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TRACK 7

ANALYTICAL CHEMISTRY AND APPLIED SPECTROSCOPY

03 November 2010 (Wednesday)

SESSION CHAIR : **DR. MASAKO NAGAI**
Research Center for Micro-Nano Technology, Hosei University, Japan

SESSION CO-CHAIR: DR. RAVI RANJAN PANDEY
Centre for Cellular and Molecular Biology, Hyderabad, India

SESSION INTRODUCTION

TITLE: STRUCTURE ELUCIDATION OF BIOACTIVE COMPOUNDS BY MEANS OF CIRCULAR DICHROISM SPECTROSCOPY

DR. JADWIGA FRELEK, Institute of Organic Chemistry of the Polish Academy of Sciences, Poland

TITLE: QUATERNARY STRUCTURE ANALYSIS OF HUMAN HEMOGLOBIN BY A NEAR-UV CD SPECTROSCOPY

DR. MASAKO NAGAI, Research Center for Micro-Nano Technology, Hosei University, Japan

TITLE: APPLICATION OF ENZYME IMMOBILIZED NANOSTRUCTURED FE DOPED TIO2 FOR ELECTROCHEMICAL QUANTIFICATION OF UREA

DR. RAVI RANJAN PANDEY, Centre for Cellular and Molecular Biology, Hyderabad, India

COFFEE BREAK & POSTER SESSION

TITLE: FABRICATION OF NOVEL NANOSTRUCTURED PD2+ DOPED TIO2 PLATFORM FOR DETECTION OF CHOLESTEROL

DR. MARSHAL DHAYAL, Centre for Cellular and Molecular Biology, Hyderabad, India

TITLE: BIOIMAGING USING MICRO-CT, DEI-CT AND X-RAY MICROPROBE WITH SYNCHROTRON X-RAYS

DR. D. VENKATESHWARA RAO, Department of Physics, Sir CRR Autonomous College, India

TITLE: USING ATR- FTIR FOR DETECTION OF IMMOBILIZED BIOMOLECULES ON AMINO ACID FUNCTIONALIZED HAP NANOPARTICLES

DR. RENU SHARMA, Centre for Cellular and Molecular Biology, Hyderabad, India

TITLE: NANO-STRUCTURED AND ATOMIC CLUSTER MATERIALS IN TRACE AND ULTRATRACE ANALYSIS OF BIO- AND ENVIROTOXIC MARKERS

DR. T.PRASADA RAO, Scientist G, CSTD, National Institute for Interdisciplinary Science & Technology (NIIST) -CSIR, Trivendrum, India

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Structure Elucidation of Bioactive Compounds by Means of Circular Dichroism Spectroscopy

Jadwiga Frelek

Institute of Organic Chemistry of the Polish Academy of Sciences, Kasprzaka, Warszawa, Poland

It is well known that the biological properties of bioactive compounds are closely related to their stereostructure as well as their physicochemical and chemical properties. Thus, the development of practical methods allowing for the unequivocal and reliable determination of the absolute configuration is actively researched. The high level of interest in this subject is, not withstanding the other reasons, related to the fact that the enantiomers of a particular bio-active compound may demonstrate differences of up to several orders of magnitude in their pharmaceutical effects and potency at the same receptor. The fact that one of the enantiomers is usually more active than its counterpart, which may even be toxic, necessitates the need to use enantiomerically pure products to avoid adverse effects. In this context, the chiroptical methods and the circular dichroism (CD) spectroscopy specifically, appear to be sensitive, fast, and convenient methods for the stereochemical assignment provided that the compounds studied are chiral and non-racemic. During the present lecture, the most recent results on the application of electronic circular dichroism spectroscopy in the structure elucidation of a broad variety of important bioactive compounds will be presented. The scope of the present lecture includes, among others, β-lactam antibiotics, amino acids, amino alcohols, vitamins, and carbohydrates. For the representative derivatives, the CD spectra computed applying the time-dependent density functional theory (TDDFT) will be compared to the experimental CD curves. The high sensitivity of the CD spectroscopy to the minor structural changes will be demonstrated.

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Quaternary Structure Analysis of Human Hemoglobin by a Near-UV CD Spectroscopy

Masako Nagai

Research Center for Micro-Nano Technology, Hosei University, Tokyo, Japan

Human adult hemoglobin (HbA) has four subunits, namely, two-α subunits and two β-subunits. X-ray crystallographic analysis have indicated that there are two distinct quaternary structures, namely, the deoxy state, represented by the tense (T), low-affinity structure, and oxy state, represented by the relaxed (R), high-affinity structure. A characteristic spectral change of HbA in the near-UV CD occurs: from a small positive band in the oxy-R form to a negative CD band with a distinct peak at 287nm in the deoxy-T form. This negative CD band of deoxyHbA known as T-state marker has been supposed to derive from the changes of Tyr and Trp residues at the α1β2 subunit interface. To identify the aromatic residue responsible for the CD band, we have synthesized five recombinant Hbs in *E. coli* in which non aromatic residue is substituted for Tyr or Trp residue; rHb (α14Trp→Leu), rHb (β15Trp→Leu), rHb (β37Trp→His), rHb (α42Tyr→Ser), and rHb (β145Tyr→Thr). We examined the near-UV CD spectra of these rHbs and a natural mutant, Hb Rouen (α140Tyr→His). The CD spectra of individual aromatic residue were extracted from the difference between Hb A and each mutant. We concluded that changes in CD bands arising froṃβ37Trp, α140Tyr, β145Tyr and α42Tyr residues contributed to the appearance of the negative CD bands at 287nm. To examine different signals of CD band among aromatic residues, the effects of environments on CD spectra were examined using model compounds of Tyr and Trp dissolved in various solvants.

