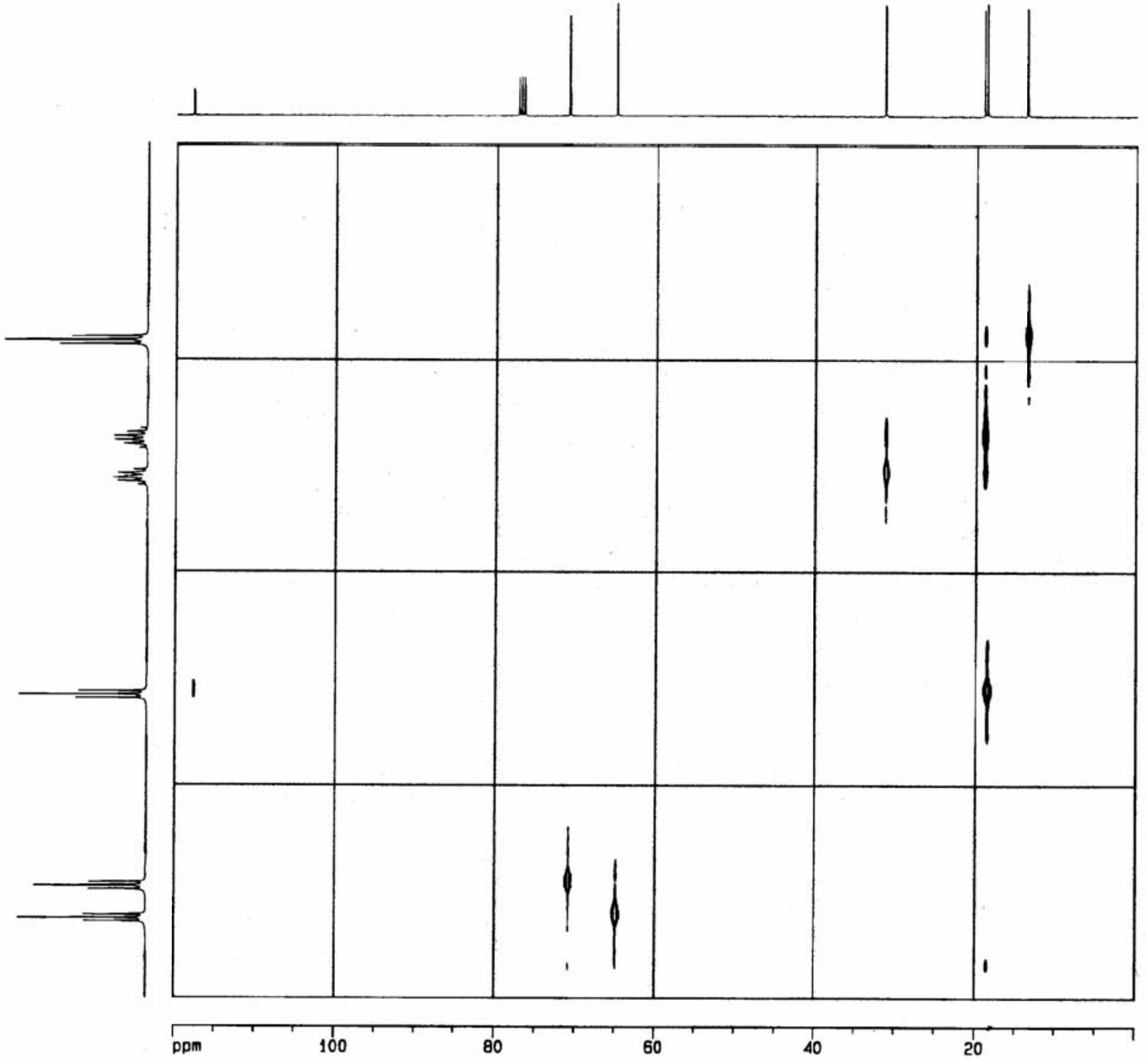
        F1 - Acquisition parameters
ND0                       1
TD                     1024
SFO1               400.1315 MHz
FIDRES             3.907618 Hz
SW                   10.000 ppm

        F2 - Processing parameters
SI                     1024
SF              400.1300038 MHz
WDW                    SINE
SSB                       0
LB                     0.00 Hz
GB                        0
PC                     1.40

        F1 - Processing parameters
SI                     1024
MC2                      QF
SF              400.1300034 MHz
WDW                    SINE
SSB                       0
LB                     0.00 Hz
GB                        0

          2D NMR plot parameters
CX2                   15.00 cm
CX1                   15.00 cm
F2PLO                 4.015 ppm
F2LO                1606.69 Hz
F2PHI                -0.003 ppm
F2HI                  -1.35 Hz
F1PLO                 4.006 ppm
F1LO                1603.07 Hz
F1PHI                -0.017 ppm
F1HI                  -6.87 Hz
F2PPMCM             0.26792 ppm/cm
F2HZCM            107.20277 Hz/cm
F1PPMCM             0.26824 ppm/cm
F1HZCM            107.32923 Hz/cm
```

Spectrum E



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NAME             BIP04xhc1
EXPNO                    1
PROCNO                   1

       F2 - Acquisition Parameters
Date_             20040916
Time                 16.56
INSTRUM            dpx_400
PROBHD        5 mm Dual 13
PULPROG               hxco
TD                    4096
SOLVENT               C6D6
NS                     105
DS                       4
SWH              22075.055 Hz
FIDRES            5.389416 Hz
AQ               0.0928244 sec
RG                   16384
DW                  22.650 usec
DE                    9.00 usec
TE                   300.0 K
CNST2          145.0000000
CNST11           3.0000000
d0              0.00000300 sec
D1              2.00000000 sec
d2              0.00344828 sec
d3              0.00229885 sec
d11             0.03000000 sec
d12             0.00002000 sec
IN0             0.00012290 sec

============ CHANNEL f1 =============
NUC1                   13C
P1                    6.70 usec
p2                   13.40 usec
PL1                  -6.00 dB
SFO1           100.6215830 MHz

============ CHANNEL f2 =============
CPDPRG2            waltz16
NUC2                    1H
P3                    9.00 usec
PCPD2               102.00 usec
PL2                  -6.00 dB
PL12                 18.00 dB
SFO2           400.1315470 MHz

       F1 - Acquisition parameters
ND0                      2
TD                     256
SFO1              400.1315 MHz
FIDRES           15.891985 Hz
SW                  10.188 ppm

       F2 - Processing parameters
SI                    4096
SF             100.6128003 MHz
WDW                   SINE
SSB                      0
LB                    0.00 Hz
GB                       0
PC                    1.40

       F1 - Processing parameters
SI                     512
MC2                     QF
SF             400.1300093 MHz
WDW                   SINE
SSB                      0
LB                    0.00 Hz
GB                       0

       2D NMR plot parameters
CX2                  17.00 cm
CX1                  15.00 cm
F2PLO              120.021 ppm
F2LO              12075.63 Hz
F2PHI               -0.021 ppm
F2HI                 -2.06 Hz
F1PLO                4.042 ppm
F1LO               1601.23 Hz
F1PHI               -0.010 ppm
F1HI                 -3.86 Hz
F2PPMCM            7.06125 ppm/cm
F2HZCM           710.45227 Hz/cm
F1PPMCM            0.26743 ppm/cm
F1HZCM           107.00805 Hz/cm
```

4. Answers tutorials

Block 1. Principles of optical spectroscopy

Answer 1.1

a) 523 nm: $3.8*10^{-19}$ J, 2.38 eV or 19184 cm^{-1}.
b) 15342 cm^{-1}: $3.0*10^{-19}$ J, 1.90 eV or 15342 cm^{-1}.
c) 300 MHz: $2.0*10^{-25}$ J, $1.2*10^{-6}$ eV or 0.01 cm^{-1}.
d) 3 cm: $6.6*10^{-24}$ J, $4.1*10^{-5}$ eV or 0.33 cm^{-1}.

Answer 1.2

0.24 W m^{-2}.

Answer 1.3

5538 K.

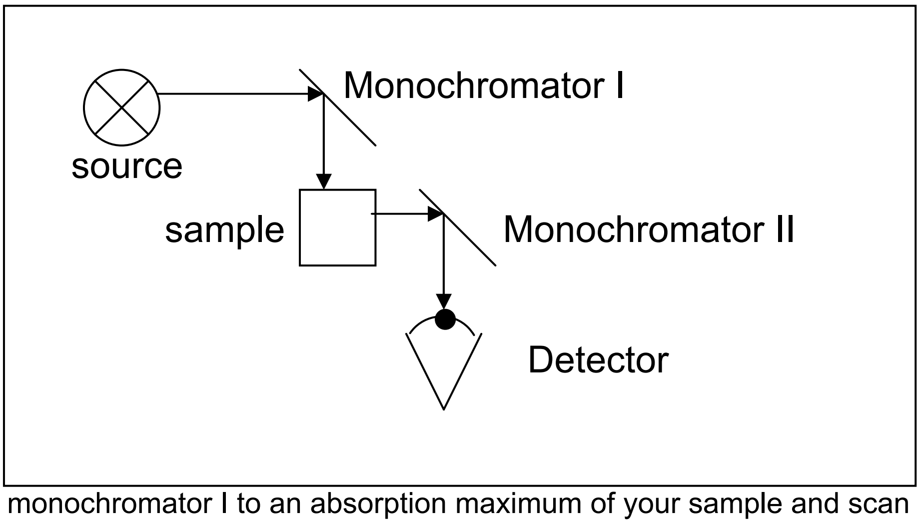
Answer 1.4

Monochromator: one wavelength at a time.
Polychromator: multiple wavelengths by multiple detectors at a time.
Fourier transform technique: multiple wavelengths at a time by one detector.

Answer 1.5

A larger distance between grating and slit gives better spectral resolution but less photons. A smaller slit gives better spectral resolution but less light. So the tradeoff is between spectral resolution and sensitivity.

Answer 1.6



First put monochromator I to an absorption maximum of your sample and scan Monochromator II to measure the fluorescence spectrum. Then use Monochromator II to select the maximum of fluorescence. Scan with monochromator I the excitation wavelength and detect the fluorescence as function of excitation wavelength. Because the fluorescence intensity will depend on the absorption intensity the resulting spectrum will give the absorption spectrum. Because you want to detect only one fluorescence wavelength a monochromator in combination with a simple detector should be used.

Answer 1.7
Doppler broadening is caused by the fact that atoms or molecules in a sample move towards or away from the source and the detector.
Lifetime broadening is caused by collisions and spontaneous emission.
See section 13.3 in Atkins.

Answer 1.8
A first estimate using equation 13.16 in Atkins results in a speed of approach of $6.34 * 10^7$ m s^{-1}. Because this speed is close to the speed of light it is more accurate to use equation 13.15 on page 436 in Atkins resulting in a speed of approach of $7.01 * 10^7$ m s^{-1}.

Answer 1.9
a) A line width of 100 MHz results from a natural lifetime of 1590 ps.
b) A line width of 2.14 cm^{-1} results from a natural lifetime of 2.48 ps.

Block 2. The theory of spectroscopic transitions

Answer 2.1
a) 5d → 2s is not allowed, for Δl=-2 (Δl must equal ±1).
b) 5p → 3s is allowed, since Δl=-1.
c) 6p → 4f is not allowed, for Δl=+2 (Δl must equal ±1).

Answer 2.2
Refer to the character table for C_{3v} in Atkins on page 1024 of the *Data Section*. Use the procedure illustrated in Example 12.7 in Atkins on page 425. Consider all three components of the electric dipole moment operator μ.

Component of μ:		x			y			z	
f3 = A_1	1	1	1	1	1	1	1	1	1
f2 = μ	2	-1	0	2	-1	0	1	1	1
f1 = A_2	1	1	-1	1	1	-1	1	1	-1
f1 * f2 * f3	2	-1	0	2	-1	0	1	1	-1
		E			E			A_2	

Since A_1 is not present in any product, the transition dipole moment must be zero.

Answer 2.3
n1 = the population of the lower level, n2 of the upper level. At 300 K:
a) 523 nm: n2 = n1 * $e^{-918.6}$ = 0.0 (upper level is empty)
b) 565 cm^{-1}: n2 = n1 * $e^{-2.72}$ = 0.07 * n1
c) 20.9 cm^{-1}: n2 = n1 * $e^{-0.1}$ = 0.90 * n1
d) 3 cm: n2 = n1 * $e^{-0.0016}$ = 0.998 * n1
e) 300 MHz: n2 = n1 * $e^{-0.000048}$ = 0.99995 * n1
Conclusion on basis of the population difference between the levels involved in the transitions: absorption spectroscopy is the most sensitive and NMR the least sensitive technique.

Answer 2.4
The reduction in intensity is 22.2%.

Answer 2.5

a) At a depth of 0.9 m.
b) At a depth of 3.0 m.

