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Oral

The Current Applied Researches in Bio-Analysis Lab

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ABSTRACT

The current applied researches in Bio-Analysis Lab are based on specificity of the bio-receptors toward their targets which are categorized to biosensors, biofuel cells, nanozymes and nanomedicine. Bio-receptors could be divided to enzymes, antibodies, nucleic acids, tissues or even single cells. Here some example of applied researches are presented: i) The nanocomposites consisting of functionalized single/multi-wall carbon

nanotubes either alone or incorporating with the metallic nano-particles such as gold and silver, or room temperature ionic liquids could be used for electrochemical biosensor development since they increase the electrical conductivity, electron transfer rate and the reversibility of the redox proteins. ii) Different types of optical biosensors especially for cancer diagnosis at early stages was developed by incorporating different types of metallic nanoparticles including gold, branched gold and porous gold nanoparticles and DNA or micro RNA probes. iii) By synthesizing different type of chemical complexes such as copper-cysteine and covering those by proteins such as albumine or appoferritine, superoxide dismutase (SOD) mimetic nanozymes were developed. iv) Some metallic nanoparticles such as silver, cadmium and copper when coated with bovine serum albumin could act as chemotherapeutic agent against invasive human breast cancer. v) To develop enzyme/microbial biofuel cell, different bioanodes was designed. Either a redox enzyme was immobilized or an electrogenic bacterium could be grown on the woven carbon fiber filament coated with a nanocomposite consisting of NH_2-MWCNTs and RTIL. As Conclusion, during last two decades different types of nanomaterials were developed in our lab to facilitate the signal transduction of bio-receptors.

Keywords: Bio-Analysis Lab, Nanozymes, Nanotechnology, Biosensors, Nanoparticles, Biofuel cell

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Oral

Type 2 to Type 3 Diabetes: Causes and Interventions

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ABSTRACT

Here, we overview our last achievements in the case of type 2 diabetic (T2D) complications including glycation-induced protein aggregation arisen from hyperglycemic and oxidative condition to change protein structure from friend to foe. Most importantly, the condition might be brought about a progressive neurodegeneration as type 3 diabetes (T3D) emphasized on sporadic Alzheimer's disease (SAD) which is likely to be related to unhealthy lifestyle or environmental-genetic factors interactions with high prevalence and growing trend. In contrast to SAD, the familial Alzheimer's disease (FAD) is linked to the autosomal gene mutations. Moreover, molecular interference in the range of exacerbation by seeding and membrane effects and amelioration by interfering small molecules are discussed.

Keywords: type2 diabetes, type 3 diabetes, Alzheimer's disease; lipid peroxidation; seeding effect

Oral

Diabetes Treatment Using Third Generation of Insulin: the Latest

Progress in Developing New Insulin Analogs

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ABSTRACT

Insulin is a key medicine for the management of hyperglycemia in type-1 diabetes and selective individuals with type-2 diabetes. Three different generations of insulin from the oldest to newest are animal insulin, human insulin and insulin analog; the latest has been recently developed through mutagenesis in human insulin gene. Three rapid-acting and two long-acting insulin analogs are currently used for the treatment of diabetes. Although these analogs cost more than traditional insulin but they indicate more effective for the patients. The insulin market still demands the new analogs with improved stability and pharmacokinetics. In this direction, we developed a novel strategy in our laboratory for the efficient production of recombinant insulin and its analogs. We introduced a novel expression and purification system for human insulin, using αB-crystallin (αB-Cry) as the fusion partner protein. This system is suitable for directing the synthesis of large amounts of the fusion proteins αB-Cry/A-chain (αB-AC) and αB-Cry/B-chain (αB-BC). After separation of the A- and B-chain from their corresponding fusion protein partners, we applied αB-Cry for the efficient folding of human insulin and its analogs. The insulin analogs produced in our laboratory indicated improved stability and significantly resist against fibrillation which is currently a big medical and biotechnological challenges, while keeping their biological activity to the acceptable levels. We believe that our procedure is also applicable for the large scale production of this highly demanding therapeutic peptide hormone and our new insulin analogs are potential candidates to be used in treatment of diabetic patients.

Keywords: Insulin analogs, Diabetes, αB-Crystallin, Insulin folding, Insulin stability

Oral

Novel Binding Models for Interaction of Small Molecules to Proteins

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ABSTRACT

In characterization, various aspects of macromolecular phenomena in living systems, reversible binding of small molecules to biological macromolecules such as proteins and DNA is of fundamental importance. This is illustrated by the binding curve that measured experimentally and analyzed by the binding models in order to characterize its physical nature and biological importance. This article represents the results of two decades study on experimental, theoretical, and computational aspects of this phenomenon in our group. Accordingly, the novel analysing models are introduced and the contribution of these works on improvement of our understanding of the functional chemistry of proteins is discussed. The concepts such as binding capacity, mean intrinsic binding quantities and binding statistical factors were presented and the experimental analyzing methods were assigned for their estimation from raw experimental data.

Keywords: ligand binding, binding model, mean intrinsic function, binding capacity

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Oral

Green and Facile Synthesis of Highly Photoluminescent Multicolor Carbon Nanocrystals for Cancer Theranostics

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ABSTRACT

A one-pot hydrothermal method was developed for the synthesis of a series of carbon dots (CDs) to use as a theranostic nano-platform against cancer. To this aim, Taxane diterpenoids were utilized as the carbon source, different diamines were used as the nitrogen source, and folic acid were used to target the nanostructures. The physico-chemical properties of nanoparticles were characterized using UV-Vis and fluorescence spectroscopy, HR-TEM, SAED, XRD, FTIR and zeta potential analyzer. High quality photo-stable and multicolor (blue and green) carbon nanocrystals with a hexagonal shape, the narrow size distribution of less than 20 nm, and high fluorescence quantum yield of up to 50.4% were obtained from Taxanes in combination with *m*-phenylenediamine and folic acid to give the best results. The nanoparticles displayed selective anticancer activity with IC_{50} values of 31.3 ± 2.73 and 34.1 ± 1.15 μg ml^{-1} for the human MCF-7 and HeLa cancer cell lines, respectively, and IC_{50} value of 120.5 ± 3.79 μg ml^{-1} on the normal human fibroblast cells. The flow cytometry studies determined apoptosis mediated cell death as the main anticancer mechanism of CDs and the molecular studies revealed the induction of both extrinsic and intrinsic apoptosis pathways. The overall results indicated the great theranostic potential of synthesized CDs for the simultaneous cancer imaging and therapy.

Keywords*:* Carbon dots, Green synthesis, Cancer theranostics, Fluorescence, Apoptosis

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Oral

Caution in Using High Sensitive Differential Scanning Calorimetry

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ABSTRACT

The analysis and interpretation of data obtained from high-sensitive (HS) differential scanning calorimetry (DSC) experiments is a creative art and great skill. In this research, we have analyzed and compared the DSC results from two different types of HS-DSC equipment. Our results showed additional peak in the native lines that differ among DSC instruments which may lead to a drastic inaccuracy in the interpretation of DSC profiles.
Literature survey on the articles in which these instruments were used showed the same disturbance in the native lines. Disturbances in the native line can be revealed by repeating the experiments and by comparing the results from different instruments. It is recommended to have good experiences and knowing the characteristic disturbances in the DSC profiles to eliminate the false interpretation.

Keywords: HS-DSC, Thermogram, DSC analysis, Native line

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Oral

Protein Interaction and Cytotoxicity of Novel Pt(II) and Pd(II) Complexes

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ABSTRACT

Metal-based drugs are effective pharmaceutical components for presence in the cancer treatment. Despite success of metal-based drugs, in particular various generations of platinum-based drugs in chemotherapy, there still remaining undesirable and toxic properties. So, there is a crucial quest to introduce new generation of metal-based drugs. Hence, in the present study the protein interaction and cytotoxicity of novel Pt(II) and Pd(II) complexes were investigated to evaluate biological aspects. The biological assessments were investigated by protein interaction study with carrier protein human serum albumin (HSA) and *in vitro* cytotoxicity studies with human colorectal carcinoma model (HCT116 cell line). Multi-spectroscopic methods as well as molecular docking were carried out to probe novel complexes binding to HSA. In addition, the cytotoxicity study was done using MTT assay. The results of fluorescence study in coherent well with molecular docking data illustrated that newly-designed complexes bind to HSA only in one position. Also, circular dichroism results and thermal stability findings revealed that the structure of HSA has no changes affected by binding to the newly-designed complexes. MTT assay showed that the growth of HCT116 cell line was inhibited by a dose-dependent response. The results demonstrated that the novel complexes with several long hydrophobic chains has negligible structural side effects along with higher cell growth inhibition effects. Therefore, the lipophilicity of metal-based drugs should be increase to have insignificant structural side effects and higher cytotoxicity. Consequently, the novel Pt(II) and Pd(II) complexes with several long hydrophobic chains are promising candidates for presence in cancer treatment.

