

transient eukaryotic-cell based biosensor which has the capability to detect hydroquinone and benzoquinone compounds in the medium. The next phase of our study has been focused on the construction of a permanent biosensor with the ability of detecting a wide variety of oxidative-stress inducing chemicals. The results will be presented.

Keywords: Bioluminescence, Oxidative-Stress, Free radical, Biosensor.

Abstract No.121

Preparation of anti-CD4 Monoclonal Antibody-Conjugated Magnetic Nanoparticles and their Application on T Lymphocyte Separation

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Detection and separation of specific classes of cell through their surface markers by Monoclonal antibodies are very valuable for research and clinical diagnosis. Nanoparticles can effectively bind to monoclonal antibodies to identify their targets in different populations of cells. In the present study, anti-human CD4 antibody was covalently immobilized on the surface of core-shell Fe₃O₄/SiO₂ nanoparticles to analyze CD4 T lymphocyte in human blood. In this study, first Fe₃O₄ nanoparticles were synthesized using a chemical precipitation method then these particles coated with Tetraethylorthosilicate. Silica coated nanoparticles were chemically modified in order to react with amino (-NH₂) groups of antibody. Attachment of antibody to nanoparticles is confirmed by various spectroscopic and biological methods. The amount of immobilized antibody was estimated by Bradford method. After conjugation, the binding ability of antibody to its receptor was investigated. An agglutination test was performed to prove the presence of fixed antibodies. The presence of nanoparticles on the lymphocytes is confirmed by iron staining and fluorescence methods. Microscopic images were taken from the culture lymphocytes clearly showed the presence of nanoparticles at the cellular level. Moreover, after each step of synthesis and antibody conjugation with

nanoparticles, spectrum of the nanoparticles confirmed the successful conjugation of functional groups. The results show that functional antibodies conjugated to nanoparticles successfully. Anti human CD4 monoclonal antibody could be conjugated to chemically modified nanoparticles efficiently. The conjugated antibody has biological activity and could be used in different application such as detection and separation of CD4 T lymphocytes. Compared with the conventional fluorescent antibody, the conjugated antibody was simple, rapid and stable method for detection and isolation of human CD4 T cells.

Keywords: Magnetic Nanoparticles, T Lymphocyte, Antibody, CD4 Molecule.

Abstract No.122

Copper/zinc-superoxide Dismutase 1 Mutants and the Propensity to Oligomerization

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Copper/zinc-superoxide dismutase 1 (SOD1), as an important antioxidant enzyme, scavenges intracellular superoxide anion radical and catalyzes the dismutation of this radical into molecular oxygen and hydrogen peroxide. More than 125 different mutations have been identified in the SOD1 gene that is related to the familial form (fALS) of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease, in which motor neurons of spinal cord and brain cortex are selectively destroyed. The presence of SOD1 aggregates in the motor neurons of fALS patients is well documented. The mechanism(s) by which these aggregates result in disease phenotype are still unknown, but, it is revealed that mutations can induce structural instability of SOD1 protein, which in turn, make the protein prone to aggregation. In this study, we selected two SOD1 mutants (Asp124His and Glu100Lys), both of them reported as pathological, and characterized them investigating their propensity to aggregation using different techniques, from ThT-binding fluorescence to Circular Dichroism spectra, light scattering and Transition Electron Microscopy (TEM). We show here that these two SOD1 mutants, only when they are under destabilizing conditions, undergo the same general mechanism of oligomerization as found for the Wilde type protein. The rates of

oligomerization are different but eventually they give rise to the same type of oligomeric species.

Keywords: Superoxide dismutase 1 (SOD1), Aggregation, ALS, Neurodegenerative Disease.

Abstract No.123

Application of Hollow fiber-Supported Liquid-Phase Microextraction Coupled with HPLC for the Determination of some Chiral Drug Enantiomers-Protein Binding

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A hollow fiber liquid-phase microextraction technique coupled with high-performance liquid chromatography was employed for determination and evaluation of the binding characteristics of drugs to human serum albumin (HSA). Enantiomers of Donepezil and Lorazepam were investigated as a model system. After optimization of some influencing parameters on microextraction, the proposed method was used for calculation of the target drug distribution coefficient between n-octanol and the buffer solution as well as study of drug-HSA binding in physiological conditions. The developed method shows a new, improved and simple procedure for determination of free drug concentration in biological fluids and the extent of drug-protein binding.

Keywords: Hollow Fiber, HSA, Donepezil, Lorazepam Enantiomer, Binding.

Abstract No.124

Chemometric Studies of Hemoglobins from the Caspian Sea Sturgeon (*Acipenser persicus* and *Acipenser stellatus*) by n-dodecyl Trimethylammonium Bromide

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Variety of hemoglobin (Hb) forms in fish is usually well adapted to the different ecological conditions or various habitats. In the current study, Hbs from two Sturgeon species of the Southern Caspian Sea Basin were investigated. After extraction and separation of hemoglobin from whole blood, we showed that although both species have variable Hbs with different isoelectric points, their dominant Hbs purified by ion-exchange on CM-cellulose chromatography. The dominant Hbs from these blood fishes were utilized for further experiments. In this study, the behavior of Hbs during the denaturation and process by n-dodecyl trimethylammonium bromide (DTAB) is investigated. In chemometric study, the interaction of DTAB as a cationic surfactant under variable concentrations, with the purified dominant hemoglobins (Hbs) was investigated using UV-visible absorption and circular dichroism (CD) spectra. The analysis of the obtained spectral data using singular value decomposition (SVD), Simple-to-use interactive self-modeling mixture Analysis (SIMPLISMA), evolving factor analysis (EFA) and multivariate curve resolution-alternative least square (MCR-ALS) as well-known chemometric techniques. The chemometric resolution techniques were used to determine the number of the components and mole fractions of the oxidized Hbs that donate the evidence for the existence of three different molecular components including native (N), intermediate (I) and denatured (D) in sturgeon Hbs. According to the distribution of intermediates broaden in a range of DTAB concentrations, the aggregation state of Hbs are slightly reduced from *Acipenser stellatus* to the *Acipenser persicus*. The results demonstrate a significant relationship between stability of fish hemoglobins and habitat depths.

Keywords: Sturgeon Hemoglobins, Intermediates, Chemometric Analysis, Aggregation.

Abstract No.125

The Concept of Hyperfold and Its Application to Evidence Theoretical Protein Fold Prediction

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