

# Dynamic Biochemistry, Process Biotechnology and Molecular Biology

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**Scope and target readership:** *Dynamic Biochemistry, Process Biotechnology and Molecular Biology* receives papers in which biochemical, molecular biology, biophysical, bioinformatic, genomic and proteomic approaches (preferably multidisciplinary) to study any aspect of biotechnology:

- 1) Biochemical and bioprocess engineering; Industrial processes/new products; Modelling and scale-up of laboratory processes;
- 2) Biominerals (metal ions, metal chelates, siderophores, metal-containing proteins) in biology, biochemistry and medicine;
- 3) Biotechniques and medical biotechnology (new techniques for cell culture or *in vitro* systems, environmental control, flow cytometry/analysis, spectroscopy and fluorescence, immunology, high throughput screening/drug discovery, DNA sequencing/arrays, genomics and proteomics); Nucleic acids;
- 4) Biotherapy and bioengineering (production of enzymes, vitamins, and other biologically active substances; studies on the processing of raw materials; and the microbiological synthesis of food and feed products);
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- 7) Metabolomics and molecular biology (metabolite target analysis, metabolic profiling and metabolic fingerprinting; improvements in data preparation, storage, curation and analyses; comparative integrated studies with transcriptomics and proteomics including within a systems biology context; and the application of metabolomics as it relates to man, animals and plants);
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- 10) Space research;
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- 12) Systems biology;
- 13) Tissue banking (quality assurance and control of banked cells/tissues, effects of preservation and sterilisation methods on cells/tissues, biotechnology, clinical applications; standards of practice in procurement, processing, storage and distribution of cells/tissues; ethical issues; medico-legal issues);
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### Guest Editor

**Dr. Mohsen Jahanshahi**

**Nanobiotechnology Research Centre, Babol University of Technology, Iran**



**Cover photos/figures:** Top row: Surfactant proteins SP-A, -B, -C and -D (Kaviratna *et al.*, pp 21-32). Center left: Operating procedure of expanded bed adsorption (Ebrahimpour *et al.*, pp 57-60). Center left: Topography of HSA NPs with AFM in 2-D (left) and 3-D (right) (Mehravari *et al.*, pp 51-56). Bottom: Preparation of albumin nanoparticles by coacervation method (Jahanshahi and Mehravari, pp 1-11).

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## **The Guest Editor**



**Dr. Mohsen Jahanshahi**  
**(Associate Professor)**

I was born in Iran, in 1971. I received a B.Sc. Degree in Chemical Engineering-petroleum from Shiraz University, Iran in 1995, and an M.Sc. in Chemical Engineering-Process Design from TMU University, Iran in 1997. Then I was awarded a three-year scholarship for my PhD study in Chemical Eng. (Bioprocess Eng.) by the Ministry of Research, Science and Technology of Iran (top 2 among more than 150 candidates in the Iranian National Exam; 1997). Finally I received my PhD in Bioprocess Engineering, from the Formulation Engineering Centre, The University of Birmingham, UK. In September 2003 I joined Babol University of Technology, Iran as an academic staff and as head of the Nanobiotechnology Research Laboratory. Since Nov. 2008 I am associate professor of Nanobiotechnology and Director of the Nanobiotechnology Research Centre in Babol University of Technology.

I have been nominated as distinguished Nanotechnology Researcher many times in Iran by the Iranian Nanotechnology Society (from 2005 to 2009). Editorial board of a number of International Journals, more than 70 papers in peer reviewed journals, 4 books as author and translator, a chapter in an edited book (Elsevier), more than 80 published papers in conference proceedings, more than 20 lectures in universities and companies in, UK, India, Holland, USA, France, Singapore, Spain, Malaysia, Germany, China, Canada, Belgium, Switzerland, Sweden, Indonesia, and Iran in the field of Nano, Nanobio and Biotechnology have made my academic career up to now.

## Foreword

Richard Feynman was the first scientist to suggest that devices and materials could someday be fabricated to atomic specifications: "The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom." Later the term nanotechnology was coined. Today nanotechnology is reshaping technology.

Nanotechnology is not only a part of future, but is all of it. Mankind history has always experienced waves of growth and development which affect societies, chances developed by mankind and employed to enhance the life quality and create wealth. Nanotechnology is one of these waves emerged to make a superior future.