Keywords: HSA, Pt(II) complex, Pd(II) complex, interaction, cytotoxicity

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Oral

Analysis of conformational changes in native and mutants human transthyretin during the folding and aggregation processes

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ABSTRACT

Transthyretin (TTR) is a homotetrameric protein that is one of 30 human proteins associated with systemic amyloidoses. In spite of its link to human pathology, an anti-amyloidogenic effect that prevents fibril formation of the amyloid β (Aβ) peptide associated with Alzheimer's

disease (AD) has been proposed for TTR. Although it has been shown that these two processes correlate with the ability of TTR to populate a monomeric state, a complete description of the conformational states populated *in vitro* by monomeric TTR at physiological pH is missing. We used an array of biochemical and biophysical methods and kinetic tests to investigate the folding and aggregation processes of monomeric TTR. Our results show that once monomers of transthyretin are released by the quaternary structure, the protein establishes an equilibrium between a set of conformational ensembles bearing different degrees of disorder. Thus, a molten globular state appears in equilibrium with the fully folded monomer, whereas an off-pathway species accumulates transiently during refolding of TTR. These two conformational ensembles are distinct in terms of structure, dynamics, kinetics and pathway of formation. Further subpopulations of the protein fold differently due to the occurrence of proline isomerism. We investigated the conformational states described above by exploiting an intramolecular Forster Resonance Energy Transfer (FRET) approach. As TTR possesses one single cysteine moiety, we labelled such residue (different mutants of transthyretin) with a probe which acts as acceptor of light emitted by tryptophan residues. This system reports on intramolecular distances and compaction of the different conformational states.

Keywords: Transthyretin, Folding, Aggregation, FRET, Amyloidoses

Oral

The Importance of Recognizing Binding Sites and Introducing Methods for Identification of them

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ABSTRACT

Determination of binding site is important for understanding the function of biological macromolecules. Understanding the specific binding site of the substrate to the enzyme, which is called active site, is essential for investigating the mechanism of the enzyme. It is important to recognize the binding site of the hormone in the receptor, drug binding site in proteins and nucleic acids, etc. from different perspectives. In order to design new drugs, it is necessary to determine the drugs binding site in the macromolecule. There are several ways to investigate binding sites. Using X-ray and NMR are appropriate for this purpose. Fluorescence, Raman and IR methods can also be used. The use of molecules with a specific binding site is an indirect method for determining the binding site. For well-known structures that have PDB files, molecular docking techniques and molecular dynamics simulations it can be used.

Keywords: Binding site, Structure, Function, Molecular dynamics simulation, Molecular docking

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Oral

Improved some Anticancer Platinum Drugs Delivery by Mesoporous Silica Microparticles Application in Chemotherapy

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ABSTRACT

Oxali-platin, carboplatin and cisplatin are the clinical anti-cancer drugs based on platinum. To date, they accompanied with 5-fluorouracil is confirmed as first line chemotherapy for advanced colorectal cancer treatment. Since the cost of them is very high, the optimization method for its green synthesis is very important. Also,

research shows drug nano-carriers could be refrained drug from undesired release before reaching tumor, and release drug in a controlled and smart manner. In this project, some Pt drugs with low solubility were synthesized and characterized for drug delivery. Then, the nano hallow sphere silica, MCM-41, was synthesized and fanctionalized by NH_2 groups and characterized by applying XRD, BET, TGA, FTIR, SEM. Drug release evaluation was done *in vitro* condition and micro dialysis memberane by UV and ICP methods. ICP results showed oxaliplatin is more loaded on MCM-41-NH_2 than other platin drugs loaded. Due to immobilization of drugs on human serum albumun as bio carrier in blood, drug release from HSA-drug and MCM-41-NH_2-drug were investigated and comparized. Data show that drugs releasing was slower in MCM-41-NH_2-drug system than HSA-drug system and also this observation is more controllable for oxaliplatin drug that cause to less toxicity and side effect in chemotherapy.

Keywords: Oxaliplatin, Anticancer drug, cisplatin, carboplatin, drug delivery, Nano hallow sphere silica (NHSS), Nano-inorganic carrier

Oral

The Effect of Tryptophan Networks in Microtubules on Learning and Memory

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ABSTRACT

There is evidence available that suggests tryptophan administration improves learning and memory. This improvement has been attributed to the role of tryptophan in the CNS, as a precursor for serotonin synthesis. Previous studies have also indicated that hydrophobic compounds which are in structure similar to tryptophan, interact with microtubules, increase the rate of microtubule polymerization, and affect memory. Therefore, the aim of the current study was to investigate the possibility that tryptophan administration could also boost microtubule polymerization and directly result in an improvement of learning and memory. Our results suggest that tryptophan enters the hydrophobic pocket inside the tubulin dimers, which consists of eight tryptophan residues. Not only does this interaction influence the structure of microtubules, but also it positively affects microtubule: assembly. The results of *in vivo* studies on rats also suggest that tryp1ophan enhances both working and long-term memory. Thereby, we propose that tryptophan ameliorates resonant energy transfer and electron tunneling in the hydrophobic networks that are present in microtubules, which possibly accounts for the observed improvement in memory.

Keywords: Microtubules, Hydrophobic pocket, Tubulin dimers, Memory improvement

Oral

Polypharmacology in Drug Discovery

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ABSTRACT

Traditional approaches for the identification of bioactive compounds use a chemical library, a single target protein, and an assay, which allows us to measure the activity of these compounds against one-target. Therefore, “one-target, one-drug, one-disease” model has long been the standard strategy for discovering new drugs in pharmaceutical research. Computational drug discovery

techniques such as quantitative structure-activity relationships (QSARs) have been carried out to design potent inhibitors against the selected target. Unfortunately QSAR model cannot consider the information of the target and in many studies has a minimal ability to predict selective inhibitors. Therefore, recently a meaningful decrease in the rate of discovery of new drug candidates has been observed. Because of complex physiological processes in the body, considering only one target in drug discovery projects is not rational. The main reason for this is that, the multiple activities of drugs against several targets might be lead to dramatic side effects and toxicity. Nowadays increasing evidence that several drugs exert their biological effects through interactions with multiple targets is boosting the development of new research such as chemogenomics, polypharmacology and proteochemometrics. Therefore the purpose of drug discovery has changed from one-drug, one-target strategy to a multi-drug, multi-target. In this lecture, I will describe the current state of drug discovery and the concepts of QSAR, polypharmacolog and proteochemometrics.

Keywords: Drug discovery, Polypharmacolog, Proteochemometrics, QSAR

Oral

Ginsenoside Rh2-, Lysine- and Arginine-treated Highly Porous Graphene Oxide Nanosheests: Novel Drug Delivery Systems with Improved Anticancer Activity

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ABSTRACT

In this study, Rh2-treated graphene oxide (GO-Rh2), lysine-treated highly porous graphene oxide nanosheets (GO-Lys), arginine-treated GO (GO-Arg), Rh2-treated GO-Lys (GO-Lys-Rh2) and Rh2–treated GO-Arg (GO-Arg-Rh2) were synthesized. MTT assay was used for evaluation of cytotoxicity of samples on ovarian cancer (OVCAR3), breast cancer (MDA-MB), Human melanoma (A375) and human mesenchymal stem cells (MSCs) cell lines. The percentage of apoptotic cells was determined by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay. The hemolysis and blood coagulation activity of nanostructures were performed. Interestingly, GO-Arg, GO-Lys, GO-Arg-Rh_2, and GO-Lys-Rh_2 were more active against cancer cell lines in comparison with their cytotoxic activity against normal cell lines (MSCs) with IC_{50} values higher than 100 µg ml^{-1}. The results of TUNEL assay indicates a significant increase in the rates of TUNEL positive cells by increasing the concentrations of nanomaterials. Results were also shown that aggregation and changes of RBCs morphology were occurred in the presence of GO, GO-Rh_2, GO-Arg, GO-Lys, GO-Arg-Rh_2 and GO-Lys-Rh_2. Note that all the samples had effect on blood coagulation system, especially on PTT. All nanostrucure act as antitumor drug so that binding of drugs to a nostructures is irresolvable and the whole structure enter to the cell as a drug.

