SYLLABUS DEL CORSO

Chimica Analitica Strumentale e Laboratorio

1920-3-E2702Q058

Aims

The main objective of the course is to provide the student with the theoretical foundations and fundamental operative tools of a variety of techniques useful in modern analytical chemistry and necessary for the qualitative and quantitative determination of the chemical nature of a sample. The knowledge of the principles and the instrumentation of the main analytical techniques will be introduced together with the ability to choose and manage the best methods suited to the purposes of the analysis. The student will then be able to evaluate the instrumental characteristics of the fundamental analytical approaches, the fields of application, the advantages and disadvantages of the individual analytical techniques and will therefore be able to suggest the choice of the analytical technique considered most suitable for a specific problem.

Contents

Spectroscopy: introduction to spectroscopy, absorption, emission and excitation spectra. Definition, details and limitations of the Lambert-Beer law. Instrumental components of UV-Visible spectrophotometers, FT-IR and spectrofluorometers: instrumentation structure, sources, monochromators, detectors and signal processing. Quantitative and qualitative applications. Fluorescence spectroscopy. Introduction to NIR spectroscopy.

Chromatography: general principles. Gas-Liquid, Liquid-Liquid, Ionic Chromatography: injectors, columns and detectors. Outline of affinity and exclusion chromatography. Chromatography applications.

Mass spectrometry: ionization methods (EI, CI, FAB, MALDI, ESI, APCI), analyzers (quadrupole, TOF, orbitrap, FT-

MS). Qualitative and quantitative applications.

NMR: nuclear spin; nuclear magnetic resonance principle; NMR spectrometer components. Spectroscopy of 1H: chemical shift, chemical shielding, chemical coupling, first order spectra and higher order spectra. Fourier transform. Spectroscopy of 13C. Examples of interpretation of spectra of organic molecules. Relaxation time T1 and T2, dynamic processes.

Practical sessions in the laboratory, to illustrate the use of the analytical instrumentation described in the course in qualitative and quantitative applications.

Detailed program

Introduction to spectroscopy, equations and properties of electromagnetic radiation. Definition of amplitude, frequency, wavelength and wave number. Overview of the energies associated with the different spectral regions. Interactions between matter and electromagnetic radiation: definitions of absorption and emission. Definition of Transmittance and Absorbance. Definition of the Lambert-Beer law, description of its parameters and definition of the fields of applicability of the law, its specifications and limitations: instrumental deviations from the law for polychromatic radiation and stray light. Experimental and theoretical absorbance and correction with reference solutions. Description of the additive behavior of the absorbance in mixtures. Definition of absorption spectra. Characteristics of UV-visible and IR absorption spectra. Vibrational transitions and model of the harmonic oscillator. Definition of the theoretical number of vibrational deformations.

Instrumental components for spectrophotometry: sources, monochromators, filters, sample cells, internal and external reflectance acquisitions, optical fiber, Michelson interferometer and Fourier transformation, detectors (photomultiplier tubes, diode arrays, charge-coupled devices). Single-beam, dual-beam, and multi-channel spectrophotometers. Relative accuracy on absorbance and dynamic range. Specifications for signal acquisition in FT-IR spectrophotometers and definition of the Singal-to-Noise ratio.

UV-visible absorption spectroscopy: qualitative and quantitative applications. Electronic transitions and absorbing species, effect of conjugation on absorption; absorption by charge transfer; quantitative determinations: calibration, limitations and characteristics, operating conditions, advantages and disadvantages; method of standard additions; determination of substances in mixtures with resolved and unresolved peaks; spectrophotometric titrations; determination of the equilibrium constant with Scatchard diagram; determination of the ionization constant of an indicator; study of the stechiometry of a reaction using the continuous variation method (Job) and the molar ratio method.

IR absorption spectroscopy: qualitative and quantitative applications. Factors that determine the increase or reduction of the number of bands in the spectrum; degeneration, coupling and overtone bands; stretching and bending vibrations; factors that determine the intensity and frequency of an absorption band; characteristic regions of the IR spectrum; introduction of the interpretation of IR spectra; background and post processing operations on IR spectra; limitations of the quantitative applications of IR spectroscopy; introduction on NIR (near-infrared) spectroscopy, NIR instrumentation, signal acquisition and industrial applications.

Fluorescence spectroscopy: excitation and emission spectra; relationship between emission spectra and absorption spectra; relationship between emission spectra and excitation spectra; characteristics of fluorescent compounds; relationship between fluorescence intensity and concentration, limits of application to maintain the linear relationships; structure of a spectrofluorometer: sources, monochromators, sample cells, detectors. Applications of fluorescence spectroscopy.

Introduction to analytical separations and chromatographic separations. Classification of chromatographic methods.

Definition of chromatogram. Characteristics of the chromatographic column; distribution constants, retention times, retention factor, selectivity factor. Efficiency of the chromatographic column and its description; definition of plate height and number of theoretical plates. Factors that determine the efficiency of the chromatographic column. Van Deemter's equation. Resolution of the chromatographic column and effect of the factors on the resolution.