Answer 2.6

Use the gas law pV = nRT
300 DU: Absorbance A = 6.37
100 DU: Absorbance A = 2.12 (this means 0.7% of all 300 nm UV-B light is transmitted, causing increased risk of skin cancer, damage to your eye's and damage to DNA of all life forms. Het gat in de ozonlaag)

Answer 2.7

a. Omdat de OD van een 1 cm veel te hoog zou zijn voor een nauwkeurige meting, bij een dergelijke OD komen er vrijwel geen fotonen meer bij de detector. Eigenlijk is zelfs de OD van de 1 mm cuvet aan de hoge kant.

b. Volgens Lambert-Beer:

$$[Tryptofaan] = \frac{A}{\varepsilon l} = \frac{0.061}{610*1.0} = 0.1mM$$

c. Twee vergelijkingen met twee onbekenden:

$$A_{200} = \left(\varepsilon_{200}^{trp}[trp] + \varepsilon_{200}^{tyr}[tyr] + \varepsilon_{200}^{fen}[fen]\right)*l_{200} = (23000 + 15000*n_{tyr} + 9300*n_{fen})*0.1*10^{-3}*0.1$$

$$A_{250} = \left(\varepsilon_{250}^{trp}[trp] + \varepsilon_{250}^{tyr}[tyr] + \varepsilon_{250}^{fen}[fen]\right)*l_{250} = (2500 + 220*n_{tyr} + 140*n_{fen})*0.1*10^{-3}$$

Uit de eerste vergelijking volgt:

$$86600 - 23000 = 15000*n_{tyr} + 9300*n_{fen} \Leftrightarrow n_{tyr} = \frac{63600 - 9300*n_{fen}}{15000} = 4.24 - 0.62*n_{fen}$$

Invullen in de tweede vergelijking:

$$3440 - 2500 = 220*n_{tyr} + 140*n_{fen} = 220*(4.24 - 0.62*n_{fen}) + 140*n_{fen} = 932.8 - 136.4*n_{fen} + 140*n_{fe}$$

$$n_{fen} = \frac{940 - 932.8}{140 - 136.4} = 2$$

$$n_{tyr} = 4.24 - 0.62*n_{fen} = 3$$

d. Zowel bij onderdeel b als c neem je aan de molaire extinctie coëfficiënten van de aminozuren in oplossing overeen komen met die van de aminozuren in het eiwit. Daarnaast neem je aan dat er geen andere aminozuren of chromoforen bij de gegeven golflengtes absorberen.

Block 3. Electronic spectra

Answer 3.1

In quantum mechanics, particles are said to have wave characteristics. The fact of the excistence of the particle then requires that the wavelengths of the waves representing them be such that the wave does not experience destructive interference upon reflection by a barier or in its motion around a closed loop. This requirement restricts the wavelengths to certain values, which restricts in turn the energies a particle can have in such bound states. See justification 9.1 on page 279 and section 9.6(a) on page 300 in Atkins for quantitative expressions of this principle.

Answer 3.2

HOMO and LUMO are acronyms (afkortingen) for Highest Occupied Molecular Orbital and Lowest Unoccupied Molecular Orbital. The difference of energy between the two levels (LUMO – HOMO) gives the lowest energy at which an electronic transition can occur (when it is allowed). Because these levels are often involved in chemical reactions their properties are considered more important than those of lower and higher levels. In Frontier Orbital theory these two levels are even the only two molecular orbitals used to explain and predict chemical and physical properties of molecules.

Answer 3.3

(a) Refer to equation 9.4a on page 280 in Atkins. Retinal consists of 12 atoms forming a conjugates system resulting in a length of the “box” of 11 * 140 pm = $1.54 * 1^{-9}$ m. In the ground state all levels up to level 6 are filled with two electrons each. In the first excited state one electron is promoted from level 6 to 7. The energy difference between these two states is given by:

$$\Delta E = \frac{h^2(7^2-6^2)}{8mL^2} = \frac{\left(6.626*10^{-12}Js\right)^2*(49-36)}{8*9.11*10^{-31}kg*(1.54*10^{-9})^2} = 3.30*10^{-19}J$$

(b) $\nu = \frac{\Delta E}{h} = 4.95*10^{14}Hz$

(c) Using the same equation as in (a) but now: with a “box” length L of 21 * 140 pm = $2.94 * 10^{-9}$ m, 22 electrons filling the ground state up to level 11, and first excited state by promoting one electron from level 11 to 12, we find:

$$\Delta E = \frac{h^2(12^2-11^2)}{8mL^2} = \frac{\left(6.626*10^{-12}Js\right)^2*(144-121)}{8*9.11*10^{-31}kg*(2.94*10^{-9})^2} = 1.60*10^{-19}J$$

giving:

$$\nu = \frac{\Delta E}{h} = 2.42*10^{14}Hz$$

So the correct line is:

The absorption spectrum of a linear polyene shifts to $\boxed{\textit{lower}}$ *frequency as the number of conjugated atoms* $\boxed{\textit{increases}}$

Extra: a more general expression for the energy difference between the HOMO and LUMO of a linear molecule of 2n conjugated atoms with internuclear distance of 140 pm and 2n pi-electrons is given by:

$$\Delta E = \frac{h^2((n+1)^2-(n)^2)}{8m(2n-1)^2*(140*10^{-12}m)^2} = Cons\tan t*\frac{2n+1}{(2n-1)^2} \cong \frac{Cons\tan t}{n}$$

This crude simplification results in the line as shown above.

Answer 3.4

When a substance absorbs visible light its color as seen by our eyes is determined by the subtraction of those wavelengths from white light. For example, absorption of red light results in the object being perceived green.

Answer 3.5

$\varepsilon = 6.28 * 10^3$ L mol^{-1} cm^{-1}.

Answer 3.6

Equation 13.5 on page 433 shows that the integrated absorbance coefficient is given by the integral of the molar absorption coefficient as function of the wave number. See figure 14.6 on page 484 in Atkins. $\lambda_{min} \approx 240$ nm → $41.7 * 10^3$ cm^{-1}, $\lambda_{max} \approx 350$ nm → $28.6 * 10^3$ cm^{-1} and $\varepsilon_{max} \approx 275$ L mol^{-1} cm^{-1}. Approaching the peak in figure 14.6 by a triangle, the integrated absorbance coefficient is estimated by:

$\frac{1}{2} * \varepsilon_{max} * (\text{width in cm}^{-1}) \approx 1.8 * 10^6 \text{ L mol}^{-1} \text{ cm}^{-2}$

Answer 3.7

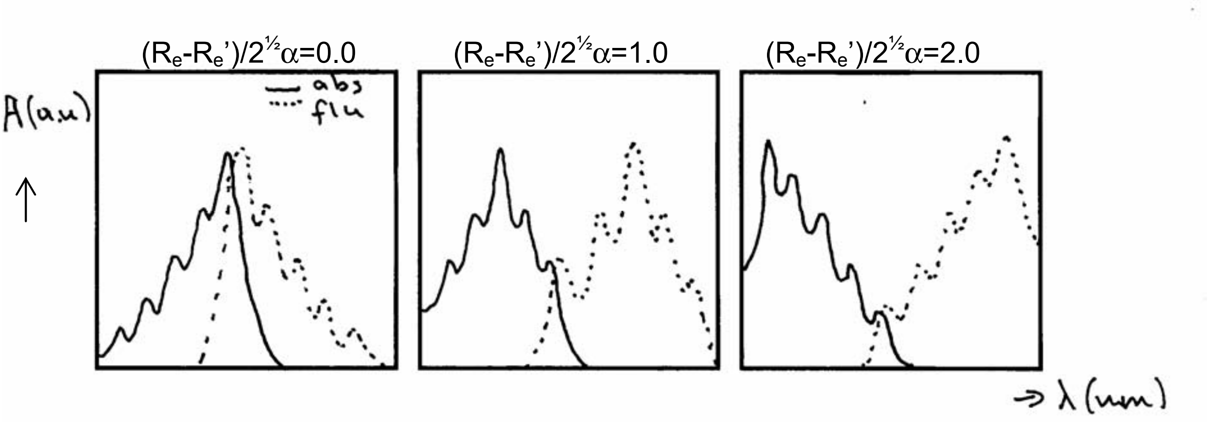
Since the dipole moments transform as $B_1(x)$, $B_2(y)$ and $A_1(z)$ under C_{2v} symmetry, excitations from A_1 to B_1, B_2 and A_1 terms are allowed (see Atkins section 12.6)

Block 4. The fate of electronically excited states

Answer 4.1

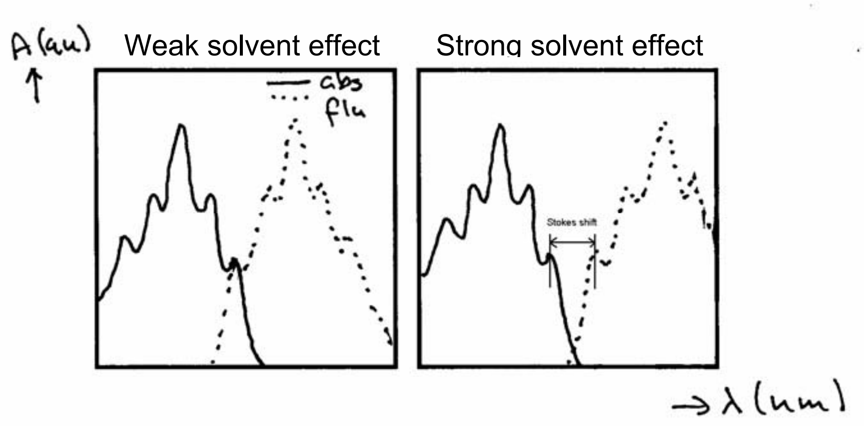
Color can arise by emission, absorption or scattering of electromagnetic radiation by an object. Many molecules have electronic transitions that have wavelengths in the visible portion of the electromagnetic spectrum. When a substance emits radiation the perceived colr will be that of the emitted radiation and it may be an additive color resulting from the emission of more than one wavelength of radiation. When a substance absorbs radiation its color is determined by the subtraction of those wavelengths from white light.
When you pour boiling water on grass it cannot perform photosynthesis anymore and the excited states start to fluoresce. Color can also be formed by scattering, including diffraction that occurs when light falls on a material with a grid of variation in texture or refractive index having dimensions comparable to the wavelength of light, for example, a bird’s plumage.

Answer 4.2



The positions on the x-axis in figure 14.9 on page 486 in Atkins correspond to the difference in equilibrium bond lengths of the ground and excited state. When there is no difference (position 0.0) 0-0 (ground electronic state, lowest vibration state) to 1-0 (first excited electronic state, lowest vibration state) transitions (abs. and flu.) are most probable.
For position 1.0 and 2.0 this transition becomes less probable as can be seen in figure 14.9, while other transitions become more probable 0-0 to 1-3 (abs.) and 1-0 to 0-3 (flu.) for position 1.0 and 0-0 to 1-5 (abs.) and 1-0 to 0-5 (flu.) transition for position 2.0.

Answer 4.3



The amount of solvent relaxation determines shift of the total fluorescence spectrum to lower energy (to the "red") with respect to the absorption spectrum that doesn't change. This shift is called the Stokes shift.

Answer 4.4

- Emission spectroscopy is more sensitive then absorption spectroscopy. It is easier and more sensitive to detect emission of photons by a detector, than to detect a small change of light intensity due to absorption on top of a large amount of light.
- Emission spectroscopy is more selective then absorption spectroscopy when one measures a mixture of molecules. Both excitation wavelength and detection wavelength can be used to select a certain type of molecules. In case of absorption spectroscopy one is dependent on the fact whether or not there is overlap of absorption bands.
- Emission spectroscopy is more sensitive for the environment of the fluorescent molecule compared to absorption spectroscopy. This "environmental" information is often very important for biological processes and.
- A disadvantage of fluorescence spectroscopy is that one is dependent on the fact that there must be emission from the molecule of interest.

Block 5. Kinetics of photophysical and photochemical processes

Answer 5.1

The fluorescence yield without a quencher is given by equation 23.33 in Atkins on page 848:

$\phi_f = \dfrac{k_f}{k_f + k_{IC} + k_{ISC}}$, with a fluorescence lifetime $\tau_0 = \dfrac{1}{k_f + k_{IC} + k_{ISC}} = \dfrac{\phi_f}{k_f}$

The fluorescence yield with a quencher is given by second equation in jusitfication 23.5 in Atkins on page 850:

$$\phi = \frac{k_f}{k_f + k_{IC} + k_{ISC} + k_Q[Q]}$$

The ratio ϕ_f / ϕ is then given by:

$$\frac{\phi_f}{\phi} = \frac{k_f + k_{IC} + k_{ISC} + k_Q[Q]}{k_f + k_{IC} + k_{ISC}} = 1 + \frac{k_Q[Q]}{k_f + k_{IC} + k_{ISC}} = 1 + \tau_0 k_Q[Q]$$

Answer 5.2
$1.5*10^{-5}$ moles of photons.

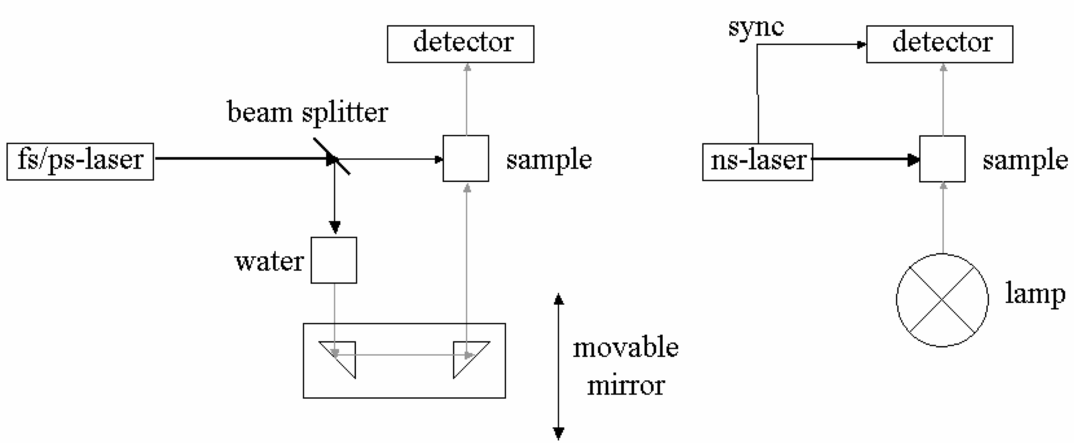
Answer 5.3
$\phi=1.11$

Answer 5.4
Make a plot of $1/I_f$ as function of [Q]. The intercept gives $1/I_a$ and the slope k_q/k_fI_a. I_a is known from the intercept and $k_f = \ln(2)/t_{½}$. The value of k_q is then calculated to be $5.1 * 10^6$ L mol^{-1} s^{-1}.