Keywords: Anti-cancer activity, Ginsenoside Rh2, Graphene oxide, Drug delivery, Hemolysis, Coagulation

Oral

Interaction of All-trans Retinoic Acid with Human Serum Albumin

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ABSTRACT

All-trans retinoic acid (ATRA) is the most effective drug in the treatment of acute promyelocytic leukemia (APL). However, the use of ATRA is not without problem. ATRA is poorly soluble in water and has limited bioavailability and its pharmacologic levels can cause retinoic acid syndrome. On the other hand, albumin is the major protein in plasma that interacts with a wide range of drugs. Often, more than 90% of the drug is bound to albumin, which has a significant effect on the drug's efficacy, the rate of drug delivery to target cells, and drug removal. In this study, ATRA binding to albumin and the effect of some compounds on the binding is investigated using different methods. Fluorescence results indicated the static type of quenching mechanism in the binding of ATRA to albumin. The association constants between ATRA and albumin and the number of binding sites as well as the thermodynamic parameters of complex were obtained at different temperatures. The calculated thermodynamic parameters revealed that the binding reaction is spontaneous and endothermic process, and hydrophobic interactions have a main role in the binding of ATRA to albumin. The effect of other compounds on the binding is under investigation.

Keywords: ATRA, All-trans retinoic acid, Albumin, interaction

Oral

The Mechanical Properties of a Single Cell Measured by Piconewton Resolution Reveal the Mechanism of Action of some Drugs

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ABSTRACT

Statins-family of drugs are commonly prescribed for hypercholesterolemia patients with both primary and secondary prevention in order to decrease the level of the blood cholesterol. Statin drugs, thus affect RBC mechanics indirectly through modulation of cholesterol content of the membrane. It is however unclear whether statin drug can directly affect RBC mechanics, or more broadly, mechanics of human cells. Optical tweezers are proven indispensable single-cell micro-manipulation and mechanical phenotyping tools. In this study we have used optical tweezers for measuring viscoelastic properties of human red blood cells (RBCs) and asked whether statin drugs, including Atorvastatin can directly affect RBC mechanics. Comparison of viscoelastic features of the healthy fresh and statin treated cells revealed that the Atorvastatin treatments soften the cells by about 25%. Using a simple modeling approach, we proposed a molecular model that explains the drug induced changes in viscoelastic properties of RBCs membrane. Our results reveal molecular interactions between drug and cytoskeleton proteins. We find that Atorvastatin and can induce protein conformational changes that may lead to dissociation of cytoskeletal junctions and significant increase in the flexibility of the cell. Our findings also suggests that direct interactions between the drug and cytoskeletal components, namely F-actin-spectrin and ankyrin-spectrin complexes, underlie the drug-induced mechanical changes of the cells.

Keywords: Optical tweezers, Red blood cell, Atorvastatin, Protein conformational change

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Hyperpigmentation Treatments: A Comprehensive Review

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ABSTRACT

Browning of fruits and vegetables and hyperpigmentation of human skin are two undesirable phenomena. Tyrosinase is the main enzyme recognized as responsible for this enzymatic browning and melanogenesis in mammals. Due to this vital role of tyrosinase, focuses on its inhibitors discovered from all sources, including synthetic compounds, extracts and active ingredients of natural products, virtual screening and structure-based molecular docking would be helpful for screening and development of effective and safe antibrowning and antimelanogenic agents. For this purpose several sensitive methods including spectrophotometric, chromatographic, electrophoretic, radiometric and electrochemical assays have been applied and developed by researchers. In addition to identification of tyrosinase inhibitors from natural sources, many researchers have designed appropriate scaffold inspired by the structure of natural compounds and developed novel synthetic inhibitors, up to now. It is interesting that among different tested compounds, some of them such as kojic acid derivatives despite their depigmenting activities didn't display tyrosinase inhibitory activitiy.

Keywords: Inhibitors, Enzyme, Mushroom Tyrosinase, Antimelanogenic

Oral

The Biophysical Characterization of the Higher-order Structures of the *C9orf72* repeat

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) and frontotempora dementia (FTD) are diseases that form a spectrum of neurodegenerative disorders. Back in 2011, it was shown that expansion of $(GGGGCC)_n$•$(GGCCCC)_n$ in the *C9orf72* gene is the main cause of ALS/FTD. Being G-rich on one strand, and C-rich on the other strand, the *C9orf72* repeats would appear to have a large propensity to form secondary structures. It has been shown that different repeats of the G-rich DNA and RNA, can form G-quadruplexes- also known as four-stranded DNA or RNA-*in vitro*. To date, the exact mechanism leading to pathogenesis is still not known. However, the two major theories regarding pathogenesis are loss-of-function or gain-of-function mechanisms. Supporting the gain-of-function mechanism for pathogenesis, multiple proteins have been shown to bind the *C9orf72* repeat. Interestingly, some of these proteins were shown to have structure-specific binding to the higher-order structures mentioned above. Structure-specific protein binding has previously been reported for other repeat expansion diseases as well. Our studies have focused on the biophysical characterization of the structure of the G-rich and C-rich strands of the *C9orf72* repeat and their binding with biologically relevant proteins and small molecules by using CD spectroscopy, UV spectroscopy and gel electrophoresis. Non-canonical structures may be important intermediates in mutagenesis; therefore, it is

important to assess the relative stability of these structures and their binding to biologically relevant molecules.

Keywords: C9orf72, G-quadruplexes, i-Motifs, Structure-specific protein binding

Oral

NMR and MD as Two Methods of Monitoring the Self-association of Small Molecules in Aqueous Solutions for Biological Application

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ABSTRACT

Small molecules, whether natural (as secondary metabolites) or synthetic (as many drugs), play an important role in regulating biological processes. Due to their low molecular weight (< 900 Da) and size of about 1 nm, they can bind to and be probes of biological macromolecules such as proteins and nucleic acids. However, since most of the molecules were found to have hydrophobic properties, from low to very high levels, and their physicochemical properties depend on their solubility in water, two problems of "How their aggregation (self-association) in aqueous solutions can be monitored?" and "How the aggregation can affect their biological properties, including the interaction with the macromolecules?" have been tried to be addressed. Form various in-vitro and computational methods, nuclear magnetic resonance (NMR) spectroscopy and molecular dynamic (MD) simulations can be used. For the former method, ^{1}H NMR spectra of a small molecule in ascending concentrations (in mM or M, depending on the power of the NMR spectrometer) and dissolved in a constant ratio of solvents (usually H_2O:DMSO) are recorded so that the chemical shift variations as a function of the concentration represent the affinity of the small molecules for self-association. For the latter one, several molecules are placed in a solvated simulation box and their behavior is monitored during the simulation time. Using NMR and MD, we showed that curcumin and quercetin are two small molecules with remarkable affinity for self-association, while rosmarinic acid has a much lower affinity.

Keywords: Small molecule, Self-association, NMR, MD, Curcumin, Quercetin

Oral

Assessment of Boron Nitride Fullerenes as the Drug Delivery Vehicles of Ifosfamide

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ABSTRACT

Osteosarcoma is a cancerous tumor that starts in the bones. The therapeutic activity of drugs is limited by their severe drug-related toxicities; therefore, a therapeutic approach which is less toxic and highly effective in tumor cell is of utmost importance. In this study, we considered density functional theory (DFT) calculations upon the adsorption of Ifosfamide (IFO) as a antitumor agent on a series of pure $B_{12}N_{12}$ and carbon-doped boron nitride including $B_{12}N_6C_6$ and $B_6N_6C_{12}$ fullerenes for both vacuum and solvent (water) environment conditions by means of PBE-1 and M06-2X functionals and 6-311+G (d,p) basis set. Our study successfully demonstrated that the adsorption of antitumor agent upon the fullerenes will increase the polarizability of boron nitride fullerenes in

comparison with C_{60} fullerene, but on the other hand, the energy of the interaction of IFO with $B_6N_6C_{12}$ is greater, which can reduce its release rate from the system.

All rights of the results of this project belong to Iran National Science Foundation, Science deputy of presidency (Code Number: 95849822*)*

Keywords: Osteosarcoma, Density functional theory (DFT), Ifosfamide

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Biophysical Approaches of Human Serum Albumin upon Interaction with a Curcumin Derivative

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ABSTRACT

Human serum albumin (HSA) is one of the most abundant blood proteins. It serves as a transport protein for several endogenous and exogenous ligands as well as various drug molecules. In addition, the levels of this protein in tumor cells are much higher than normal cells, and acts as carrier conjugate of many antitumor drugs. Therefore, interaction studies of HSA to comprehension of the pharmacokinetics of drugs and the function of HSA would be interesting. In the current study, the interaction between HSA and curcumin derivative (DVH) was systematically investigated in details for the first time by UV-Vis absorption, fluorescence and circular dichroism methods. The results proved that the DVH could slightly change the secondary structure of protein. Fluorescence spectroscopic studies demonstrate that DVH binds to HSA through a static quenching mechanism. The binding constant (K_b) and the number of binding sites (n) were obtained based on the results of fluorescence measurements. The results of site marker investigations showed that DVH is embedded into subdomain IIA of HSA. The thermodynamic parameters indicate that the binding process was spontaneous and that hydrogen bonds and van der Waals forces play a major role in the interaction. Furthermore, the apparent distance between donor (HSA) and acceptor (DVH) was determined using fluorescence resonance energy transfer (FRET). The obtained results give us key insights into the performance of effective anti-cancer drugs when faced with proteins such as HSA.

