

# INSTRUMENTAL TECHNIQUES APPLIED TO BIOTECHNOLOGY

MODULE	CONTENT	YEAR	TERM	CREDITS	TYPE
Technological	Instrumental techniques applied to biotechnology	1st	2nd	6.0	Compulsory
<b>LECTURER(S)</b>			<b>Postal address, telephone n°, e-mail address</b>		
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<b>DEGREE WITHIN WHICH THE SUBJECT IS TAUGHT</b>					
Biotechnology					
<b>PREREQUISITES and/or RECOMMENDATIONS (if necessary)</b>					
<ul style="list-style-type: none"> <li>Suitable knowledge of Chemistry, high-school level.</li> <li>Basic knowledge on mathematical operations (logarithmic and exponential functions, use of calculator, etc.).</li> </ul>					
<b>BRIEF ACCOUNT OF THE SUBJECT PROGRAMME</b>					
Interactions between radiation and matter. Main components in spectroscopic techniques. Atomic absorption and emission spectroscopy. Molecular absorption spectroscopy. Emission spectroscopy. Nuclear magnetic resonance spectroscopy. Mass spectrometry.					
<b>GENERAL AND PARTICULAR ABILITIES</b>					
Basic: CB2, CB3, CB5 General: CG1, CG2, CG4, CG5, CG7 Transversal: CT3, CT5, CT8, CT9 Specific: CE28					



## DETAILED SUBJECT SYLLABUS

### LECTURES:

#### **Unit 1.- Absorption of light and main components for optic spectroscopy.**

Nature of the electromagnetic radiation. Regions of the electromagnetic spectrum. Molecular energy levels. Selection rules. Lambert-Beer's Law. Deviations from Beer's Law. Range of minimum error. Components and configurations of instruments for optic spectroscopy. Light sources. Wavelength selectors. Light detectors.

#### **Unit 2.- Atomic spectroscopy.**

Introduction to atomic spectroscopy. Atomic spectra and selection rules. Effect of the temperature on the atomic spectra. Sample atomization. Sample introduction. Atomic absorption spectroscopy. Radiation sources. Flame atomizers. Electrothermal atomizers. Types of spectrophotometers. Interferences. Atomic emission spectroscopy. Atomizers. Inductively coupled plasma (ICP). Types of spectrophotometers. Applications. Flame photometry. Comparison between the different atomic spectroscopy techniques.

#### **Unit 3.- IR spectroscopy.**

IR radiation. Vibration of diatomic molecules and potential energy curves. Absorption of IR radiation. Selection rules. IR spectra and force constant in diatomic molecules. Anharmonicity. Vibration of polyatomic molecules. Instrumentation for IR spectroscopy. IR spectra of biopolymers.

#### **Unit 4.- UV-vis absorption spectroscopy.**

Electronic spectra: vibration structure of the electronic bands. Selection rules. Electronic transitions in polyatomic molecules. Chromophore and auxochrome groups. Instrumentation. UV-vis spectra of biopolymers: proteins and nucleic acids. Conformational effects on the absorption: sensitivity to the local environment, and chromophore interactions. Optical rotatory dispersion. Circular dichroism and protein structure.

#### **Unit 5.- Emission spectroscopy I.**

Introduction to fluorescence emission. Features of the fluorescence emission. Monomolecular processes of excited-state deactivation. Fluorescence quantum yield and lifetime. Steady-state fluorescence spectra and time-resolved fluorimetry. Instrumentation. Factors affecting the fluorescence emission: Kavanagh's Law. Intrinsic and extrinsic fluorophores. Labelling of molecules, biomolecules, and macrostructures. Quimioluminescence and bioluminescence.

#### **Unit 6.- Emission spectroscopy II.**

Solvent effects. Determination of the environment polarity. Solvent relaxation. Time-Resolved Emission Spectroscopy (TRES). Fluorescence quenching: static, dynamic, and sphere of action quenching. Fluorescence Resonance Energy Transfer (FRET). FRET pairs. FRET as a spectroscopic ruler. FRET for determining molecular associations. FRET for studying membranes. Fluorescence polarization and anisotropy. Fluorescence anisotropy to study molecular associations and membranes. Fluorescence correlation spectroscopy (FCS). FCS to study molecular diffusion and reactions. Fluorescence microscopy and fluorescence lifetime imaging microscopy (FLIM). Single-molecule fluorescence spectroscopy. Semiconductor nanocrystals: quantum dots. DNA sequencing.

#### **Unit 7.- Nuclear Magnetic Resonance.**

Principles of NMR. Instrumentation. Features of NMR spectra. Chemical shift. Multiplicity. Proton NMR spectra of biological systems.  $^{13}\text{C}$ -NMR spectra of proteins.  $^{31}\text{P}$ -NMR spectra.  $^{19}\text{F}$ -NMR spectra of biological systems. NMR spectra of nucleic acids.

#### **Unit 8.- Mass spectrometry.**

Physical fundamentals. Ionization and fragmentation. Ionization methods: gas phase and desorption (electrospray and MALDI). Mass analyzers. Detectors. Mass spectra. Molecular and structural information.



## LABORATORY SESSIONS AND SEMINARS:

### Seminars

- Problems solving

### Laboratory sessions

- **Session 1. Protein determination by UV-vis spectroscopy.**
- **Session 2. Dynamic quenching.**
- **Session 3. Molecular distances measured by FRET.**
- **Session 4. FTIR-ATR for structural information of organic molecules and proteins.**

## READING

- **Principios de Análisis Instrumental.** (6ª Edición). D.A. Skoog, F.J. Holler, S. R. Crouch. Ed. McGraw-Hill.
- **Methods in Molecular Biophysics. Structure, Dynamics, Function.** I.N. Serdyuk, N.R. Zaccai, J. Zaccai. Cambridge University Press.
- **Principles of Fluorescence Spectroscopy.** (3rd Ed.). J. R. Lakowicz. Springer.
- **Análisis Instrumental.** K.A. Rubinson, J.F. Rubinson. Prentice-Hall.
- **Chemical Analysis. Modern Instrumentation, Methods and Techniques.** (2nd Ed.). F. Rouessac, A. Rouessac. Ed. Wiley.

## RECOMMENDED INTERNET LINKS

### UC Davis ChemWiki online resources

<http://chemwiki.ucdavis.edu>

### William Reusch Virtual Textbook of Organic Chemistry

<http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/intro1.htm>

### IR, RMN and mass spectrometry database: SDBSWeb:

<http://sdfs.db.aist.go.jp>

(National Institute of Advanced Industrial Science and Technology, accessed 5/12/2014)

### Beer's Law

<http://www.chm.davidson.edu/ChemistryApplets/spectrophotometry/BeersLaw.html>

### IR spectroscopy:

<http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm#ir1>

### UV-vis spectroscopy:

<http://teaching.shu.ac.uk/hwb/chemistry/tutorials/>

### NMR spectroscopy:

<http://www.cis.rit.edu/htbooks/nmr/inside.htm>

### NMR exercises:

<http://www.chem.ucla.edu/~webspectra/#Problems>



**Mass spectroscopy:**

<http://www.astbury.leeds.ac.uk/facil/MStut/mstutorial.htm>