Nanotechnology is a group of emerging technologies in which the structure of matter is controlled at the nanometer scale, the scale of small numbers of atoms, to produce novel materials and devices that have useful and unique properties. Some of these technologies impose only limited control of structure at the nanometer scale, but they are already in use, producing useful products. They are also being further developed to produce even more sophisticated products in which the structure of matter is more precisely controlled. The Foresight Nanotechnology Challenges focus on applying these developing technologies to solving important world problems. Foresight has had a long-standing interest in the capabilities that wait at the other end of this development process, when advanced nanotechnology will enable construction of complex systems in which each individual atom is specified and serves a designed function in the system.

However, Nanobiotechnology is the branch of nanotechnology with biological and biochemical applications or uses and is application of nanotechnology in life science in general. Nanobiotechnology often studies existing elements of nature in order to fabricate new devices. Nanobiotechnology is that branch of one, which deals with the study and application of biological and biochemical activities from elements of nature to fabricate new devices like biosensors. It is application of nano-scaled tools to biological system and the use of biological systems as templates in the development of novel nano-scaled products.

The term bionanotechnology is often used interchangeably with nanobiotechnology, though a distinction is sometimes drawn between the two. If the two are distinguished, nanobiotechnology usually refers to the use of nanotechnology to further the goals of biotechnology, while bionanotechnology might refer to any overlap between biology and nanotechnology, including the use of biomolecules as part of or as an inspiration for nanotechnological devices and is more based on Biotechnology.

In addition, from a more specific point of views, *second generation* of biotechnological products are nanoparticulate in nature. Examples include viruses, plasmids, virus-like particles and nano-protein assemblies variously in manufacture or development as vaccines, drug delivery vehicles and diagnostic tools, or as the components of nano-engineered therapies, implants, nano-motors or nano-sensors. Such products must be manufactured in advanced states of purity, material definition and sophisticated formulation to rival those demanded of the pharmaceutical macromolecules which dominate as *first generation* products. Nanoparticulates are characterised by a critical size range (20-300 nm diameter) and complexity of surface chemistry and internal organisation which pose new challenges in separation science and engineering, controlled chemistries of modification and material measurement not readily addressed by extant technologies.

However nanoparticles of biodegradable polymers can provide a way of sustained, controlled and targeted drug delivery to improve the therapeutic effects and reduce the side effects of the formulated drugs. Protein nanoparticles generally hold certain advantages such as greater stability during storage, stability *in vivo*, non-toxicity, non-antigen and ease to scale up during manufacture over the other drug delivery systems. Such assemblies can be manufactured to act as surrogate mimics, or as bona fide nanoparticulate products in their own right (i.e. drug delivery vehicles).

In conclusion, nanobiotechnology is often used to describe the overlapping multidisciplinary activities associated with photonics, chemistry, biology, biophysics nanomedicine and engineering converge. Application scope of nanotechnology are usually divided into six general sections namely; Food and Agricultural, Water, Energy and Environment, Medicine, Pharmacology, Biology and Neural Science.

### **Dr. Mohsen Jahanshahi**

Associate Professor in Nanobiotechnology  
Director of Nanobiotechnology Research Group  
Babol University of Technology (BUT)

PO.Box:484, Babol, Iran.

Tel. & Fax NO.: 0098-111-3220342

E-mail: mjahan@nit.ac.ir, mmohse@yahoo.com

Website: <http://www.nano.nit.ac.ir/IndexEn.aspx>.

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**Mohsen Jahanshahi, Rabeah Mehravar (Iran)** Protein Nanoparticles as a Novel System for Food Science and Technology (pp 1-11)

**ABSTRACT**

**Invited Review:** In recent years, the concept of controlled release of encapsulated ingredients at the right place and the right time has become of more and more interest to the food and pharmaceutical industry. A timely and targeted release improves the effectiveness of food additives, broadens the application range of food ingredients and ensures optimal dosage, thereby improving cost-effectiveness for the food manufacturer. Incorporation of bioactive compounds – such as vitamins, probiotics, bioactive peptides, antioxidants, etc. – into food systems provide a simple way to develop novel functional foods that may have physiological benefits or reduce the risks of diseases. This review focuses mainly on potential applications of protein nanoparticles in the food industry, which may equally be applied in the feed industry.

**Rabeah Rawashdeh, Yousef Haik (USA)** Antibacterial Mechanisms of Metallic Nanoparticles: A Review (pp 12-20)

**ABSTRACT**

**Invited Review:** Given the slow approval rate for new antibiotics and the inability of current antibiotics to fully control bacterial infection, it is obvious that there is a great demand for unconventional biocides. Metallic nanoparticles, another possible route for fighting bacteria, should be considered. Metallic bactericidals have been in use for several years as external sanitizers and disinfectants and have shown biocidal effectiveness against both Gram-positive and Gram-negative bacteria, as well as against fungi. The mechanism of interaction of these metallic biocides includes protein membrane damage, production of superoxide radicals, and ions release that interact with the cellular granules and form condensed molecules. This article presents a review of the metallic nanoparticles antimicrobial mode of interaction against bacteria.