Keywords: Human serum albumin, Curcumin, 3D florescence, Thermodynamic, FRET

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Purification and Charactrization of Protease by Pleurotus Eryngii

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ABSTRACT

This work focuses to protease production by *Pleurotus eryngii* using different carbon and nitrogen source with 0.5 and 1.0% concentrations employing submerged fermentation. The highest production rate was recorded with 0.1% sucrose and 1.0% casein as a carbon and nitrogen source.The enzyme was purified 1.377- fold by gel chromatography on Sephadex G-100, and Fraction-1, F2 and F3 were purified 1.379, 1.419, and 1.322- fold on anion exchange CM A-50 and purified enzyme showed high specific activity of 2.420, 2.49 and 2.32 respectively. The molecular weight of purified fraction F1, F2 and F3 were found to be 30, 30 and 35 kDa by SDS-PAGE.The optimal pH and temperature for protease activity was 8 and 65 °C, respectively. But from pH 7 to 9 the protease activity remains constant in two fractions after 9 pH activity slowly

decline. The temperature profile showed that purified protease activity increases with increase of temperature up to 65 °C and activity gradually lost with the increases of temperature due to enzyme denaturation at high temperature. The temperature stability was checked by heating enzyme for 10 min prior the addition of substrate and enzyme activity was checked by following procedure of enzyme activity. But still 25% activity was retained at 80 °C for 10 min. The Km and V_{max} values of purified protease activity of F1, F2 and F3 were observed 0.65mg/ml/0.157units/min; 0.5mg/ml/ 0.157units/min and 0.9mg/ml/1.00units/min, respectively. The activation energy of fraction F1, F2 and F3 was calculated 1.1157, 1.1269 and 0.9602 kJ mol^{-1} respectively. The half-life of *Pleurotus eryngii* protease was noted 75, 83 and 76 °C for fraction F1, F2 and F3 respectively. Although 5 mM concentration of $AgNO_3$ reduced the 60% activity of F1, $CuSO_4$ reduced the 64% activity of F2 and $MnSO_4$ reduced 79% activity of F3. As shown in result $CaCl_2$, Cysteine, $MgCl_2$, $ZnCl_2$, $CoCl_2$, mercaptoethanol stimulated the protease activity with different rate, which suggest two or more active sites.

Keywords: Protease, Production, Purificaion, Characterization, *Pleurotus eryngii*

The Interaction of Aspirin with Human Serum Albumin: A Molecular Dynamics Simulation Study

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ABSTRACT

The most important protein in the blood plasma is Human serum albumin (HSA). Molecular dynamics simulations of subdomain IIA of HSA and its complex with salicylic acid (SAL) were performed to investigate structural changes induced by the ligand binding. To estimate the binding affinity of SAL molecule to subdomains IB and IIA in HSA protein, binding free energies were calculated using the Molecular Mechanics-Generalized Born Surface Area (MM-GBSA). It was found that the presence of SAL molecule leads to the stability of HSA. Also, ligand binding decreases the α-helix content of HSA. Binding free energy calculations demonstrate that the binding affinity of the SAL molecule to subdomain IIA of HSA is more than that of subdomain IB of HSA and the contributions of van der Waals interactions are more than that of electrostatics interactions. Our important finding is that the subdomain IIA of HSA is the main HSA-SAL binding site. The results obtained are in good agreement with the corresponding experimental data.

Keywords: Molecular dynamics simulations, Binding free energy, Human serum albumin, Salicylic acid, Molecular mechanics-generalized Born surface area

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Thiamin Pyrophosphate Riboswitch is Going to Accept New Compounds as Antibacterial to Repress Gene Expression

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ABSTRACT

Thiamine pyrophosphate (TPP) riboswitch controls gene expression through binding of small molecules to the

aptamer sequence. As this sequence is found in bacteria, it can be one target of antibacterial. A QSAR study in our laboratory using experimentally tested compounds indicated that among 1875 descriptors, four of them (Alogp^2 with lipophilic (hydrophobicity) property, ionization energy attributed to AATSC6i; charge indicated by ATSC0c and βLUMO indicative of sensitivity of a molecule to be attacked by nucleophiles) are the most important properties which altogether consist the driving force of ligand binding to the binding site. Approximately 10000 compounds were generated followed by applying the QSAR model, docking and ADME (absorption, distribution, metabolism, and excretion) criteria to predict the activity and quality of the compounds respectively. pK_d prediction of the compounds using QSAR indicated that the activity of the some of the designed ligands are between 8.00 to 9.00, therefore these were selected to predict ΔG by docking the molecules in AutoDock Vina which offered some ligands with ΔG between -9.7 to -11.0. kcal mol^{-1}. The quality of ligands from ADME standing point was verified using SWISSADME. ADME quality study indicated that these compounds are not P-gp substrate, molecular weight is less than 500 Da and the selected compounds passed Lipinski rule of five. The best compounds are under synthesis in our Lab.

Keywords: QSAR, TPP Riboswitch, Ligand design

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on average, 5.4 kJ per mole. The stability depends on a number of factors. Intramolecular forces such as chemical bonds, charge-charge interactions, etc. and intermolecular interactions such as solvent effects, pH changes, *etc.* Most sustainability tests are carried out with complete sedimentation of proteins, which can indicate the required amount of bentonite needed to prevent protein instability.

Protective Effects of Silibinin Against Insulin Amyloid Fibril-induced Toxicity in SH-SY5Y Human Neuroblastoma Cells

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ABSTRACT

Misfolding and deposition of various peptides and proteins is a key pathogenic feature of more than 20 amyloid-related diseases. Inhibition of protein aggregation is therefore viewed as a potential method to halt or slow the progression of neurodegenerative disorders. In this regard, numerous reports have demonstrated the effectiveness of some of the naturally occurring small molecules, and specially certain natural polyphenols, on prevention of protein aggregation and their associated cytotoxicity. In the present study, the effect of silibinin, as a major biologically active component extracting from the seeds of milk thistle plant named Silybum marianum, on the cytotoxic action of insulin fibrils was tested with human neuroblastoma SH-SY5Y cells. Insulin fibril-induced neuronal toxicity was verified by the MTT viability assay, intracellular ROS measurement, and morphological analysis. We found that the cell exposure to the co-incubated insulin amyloids with silibinin led to the increased cell viability and decreased ROS content dose-dependently, compared to cells exposed to insulin fibrils alone. Co-incubation with silibinin also attenuated the extent of morphological alterations induced by insulin fibrils. These results suggest that silibinin has protective effects against insulin fibrils induced cytotoxicity, which might be a potential therapeutic agent for treating or preventing neurodegenerative diseases.

Keywords: Insulin, Amyloid fibril, Silibinin, SH-SY5Y cells, Cytotoxicity

Investigating the Effect of X-Ray and UV Light on the Conjugation of Lysozyme with Dextran

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ABSTRACT

The radiation damage (X-ray, UV light) of many proteins and other biomolecules has been investigated by various physicochemical techniques. These studies have revealed numerous changes in the local and global structure of the proteins studied, together with alterations of their functional abilities, such as inactivation, aggregation, fragmentation, destruction of aromatic amino acids and disulfide bonds, formation of crosslinks and new chromophores, partial unfolding, etc. X-irradiation radiolysis products of water ($OH^{\bullet}$, $O_2^{\bullet-}$ and H2O2), are the true reactants and UV light can also produce reactive free radical. Lysozyme has been subjected to many radio and photobiological treatments, particularly aiming at the damage mechanics. The aims of this study was to investigate the possibility of using X-ray and UV light to create new stable covalent bond between dextran and lysozyme and to monitor the structural changes incurred in the conjugated products. Lysozyme and dextran were mixed with a weight ratio 1 to 5 in phosphate buffer (pH 7.4). Sample were exposed to UV-C light (250-280 nm) for 4 h. Another sample was exposed to X-ray (50 KeV) for 0.04 s. The lytic activity of all treated samples was reduced compared to the control (no treatment) sample. However, no significant changes were observed in the SDS- PAGE pattern of all samples. Our data suggests that both X-ray and UV light cause structural changes in lysozyme but, unlike other physical treatments such as heat or microwave, do not bring about significant degree of conjugation.