Answer 5.5
Plot $1/E_T$ as function of R^6. This should be a straight line with intercept 1, because we can derive from equation 23.38 in Atkins on page 852 that $\frac{1}{E_T}=1+\left(\frac{R}{R_0}\right)^6$. The slope is given by $\left(\frac{1}{R_0}\right)^6 \Rightarrow R_0 \approx 3.52nm$.

Block 6. Lasers and their application in spectroscopy

Answer 6.1
Setup **A** is used for ps-absorption spectroscopy of excited singlet states. Setup **B** is used for µm and ms-absorption spectroscopy of metastable triplet states. Also see section 14.6(e) in Atkins.



Setup A Setup B

Answer 6.2
The basic requirement for a laser is that it has at least three energy levels. Of these levels, the highest lying state must be capable of being efficiently populated above its thermal equilibrium value by a pulse of radiation. A second state, lower in energy, must be a metastable state with a long enough lifetime for it to accumulate a population greater than its thermal equilibrium value by spontaneous transitions from the higher overpopulated state.
The metastable state must than be capable of undergoing stimulated transitions to a third lower lying state. This last requirements implies not only that the metastable state have more than its thermal equilibrium population, but also that it have a higher population than the third lower lying state, namely that it achieve population inversion. See Figs 14.28 and 14.29 in Atkins on page 496 for a description of the three- and four-level laser. The amplification process occurs when low intensity radiation of frequency equal to the transition frequency between the metastable state and the lower

lying state stimulates the transition to the lower lying state and many more photons (higher intensity of the radiation) of that frequency are created.

Answer 6.3
The ratio of the transition probabilities of spontaneous emission A and stimulated emission B at frequency v is given by equation 13.11 on page 434 in Atkins:

$$A = (\frac{8\pi h\nu^3}{c^3})B = \frac{k}{\lambda^3}B$$

where k is a constant and we have used $\nu = \frac{c}{\lambda}$

Thus at 400 nm: $A(400nm) = \frac{k}{\left(400*10^{-9}\right)^3}B(400nm)$, and at 500 nm:

$$A(500nm) = \frac{k}{\left(500*10^{-9}\right)^3}B(500nm)$$

Then, $\frac{A(500nm)}{A(400nm)} = \frac{(400*10^{-9})^3}{\left(500*10^{-9}\right)^3}*\frac{B(500nm)}{B(400nm)} = 5*10^{-6}$

Given is the ratio $\frac{B(400nm)}{B(500nm)} = 1*10^5$ this results in

$$\frac{A(500nm)}{A(400nm)} = \frac{(400*10^{-9})^3}{\left(500*10^{-9}\right)^3}*\frac{1}{1*10^5} = 5*10^{-6}$$

Lifetimes and half-lives (not the same!) are inversely proportional to transition probabilities (rate constants) and hence:

$$\frac{\tau_{1/2}(400nm)}{\tau_{1/2}(500nm)} = \frac{\tau_{1/2}(S^* \to S)}{\tau_{1/2}(T \to S)} = 5*10^{-6} \Leftrightarrow$$

$$\tau_{1/2}(T \to S) = \frac{1}{5*10^{-6}}\tau_{1/2}(S^* \to S) = 2*10^{-4}s$$

Block 7. Energies of electrons and nuclei in magnetic fields

Answer 7.1
^{19}F in a magnetic field of 16.2 T has a resonance frequency of 649 MHz.

Answer 7.2
^{33}S has four nuclear magnetic spin states m$_I$ = -3/2, -1/2, 1/2, and 3/2. The energies of these four states at 7.5 T is given by:

$$E_{m_I} = -g_{I_{^{33}S}}\mu_N B_0 m_I = -1.626*10^{-26}J*m_I$$

Answer 7.3
a) ^{14}N has three nuclear magnetic spin states m$_I$ = -1, 0, 1. A 600 MHz NMR spectrometer refers to the resonance frequency of ^{1}H, which corresponds to a magnetic field strength of 14.1 T. The energy of the ^{14}N states at 14.1 T is given by:

$$E_{m_I} = -g_{I_{^{14}N}}\mu_N B_0 m_I = -2.874*10^{-26}J*m_I$$

Given the selection rule $\Delta m_I = \pm 1$, the energy difference for allowed transitions is:

$$\Delta E = g_{I_{^{14}N}}\mu_N B_0 |\Delta m_I| = 2.874*10^{-26}J$$

b) The energy difference for a free electron at 0.3 T is:

$$\Delta E = g_e \mu_B B_0 = 5.571*10^{-24} J$$

Answer 7.4

This is an incomplete question, but lets give it a try. Sensitivity is, amongst other factors, determined by the population difference between the lower and upper energy level; the bigger the difference the stronger the absorption of energy, the stronger the signal. From justification 15.1 on page 518 in Atkins we find for spin 1/2 systems at a certain B_0 field that:

$$\frac{N_{high} - N_{low}}{N_{high} + N_{low}} = \frac{\Delta N}{N_{total}} \approx \frac{\Delta E}{2kT}$$

For free electrons and spin 1/2 nuclei the energy difference is respectively given by:

$$\Delta E_{ESR} = g_e \mu_B B_0 = 2.00319*9.724*10^{-24} * B_0 (J)$$

$$\Delta E_{NMR} = g_I \mu_N B_0 = g_I 5.051*10^{-27} * B_0 (J)$$

The largest g_I from table 15.2 on page 516 and table 15.2 on page 1014 in Atkins is the one for ^{1}H, which will give the largest population difference. Given the same amount of free electrons and protons, the same magnetic field and the same temperature the sensitivity ratio is then:

$$\frac{\Delta N_{ESR}}{\Delta N_{NMR}} \approx \frac{2.00319*9.724*10^{-24}}{5.586*5.051*10^{-27}} = 690$$

This answer is only part of the truth and open for discussion and criticism.

Answer 7.5

Given the selection rule $\Delta m_I = \pm 1$ the first equation in answer 7.3 can be generalized to:

$$\Delta E = g_I \mu_N B_0 = h\nu \text{ , which leads to } B_0 = \frac{h\nu}{g_I \mu_N} = 1.312*10^7 * \frac{\nu}{g_I} (T)$$

Using the data from table 15.2 on page 516 and table 15.2 on page 1014 in Atkins, the following magnetic field strengths to achieve resonance at 300 and 750 MHz are calculated:

B_0 (T)	**Nucleus**	**a) ^{14}N**	b) **^{19}F**	**c) ^{31}P**
	g_I	**0.40356**	**5.2567**	**2.2634**
ν (MHz)				
i) 300		**97.5**	**7.49**	**17.4**
ii) 750		**244**	**18.7**	**43.5**

Answer 7.6

The second equation in justification 15.1 on page 518 in Atkins can be rewritten to equation 15.14b on page 517:

$$N_\alpha - N_\beta = \Delta N \approx \frac{(N_\alpha + N_\beta)\Delta E}{2kT} = \frac{Nh\nu}{2kT}$$

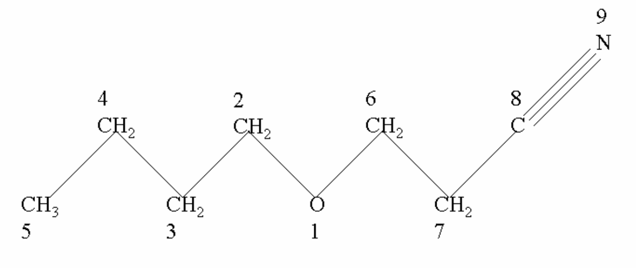
Thus ΔN∝ν. Comparing 60 MHz ^{1}H-NMR to 800 MHz ^{1}H-NMR gives for all nuclei a relative population difference of:

$$\frac{\Delta N(800MHz)}{\Delta N(60MHz)} = \frac{800MHz}{60MHz} = 13.0$$

This ratio is independent of temperature and nuclei as long as the approximation as given by the first equation in justification 15.1 is valid.

Block 8. Nuclear magnetic resonance spectroscopy

Answer 8.1



3-butoxypropionitril

See the ^{1}H-spectrum (spectrum A in the appendix of chapter 3):

- On basis of the integrals is the peak at 0.9 ppm caused by 1.5 times as many protons as all other peaks. Given the structure this must be CH_3 (5) while all other peaks belong to CH_2-groups.
- On basis of chemical shifts (see section 15.5(a) in Atkins) no unambiguous assignments can be made.
  One expects neighbouring group contributions of oxygen group O1 on the 2 H2-nuclei, and 2 H6-nuclei and of the nitril group (N9≡C8) on the 2 H7-nuclei. One could on basis of this tentatively assign the peaks at 3.5 and 3.6 ppm to the 2 H2-nuclei and 2 H6-nuclei, because the effect is expected to be comparable, and the peak at 2.6 ppm to the 2 H7-nuclei. These assumptions, however, have to be confirmed by other observations.
- On basis of fine structure one expects the following multiplets (assuming all ^{1}H-couplings equally strong):
  2 H2's couple to 2 H3's → 1:2:1 triplet
  2 H3's couple to 2 H2's and 2 H4's → 1:4:6:4:1 quintet at 1.6 ppm
  2 H4's couple to 2 H3's and 3 H5's → 1:5:10:10:5:1 sextet at 1.4 ppm
  3 H5's couple to 2 H4's → 1:2:1 triplet
  2 H6's couple to 2 H7's → 1:2:1 triplet
  2 H7's couple to 2 H6's → 1:2:1 triplet

Conclusion:

Triplet at 0.9 ppm	CH_3 5
Sextet at 1.4 ppm	CH_2 4
Quintet at 1.6 ppm	CH_2 3
Triplet at 2.7 ppm	probably CH_2 7 (CH_2 2 and 6 cannot be excluded)
Triplet at 3.5 ppm	CH_2 2 or 6 (even CH_2 7 cannot be excluded)
Triplet at 3.6 ppm	CH_2 2 or 6 (even CH_2 7 cannot be excluded)

Answer 8.2

The difference between ^{13}C-spectrum B and C is that spectrum C is proton decoupled (see section 15.10 in Atkins). This means that although ^{1}H-nuclei are present, they do not split the ^{13}C-resonances. The effect is clearly demonstrated, because almost all multiplets in spectrum B become single peaks in spectrum C.
The ^{13}C-multiplet at 77 ppm is attributed to $CDCl_3$ (deuterated chloroform). This solvent is used instead of $CHCl_3$ to avoid a large solvent peak in the ^{1}H-spectrum. The "D" refers to ^{2}H-nuclei (deuterium), which is an isotope of proton and is a spin-1 nucleus (see table 15.2 on page 516 in Atkins).
Of all $CDCl_3$ carbons 1% are spin-½ ^{13}C-nuclei that give to a resonance at 77 ppm. All chloroform ^{13}C-nuclei feel the spin-1 ^{2}D-nuclei and will split into a 1:1:1 triplet. The multiplet is still present in spectrum C, because proton decoupling only affects coupling to ^{1}H-nuclei and not to other nuclei with a nuclear spin larger than 0.

The multiplet caused by $CDCl_3$ is a 1:1:1 triplet, because is originates from a coupling of the spin-½ ^{13}C-nucleus to a spin-1 ^{2}H-nucleus. This is completely different from the 1:2:1 triplet that originates from a coupling of a spin-½ nucleus to 2 spin-½ nuclei. Compare figures 15.17 and 15.18 on page 527 in Atkins.