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Application of Enzyme Immobilized Nanostructured Fe doped TiO_2 for Electrochemical Quantification of Urea

Ravi Ranjan Pandey[1], Krishan Kumar Saini[2], Marshal Dhayal[1]

[1]Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, India
[2]National Physical Laboratory, Dr K S Krishnan Marg, New Delhi, India

Electrochemical enzymatic biosensors detect their targets based on enzymatic catalysis of a reaction that produces or consumes electrons. Electron communication between the enzymes and the electrode is a major barrier in the development of electrochemical enzymatic sensors. One of the reasons for this is that the active redox centers of enzymes are deeply embedded in an electrically insulate protein shell. In order to address this problem, we have utilized Fe doped metal oxide (TiO_2) films as the shuttles to transport electrons between the electrode and the redox centers of enzymes. An electrochemical biosensor was fabricated for the quantitative determination of urea in aqueous medium using PBS, at pH-7.2. The urease (Urs) was immobilized onto an electrode made of Fe doped TiO_2 films onto an indium-tin oxide (ITO) coated glass substrate using sol-gel technique. The linkage between the Urs enzyme and Fe doped TiO_2 films provided the resulting enzyme electrode (Urs/Fe-TiO_2/ITO) with a high level of enzyme immobilization and excellent lifetime stability. The response studies were carried out as a function of urea concentration with amperometric measurements. The biosensor based on Urs/Fe-TiO_2/ITO as the working electrode showed an enhanced sensitivity of urea, indicating the Urs enzyme immobilized on the electrode surface had a high affinity to urea.

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Fabrication of Novel Nanostructured Pd^{2+} Doped Tio_2 Platform for Detection of Cholesterol

Marshal Dhayal[1], Ravi Ranjan Pandey[1], Krishan Kumar Saini[2]

[1]Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, India
[2]National Physical Laboratory, Dr K S Krishnan Marg, New Delhi, India

With recent advances in nanotechnology, rapid progress has been made in biosensors based on nanomaterials, however there are still challenges to overcome with practical applicability of such systems. We describe nanomaterial-based biosensors with unique properties, provides a promising platform, which is simple, cost-effective, and requires no external modification to biomolecules. The unique catalytic, amperometric cholesterol biosensors have been developed based on the immobilization of cholesterol oxidase (ChOx) in the nonostructured films of sol–gel-derived Pd doped TiO_2. The presence of Pd in the sol–gel-derived TiO_2 onto ITO providing more surface energy as well more surface area which improves the sensitivity and long-term stability of the biosensor. Analytical performance of the cholesterol biosensor based on the Pd doped TiO_2 films is superior to that of the biosensor based on undoped TiO_2 films in terms of response time, sensitivity, and long-term stability. The Pd doped TiO_2 nanostructured platform offer the pathway for direct electron transfer between the electrode surface and the active redox centers of Cholesterol oxidase (ChOx) which enables the biosensor to operate at a low working potential and to avoid the influence of the O_2 presence on the amperometric current response. This work offers a unique platform for development of enzymatic electrochemical biosensors.

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Bioimaging Using Micro-Ct, Dei-Ct and X-Ray Microprobe with Synchrotron X-Rays

D. Venkateshwara Rao

Department of Physics, Sir CRR Autonomous College, Eluru, India

The author focus on research topics related to synchrotron-based tomography, mainly on medical, biomedical, biological, and environmental science imaging with synchrotron X-rays. Also highlight on nanoscience by utilizing the nanosized high energy photon beam with light source / accelerator facilities to investigate the properties of novel biomaterials and nanomaterials and imaging tools for medical, biomedical, and environmental science imaging research. New and Improved Tools: Light source / accelerator facilities will provide unprecedented capabilities for coherence-sensitive approaches, including scanning microscopes and microprobes. This will reshape the technical choices one would make compared to other facilities, so that whole-cell tomography would be done with a tenfold reduction in radiation dose, and chemical state mapping and trace element mapping would be done with improved sensitivity, spatial resolution, and speed. Extensive and innovative investigations related to biomaterials and nanomaterials with nanosized high energy photon beam are valuable. Multi-Technique Integration: Because scientific questions are often not completely answered by just one technique, an integrated suite of beamlines ranging from infrared, to soft and hard X-ray, should be developed with common sample preparation facilities and maximum compatibility of sample handling and mounting schemes. Cross-Disciplinary Approaches: A significant part of environmental science today addresses questions of the role of bacterial exudates and organic coatings on metal and radionuclide transport and reactivity in hydrated systems, as well as the health effects of contaminants. The overlap between this area of environmental science and bioimaging involves both scientific insight and technical approaches, so that this overlap should be embraced.