Gas-liquid chromatography; introduction to Gas-Liquid chromatography, the separation process in gas chromatography; injection system, columns and their characteristics, capillary and packed columns, liquid stationary phases, flame ionization detectors (FID), thermal conductivity detectors (TCD), electron capture detectors (ECD). Applications of Gas-Liquid chromatography.

Liquid - Liquid Chromatography: characteristics of the chromatograph; sample pumping and injection systems. Types of columns. Characteristics of the stationary phase. The process of elution (isocratic and gradient). Detectors. Ionic Chromatography. Overview of partition, adsorption, size-exclusion and affinity chromatography.

Mass spectrometry: principles of mass spectrometry, electronic ionization, definition of mass spectrum; types of mass spectrometers (quadrupole, flight time, ion trap, orbitrap); components of a mass spectrometer: injection system, ionization methods (EI, CI, FAB, MALDI, ESI, APCI), mass analyzer (quadrupole, TOF, ion trap, FT-MS), detector. interfaces Chromatography - mass spectrometry. Resolution of mass spectrometers and types of mass analyzers. Introduction to atomic mass spectrometry and molecular mass spectrometry. Qualitative applications of mass spectrometry (molecular recognition) and quantitative (ifenated techniques with chromatographs or ICP-MS).

Background. Physical bases of nuclear magnetic resonance, concept of nuclear spin, spin quantum number, main magnetic field, Larmor frequency, population of spin levels. Laboratory reference system, rotating reference system, radiofrequency pulse, 90° pulse, Free Induction Decay (FID). Diagram of an NMR spectrometer. Sample preparation, tuning, shimming. Choice of deuterated solvent. Acquisition of FID and Fourier transform.

^{1}H NMR Spectroscopy. Definition of chemical shift and determining factors: diamagnetic contribution and contribution of magnetic anisotropy. spin-orbit interaction. Concept of spin system. Multiplets of the 1st order and relative intensity (Pascal triangle) Examples of higher order spin systems. Karplus curve for J coupling on three bonds. Coupling with heteronuclei. Homotopic, enantiotopic and diastereotopic atoms. Magnetic equivalence concept. Exercises for the determination of spin systems generated by organic compounds. Correlation between relaxation time T2 * and line width. Definition of T2. Contributions to T2*: inhomogeneity and magnetic susceptibility. Spin coherence and de-phasing concepts. Spin Echo. Pulse sequence "Hahn Echo". Dipolar coupling and Nuclear Overhauser Effect (NOE). NOE sequence to determine spatial proximity in rigid or flexible molecules.

^{13}C NMR spectroscopy. Chemical shift of the various functional groups, coupling with protons, decoupled spectra. Non-quantitative spectra and quantitative spectra. Description of the INEPT pulse sequence and the involved nuclear spin levels. DEPT 90 and DEPT 135. 2D NMR. Description of the two-dimensional NMR experiment. Examples of COSY spectra. Finally some lessons will be entirely dedicated to the interpretation of NMR spectra of organic molecules.

Practical lab sessions include the following six activities: determination of caffeine in coca-cola by high performance liquid chromatography (HPLC), spectrophotometric determination of the ionization constant of an indicator, fluorimetric determination of mercury, monitoring the transformation of barium sulfate into barium carbonate with FTIR, spectrophotometric titration of para and meta-nitrophenol, 1 H NMR determination of acetone and ethyl acetate mixture.

Prerequisites

Basic knowledge on the theoretical and operational foundations of analytical chemistry. Basic manual skills and

operational skills in practical laboratory experiences.

Teaching form

The course is divided into a part of lectures and frontal exercises, in which the theoretical background on the topics are given. During the course, the students follow six different practical experiences in the laboratory, where they learn directly the use of the analytical instrumentation described in the course for qualitative and quantitative applications. On the e-learning page of the course, the slides of the lessons are constantly updated and additional contents are available for further information on specific topics.

Textbook and teaching resource

For each laboratory experience, a laboratory sheet is provided through the e-learning platform. It describes the experience in a synthetic way; furthermore, additional information material is provided, which includes a detailed document with a description of the theoretical foundations and operational methods of the experience, scientific articles for a deepening of the experience and the operating instructions of the instruments.

Semester

First semester

Assessment method

The exam consists of an oral examination in which the topics described in the lectures and the laboratory experiences are discussed. In addition to knowledge on the fundamentals presented in the course, students' skills and aptitudes are also assessed to adapt the theoretical foundations of analytical instrumental chemistry to particular operational and practical conditions; the expositive ability and adequacy of the student's language are also assessed.

In addition, two intermediate tests (with multiple choice tests in the informatic lab) are carried out at half of the course and at the end of the course; each trial includes 30 questions; the first test includes questions on the topics presented in the first part of the course, the second test includes questions on topics presented in the second part of the course and on laboratory experiences; students who obtain positive results in both tests (at least 20 questions answered correctly) may take a reduced oral exam, in which the laboratory reports and their connection to the fundamental topics of the course are discussed. The starting mark in the reduced oral exam consists of the average number of correct answers provided in the two intermediate tests.

For admission to the oral examination it is necessary to have attended at least five of the six laboratory experiences and to have delivered the relative reports.

Students who fail an exam can repeat it at the successive exam date

Office hours

Teachers are always available to receive students in their offices upon an e-mail request.