Answer 8.3

See the ^{13}C-spectra (spectrum B and C):

- On basis of chemical shifts (see figure 15.5(b) on page 520 in Atkins) the following assignments can be made (best seen in spectrum C):
  R-**C**-H: ^{13}C-resonances between 0-60 → peaks at 13.6, 18.7, 19.0 and 31.4 belong to either C3, C4, C5 or C7
  R-**C**≡N: ^{13}C-resonances between 120-150 ppm → peak at 118 must be C8
  R-**C**-O: not shown in figure 15.5(b). But on basis of exclusion peaks at 65.1 and 71.0 must belong to either C2 or C6. The deshielding effect of O1 is the same as seen for H2 and H6 in the ^{1}H-spectrum A.
- On basis of fine structure one expects the following multiplets in spectrum B:
  2 H2's couple to C2 → 1:2:1 triplet
  2 H3's couple to C3 → 1:2:1 triplet
  2 H4's couple to C4 → 1:2:1 triplet
  3 H5's couple to C5 → 1:3:3:1 quartet (multiplet 13.7 ppm)
  2 H6's couple to C6 → 1:2:1 triplet
  2 H7's couple to C7 → 1:2:1 triplet
  no protons couple to C8 → singlet (peak at 118 ppm)

Conclusion:

• Quartet at 13.7 ppm	CH_3 5
• Triplet at 18.7, 19.0, and 31.2 ppm	CH_2 3, CH_2 4 or CH_2 7
• Triplet at 65.1 and 71.0 ppm	CH_2 2, CH_2 6
• Singlet at 118 ppm	C 8

Answer 8.4

See equations 15.15 on page 518, and 15.16 and 15.19 on page 519 in Atkins:

$$\left|\Delta B_{loc}\right| = |\Delta\sigma| B_0 \approx \left|\delta(CH_3)-\delta(CH_2)\right| * 10^{-6} * B = |1.16-3.36| * 10^{-6} * B = 2.20 * 10^{-6} B$$

a) $B = 1.5T \rightarrow \left|\Delta B_{loc}\right| \approx 4.2 * 10^{-6} T$

b) $B = 16.5T \rightarrow \left|\Delta B_{loc}\right| \approx 3.63 * 10^{-5} T$

Block 9. CW and FT NMR spectroscopy

Answer 9.1

The chemical difference between the two peaks is 1.2 ppm. This corresponds to a frequency difference $\delta\nu$ at 60 MHz of 1.2 * 10^{-6} * 60 * 10^{6} Hz = 72 Hz, and at 300 MHz of 1.2 * 10^{-6} * 300 * 10^{6} Hz = 360 Hz. Using equation 15.29 on page 532 in Atkins one can calculate the following lifetimes and transition rates:

At 60 MHz: $\tau = \frac{\sqrt{2}}{\pi\delta\nu} = \frac{\sqrt{2}}{\pi 72 Hz} = 6.3 ms \rightarrow rate = \frac{1}{\tau} = 160 s^{-1}$

At 300 MHz: $\tau = \frac{\sqrt{2}}{\pi\delta\nu} = \frac{\sqrt{2}}{\pi 360 Hz} = 1.3 ms \rightarrow rate = \frac{1}{\tau} = 800 s^{-1}$

EXTRA:

This information is often used to calculate the activation energy of the jumping process using equation 22.29 on page 807 in Atkins:

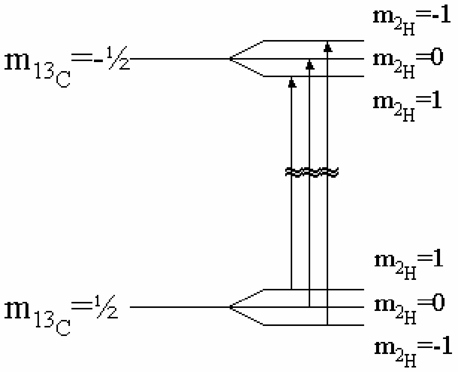
$$\ln(k) = \ln(A) - \frac{E_a}{RT} \rightarrow k \propto e^{E_a/RT}$$

From this we can derive for two rates at two temperatures the following:

$$\ln\left[\frac{k_{300K}}{k_{280K}}\right] = \frac{-E_a}{R}\left(\frac{1}{300K} - \frac{1}{280K}\right) \Leftrightarrow E_a = \frac{R\ln(k_{280K}/k_{300K})}{\left(\frac{1}{280K} - \frac{1}{300K}\right)} = 56.2kJ/mol$$

Answer 9.2

^{13}C is a spin-½ that couples to one ^{2}H, a spin-1 nucleus. It is assumed that the coupling constant is positive. In ^{13}C-spectroscopy $\Delta m_{13_C} = -1, \Delta m_{2_H} = 0$ transitions are observed. The nuclear spin energy levels of ^{13}C with observed transitions are given by:



These results in the 1:1:1 triplet found at 77 ppm in spectrum B and C.
Please notice that the energy difference between the ^{13}C-levels is much larger then between the levels due to the ^{2}D-coupling.

Answer 9.3

See equation 15.19 on page 519 in Atkins. Resonance at δ=0.0 results from a larger shielding compared to δ=3.5. In CW-NMR one measures at a fixed radio frequency while scanning the magnetic field from low to a high field strength (left to right). The least shielded nuclei “feel” the smallest opposing local field, so the will resonate at a lower B-field than more shielded nuclei. So the low field end (left-hand side) of the spectrum corresponds to high chemical shift values, while the high field end (right-hand side) corresponds to smaller or even negative chemical shifts.
See equation 15.17 on page 519 in Atkins: the higher the shielding the lower the resonance or Lamor frequency. So the low field end of the spectrum (left-hand end) corresponds to larger resonance frequencies compared to the high field end (right-hand end) of the spectrum.

Block 10. Time domain NMR spectroscopy

Answer 10.1

T_1 characterizes the time a nuclear spin-½ system with a non-equilibrium population difference of spin-up and spin-down needs to restore the Boltzmann equilibrium. The process is driven by energy TRANSFER (so LOSS of energy) from the spin system to the surroundings. This process is called spin-lattice relaxation.
It can be determined by a series of 180°-tau-90°-detection experiments with different tau’s. The signal as function of tau is given by:

$$M_z(\tau) = M_0(1 - 2e^{-\frac{\tau}{T_1}})$$

T_2 characterizes the time in which a nuclear spin-½ system with its net magnetization perpendicular to the main magnetic field looses its coherence. There is NO LOSS of energy from the spin system to the surroundings. This process is called spin-spin relaxation

It can be determined by a series of 90°-tau-180°-tau-detection experiments with different tau's. The signal as function tau is given by:

$$M_{xy}(2\tau) = M_0 e^{-\frac{2\tau}{T_2}}$$

T_2^* characterizes the time in which a nuclear spin-½ system with its net magnetization perpendicular to the main magnetic field, looses its coherence due to both spin-spin relaxation and inhomogeneities in the magnetic field. It can by determined by a simple 90°-detection experiment. The FID as function of time is given by:

$$M_{xy}(t) = M_0 \cos(2\pi \nu_L t) e^{-\frac{t}{T_2^*}}$$

Answer 10.2

See section 15.9(a) in Atkins.

At, say, room temperature, the tumbling rate of benzene, the small molecule, in a mobile solvent, may be close to the Lamor frequency, and hence its spin-lattice relaxation time will be small because the relaxation process is very effective. As the temperature increases, the tumbling rate may increase well beyond the Lamor frequency, resulting in an increased spin-lattice relaxation time due to a less effective relaxation process.

For the large oligopeptide at room temperature, the tumbling rate may be well below Lamor frequency, but with increasing temperature it will approach the Lamor frequency due to the increased thermal motion of the molecule combined with decreased viscosity of the solvent. Therefore, the spin-lattice relaxation time may decrease, because the relaxation mechanism becomes more effective.

Answer 10.3

See equation 15.13b on page 517 in Atkins. It gives the precession frequency due to a magnetic field B_0. The same equation can be used for the B_1-field.

A 90° rotation in 10 μs corresponds to a radial frequency of:

$$\omega = 0.5 rad / 10 \mu s = 1.57 * 10^5 rad/s$$

Equation 15.13b can be rewritten to radial frequency as follows:

$$\omega_L = 2\pi \nu_L = \gamma B_1 \Leftrightarrow B_1 = \frac{\omega_L}{\gamma} = \frac{1.57*10^5}{26.75*10^7} = 5.9*10^{-4} T$$

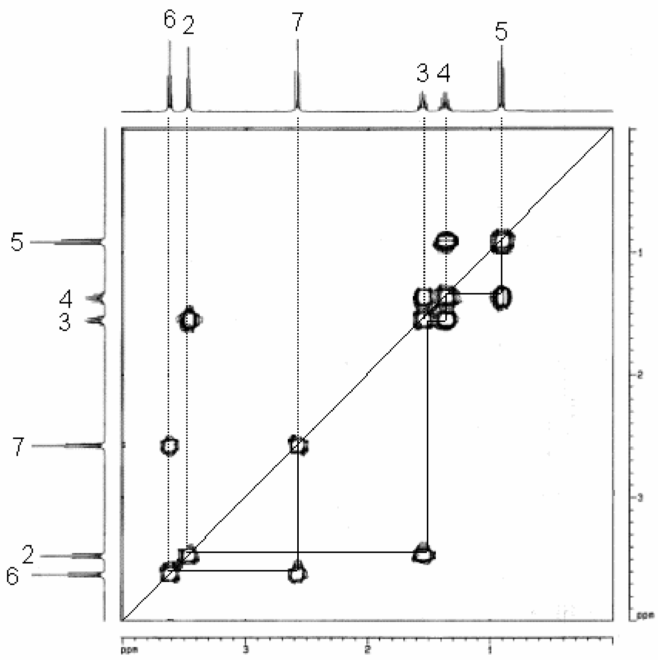
A 180° pulse takes twice as long as the 90° pulse, so 20 μs.

Block 11. Two-dimensional NMR spectroscopy

Answer 11.1

Conclusions from question 8.1 (spectrum A):

• Triplet at 0.9 ppm	CH_3 5
• Sextet at 1.4 ppm	CH_2 4
• Quintet at 1.6 ppm	CH_2 3
• Triplet at 2.7 ppm	probably CH_2 7 (CH_2 2 and 6 cannot be excluded)
• Triplet at 3.5 ppm	CH_2 2 or 6 (even CH_2 7 cannot be excluded)
• Triplet at 3.6 ppm	CH_2 2 or 6 (even CH_2 7 cannot be excluded)



The diagonal in spectrum D from bottom left corner to the top right corner gives the same resonances as spectrum A. Off-diagonal peaks (or cross peaks) show presence of scalar through-bound (or spin-spin) couplings. Starting at CH_3 5 at 0.9 ppm in spectrum E we find a cross peak with CH_2 4 at 1.4 ppm. The peak at 1.4 ppm on his part has cross peaks with CH_3 5 at 0.9 ppm and CH_2 3 at 1.6 ppm. CH_2 3 at 1.6 shows cross peaks with CH_2 4 at 1.6 and CH_2 2 at 3.5 ppm. The last assignment removes an uncertainty in the assignment of spectrum A. The other assignments confirm previous assignments in spectrum A.

There is also a cross peak between the peak at 2.7 and 3.6 ppm. So these must belong to neighbouring groups with a scalar spin coupling. Indeed CH_2 7 and CH_2 6 are neighbouring groups, however, it is still not possible to make a 100% certain assignment of the resonance at 2.7 and 3.6 ppm. Only on basis of chemical similarity one expects CH_2 6 to give a resonance near CH_2 2.

Conclusion:

• Triplet at 0.9 ppm	CH_3 5
• Sextet at 1.4 ppm	CH_2 4
• Quintet at 1.6 ppm	CH_2 3
• Triplet at 2.7 ppm	CH_2 7 (CH_2 6 cannot be excluded for 100%)
• Triplet at 3.5 ppm	CH_2 2
• Triplet at 3.6 ppm	CH_2 6 (CH_2 7 cannot be excluded for 100%)

Answer 11.2

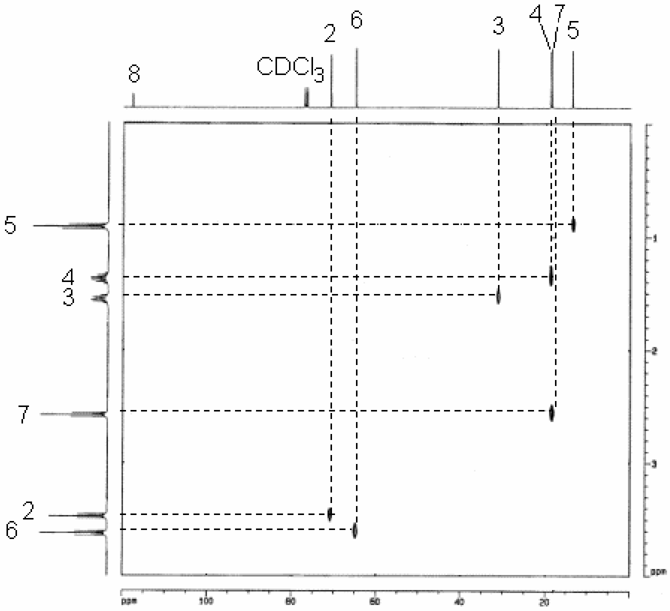
On the horizontal axis in spectrum E the ^{13}C-spectrum is shown, on the vertical axis the ^{1}H-spectrum. Peaks show scalar through bound (or spin-spin) couplings between ^{1}H-nuclei and ^{13}C-nuclei. So all previous conclusions from ^{1}H- and ^{13}C-spectra should be confirmed by this spectrum.

Conclusions from question 8.3 (spectrum B and C):

• Quartet at 13.7 ppm	CH_3 5
• Triplet at 18.7, 19.0, and 31.2 ppm	CH_2 3, CH_2 4 or CH_2 7
• Triplet at 65.1 and 71.0 ppm	CH_2 2, CH_2 6
• Singlet at 118 ppm	C 8

Conclusions from question 11.1 (spectrum A and D):

Triplet at 0.9 ppm	CH_3 5
Sextet at 1.4 ppm	CH_2 4
Quintet at 1.6 ppm	CH_2 3
Triplet at 2.7 ppm	CH_2 7 (CH_2 6 cannot be excluded for 100%)
Triplet at 3.5 ppm	CH_2 2
Triplet at 3.6 ppm	CH_2 6 (CH_2 7 cannot be excluded for 100%)



This time all uncertainties disappear because CH2 7 on basis of ^{13}C-chemical shift (see figure 15.5(b) on page 520 in Atkins) is expected at 18.7, 19.0, or 31.2 ppm. The peaks at 19.0 and 31.2 are unambiguously attributed to CH_2 4 and CH_2 3 via the ^1H-resonances. So the ^1H-resonance at 2.7 ppm must be CH_2 7 and therefore the ^1H-resonance at 3.6 ppm must be CH_2 6.