Keywords: Lysozyme, Dextran, X-ray, UV light, Conjugation

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Effects of Lead-HSA Interactions on Fibrillation of Protein

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ABSTRACT

These days, general population is exposed to lead from air, food and other resources. So, lead (Pb) deserves special attention since this metal is the most toxic biochemical agent responsible for the world's most common environmental caused disease. Pb has high-affinity metal-binding proteins. It is reported that lead is a potent neurotoxin for human being especially for the developing children, and in the brains of patients with Alzheimer disease, Pb^{2+} at high concentrations is found [1]. Protein-lead interaction shows that lead is capable of affecting the functional properties of proteins. In this study, the samples of protein were incubated at 68 °C under physiologic pH in

a water bath and the kinetic of fibril formation, changes in protein structure and reactive oxygen species generation were determined. Our studies showed the binding of Pb to HSA induced aggregation, fibril formation and caused rise to reactive oxygen species(ROS). Taking together our results and those of numerous other studies, we hypothesize that Pb-induced conformational changes which enhanced the neurotoxicity of proteins fibrils and lead to development of amyloidogenesis disease.

Keywords: Lead, HSA, Fibrillation, reactive oxygen species(ROS), Amyloidogenesis

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Aggregation of Aβ42 and Lysozyme in the Presence & Absence of the Natural Peptide Fasciculin-2 & the Synthetic Peptide KLVFF

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ABSTRACT

Presence of insoluble proteinaceous deposits in human tissues is associated with the development of disorders such as amyloidosis and neurodegenerative diseases. These proteinaceous deposits might generate through the effects of intracellular agents or induced environmental conditions which ultimately leads to covalent modification, consequent protein misfolding and its aggregation. Thus finding a way to inhibit these aggregates could have a great impact in prevention and therapy of the devastating diseases. In this study, we examined, the effects of fasciculin II (Fas II) (a short, highly toxic peptide in the venom of Mamba snakes), and a short synthetic peptide, named KLVFF, (derived from 16-20 residues of Aβ42), on the aggregation of lysozyme and Aβ42 through exertion of different experimental setups. The aggregates were detected by techniques such as fluorescence spectroscopy, atomic force microscopy, electrophoresis and rheology. Our results showed that Fas II reduced the aggregation potency of both lysozyme and Aβ, incubated for different lengths of incubation times. Despite KLVFF showed no significant effects on lysozyme aggregation, it could reduce Aβ42 aggregation considerably. Each of the above-mentioned experimental setups were also examined after proteolytic cleavage of lysozyme and Aβ by trypsin, which showed impressive effects on lysozyme and Aβ42 aggregates inhibition. These preliminary and promising results could be considered in the framework of using short length peptides as potential candidate drugs in the treatment of amyloidogenic diseases. Our rheological results demonstrated significant increment of incubated Lysozyme's viscosity compared with monomeric lysozyme and efficacy of rheology as a novel technique for aggregation assay.

Keywords: Protein aggregation, Protein aggregation inhibition, Amyloid beta peptide, Lysozyme, Fasciculin II, KLVFF peptide

Optimization of Phycocyanin Extraction from Wett Biomass of *Chlorella sp*

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ABSTRACT

Phycocyanin (PC) is a major light harvesting pigment-protein of cyanobacteria. PC and other phycobiliproteins have many properties in the food industry, cosmetic

industry, biotechnology, diagnosis and treatment. There are many methods for phycocyanin extraction such as organic and inorganic acid treatment, freezing and thowing, homogenization and lysozyme treatment. In the present study, optimization, extraction and purification of phycocyanin from wet biomass *of Chlorella sp* has been reported. *Chlorella sp* was cultured in BG-11 medium at 25 ºC ± 2.The extraction was carried out using different conditions, including temperature, biomass-solvent ratio and different chemical treatments. Then, glycerol was added to the suspension of algae and placed in the refrigerator for 3-4 days. Distilled water and acetate were added to the cell to break down them. Then, the solution was placed in the refrigerator and darkness for two days. Based on the results, the extraction by Sodium acetate 10 mM at 0 °C proved to be the most efficient method. Crude extract of phycocyanin was purified by ammonium sulphate precipitation and dialysis. Pure C-Phycocyanin was finally obtained from *Chlorella sp* with concentraton of 0.079 mg/ml and purity ratio (A_{615}/A_{280}) of 0.5.

Keywords: Phycocyanin, Extraction, Cyanobacteria, chlorella sp

Substitution of His by Asp Modify Thermal Stability of Firefly Luciferase

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ABSTRACT

Light emission of luciferase is used in a wide variety of biochemical assays in clinical, industrial and scientific research applications. However, several factors limit further applications of this technology, one of them is the low stability of luciferase enzyme in the room and human physiological temperature. A common strategy to improve thermal stability of proteins is to introduce new salt bridges or increase the number of hydrogen bonds in the solvent exposed flexible region of proteins. By calculating the root mean square fluctuation (RMSF) of backbone atoms at two temperatures 300 and 340 K, thermal sensitive (flexible) regions of luciferase were identified. Histidine461 and histidine489 are located in the flexible regions, therefore we decided to substitute them with a negatively charged residue, aspartate. Substitution of His461 by aspartate in H461D decreased ATP binding affinity, reduced the melting temperature of protein by around 26 °C and shifted its optimum temperature of activity to 10 °C. In line with the common feature of psychrophilic enzymes, the MD data showed that the overall flexibility of H461D was relatively high at low temperature, probably due to a decrease in the number of salt bridges around the mutation site. On the other hand, substitution of His489 by aspartate in H489D introduced a new salt bridge between the C-terminal and N-terminal domains and increased protein rigidity but only slightly improved its thermal stability.

Keywords: Luciferase, Thermal stability, Flexibility, Molecular dynamics simulation, Histidine

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An Alpha Synuclein- Peptide Docking Study to Select Appropriate Peptides Interacting with the Protein NAC Domain

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A Spectroscopy Study on the Interaction between SiO_2 Nanoparticle and Bovine Alkaline Phosphatase

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ABSTRACT

The improvement of enzymes activity and stability is crucially important for their industrial applications. Nanoparticles have recently been developed to improve enzymes performance. In this study, SiO_2 nanoparticles were used to stabilize bovine intestinal alkaline phosphatase (BIALP) as an enzyme system. Different spectroscopic method were performed to study enzyme-SiO_2 nanoparticle interaction, including fluorescence, ultraviolet-visible absorption spectroscopy, and circular dichroism spectrum. Moreover, activity of enzyme in the presence of SiO_2 nanoparticles was assayed. SiO_2 nanoparticles quenched the enzyme spectra and ground state complex was formed. The results of current study showed that SiO_2 nanoparticles bound to the BIALP through hydrogen bonds and van der Waals force. The Stern-Volmer quenching constant (K_{SV}) and corresponding thermodynamic parameters of $\Delta H°$, $\Delta G°$ and $\Delta S°$ were calculated. In addition, the binding constant (K_b) and number of binding sites (n) were performed by fluorescence quenching method at 298 K and 308 K. Additionally, secondary and tertiary structure of BIALP in the absence and presence of SiO_2 nanoparticle were analyzed by UV-Vis absorption and circular dichroism spectrum. The results showed that SiO_2 nanoparticle altered the secondary structure of enzyme by changing α-helix and β-sheet content of BIALP. The activity of BIALP is increased by adding various concentrations of

ABSTRACT

α-Synuclein (AS) is one of the presynaptic intrinsically disordered proteins. However, the change in environmental factors (such as pH, ionic strength, severe shaking) induces the formation of AS aggregations and amyloid fibrils *in vitro*. Lewy bodies are aggregates that are seen in Parkinson's patients. The main component of the Lewy bodies are the amyloid fibers of AS. The exact function of this protein and the mechanisms that cause toxicity and cell death are still not well defined. Inhibition AS aggregations may be an approach to preventing and treating Parkinson's disease. A number of polyphenol antioxidants have been discovered that inhibit AS fibers. However, their effects are non-specific. Generally, protein-protein interactions are highly specific and well-regulated. In this study, we attempted to design using computational methods and synthesize a new peptide according to AS sequence 68-83, and its inhibitory effects on AS accumulations will be examined. Approximately 78 modified linear peptides were designed in sequences AS (68-83). The circular forms of these peptides (N to C terminal cyclization) were also considered. The protein-peptide docking with the HADDOCK web server performed on these linear and circular peptides and their binding energy and interaction with the protein were determined. Based on HADDOCK scores, RMSD and Pose, the proper linear and cyclic peptides were selected and will be synthesized. A scramble peptide with similar amino acid composition with different sequence will be synthesized to examine the significance of the sequence in the future.

Keywords: Alpha synuclein, Parkinson's disease, HADDOCK, Peptide- based inhibitors

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SiO_2 nanoparticle. In overall, our results suggest that SiO_2 nanoparticle can bind to the BIALP and improve its activity and stability.