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Using ATR- FTIR for Detection of Immobilized Biomolecules on Amino Acid Functionalized HAp Nanoparticles

Renu Sharma, Ravi Ranjan Pandey, Satabhisa Kar, Marshal Dhayal

Centre for Cellular and Molecular Biology, Hyderabad, India

Surface functionalization nanoparticles (size <100 nm) with bioactive molecules is a rapidly expanding field in current biomaterial research and it should be of great interest to fine-tune the bioactivity of such nanoparticles by surface functionalization using water-soluble biomolecules. Calcium hydroxyapatite or calcium phosphate (HAp, $Ca_{10}(PO_4)_6(OH)_2$) nano-particles which is the main inorganic constituent of the bones and teeth has been used as a model system. In this regard, amino acids were selected as ideal candidates for surface functionalization for production of bioinorganic HAp nanoparticles and bionanocomposites due to their relative low cost, intrinsic biocompatibility and ability to interact with HAp surfaces. This paper highlights, in the first part, synthesis of amino acid-functionalized hydroxyapatite (HAp-AA) nanoparticles with uniform size and having rod-like morphology which was achieved by wet chemical process with $Ca(OH)_2$: H_3PO_4 : amino acid. Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectroscopy had been used to quantify to determine carboxylate group of the amino acid which was preferentially bound to the surfaces of hydroxyapatite nanoparticles. Variations in the size and shape of the HAp nanoparticles functionalized with different amino acid were consistent with differences in the strength of binding at the HAp surfaces. Second part of the paper describes enzyme immobilization on these functionalized HAp nanoparticles and FTIR-ATR spectroscopy was used for detection of relative proportion of enzyme loading on HAp.

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Nano-Structured and Atomic Cluster Materials in Trace and Ultratrace Analysis of Bio- and Envirotoxic Markers

T. Prasada Rao

Scientist G, Chemical Sciences & Technology Division, National Institute for Interdisciplinary Science & Technology (NIIST)-CSIR, Trivandrum, India.

The selection of appropriate analytical technique for detection and quantification of ultra trace amounts of bio- and envirotoxic markers depends on primary criteria such as sensitivity, selectivity, precision and accuracy in addition to auxiliary criteria like availability, cost of equipment, time of analysis, sampling and standards requirements. Various singular and hyphenated techniques have been developed over the years which include spectral, electrochemical, radiochemical, X-ray and mass spectrometric. In spite of such significant advances in analytical instrumentation, analytical chemists often resort to either off-line or on-line pre-concentration to reliably quantify bio- and envirotoxic markers present in complex matrix and several other analogous co-existing species.The invited talk consists of 2 parts namely gold atomic clusters and nano-structured molecularly imprinted polymer materials for ultra trace detection and quantification of biomarkers(Cysteine and Tyrosine) and envirotoxic markers(Uranium). In addition, the electrocatalytic red-ox behaviour of atomic cluster scaffolds and functional nano-material modified gold/glassy carbon electrodes will be described. Furthermore, the synthesis and characterization of surface imprinted nano-spheres for pre-concentrative separation of uranium and successful utilization of these materials for harnessing or decontamination of uranium from various natural water simulants including SAMBHAR SALT LAKE will be touched upon.

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Bioanalytical Method Validation: A Review Article

Darshan R. Telange

SVKM's, NMIMS University, Babulde, Shirpur, India

Method validation is a process that demonstrates that a method will successfully meet or exceed the minimum standards recommended in the Food and Drug Administration (FDA) guidance for accuracy, precision, selectivity, sensitivity, reproducibility, and stability. This article discusses the validation of bioanalytical methods for small molecules with emphasis on chromatographic techniques. Bioanalytical methods are used for the quantitation of drugs and their metabolites in biological matrices. In today's drug development environment, highly sensitive and selective methods are required to quantify drugs in matrices such as blood, plasma, serum, or urine. Chromatographic methods (high-performance liquid chromatography [HPLC] or gas chromatography [GC] have been widely used for the bioanalysis of small molecules, with liquid chromatography coupled to triple quadrupole mass spectrometry (LC/MS/MS) being the single most commonly used technology. After developing a method with desired attributes, the method is validated to establish that it will continue to provide accurate, precise, and reproducible data during study-sample analysis. The validation is performed using a control matrix spiked with the compounds to be quantified. When validation begins, chances for its successful completion (and more important, successful sample analysis) are high. During method validation, values for validation parameters are obtained. While obtaining above validation parameters, other parameters are also determined during validation (eg, extraction efficiency, calibration range and response function [linear or nonlinear], positional differences within an analytical run.