Conclusion:

^1H-assignments

Triplet at 0.9 ppm	CH_3 5
Sextet at 1.4 ppm	CH_2 4
Quintet at 1.6 ppm	CH_2 3
Triplet at 2.7 ppm	CH_2 7
Triplet at 3.5 ppm	CH_2 2
Triplet at 3.6 ppm	CH_2 6

^{13}C-assignments

Quartet at 13.7 ppm	CH_3 5
Triplet at 18.7 ppm	CH_2 7
Triplet at 19.0 ppm	CH_2 4
Triplet at 31.2 ppm	CH_2 3
Triplet at 65.1 ppm	CH_2 6
Triplet at 71.0 ppm	CH_2 2
Singlet at 118 ppm	C 8

Answer 11.3

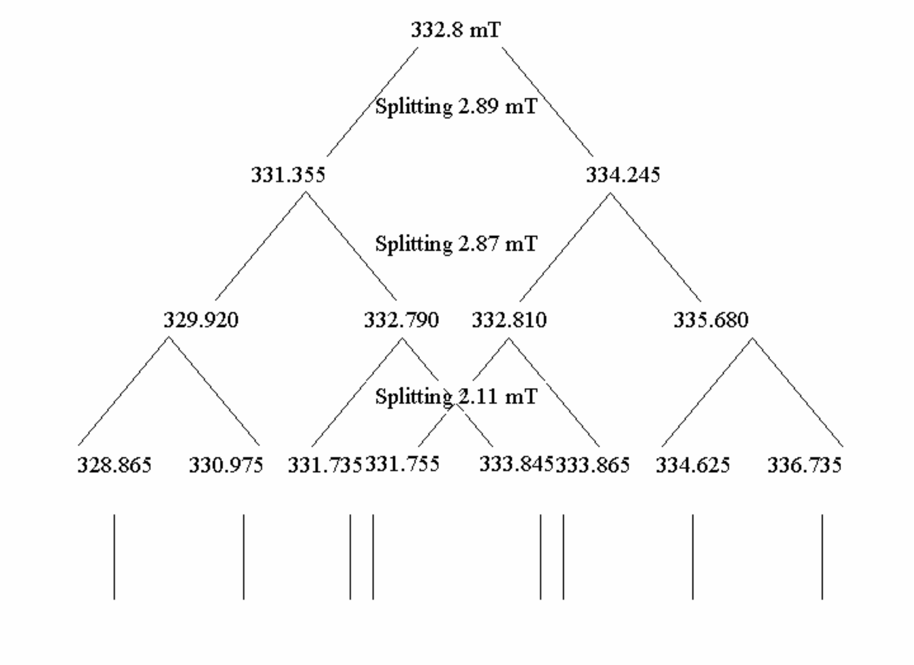
The ^{13}C-spectrum along the horizontal axis in spectrum E is proton decoupled as in spectrum C.

Block 12. Electron spin resonance spectroscopy

Answer 12.1
B = 1.3 T

Answer 12.2
The hyperfine coupling is $2.2 \cdot 10^{-3}$ T. The g-value is 1.992.

Answer 12.3
The diagram below is not put in proportion (niet op schaal)!



Depending on the spectral resolution of the ESR spectrometer the four central peaks are seen as two peaks (total spectrum 1:1:2:2:1:1 sextet) or as four separate peaks (total spectrum 1:1:1:1:1:1:1:1 octet).

Answer 12.4
a) At B = 332.3 mT.
b) At B = 1209 mT.

Answer 12.5
The Lamor frequency of a proton in a field with a gradient is given by:

$$\nu_L(x) = \frac{\gamma}{2\pi}(B_0 + G_x x)$$

x is the distance from the pivot point of the gradient (where it is zero), G is the slope of the gradient in T/m. This equation can be used to calculate the difference in Lamor frequency $\Delta\nu_L$ due to a difference in position Δx:

$$\Delta\nu_L(\Delta x) = \frac{\gamma}{2\pi} G_x \Delta x \Leftrightarrow G_x = \frac{2\pi\Delta\nu_L(\Delta x)}{\gamma\Delta x} = \frac{2\pi * 100 s^{-1}}{26.75 * 10^7 T^{-1} s^{-1} * 0.08 m} = 29.4 \mu T m^{-1}$$

As you can see a lot of the information given in the question is not necessary for the answer.

5. Proef tentamen

Opgave 1 (33/100 punten)

Twee verbindingen A en B worden afzonderlijk opgelost in benzeen, een apolair oplosmiddel (solvent). Van beide oplossingen wordt het absorptiespectrum gemeten. Beide spectra laten één absorptiepiek bij 535 nm zien. Vervolgens worden de twee verbindingen opgelost in ether, een meer polair oplosmiddel. Opnieuw worden de absorptiespectra gemeten. Verbinding A laat in ether een blauwverschuiving (blue shift) van de absorptiepiek zien, in tegenstelling tot verbinding B in ether, die een roodverschuiving (red shift) geeft, beide ten opzichte van de absorptiepiek in benzeen. De blauw- en roodverschuiving zijn, uitgedrukt in golflengtes (wavelengths), even groot. Uitgedrukt in golfgetallen (wavenumbers) bedraagt het verschil tussen de twee absorptiepieken 300 cm^{-1}.

a. Bereken de golflengtes van beide absorptiepieken.

Het is bekend dat het in alle gevallen om een $S_{0,0} \rightarrow S_{1,0}$ overgang gaat. Bovendien is bekend dat het oplosmiddel ether voor beide moleculen stabiliserend werkt.

b. Wat concludeer je uit je uit bovengenoemde waarnemingen?

De afzonderlijke verbindingen A en B laten in beide oplosmiddelen een fluorescentiespectrum met één piek van gelijke intensiteit zien. Hierna is in beide oplosmiddelen een 1:1 mengsel van verbinding A en B gemaakt. In het meer polaire ether wordt nu energieoverdracht geconstateerd met behulp van een fluorescentiespectrometer.

c. Welke verbinding is de energiedonor en welke de energie-acceptor? Motiveer je antwoord.
d. Er staan zowel een absorptiespectrometer als een fluorescentiespectrometer ter beschikking. Beschrijf de metingen die minimaal nodig zijn om te **bewijzen** dat er sprake is van energieoverdracht. Geef schetsen van alle spectra die gemeten worden.

Opgave 2 (33/100 punten)

Tip: lees de **hele** opgave alvorens vraag a t/m c te beantwoorden.

Van een verbinding is bekend dat de fluorescentie-opbrengst (fluorescence quantum yield) 0.8 bedraagt, de tripletopbrengst (triplet quantum yield) is 0.1 en de fosforescentie-opbrengst (phosphorescence quantum yield) 0.05.

a. Geef de definitie van het begrip “quantum yield” en leg dit uit in eigen bewoordingen.
b. Wat concludeer je uit het feit dat som van de fluorecentie-opbrengst en de tripletopbrengst samen niet 1.0 is?
c. Wat concludeer je uit het feit dat de tripletopbrengst en forforescentie-opbrengst verschillend zijn?

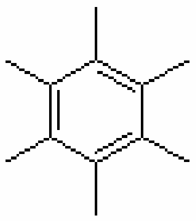
Met behulp van een monochrome lichtbron slaagt men erin alleen de $S_{0,0} \rightarrow S_{2,0}$ overgang te exciteren (de verbinding wordt dus aangeslagen naar de laagste vibratietoestand van het tweede aangeslagen singlet niveau).

d. Heeft dit wel of niet effect op bovengenoemde opbrengsten? Beargumenteer je antwoord.
e. Schets een energiediagram met daarin alle relevante niveaus en processen.

In “Atkins’ Physical Chemistry, 8th edition” staat op bladzijde 847 een fout: in de laatste formule moet staan $\phi_f + \phi_{IC} + \phi_{ISC} = 1$, om dezelfde redenen moet in de eerste formule op pagina 848 staan $\phi_f + \phi_{IC} + \phi_{ISC} + \phi_R = 1$.

f. Leg uit waarom Atkins het fout heeft. **Dit is een bonusvraag**!

Opgave 3 (33/100 punten).



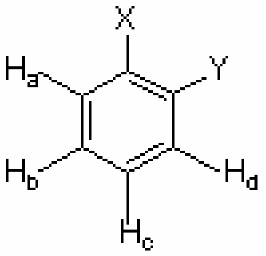
A

Van het radicaal anion van benzeen (verbinding A) wordt een “continuous-wave” ESR-spectrum (excitatie bij 9.0 GHz) gemeten. De g-waarde van het ongepaarde elektron in dit radicaal is 2.0026. De hyperfijnkoppelingsconstante (hyperfine coupling constant) tussen het ongepaarde elektron en alle protonen bedraagt 0.375 mT.

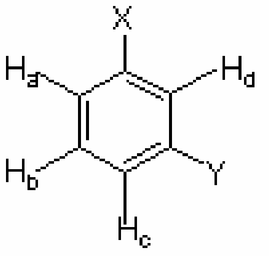
a. Bereken de posities van alle resonantielijnen in het ESR-spectrum.
b. Geef de verhoudingen tussen de intensiteiten van alle resonantielijnen. Beargumenteer je antwoord.
c. Maak een schets van het spectrum.

Van benzeen (verbinding A) wordt ook het Fourier-transform ^{1}H-NMR spectrum gemeten. De chemische verschuiving δ (chemical shift) van benzeen ten opzichte van een referentieverbinding (TMS) bedraagt 7.25. De resonantiefrequentie van TMS is 500 MHz (500 * 10^6 Hz).

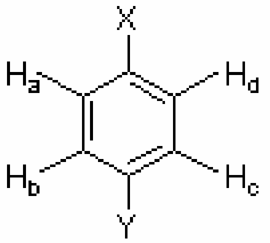
d. Bereken de frequentie van het centrum van het ^{1}H-NMR spectrum van benzeen.
e. Maak een schets van ^{1}H-NMR spectrum van benzeen.



B



C



D

Verbinding B, C en D bevatten groepen X en Y die geen protonen bevatten. Wel beïnvloeden beide groepen de chemische verschuiving van alle protonen. Ga er vanuit dat alle chemisch verschillende protonen een zodanig verschillende Lamor frequentie hebben, dat ze in het ^{1}H-NMR spectrum geen overlap vertonen.

f. Is het mogelijk verbinding B en C met behulp van ^{1}H-NMR van elkaar te onderscheiden? Motiveer je antwoord.
g. Schets het ^{1}H-NMR spectrum van verbinding D. Geef bij alle resonantielijnen en/of resonantiepatronen (multipletten) aan door welk proton of welke protonen ze zijn veroorzaakt. Zorg er ook voor dat de verhoudingen van de lijnen onderling kloppen. Let op! De gegeven informatie is niet voldoende om het exacte spectrum te geven, het gaat om een schematisch spectrum.

6. Uitwerking proeftentamen

Opgave 1

a. Druk de golflengte van de absorptiepiek uit in reciproque centimeters (cm^{-1}):

$$\frac{1}{535*10^{-9}m*100} = 18691.6cm^{-1}$$

Een piek is 150 cm^{-1} roodverschoven (lagere energie):

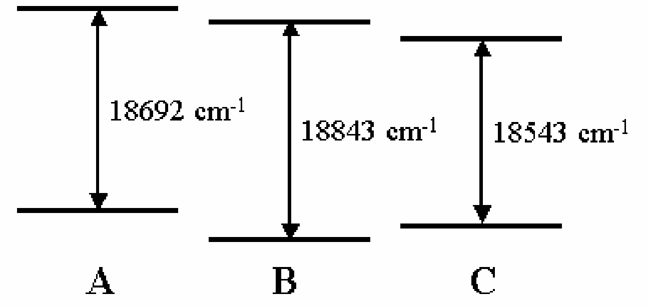
$$\frac{1}{\left(18691.6cm^{-1} - 150cm^{-1}\right)*100} = 539.3nm$$