**Anubhav Kaviratna, Apurva Shah, Shailendra Singh Rao, Rinti Banerjee (India)** Pulmonary Surfactant Nanostructures and their Implications (pp 21-32)

**ABSTRACT**

**Invited Review:** A surface active material that lines our lungs is referred to as pulmonary surfactant (PS) and consists of a self-assembled complex of nanostructures (NSs) rich in phospholipids (PLs) and proteins that lie at the air-liquid interface of the pulmonary alveoli. It serves a very critical function during respiration by dynamically modifying surface tension. It is responsible for the attainment of near zero surface tension at the end of expiration. PLs are assisted by surfactant proteins to form lamellar bodies of 500 nm to 1-2  $\mu\text{m}$  in size, nanotubes of 2-5 nm height, monolayer films of domain heights of 0.8 to 5 nm and multilayered stacked reservoir phases during the surfactant life cycle. During respiration, the PL molecules present in the surfactant film undergo molecular rearrangement to alter surface tension and maintain high lung compliance. Destruction or absence of PS and/or the above mentioned NSs can occur due to genetic variations, direct or indirect lung injuries and lead to many respiratory diseases. Surfactant NSs, their composition and the packing of the surfactant monolayer are altered in diseased states. Transmission electron microscopy and atomic force microscopy are useful techniques to evaluate pulmonary NSs and confirm their alterations in diseased states. Drug-loaded nanoparticles (NPs), when delivered in the respiratory system, first interact with pulmonary surfactant. These interactions can alter drug release, residence time and cellular interactions of the NPs. Similarly, pulmonary surfactant also influences the cellular response and toxicity of respirable environmental fine particles. This review describes the various nanostructures formed by PS, the interactions NPs with PS and their implications.

**Deepti Dyondi, Rahul Lakhawat, Rinti Banerjee (India)** Biodegradable Nanoparticles for Intra-articular Therapy (pp 33-41)

**ABSTRACT**

**Invited Review:** Osteoarthritis (OA) is a chronic degenerative joint disease characterized by progressive loss of articular cartilage which leads to severe pain and restricted mobility in the patients. Age, excessive joint loading, or sports injury are the factors known to increase the risk of joint degeneration thus leading to OA. Many techniques have been employed in OA therapy but none of them has given satisfactory results in long term. Oral drugs have the disadvantage of having several side effects such as gastrointestinal problems, heart attack, and stroke associated with them. Surgical techniques lead to significant

donor site morbidity. Intra-articular (IA) injections of hyaluronic acid (HA), glucocorticoids, have shown symptomatic relief but none of these treatments have been able to show disease modifying effects to an appreciable extent. Nanostructured drug delivery systems using liposomes and nanoparticles (NPs) can be administered as IA injections for sustained release and increased local concentrations of drugs in arthritis. A liposomal formulation of dexamethasone palmitate is currently available for IA drug delivery. Several other NPs of chitosan and biodegradable synthetic polymers like PLGA are in the developmental stages for delivery of steroids, NSAIDs and clodronate. This review highlights some of the promising nanostructured drug delivery systems for IA therapy, the issues involved in developing such systems and the future potential of such therapies for degenerative and inflammatory joint diseases.

**Karishma Fernandes (USA), Yousef Haik (USA/United Arab Emirates)** Detection of Peanut-Specific IgE using Functionalized Nanoparticles (pp 42-47)

#### ABSTRACT

**Original Research Paper:** The diagnosis of patients with clinically reactive food allergies is extremely crucial and remains a challenge for all allergists. The current technology available to diagnose the presence of peanut-specific immunoglobulin E (IgE) are highly invasive, expensive, time consuming and need trained personnel and specialized equipment for the test to be conducted. In this study we present a new technique for detecting the presence of peanut specific IgE by coating allergens on magnetic nanoparticles. Upon the isolation of the peanut specific IgEs from the sample, a colorimetric detection is utilized to assess the severity of the allergy. To evaluate the feasibility of the new technique, the functionalized particles were utilized to detect the presence of peanut specific IgE in 50  $\mu$ l plasma of an allergic individual using only 100  $\mu$ g of functionalized nanoparticles. Other evaluations were conducted in spiked plasma samples. The selectivity and sensitivity of the developed assay was highly specific for the peanut-specific IgE in plasma and more sensitive than conventional ELISA.