Keywords: Alkaline phosphatase, SiO_2 nanoparticle, Circular dichroism, Quenching mechanism, Fluorescence

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Inhibitory Effect of Graphene Oxides on Hen Egg White Lysozyme Amyloid Fibril Formation

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ABSTRACT

Protein misfolding and aggregation is related to a large number of neurodegenerative disorders including Alzheimer's, Parkinson's, and Huntington's diseases. Here, we exploited Graphene Oxides (GO) as an efficient inhibitor of hen egg white lysozyme (HEWL) amyloid fibrillation. A range of amyloid-specific techniques, including thioflavin T (ThT) and 1-anilino-naphthalene 8-sulfonate (ANS) fluorescence measurements and Congo red binding assay were employed to investigate the efficacy of GO for inhibition of HEWL amyloid fibrillation. Additionally, the morphology of resultant aggregates in the absence and presence of GO were analyzed by Atomic Force Microscopy (AFM). Kinetics of HEWL amyloid formation in the absence and presence of GO demonstrated a concentration-dependent decrease in ThT fluorescence. Similarly, a pronounced dose-dependent decrease in ANS fluorescence intensity and Congo red absorbance were also observed. These results were more confirmed by AFM images, suggesting that in the presence of GO formation of mature fibrillar structures was strongly inhibited. Based on obtained results, it is concluded that GO are able to act as an effective inhibitor of HEWL amyloid fibrillation.

Keywords: Hen egg white lysozyme, Graphene Oxides, Thioflavin T, Atomic force microscopy

Conformation and Dynamic of Versatile Peroxidase in the Presence of the Glycerol Based Deep Eutectic Solvent

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ABSTRACT

Ionic liquids are considered as green solvents and promising candidates to replace organic solvents. A subclass of ionic liquids called deep eutectic solvents are synthesized by mixing a quaternary ammonium salt, the most conventional among them choline chloride, and a hydrogen bond donor such as glycerol. The deep eutectic solvents are simple to synthesize, biodegradable and biocompatible. On the other hand, versatile peroxidase, capable of degrading a wide range of substrates high or low potential substrates, has attracted attention to be applied in industry. In the present study, the effect of hydrated choline chloride: glycerol on the conformation and dynamic of versatile peroxidase were studied using extrinsic fluorescence, steady-state anisotropy, and fluorescence

lifetime measurements. According to the results of extrinsic fluorescence studies, presence of choline chloride: glycerol caused higher exposure of hydrophobic patches of versatile peroxidase. Furthermore, decreased values of steady-state anisotropy in the presence of increasing concentrations of choline chloride: glycerol revealed formation of versatile peroxidase assemblies. Moreover, higher fluorescence lifetime values in the presence of growing concentrations of the choline chloride: glycerol implied adoption of more compact conformation and consequently less flexibility of versatile peroxidase. In general, it could be concluded that hydrated choline chloride: glycerol can alter versatile peroxidase's conformation and dynamic.

Keywords: Choline chloride: glycerol, Deep eutectic solvents, Versatile peroxidase, Conformation, Dynamic

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Release of Brain Hexokinase 1 from Mitochondria Induced by Amyloid Aggregates of α-Synuclein

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ABSTRACT

Recent studies suggest that accumulation of alpha-synuclein amyloid aggregates on mitochondria is associated with Parkinson disease and other Lewy body disorders. Clearly, these amyloid aggregates disrupt mitochondrial function and initiate a pathophysiological cascade leading to neuronal degeneration. However, the molecular mechanism(s) of alpha-synuclein toxicity and its effect on mitochondria, in particular, remain elusive. Hexokinase 1 (HK 1) is a key glycolytic enzyme that plays important roles by reducing mitochondrial reactive oxygen species (ROS) generation and preventing apoptosis in neurons and other cell types. In this study, we have investigated the effect of alpha-synuclein amyloid intermediates on the release of HK1 from rat brain mitochondria. We found that alpha-synuclein amyloid intermediates, including oligomer, protofibril, and mature fibril, triggered HK 1 detachment from mitochondrial membrane in a dose-dependent manner. Moreover, a significant decrease in HK 1 activity was observed upon addition of alpha-synuclein aggregates. Based on obtained results, we suggest that inactivation and detachment of HK 1 from mitochondrial membrane by alpha-synuclein aggregates may play important roles in oxidative stress and neurodegeneration of Parkinson disease.

Keywords: α-Synuclein, Protein aggregation, Mitochondria, Hexokinase 1, Parkinson disease

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Investigation on the Effect of Kosmotropic and Chaotropic of Zinc and Copper Ions *in Vitro* Fibrillization of Human Serum Albumin

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ABSTRACT

Amyloid fibrils, formed by aggregation of improperly folded or intrinsically disordered proteins, are closely related with the pathology of the number of devastating amyloid diseases, such as Alzheimer's, Parkinson's, and

Creutzfeldt-Jakob diseases. Occupational exposure to specific metals, especially copper, lead, iron, zinc, appears to be a risk factor for some of these disease based on epidemiological studies. The interaction of proteins with aqueous solutions of ionic ligands has attracted considerable recent attention. We investigated the interaction of Zn^{2+} and Cu^{2+} in chloride salt forms with human serum albumin (HSA) fibrils through ThT fluorescence method under physiological pH. Also, in this work we studied kosmotropic and chaotropic effects of these ions on the protein fibrils. A comparison of the effects of these chloride salts revealed that anions don't obserable response in stabilizing HSA fibrils.

Keywords: Zinc ion, Copper ion, Human serum albumin (HSA), Amyloid disease, Protein fibrillation

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Production of Casein Protein Stabiliy Fibers from Dairy Products (Milk)

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ABSTRACT

Casein is a protein that exists in large amounts in milk. Protein and peptides are made by a sequence of various amino acids, the most important of which are Glycine, Cysteine and Threonine. In the present research, initially the cream, which is an important nutrient, is separated from the milk. Then the remaining milk is warmed to 40 °C and the protein content is coagulated by acidifying the milk. The coagulated protein is extracted from the aqueous part of the milk, washed to remove the acid and salt and then dried. The collected Casein is fully mixed and dissolved in Sodium Hydroxide solution. After passing through special filters, it is entered into the Spinneret fiber making machine. The next stage is the coagulation bath containing sulphuric acid, formaldehyde, and glucose. At the end the spinning operation and casein fibers production is performed.

Keywords: Fibers, Protein, Polymer, Milk, Casein

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Investigation the Structure and Stability of Carboxypeptidase A on Presence of Putrescine

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ABSTRACT

Polyamines such as putrescine can have interaction with proteins. The aim of the present study was to investigate how putrescine could influence the structure and thermal stability of carboxypeptidase A (CPA). CPA (EC 3.4.17.1) is a zinc-containing proteolytic enzyme that was isolated in the pure crystalline form in 1937 and was the first metalloprotease and second zinc enzyme to be identified. The UV-spectroscopy, thermal denaturation and thermodynamics studies were conducted to investigate the effect of putrescine on the structure and thermal stability of CPA, in 25 mM Tris-HCl buffer, with the pH 7.5. The UV-spectroscopy results show that the stability (T_m) of CPA was increased in the presence of putrescine in the range 303-353 K in the tris-HCl buffer and pH 7.5. The analysis of UV-Vis indicates that by increasing concentration of putrescine, absorption was decreased. Thermodynamic parameters showed static quenching during putrescine binding. The thermodynamic parameters changes,

including Gibbs free-energy (ΔG°), entropy (ΔS°) and enthalpy (ΔH°), showed that the binding of putrescine to CPA was spontaneous and the hydrogen bonding and van der Waals interactions played a major role in stabilizing the CPA-putrescine complex. The value of *n* was approximately equal to 1, indicating that it was one single binding site in CPA for putrescine during their interaction. As a result, putrescine could be considered as a stabilizer for CPA.

Keywords: Carboxypeptidase A, Putrescine, Stabilizer Spectroscopic techniques, Thermodynamics

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Generation of Reactive Oxygen Species by Alpha-synuclein Fibrils and Oligomers

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ABSTRACT

The formation of toxic oligomers and fibrils is the hallmark of Parkinson's disease (PD) pathology. In PD, the procedure of aggregation of distinct protein such as alpha-synuclein from monomers to oligomeric intermediates and amyloid fibrils is accounted as the disease-causing agent of toxic mechanism. Mitochondria are a major source of ROS (reactive oxygen species) within most mammalian cells. This ROS production contributes to mitochondrial damage in a range of pathologies and is also important in redox signalling from the organelle to the rest of the cell. Furthermore, the interaction and internalization of toxic aggregated alpha-synuclein to mitochondrial membrane can cause major impairments in this organel. Subsequently, mitochondrial dysfunction may lead to increased oxidative stress and consequent cytotoxicity. Therefore, the present study was undertaken to compare and contrast the percentage of ROS production as a consequences of the interaction of alpha-synuclein amyloid aggregates, produced in the absence and presence of dopamine, with rat brain mitochondria. The ROS production was evaluated by 2,7-dichlorofluorescein diacetate (DCFH-DA), which readily diffuses into cells. Our results obviously demonstrated both fibrils and oligomers of alpha-synuclein induce ROS generation.