De andere piek is 150 cm^{-1} blauwverschoven (hogere energie):

$$\frac{1}{\left(18691.6cm^{-1} + 150^{-1}\right)*100} = 530.7nm$$

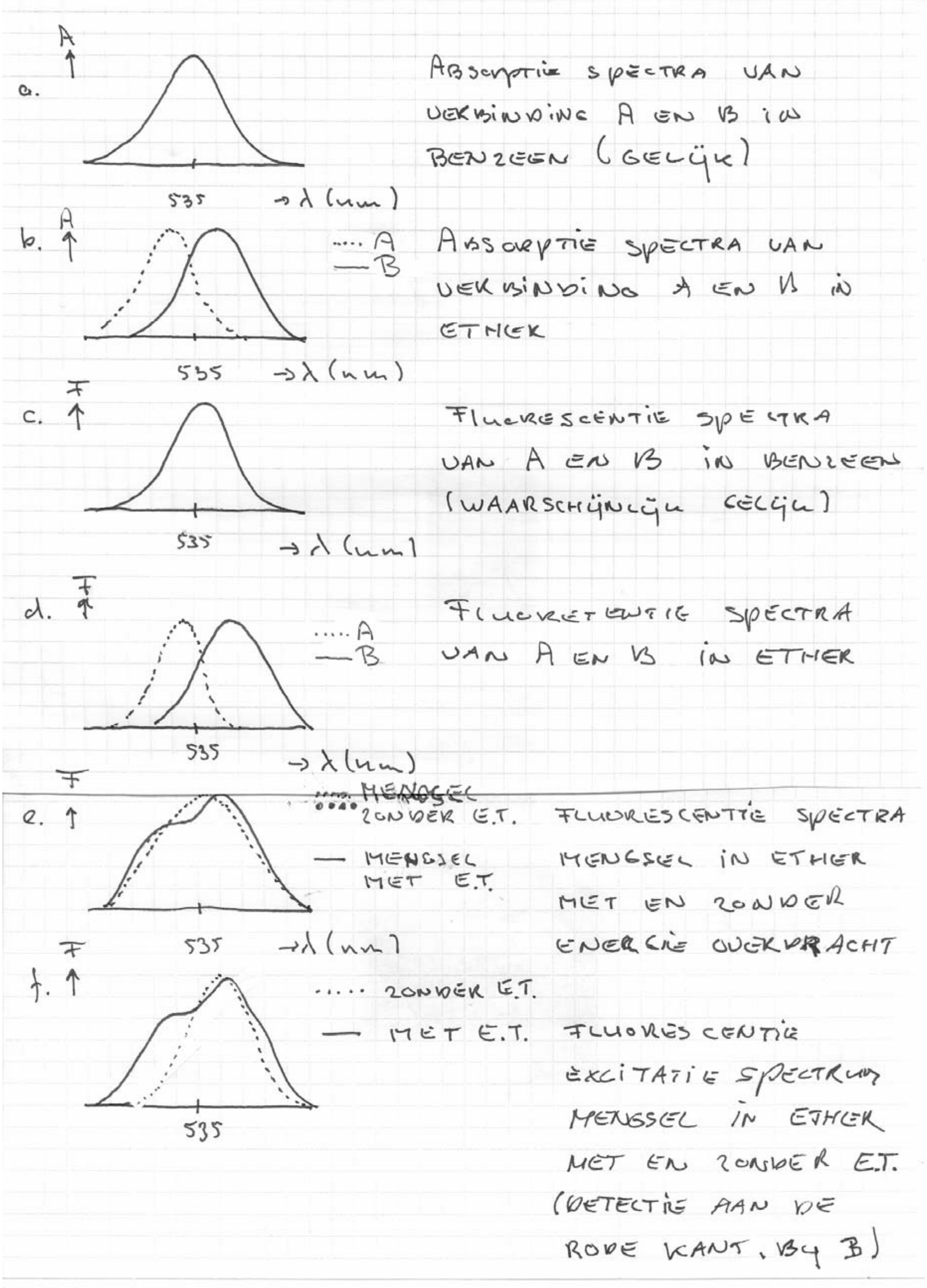
Let goed op bij het omrekenen van nm naar cm^{-1} en omgekeerd!

b. De oplosmiddel-stabilisatie van de grondtoestand van verbinding A is groter dan die van de aangeslagentoestand. Voor verbinding B is dit andersom.
Figuur A geeft de situatie voor beide verbindingen in het apolaire oplosmiddel. Figuur B geeft de situatie voor verbinding B in het polaire oplosmiddel, figuur C voor verbinding A.



c. Fluorescentie is altijd roodverschoven ten opzichte van de absorptiepiek. Dat wil zeggen dat alleen de blauwverschoven verbinding A fluorescentie geeft met voldoende energie om de roodverschoven verbinding B te exciteren. Andersom is energetisch onmogelijk. Dus verbinding A is de energie-donor en verbinding B de energie-acceptor.

d. Meet de absorptiespectra van A en B in ether (zie figuur b). De piek voor A ligt bij 530.7 nm en die voor B bij 539.3 nm. Meet vervolgens de fluorescentie spectra voor beide verbindingen afzonderlijk (figuur c. en d.) en de 1:1 mengsels (figuur c. voor benzeen en e. voor ether), in beide oplosmiddelen. Voor benzeen verwacht je dat het mengsel het somspectra van de afzonderlijke fluorescentiespectra in benzeen geeft. Voor ether verwacht je een verschil tussen het somspectra van de fluorescentiespectra en het fluorescentiespectrum van het 1:1 mengsel. Bij energie overdracht (energy transfer ET) verwacht je minder bijdrage van verbinding A aan het fluorescentiespectrum dan verbinding B. Meet vervolgens een fluorescentie excitatie spectrum van het mengsel in ether, waarbij je selectief detecteert op fluorescentie van de acceptor (figuur f.). Indien er sprake is van energie overdracht moet je dan ook absorptie bij 530.7 door verbinding A meten. In dat geval “zie” je de donor namelijk via de acceptor en is er dus sprake van energieoverdracht. Het fluorescentie excitatie spectrum geeft het definitieve bewijs.



a. ABSORPTIE SPECTRA VAN VERBINDING A EN B IN BENZEEN (GELIJK)

b. ABSORPTIE SPECTRA VAN VERBINDING A EN B IN ETHER

c. FLUORESCENTIE SPECTRA VAN A EN B IN BENZEEN (WAARSCHIJNLIJK GELIJK)

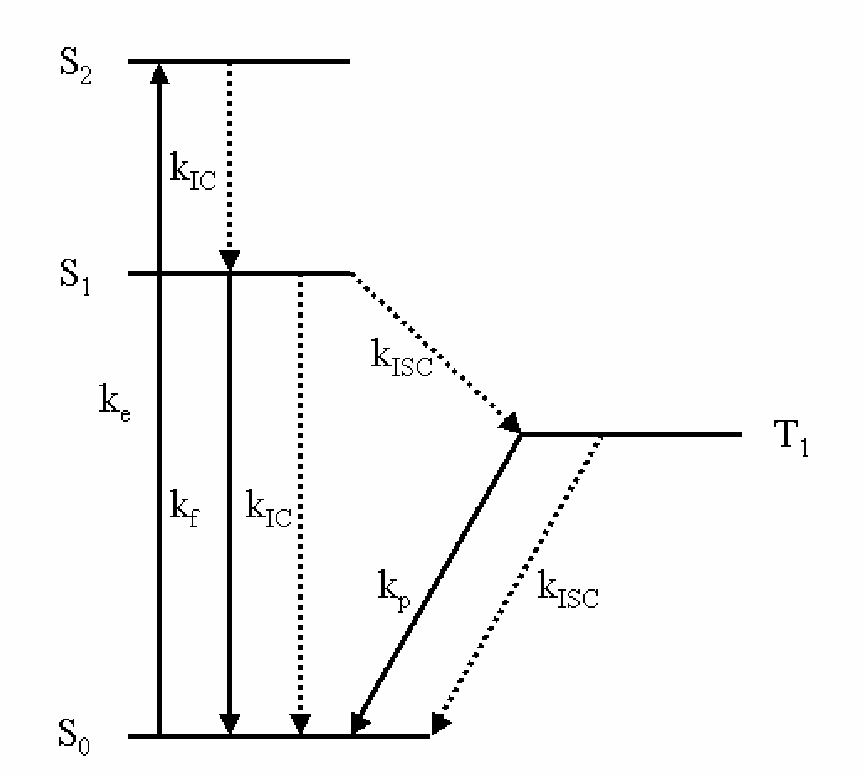
d. FLUORESCENTIE SPECTRA VAN A EN B IN ETHER

e. FLUORESCENTIE SPECTRA MENGSEL IN ETHER MET EN ZONDER ENERGIE OVERDRACHT

f. FLUORESCENTIE EXCITATIE SPECTRUM MENGSEL IN ETHER MET EN ZONDER E.T. (DETECTIE AAN DE RODE KANT, BIJ B)

Opgave 2

a. De “quantum yield” of kwantum opbrengst van een proces geeft de verhouding van het aantal tussen het aantal keren dat het proces optreedt en het aantal fotonen waarmee het systeem per wordt geëxciteerd.
b. Dat er ook nog stralingsloos verval is vanuit S_1 naar S_0 met een quantum yield van 0.1.
c. Dat er ook nog stralingsloos verval is vanuit T_1 naar S_0 met een quantum yield van 0.05.
d. Dit heeft geen effect omdat hogere singlet toestanden zeer snel via stralingsloosverval naar de laagste aanslagen singlet toestand vervallen.
e.



f. Fotonen die leiden tot vorming van de laagste triplet toestand, die vervolgens via fosforescentie naar de grondtoestand vervalt, worden dubbel geteld.

Opgave 3

a. Het ongepaarde electron koppelt met 6 protonen met hyperfijnkoppelingsconstante A_H = 0.375mT. Hierdoor splitst de ESR resonantie in 6+1 lijnen, waarvan de posities met de volgende formule kunnen worden berekend:

$$B_{m_I} = \frac{h\nu}{g_e\mu_B} + m_I * A_H = \frac{6.62608*10^{-34} Js * 9.0*10^9 s^{-1}}{2.0026*9.724*10^{-24} JT^{-1}} + m_I * 0.375 mT$$

Voor m_I = -3, -2, …, 3. Toepassen van deze formule geeft 7 veldsterktes waarbij overgangen plaatsvinden, te weten:

319.9732 320.3482 320.7232 321.0982 321.4732 321.8482 322.2232

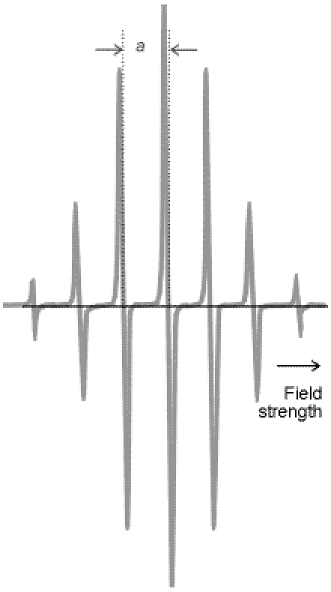
Alle waarden in mT.

b. Gebruik de driehoek van Pascal

	1
1	1 1
2	1 2 1
3	1 3 3 1
4	1 4 6 4 1
5	1 5 10 10 5 1
6	1 6 15 20 15 6 1

Dus de verhouding van de resonantielijnen is 1:6:15:20:15:6:1. De verklaring van dit patroon ligt in het feit dat veel meer combinaties de middelste overgangen mogelijk maken dan de kleinste en grootste overgang.

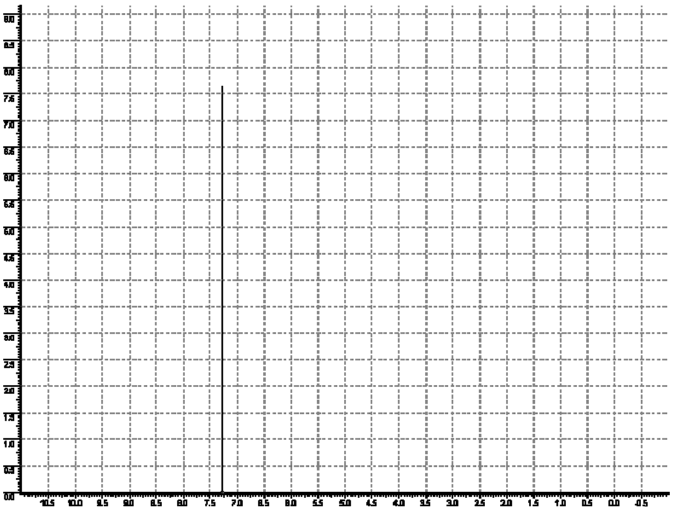
c. Een tekening met “paaltjes” is ook goed.



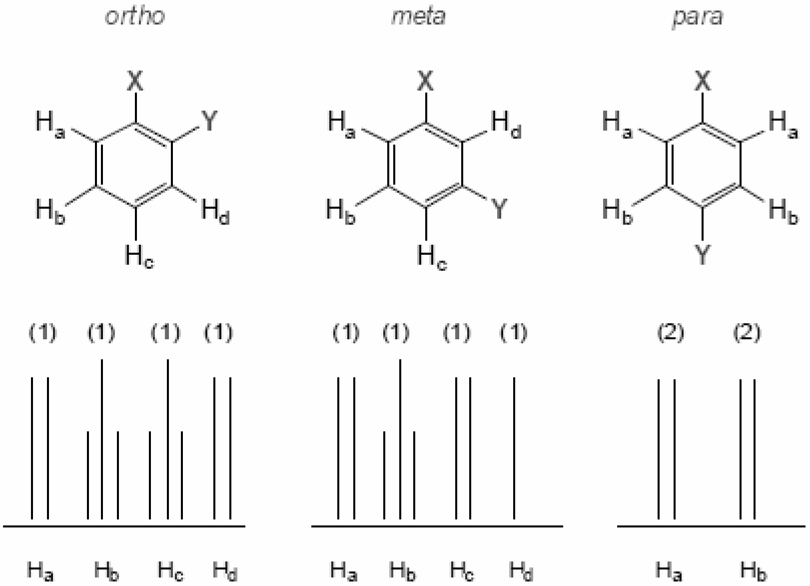
d. Gebruik formule 15.18 op pagina 519 in Atkins om de verschuiving gegeven in ppm om te rekenen naar frequentie:

$$\nu = \frac{\delta \nu_0}{1*10^6} + \nu_0 = \frac{7.25*500*10^6}{1*10^6} + 500*10^6 = 500.003.625 Hz$$

e. Alle protonen in benzeen zijn equivalent en geven daarom GEEN opsplitsing.



f. Alle 4 protonen van zowel verbinding B (ortho hieronder) als verbinding C (meta hieronder) zijn niet-equivalente met 4 verschillende chemische verschuivingen. Proton b en c van verbinding B koppelen met respectievelijk proton a en c en proton b en d. Omdat het om naastgelegen protonen gaat zal de koppeling ongeveer even groot zijn wat resulteert in twee tripletpatronen. Proton a koppelt met b en proton d met c wat resulteert in twee doubletten.
Proton d van verbinding D koppelt met geen enkel proton en geeft een singletpiek. Proton b voelt zowel a als c ongeveer even sterk wat een triplet oplevert. Proton a en c voelen beiden alleen proton b en geven daarom een doublet.
Verbinding B en C zijn dus van elkaar te onderscheiden door de opsplitsingpatronen.
Let op! de getallen tussen haakjes bij de spectra staan voor de integraal van het hele patroon. De verhoudingen in de tekeningen deugen dus niet.



g. Zie het spectrum horende bij de para verbinding hierboven. Proton a en d in figuur D zijn chemisch equivalent, net als protonen b en c, de equivalente protonen hebben daarom hierboven respectievelijk het label a en b gekregen. De equivalente protonen geven twee verschillende chemische verschuivingen. Elk proton a voelt één proton b en andersom. Dit resulteert in twee doubletten met de dubbele integraal in vergelijking met de opsplitsingpatronen van verbinding B en C (ortho en meta hierboven).