**Alireza Badiel, Ismayil Haririan, Ali Jahangir, Ghodsi Mohammadi Ziarani (Iran)** Incorporation of Ibuprofen into SBA-15; Drug Loading and Release Properties (pp 48-50)

#### ABSTRACT

**Short Communication:** The development of mesoporous materials like SBA-15 offers new possibilities for incorporating biological agents into silica structures and controlling the release kinetics from the matrix due to its well-arranged pore architecture. These materials show significant mesoporosity associated with their hexagonally organized channels, narrow pore size distribution, and large surface area. Ibuprofen was selected as a model molecule since it is a well-documented and highly used anti-inflammatory drug. Furthermore, its molecular size (about 1.0 nm) is suitable for incorporation into the mesopores of the SBA-15 material (pore size is 4.6-10 nm). Mesoporous silica SBA-15 was prepared to evaluate its application as a carrier for Ibuprofen drug delivery. The loaded SBA-15 was characterized by thermo gravimeter analysis, X-ray diffraction, scanning electron microscopy, and N<sub>2</sub> adsorption/desorption isotherm. The incorporation procedure resulted in a significant improvement of the amount of Ibuprofen loaded into SBA-15, and *in vitro* drug release was investigated.

**Rabeah Mehravar, Mohsen Jahanshahi, Naser Saghatoleslami (Iran)** Human Serum Albumin (HSA) Nanoparticles as Drug Delivery System: Preparation, Optimization and Characterization Study (pp 51-56)

#### ABSTRACT

**Original Research Paper:** Nanoparticles have been developed as an important strategy to deliver low molecular-weight drugs, as well as biomacromolecules such as proteins or DNA. The body distribution of colloidal drug delivery systems was mainly influenced by two physicochemical properties namely particle size and surface characteristics. Particle size is a crucial parameter, in particular for the *in vivo* behavior of nanoparticles after intravenous injection. The objective of the present study was the preparation of human serum albumin (HSA) nanoparticle by desolvation method and optimization of nanoparticle by applying the Taguchi method together with characterization of the nanoparticle bioproducts for drug delivery application. Several process parameters were examined to achieve a suitable size of nanoparticle such as pH, HSA concentration, organic solvent adding rate and the ratio of organic solvent/HSA solution. Taguchi method with L<sub>16</sub> orthogonal array robust design was implemented to optimize experimental conditions of the purpose. This approach facilitates the study of interaction of a large number of variables spanned by factors and their settings with a small number of experiments leading to considerable saving in time and cost for the process optimization. As a result of Taguchi analysis in this study, pH and ratio of organic solvent/HSA solution were the most influencing parameters on the particle size. The minimum size of nanoparticles (53 nm) were obtained at

pH 9, 75 mg.ml<sup>-1</sup> HSA concentration, ratio of organic solvent/HSA solution of 4 and organic solvent adding rate of 1.5 ml.min<sup>-1</sup>. The mechanistic of the optimum conditions for preparing protein nanoparticles and their characterization as a drug delivery vehicles are discussed.

**Melika Ebrahimpour, Mohamad Hassan Shahavi, Mohsen Jahanshahi, Ghasem Najafpour (Iran)** Nanotechnology in Process Biotechnology: Recovery and Purification of Nanoparticulate Bioproducts Using Expanded Bed Adsorption (pp 57-60)

#### ABSTRACT

**Original Research Paper:** In recent years developments in production of pharmaceutical and biotechnological products such as plasmid DNA (pDNA) as putative gene therapy vectors and protein nanoparticles as drug delivery vehicles have increased. In this study, a rapid and efficient scaleable purification protocol allowing obtaining concentrated, pure nanobioproduct was developed. However, expanded bed adsorption (EBA) of nanobioproducts was carried out and the dynamic binding capacity was calculated. The overall process yield of recovery of the nanoparticle bioproduct was more than 80%, which was a superior result in expanded bed chromatography. The generic application of expanded bed adsorption for the recovery and adsorption of nanoparticulate bioproducts is strongly indicated.

**Mohsen Jahanshahi, Sayed Mahmood Rabiee, Roya Ravarian, Nima Nabian (Iran)** Preparation and Characterization of Nano Bioactive Glass based on the CaO–P<sub>2</sub>O<sub>5</sub>–SiO<sub>2</sub> System (pp 61-63)

#### ABSTRACT

**Research Note:** Nano Bioactive glass material of the type CaO–P<sub>2</sub>O<sub>5</sub>–SiO<sub>2</sub> was obtained by the sol-gel processing method. The obtained material was characterized by X-ray powder diffraction (XRD) and surface electron microscopy. The bioactivity was examined *in vitro* with respect to the ability of a hydroxyapatite layer to form on the surface as a result of contact with simulated body fluid (SBF). The XRD studies were conducted before and after contact of the material with SBF.