Keywords: Parkinson disease, Alpha-synuclein, Mitochondria, Free radicals

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Design and Synthesis of Erythropoietin Nanoparticle Coated with Gemini Cationic Surfactant (as a Carrier) for Delivery of Human Erythropoietin to Brain

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ABSTRACT

Erythropoietin (EPO) is a hematopoietic growth factor. Also EPO as a neuroprotective agent can prevent neuronal death in brain damage. But this protein has no ability to across the blood-brain barrier, Because of its large size. For this reason, high doses are used to treat brain damage, which leads to extensive side effects. Therefore, the use of

this drug is limited .The purpose of this study was design of new Nano drug delivery system for increase the half-life of the drug and reduces the dose of the drug and delivering of EPO to the brain to skip the blood brain barrier. So, anew quaternary ammonium-based cationic Gemini surfactant has been used in this study for preparation of EPO-loaded Gemini micelles. In this study, we studied the physicochemical characteristics of Synthetic Nano drug whit using Dynamic light scattering (DLS) and ζ-potential measurement, Morphology studies and in vitro drug release. The result of this study showed that there are appropriate interactions between EPO and Gemini nanoparticles and EPO loaded in to the Gemini nanoparticles. DLS results and Morphology studies indicate that size of the Gemini-EPO Nano micelle is smaller than 150 nm and Nano micelles (EPO-loaded) having smooth regular surface. TEM micrograph results confirm DLS results sizes of Nano micelles whit were reported between 100-150 nm. On the other hands, in vitro drug release studies indicate that Nano micelles has been degraded and the drug released of Nano micelles into a simulated solution in simulated early-time periods. Also results display decreasing stability structure of Nano micelles (EPO-Gemini) against temperature. In conclusion, the obtained results proposed that Gemini nanoparticles can bind to EPO as a Nano carrier with smooth surface micelles and size of between 100-150 nm. So, Gemini cationic surfactant can be considered as a candidate for drug delivery (EPO).

Keywords: Erythropoietin, Blood-brain barrier, Gemini, DLS, TEM, Drug release

Increase of Casein Hydrophobicity and its Effect on Surface Activity

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ABSTRACT

The major part of the proteins in the milk of the cow and the camel are caseins. Caseins are the most important group of milk phosphoryl proteins, and their relative proportions in most mammals are generally higher than serum milk proteins. Structural indicators of caseins are open and flexible buildings. Their three-dimensional structure varies in terms of temperature, pH and environmental conditions. In fact, their structure results in foaming properties, high resistance to heat and formation of gel-like networks. A great attempt has been made to define the hydrophobicity of proteins due to the importance of hydrophobic interactions for their stability, compatibility and performance. However, effective hydrophobicity, that is, the actual surface hydrophobicity, should be more important in justifying the function of the protein. A lot of hydrophobic groups would be exposed at the molecular surface as protein heating proceeds. In this work we attempt to report the physical parameters relating to interfacial tension and emulsifying activity of beta-casein. There is good correlation between emulsifying activity and interfacial tension for casein. The more hydrophobicity relates to the decrease of interfacial tension and increase the activity of emulsion for beta casein.

Keywords: Effective hydrophobicity, Fluorescence probe, Surface activity, Casein

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Experimental Analyses of R12C Mutant αA-crystallin Aggregation and Chaperone Activity in the Presence of Calcium: The Mechanistic Interpretation of Diabetes-induced Hypercalcemia and Cataractogenesis

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ABSTRACT

Calcium level increases significantly in the eye lenses of diabetic patients. This is due to the impairment in the proper membrane influx/efflux of calcium ions occurring as a result of oxidative environment produced by chronic hyperglycemia in diabetes. Therefore, a large amount of calcium ion will accumulate in the intra cellular of the lenticular tissues, inducing different pathogenic reactions in eye lenses which ultimately result in development of cataract diseases. In our study, we evaluated the impact of calcium ion on structure, aggregation propensity and chaperon-like activity of R12C mutant αA-crystallins, using different spectroscopic assessments and SDS-PAGE analysis. A significant reduction in chaperone activity of the mutant αA-crystallin was observed in the presence of calcium ion. Moreover, R12C mutant αA-crystallin demonstrated significant propensity for disulfide-mediated dimerization and aggregation in the presence of calcium, particularly at the elevated temperature. The elevation of calcium levels may also result in activation of calpain which subsequently induced partial hydrolysis of lens crystallins, resulting in protein kinase C inhibition and subsequent increment of the reactive oxygen species level in the lenticular tissues. All these pathological events may contribute in development of lens opacity and cataract diseases in diabetic patients. Moreover, the raise of calcium level in eye lenses is an additional contributory factor accelerating the development of cataract diseases in patients with R12C mutation.

Keywords: Calcium, αA-crystallin, Aggregation, Chaperone activity, Diabetic cataract

Effect of Various Additives on Carboxypeptidase G2 G2 Thermal Stability

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ABSTRACT

Carboxypeptidase is a bacterial dimeric zinc-dependent exopeptidase (derived from *Pseudomonas* sp. strain RS-16) that hydrolyze the C-terminal glutamate moiety from folic acid and its analogues, such as methotrexate[1]. High-dose methotrexate has been particularly useful in the treatment of leukemias and lymphomas. Because methotrexate is largely excreted by the kidneys, this can greatly potentiate tissue damage. Toxic levels of blood methotrexate can be rapidly and effectively decreased by intravenous administration of carboxypeptidase G2. However, the utilization of carboxypeptidase G2 as therapeutics is notably restricted due to its thermal instability[2]. Therefore, in this study, we decided to examine the enzyme stability in different additives such as glycerol, sorbitol, trehalose, sucrose and fructose monohydrate. For this reason pET21a containing SUMO-carboxypeptidase G2 gene was transformed into *E.coli* strain BL21 (DE3). Carboxypeptidase G2 was then expressed in LB medium induced with 1 mM IPTG at 20 °C. The recombinant protein was over-expressed as soluble protein and SUMO tag was removed by SUMO protease. The purity of carboxypeptidase G2 and its complete excision of the SUMO tag were confirmed by SDS-PAGE. The enzyme activity was analyzed using methotrexate as a substrate.

The *K*m and V_{max} values for this enzyme, respectively was 0.012 mM and 0.016 µmol min. Irreversible thermoinactivation of carboxypeptidase G2 has been evaluated at 50 °C in the presence and absence of glycerol, sorbitol, and sucrose. Among examined additives, sorbitol had the most thermostabilizing effect.

Keywords: Carboxypeptidase G2, SUMO tag, Expression, Thermal stability, Trehalose

Inhibitory Effect of Apigenin on TGFβ Receptor II Using Bioinformatics Analysis

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ABSTRACT

Transforming growth factor beta receptor II (TGFβRII) is a multifunctional regulatory polypeptide that controls many cellular functions including cellular proliferation, differentiation, migration, apoptosis, angiogenesis and survival[1]. Therefore, blocking the signaling pathway related to this receptor could be a prominent manner to inhibit cancer cells. Many compounds have been investigated recently and flavonoid compounds are found to be more efficient[2]. The aim of this study was to find a potent compound to inhibit the receptor using molecular docking and simulation approaches. Six compounds (apigenin, hesperetin, epigulactochin gallate, nobilitin and K3 vitamin) were chosen and docked with ectodomain of TGFβRII using AutoDock 4.2. Due to the best binding energy, the best receptor-ligand complex file was chosen as a candidate for simulation. The simulation was carried out using GROMACS package 5.1.1 with Anmber99sb force field for 100 ns. To equilibrate the system at a constant temperature and pressure at 300 K and 1 bar, the NVT and NPT were performed for 50 ps and 80 ps, respectively. Among mentioned compounds, apigenin showed the best binding energy of -7.4 kcal mol^{-1} with nine number of structures in this binding energy. The Root Mean Square Deviation (RMSD) and Fluctuation (RMSF), gyrate diameter, H-bond and secondary structure have been analyzed for TGFβRII and TGFβRII-apigenin complex. Results revealed that apigenin made the TGFβRII more stable. The secondary structure of TGFβRII-apigenin complex was increased leading to a higher stability. These data suggest that apigenin may be a promising candidate as a drug inhibitor for TGFβRII.