7. Laboratory Experiments

Contents

Experiment OS	Emission of biacetyl
Experiment ESR	Introduction to the ESR spectrometer
Experiment NMR	The exchange rate of protons in vitamin B_1

General remarks

- Before leaving the laboratory after the experiment, the answers to the questions and experimental results should be **authorized by the instructor**.
- For every experiment a written **measuring report** is required in which the results are worked out and all questions are answered.
- The measuring report of the laboratory experiments should conform to the guidelines as given in the brochure “Recording, reporting and presenting your work” by the former School of Technology and Nutrition (see “Guidelines for reports” on the Spectroscopy Blackboard site)

Experiment OS: Emission of biacetyl

Aim

To determine the emission properties of biacetyl and to study the effect of oxygen on these properties.

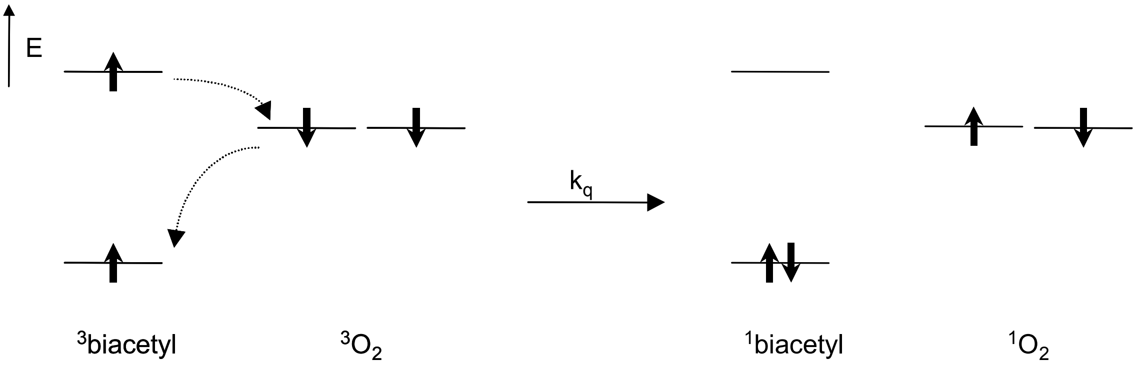
Theory

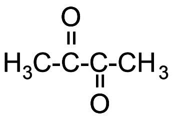
Refer to the course book: Atkins, P. and De Paula, J., Physical Chemistry, 8th ed., 2006 (Oxford University Press, ISBN 0-19-870072-5).

- The structures of diatomic molecules (section 11.4)
- The intensity of spectral lines (section 13.2)
- Fluorescence and phosphorescence (section 14.3)
- Kinetics of photophysical and photochemical processes (section 23.7)

Background information

The purpose of this experiment is to study the emission properties of biacetyl (official name 2,3-butanedione) in the presence and absence of oxygen. Oxygen is a so-called triplet quencher (to quench = *uitdoven*). Oxygen is able to react with molecules in the excited triplet state. After this reaction, these molecules will then be in the ground state and the oxygen molecules will be in an excited state. During to this process there will be energy transfer from biacetyl to oxygen. The mechanism for this is a simultaneous exchange of electrons from biacetyl and oxygen (see Figure 1).




$H_3C-\overset{\overset{O}{\|}}{C}-\underset{\underset{O}{\|}}{C}-CH_3$

Chemical structure of biacetyl

Figure 1. Mechanism of energy transfer from biacetyl to oxygen.

Procedure

1. Dissolve 1 µl biacetyl in 3 ml *n*-hexane in a fluorescence cuvette. Work in the fume cupboard and use a pipette balloon for the hexane. Micropipettes can be obtained from the instructor. Close the cuvette with a septum and shake it gently.

2. Inspect the colour of the solution and based on this select the wavelength range for the absorption spectrum. Record the absorption spectrum. Explain the absorption bands in this spectrum.
   Argue on basis of the absorption spectrum (consider: ground state, excited state, vibration bands) at which wavelength fluorescence can be expected, and what will

be the optimal excitation wavelength.

3. Record an emission spectrum up to at least 600 nm.

4. Record the excitation spectrum of the highest emission peak.

5. Ask the help of the instructor and, by using a hollow needle through the septum, gently bubble for about 2 min the biacetyl solution with nitrogen gas. The N_2 gas is drained away via a second needle that stays above the liquid.

6. Quickly record an emission spectrum.

7. After the experiment, discard the solution in a special container for hazardous compounds. **It is strictly forbidden to throw it down the sink**.

Working out

1. Compare the absorption spectrum to the excitation spectrum. Explain similarities and differences.

2. What is the origin of the extra emission band, that is well observable in the second, but not in the first emission spectrum?

3. Refer to table 23.2 on page 846 in Atkins. Which of the shown photochemical processes in that table is represented in figure 1?

4. Schematically draw the energy level scheme for the biacetyl molecule. Indicate in this drawing the kinetic parameters. Also include in the scheme the effect of oxygen.

5. Write down the electronic states of molecular oxygen. What is the spin state of the ground state of oxygen? What conclusion can you draw from this for the quenching properties of oxygen?

6. Why is the quenching of the triplet state of biacetyl by oxygen so effective, as compared, for example, with the phosphorescence process?

7. Why will nitrogen gas not quench the triplet state of biacetyl?

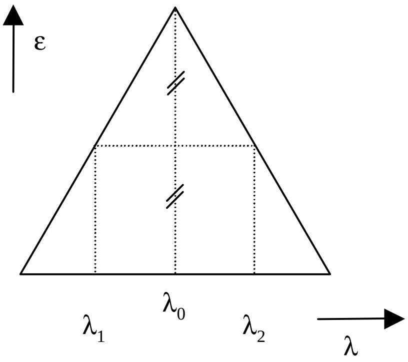
8. Explain in your own words the meaning of the term quantum yield.

9. Experimentally determine the ratio between the quantum yield of fluorescence and phosphorescence.

Now we want to extract kinetic parameters from the data, for instance the rate constant of fluorescence k_f and intersystem crossing k_{ISC}. The intensity of fluorescence, i.e. the spontaneous emission of radiation, is related to the initial absorption, which takes the molecule to an excited electronic state. As shown in equation 13.11 on page 434 of Atkins there is a relation between the intensity of spontaneous emission and the intensity of stimulated absorption. Using this relation it can be shown that the following relation exists between the rate constant of fluorescence (also called intrinsic radiation decay rate) and the absorption spectrum:

$$k_f = 3*10^{-9}\tilde{\nu}_0^2\int\varepsilon(\tilde{\nu})d\tilde{\nu}$$

In this equation $\tilde{\nu}_0$ (in cm^{-1}) is the position of the maximum of the absorption band. In the constant 0.69 all conversion factors are taken into account. The extinction coefficient ε (in $l \cdot mol^{-1} \cdot cm^{-1}$) is integrated over the absorption band as a function of the wave number $\tilde{\nu}$ (in cm^{-1}). Note that the experimental absorption spectrum is normally plotted as a function of the wavelength λ, whereas the equation uses the wave number: $\tilde{\nu} = 1/\lambda$. The integral can be approximated by assuming that the absorption band is a triangle with height ε_{max} and width given by the width $\Delta\tilde{\nu}$ (in cm^{-1}) of the absorption band at half height ($½\varepsilon_{max}$). The integral is then about equal to the area of the triangle $\varepsilon_{max} \times \Delta\tilde{\nu}$.



The intrinsic radiation lifetime τ_f, is expressed as:

$$\tau_f = 1/k_f.$$

This would be the lifetime of fluoresence as observed in a fluorescence lifetime measurement in absence of of nonradiative processes like interconversion and intersystem crossing.

10. Based on the equations above, calculate the rate constant of fluorescence, k_f, and intrinsic radiation lifetime τ_f, of biacetyl. Argue about which peak from the absorption spectrum should be taken.

11. Figure 2 shows the results of an experiment in which the decay of the fluorescence of biacetyl is measured. In this experiment the fluorescence intensity is measured after an excitation light pulse of a few nanoseconds (see the peak on the left part in Figure 2). The fluorescence intensity is shown on a logarithmic scale. Calculate from Figure 2 the observed fluorescence lifetime τ_0. Explain the difference between the observed fluorescence lifetime τ_0 and the intrinsic radiation lifetime τ_f.

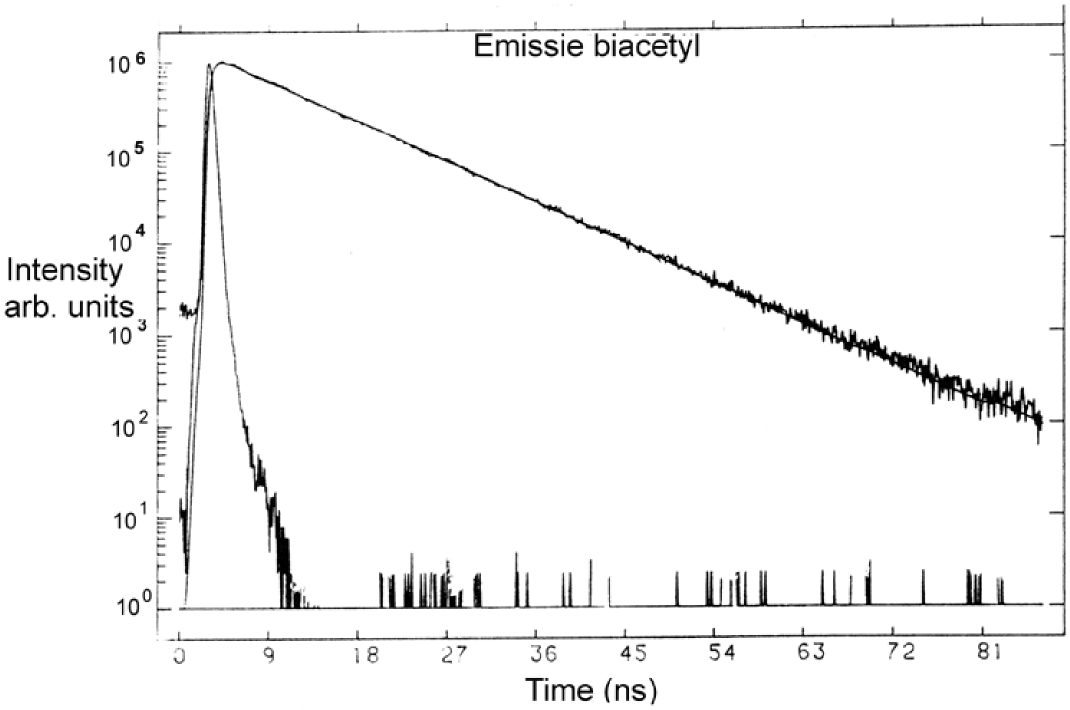


Figure 2. Fluorescence decay of biacetyl.

12. Starting from the results found before, calculate the rate constant for the intersystem crossing from the excited singlet to the triplet state (k_{ISC}), assuming that internal conversion from the excited singlet state to the ground state (k_{IC}) can be neglected

13. Calculate the quantum yield of fluorescence and phosphorescence, assuming again that internal conversion from the excited singlet state to the ground state (k_{IC}) can be neglected. Compare the ration of the calculated quantum yield of phosphorescence with that obtained experimentally in question 6. Can k_{IC} really be neglected?

Experiment ESR: Introduction to the ESR spectrometer

Aim

Getting used to the ESR spectrometer and analysis of ESR spectra.

Theory

Refer to the course book: Atkins, P. and De Paula, J., Physical Chemistry, 8th ed., 2006 (Oxford University Press, ISBN 0-19-870072-5).

- The ESR spectrometer (section 15.14)
- Spin probes (I15.2 on page 553)
- The g-value (section 15.15)

Background information

The hyperfine coupling constant for the nitrogen nucleus in a nitroxide radical is proportional to the electron spin density at the nitrogen nucleus. This electron spin density is dependent on the local polarity and hydrogen bond formation by the radical, and thus of the physico-chemical properties of the solvent. For certain resonance structure of the molecule, hydrogen bond formation can allow for an extra stabilisation.

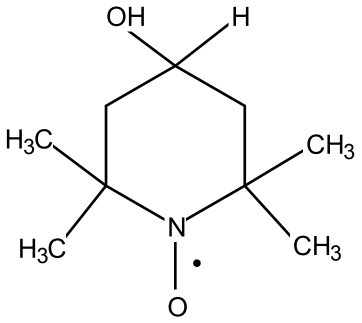


Figure 3. Molecular structure of TEMPOL.