**Camila Bitencourt Mendes, Lucas Rossi Sartori, Arnaldo César Pereira, Mariana Gava Segatelli, César Ricardo Teixeira Tarley (Brazil)** Assessment of Multiwalled Carbon Nanotube Paste Electrode for Square-Wave Adsorptive Cathodic Stripping Voltammetric (SWAdCSV) Determination of Methyl Parathion in Water Samples (pp 64-70)

#### ABSTRACT

**Original Research Paper:** In this paper the assessment of an electrode composed by multiwalled carbon nanotube (MWCNT) dispersed in mineral oil as well as its application on the electrochemical determination of methyl parathion (MP) in environmental water samples by square-wave adsorptive cathodic stripping voltammetry (SWAdCSV) is described. The suitability of the electrode for this purpose was confirmed by comparing it to a glassy carbon electrode (GCE) and to a carbon paste electrode (CPE). A 7.1- and 3.4-fold increase of the signal, respectively, was achieved. In order to obtain the best performance of the method, significant factors were established by a factorial design and the method optimized by employing the Doehlert matrix. Based on these chemometric tools the following experimental conditions were selected: 7.95, 70 mV, 205 Hz and 0.3 mol L<sup>-1</sup>, respectively, for sample pH, PA, F and BC. A study of interferences was conducted through the addition of inorganic ions NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup> and Mn<sup>2+</sup> during MP analysis and no interference was noted. The method presented a linear range between 0.56 and 18.00 μmol L<sup>-1</sup> (r = 0.995), limit of detection (LD) of 0.15 μmol.L<sup>-1</sup> and limit of quantification (LQ) of 0.49 μmol.L<sup>-1</sup>. The determination of MP on environmental spiked samples showed good recovery values and repeatability.

**Forough Toubi, Mohsen Jahanshahi, Abas Ali Rostami (Iran), Solmaz Hajizadeh (Iran/Sweden)** Voltammetric Tests on Different Carbon Nanotubes as Nanobiosensor Devices (pp 71-74)

#### ABSTRACT

**Original Research Paper:** This paper addresses a recent advance in electrochemical pretreatment of carbon nanotube (CNT)-based nanobiosensors. The unique chemical and physical properties of CNTs have paved the way to new and improved sensing devices. CNTs which are produced by different processes, i.e. arc-discharge (ARC) in solution and/or gas and chemical vapor deposition (CVD), have different effects on sensors efficiency. Various samples of ARC-CNT and CVD-CNT were chosen herein and ARC-CNT anodic pretreatment resulted in a dramatic improvement in the electrochemical reactivity (cycle voltammetric tests in the range of 0.0 to 1.5 V). In contrast, CVD-CNT appeared to be resistant to the anodic activation based on their structure as well as purification. However, in a separate experiment, different samples of multi-and single-wall

CVD-CNTs were compared. The high purity multi-walled CNT synthesized by ARC showed the best outcome on the electrochemical behavior of glassy carbon electrode when compared to the other samples. The generic application of CNT and the effect of its structure and purity as a nano biosensor for electrochemical responses are widely discussed.

**Arnaldo C. Pereira, Alexandre Kisner, Cesar Ricardo T. Tarley, Nelson Durán, Lauro T. Kubota (Brazil)** Determination of Phenol Compounds Based on Electrodes with HRP Immobilized on Oxidized Multi-Wall Carbon Nanotubes (pp 75-79)

#### **ABSTRACT**

**Original Research Paper:** In the present work the development of an amperometric biosensor for phenol detection based on oxidized multi-wall carbon nanotubes (MWCToxi) and horseradish peroxidase (HRP) is reported. The variables that exert influence on the performance of the biosensor response, including enzyme immobilization procedure, HRP amounts, pH, and working potential were investigated. Furthermore, the feasibility of the biosensor response for various phenol compounds was also investigated. The amperometric response for catechol using the proposed biosensor showed a wide linear response range (1 to 150  $\mu\text{mol L}^{-1}$ ), good sensitivity (53  $\mu\text{A cm}^{-2} \mu\text{mol L}^{-1}$ ), excellent operational stability (after 200 determinations the response remained at 97%) and very good storage stability (lifetime > 3 months). The results were compared with HRP immobilized on graphite powder, highlighting the remarkable features of MWCToxi in the biosensor performance. According to these features, it is possible to affirm that the developed biosensor is a promising tool for phenol detection due to its good electrochemical response and enzyme stabilization.