Procedure

1. Together with the instructor, record the ESR spectrum of the TEMPOL spin label (Figure 3) in water. Consider the following parameters of the spectrometer and optimise them:

   Sweep width (*Gauss*)[1]
   Centre field (*Gauss*)
   Time constant (*ms*)
   Conversion time (time to convert the signal form analogue to digital) (*ms*)
   Modulation amplitude (modulation width of the magnetic field during the scan) (*Gauss (peak-to-peak)*)
   Gain (sensitivity, needed to observe a sufficiently strong ESR signal)
   Attenuation (microwave power) (*mW*).
   Frequency (frequency of the microwave) (*GHz*)

   1.1 Analyse the ESR spectrum: Determine the hyperfine coupling constant (A) in mT and the line width in µT. Also determine the g-value.
   1.2. Draw the energy diagram for the hyperfine interaction and indicate the allowed transitions.

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[1] On the ESR spectrometer the magnetic field strength is expressed in unit of Gauss. This is based on tradition. The conversion from Gauss (G) to Tesla (T), which is the proper SI unit, is 1 G = 10^{-4} T.

2. Using the optimized instrumental parameters, record the ESR spectrum of TEMPOL in diethylether, acetonitrile, and 1-propanol. The dielectric constants are for diethylether: 4.34, acetonitrile: 38.8, 1-propanol: 20.1, and water: 80.4.
    - 2.1. Explain the differences in hyperfine coupling based on the electron distribution over the nitroxide group of the TEMPOL molecule. For this, draw the two resonance structures of the nitroxide group, taking into account all electrons involved in the N-O bond including the free unpaired electron. In which orbital is this electron located?
    - 2.2. With the so-called "Q band" ESR spectrometer, a microwave frequency is used of 35 GHz instead of 9.5 GHz ("X-band" ESR) of the spectrometer used in this experiment. At what value of the magnetic field will a free electron have a transition at Q band ESR? What will be the hyperfine coupling constant for TEMPOL at this value of the magnetic field?
3. Membranes are highly selective barriers. The principal structural element of a membrane is the phospholipid bilayer (see figure 4). A phospholipid contains a hydrophilic head group and two hydrophobic tails. In a test tube of water phospholipids will self-organize to form a bilayer.

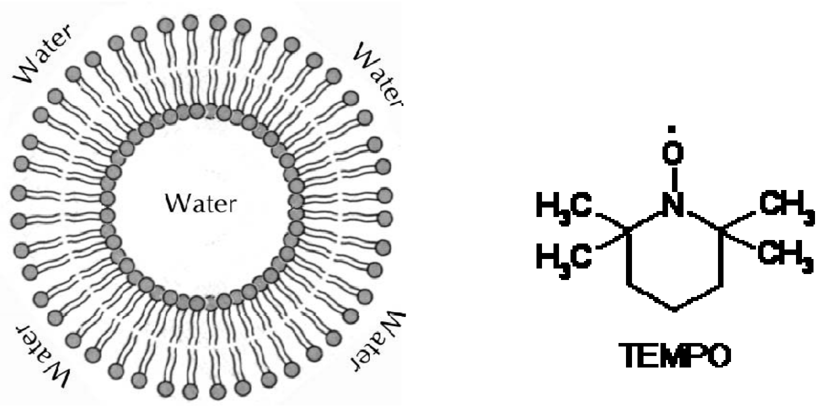

Figure 4. Model of a bilayer and the chemical structure of TEMPO.

The spin label TEMPO (see figure 4) will partition between the hydrophilic water environment and the hydrophobic lipid environment. The assistant will give you a sample of a phospholipid solution with the TEMPO spin label already added at a concentration of 1% relative to the phospholipid molar concentration.

- 3.1. Record a spectrum and explain the result.
- 3.2. Determine the value of the hyperfine splitting of TEMPO in water and within the bilayer. Schematically draw the final spectrum (summation of the two environmental spectra) using these experimental values.
- 3.3. Estimate the amount of spin label (in percentage) present within the bilayer.

Experiment NMR: The exchange rate of protons in vitamin B_1

Aim

Determination of the proton exchange rate in vitamin B_1 (thiamine) by measuring the NMR spectrum as a function of time.

Theory

Refer to the course book: Atkins, P. and De Paula, J., Physical Chemistry, 8th ed., 2006 (Oxford University Press, ISBN 0-19-870072-5).

- The chemical shift (section 15.5)
- http://www.chemistry.nmsu.edu/studntres/chem435/Lab8/index.html

Background information

Vitamin B_1 (in the form of the cofactor thiamine pyrophosphate, see Figure 5) is a nutrient with a critical role in maintaining a healthy central nervous system. Thiamine pyrophosphate is a cofactor for a large number of metabolic reactions. This involves enzymes that catalyse aldol-type addition reactions.

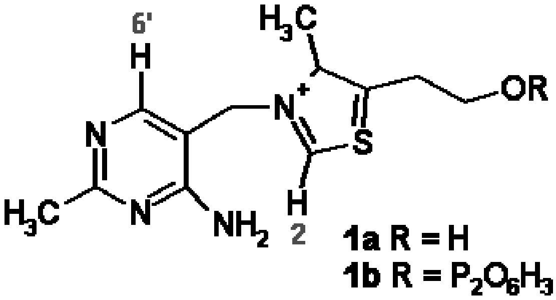

Figure 5. Molecular structure of vitamin B_1 (thiamine, **1a**) and thiamine pyrophosphate (**1b**).

The molecular basis of the catalysis by thiamine arises from the base-catalysed removal of the C(2) proton of the thiazonium ring (this is the proton on the C between the N and S). In this process the thiazonium ion is formed (see Figure 6).

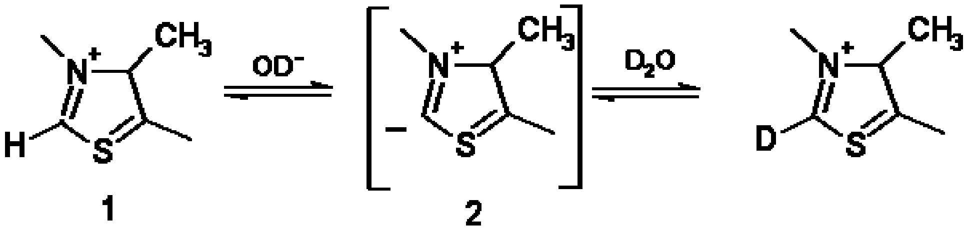

Figure 6. The base-catalysed exchange of the C(2) proton of thiamine (**1**) takes place via the thiazonium ion (**2**). The neighbouring positively charged nitrogen stabilizes the thiazonium carbanion.

Procedure

1. Prepare an acetate/acetic acid buffer of 2 ml in D_2O at pH ~4.5 (use NaAc and acetic acid (d_4)) with an ionic strength of 0.05 M (use NaCl).

2. Dissolve in this buffer 20 mg thiamine-HCl. Determine the pH of the solution using a pH meter.

3. Record at room temperature during 30 min the ^1H NMR spectrum at 3-min intervals.

Working out

1. Plot the complete NMR spectrum of thiamine-HCl and assign the peaks.

2. Plot all spectra and determine the integrated intensities of the C-(2)-H (I_2) and C-(6')-H (I_6) protons. Limit yourself to the range from 8 to 10 ppm.

3. Explain why these intensities are monitored.

4. Explain why it is necessary to use a buffer and why the acetate/acetic acid buffer is used.

5. Prepare a semi-logarithmic plot of the normalised intensity of the C-(2)-H proton (I_2/I_6) as a function of time. Determine the pseudo-first order exchange rate, k_{obs}, from the slope of the plot.

6. Calculate k_{OD} from the k_{obs} and the concentration of the OD^- ion:

$$k_{OD} = k_{obs}/[OD^-]$$

The concentration of the OD^- ion can be calculated from:

$$[OD^-] = 10^{(pD - pKw)}$$

The value for pK_w can be found from the table below:

t/°C	pK_w	
	H_2O	D_2O
10	14,53	15,53
15	14,34	15,32
20	14,16	15,13
25	14,00	14,95
30	13,84	14,78
35	13,69	14,62

The pD can be calculated from the measured pH in the following way:

$$pD = pH + 0.4$$

8. Projects

Contents

General remarks

- Prepare the project using the checklist in your discussion with the instructor, informants and supervisors to find out the best approach for your project.
- The project group should prepare **a full written report** according to the following outline:
  Summary – introduction – theory – materials and methods – results – discussion – conclusions – literature.
- Only for **Week 5**: Following the same outline, an **oral presentation** is prepared supported by PowerPoint slides.
- Both the project reports and oral presentation should conform to the guidelines as given in the brochure “Recording, reporting and presenting your work” by the former School of Technology and Nutrition (see “Guidelines for reports” on the Spectroscopy Blackboard site)
- Every group member prepares **a reflection report** for both projects

Checklist

Use the checklist in your discussion with the instructor, informants and supervisors to find out the best approach for your project[1].

In preparing the checklist take into account the following points:

- How much material is available?
- Is the sample transparent for the electromagnetic radiation used?
- What kind of pre-treatment of the sample is needed?
- Can the project be finished within the planning and deadlines?
- Use the following qualifications: ++ = very good; + = good; +/- = reasonable; - = moderate; -- = bad.
- Provide your arguments in a complete line of reasoning.

Spectroscopic technique	*Sensitivity*	*Information*	*Suitability*	*Arguments*
^{1}H NMR				
^{13}C NMR				
^{31}P NMR				
NMR-imaging				
ESR on spin probes				
ESR on free radicals				
IR and FT-IR				
Laser-Raman spectroscopy				
Circular dichroism				
UV/VIS spectroscopy on natural compounds				
UV/VIS spectroscopy on probes				
Fluorescence spectroscopy on natural compounds				
Fluorescence spectroscopy on probes				
Fluorescence imaging on natural compounds				
Fluorescence imaging on probes				

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[1] Background material is available on the Spectroscopy Blackboard site.

Project 1: Energy transport and energy conversion in spinach

Introduction

Green plants convert sunlight into chemical energy. Various pigments absorb light of particular color. 'Working together' of the different pigments is necessary in order to get all the collected energy into the water splitting engine (reaction center) of the plant cell. Measuring of energy transport will provide information about the organization of pigments in the living cell.

Aim

A qualitative determination of 'photosynthetic activity' (energy transport or energy conversion) in i) intact chloroplasts of spinach leaves, ii) disrupted chloroplasts (by detergent), and iii) isolated pigment-protein complexes. For comparison 'photosynthetic activity' will also be determined of the free pigments (obtained by dilution of the chloroplast suspension in organic solvent).

Approach

Make a schedule and planning for the research and work this out based on the starting literature and your acquired knowledge about spectroscopy. Follow the checklist at the end of this chapter to find the best possible approach.
Take into account the following points:

- Which spectroscopic technique could be suitable for the determination of energy transport in plant cells?
- Explicitly take into account the use of spectroscopic probes.

Literature[1]

- http://photoscience.la.asu.edu/photosyn/education/photointro.html
- Nathan Nelson and Charles F. Yocum, Structure and Function of Photosystems I and II, The Annual Review of Plant Biology (2006), 57: p521-65

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[1] Reprints of the papers are available on the Spectroscopy Blackboard site.

Project 2: Spectroscopy of water.

Introduction

Water is a common chemical substance that is essential to all known forms of life. The omnipresence of water makes it an ideal internal probe. One of the more successful exploits of this concept has led to the routinely use of Magnetic Resonance Imaging (MRI) in medicine. Not only the fact that water can be spatially encoded within an object or subject, but also that it easily can be discriminated due to its local environment, are key features which make this technique so versatile and powerful.

Aim

Prepare a practical/laboratory experiment for one daily period (4 hours, een dagdeel), which illustrates the main principles of MRI to students of your level.

Approach

Explore the available hardware and methods with some simple demonstration experiments. Consider, given the limitations of the setup, experimental complexity and available time, what are realistic learning goals for the practical. Prepare an instruction for both students with an introduction, assignments and questions. The format should be comparable to the laboratory experiment of the course Spectroscopy (see chapter 7). Also prepare an instructors version with guidelines, answers and backgrounds.

Literature[1]

- Atkins, P. and De Paula, J., Physical Chemistry, 8th ed., 2006 (Oxford University Press, ISBN 0-19-870072-5). Pulse techniques in NMR (section 15.8 and 15 9).
- The Basics of MRI by Joseph Hornak (http://www.cis.rit.edu/htbooks/mri/).

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[1] Reprints of the papers are available on the Spectroscopy Blackboard site.

Reflection

Reflection report project Spectroscopy

The aim of the reflection report is to obtain a balanced impression of the **project part** of the course Spectroscopy (BIP-31306). It is possible that you are satisfied; rather, you are not pleased with your results, your own efforts, or the efforts of the people within and outside your project. You should report these findings in your reflection report.

Content reflection report

The reflection report should include the following points:

- Name
- Title
- Date
- Setting of the project (use at most five sentences)
- Analysis of the behaviour of the people involved
  1. Of yourself – what was your own contribution?
  2. Of your team members in the group
  3. Of the group as a whole
  4. Of the instructors (staff people of the laboratories)
- What do you conclude and learn from this analysis?
- What possible adaptations of your own behaviour and activities could you suggest?

Submission dates

Submit your reflection report by e-mail **not later than 18:00 hr** to Frank Vergeldt (Frank.Vergeldt@wur.nl) at the following dates:

- Projects in week 5: **Friday 5-10-2007**
- Projects in week 6: **Thursday 11-10-2007**

Without giving one's reasons late submission of the reflection reports will lead to exclusion at the written exam ("*Zonder opgave van redenen leidt te late inlevering van de reflectieverslagen tot uitsluiting van het schriftelijke examen*")