Keywords: TGFβRII, Flavonoid compounds, Apigenin, Docking, Simulation

Atomic force Microscopic Characterization of Aloe Vera/ garphene Oxide Based Electrospun Nanofibers

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ABSTRACT

There has been increasing interest in polymeric fibers for biomedical and biotechnological applications. Nanofibrous structures developed by electrospinning technology provide suitable extracellular matrix for regenerative medicine. Aloe vera is one of the medicinal herbs with a well-established spectrum of wound healing, antimicrobial and anti-inflammatory property. The purpose of the present work was to combine the biological properties of Aloe vera and the advantages of electrospun nanofibers. Also, garphene oxide was produced by the modified Hummers method and was then dispersed in water by sonication for about 2 h. It was synthesized from

natural graphite by the modified Hummers method. The samples were then air dried under ambient lab conditions. With amounts of 6% w/v for total concentration of solid powder of polyvinylpyrrolidone (PVP) and Aloe vera in the solution; 30% w/w of Aloe vera with respect to total concentration; 18 kV for applied voltage. After completely dissolving PVP in Ethanol: DMF: H2O (45:45:10) Aloe vera powder was dissolved in the solution. Stable graphene oxide nanosheets 1.5% w/w with respect to total concentration were prepared by adding these particles to Ethanol: DMF: H2O followed by sonication. The morphology of the resulting PVP/Aloe vera/graphene oxide electrospun nanofibers was analyzed using Atomic force microscope (AFM).

Keywords: Electrospun nanofibers, Garphene oxide, Atomic force microscope, Polyvinylpyrrolidone (PVP)

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Spectroscopic Studies of the Nteraction between Maltose and Trypsin

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ABSTRACT

Bovine trypsin is one of the most important enzymes which have many applications. This enzyme is a soluble globular protein that is composed of 223 residues. Disaccharides in the human diet, especially in fruits and vegetables are extraordinary sugars. The great attraction of disaccharides toward digestive proteases in various investigations have pointed out that the interaction of intestinal proteases with disaccharides is considerable. The conformation and activity changes were studied by various experimental and computational methods. UV-Vis spectroscopy results indicate an interaction between maltose and trypsin and forms a protein-ligand complex with certain new conformation. The fluorescence emission spectrum was recorded in the range of 295-400 nm upon excitation at 290 nm. As revealed by the data, trypsin had strong fluorescence with an emission peak at 335 nm due to its Trp residue. When maltose was added into the trypsin solution, the intrinsic fluorescence intensity of trypsin decreases regularly and a small red shift is seen by increasing of maltose concentration. These results suggest that maltose interact with trypsin and cause quench its intrinsic fluorescence. The activity of Trypsin was decreased in the presence of maltose. The inhibition of trypsin by maltose was mixed. Molecular docking results indicate the presence of one binding site with a negative value for the Gibbs free energy. Dynamic simulation and CD spectroscopy results present that the complex (Try-Mal) become more stable than trypsin alone. generally, maltose act as an inhibitor and dynamic quencher that can change the structure of trypsin.

K**eywords:** Maltose, Trypsin, Circular dichroism, Fluorescence intensity, Molecular dynamic simulation

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Effect of Chitosan on the Fibrinogen Structure and Conformation

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ABSTRACT

Chitosan is a beneficial biopolymer that recognized as functional biomaterials because of biocompatibility, biodegradability and non-toxicity. Chitosan that carry out *in vivo* would contact with blood components like fibrinogen. Fibrinogen is a crucial clotting protein that plays an important role in the anticoagulant control in controlling bleeding and accelerating the process of clotting and repairing the skin at the wound site. Also chitosan with antimicrobial and wound-healing effect is used for wound dressing for accelerating the wound healing. So the interaction study of chitosan with fibrinogen and investigation on the structural change of protein to determine the appropriate function of fibrinogen for wound healing applications can be important. The present study was aimed to investigate the structural change of fibrinogen by using fluorescence and circular dichroism spectroscopic techniques. As a result of CD spectroscopy, the conformation and secondary structure of fibrinogen are changed by chitosan. The fluorescence study shows that addition of chitosan to fibrinogen give rise to form complex and cause intensity reduction. This result is due to the coating of tryptophan in the hydrophobic part of the protein by chitosan. These conclusion provide an important vision into the determining the sufficiency and safety of the chitosan for biomedical applications.

Keywords: Chitosan, Fibrinogen, Conformation, Biopolymer

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Esterification of Chrysin with n-3 Poly Unsaturated Fatty Acids Pproposed as Stronger Innovative Tyrosinase Inhibitor

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ABSTRACT

The esreification chrysin with n-3 poly unsaturated fatty acids (complex I) resulted to design of new MT inhibitor. Thermodynamic parameters of enzyme including melting point (T_m) and ΔG values also obtained from thermal and chemical denaturation curves. Complex I showed a competitive inhibitory effect on mushroom tyrosinase with a Ki of 0.45. The T_m values were calculated 328.6, 322.4 K and ΔG values of 62.8, 52.9 KJ.mol^{-1} were recorded for sole enzyme and its interaction with complex I, respectively. The intrinsic and extrinsic fluorescence techniques, showed structural instability of enzyme in concomitance with reduction in regular secondary structure acquired using CD spectrometry. Our data clearly proved that new derivative showed stronger inhibitory effect than the separate compounds. The Molecular docking analysis showed that the interaction between enzyme and complex I obeyed from hydrophobic nature.

Keywords: Mushroom Tyrosinase, Chrysin, Fatty acid, Molecular docking, Inhibition

The Effect of Glyphosate on the Fibrillation Mechanism of Bovine Serum Albumin

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Aptamers Can Monitor Ionization Radiation

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ABSTRACT

Ionization radiation, even at low doses, causes various diseases and is one of the main reasons for many kinds of cancers. Therefore, the detection and control of low doses of ionizing radiation are very important in nuclear medicine, radiation protection, and environmental problem. Gold nanoparticles are one of the most stable metal nanoparticles which theirs colour variation depends on size, due to the localized resonance surface plasmon. This colour change, which is in the visible region and change from red to blue range, has greatly favourable to the use of gold nanoparticles for colour-based diagnosis. Aptamers are synthetic single-strand sequences of oligonucleotides that produced by so-called SELEX process. The interaction of aptamers with gold nanoparticles improves electrostatic repulsion between nanoparticles and enhance theirs colloidal stability. The binding of aptamer to the target molecule with high specificity and affinity changes the stability of gold nanoparticles and then changes the colour of the colloidal solution. In this study, the effect of very low doses of gamma radiation with ^{60}Co source on the plasmonic behavior of gold nanoparticles protected by aptamers was investigated. The results show that the presence of radiation reduces the colour intensity of the Gold

ABSTRACT

Glyphosate, the active ingredient of some commercial herbicides, has been the most widely used herbicide in worldwide agricultural crop productions. Glyphosate inhibits the biosynthesis of aromatic amino acids (i.e., phenylalanine, tyrosine, and tryptophan) and leads to several metabolic disturbances, including interruption of protein production, secondary product biosynthesis, and a general metabolic disruption of the phenylpropanoid pathway owing to reduction in the biosynthesis of aromatic amino acids. Protein aggregation is impacted by many factors including temperature, pH, and the presence of surfactants, electrolytes, and metal ions. Fibrillation generally develops under conditions in which the protein is in a partially destabilized or misfolded/unfolded statecompeting with the normal folding pathways.Bovine serum albumin is an important transport protein of the blood and its aggregation/fibrillation would adversely affect its transport ability leading to metabolic disorder. In this report the qualitative and quantitative aspects of the effect of glyphosateon heat induced aggregation/fibrillation of BSA at physiological pH (pH 7.4) have been studied employing a combination of ultraviolet-visible fluorescence spectroscopy, differential scanning calorimetry (DSC), dynamic light scattering (DLS) and atomic force microscopy (AFM).The objectives of this study were to examine the impacts of glyphosate applications on fibrillation pathway of bovine serum albumin and its impacts on metabolism of animals and end users' health, using meat as a food.

Keywords: Glyphosate, Bovine serum albumin, Fibrillation, Aggregation

nanoparticles solution and therefore, can simply detect the ionizing radiation.

Keywords: Radiation detection, Gold nanoparticles, Plasmonic, Aptamer, Gamma ray

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Determination of the Effect of Lawsone on Catalase Activity

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ABSTRACT

Catalase is one of the antioxidant enzymes that converts hydrogen peroxide into water and molecular oxygen and protects cells from oxidative damage. Studies have shown that inhibition of this enzyme can help to treat Alzheimer's and cancer. On the other hand, catalase-positive pathogens produce catalase to inactivate peroxide radicals and survive in the host's environment .Henna is used as an herbal medicine and decreases growth of tumors, inflammation and relieve pain. Lawsone is one of the hennas derivatives which is responsible for the red-orange color of the henna powder. Lawsone is used for treatment of dermatitis and acts against microbes and bacteria such as staphylococcus aureus. Due to antimicrobial and antioxidant properties of lawsone, it is used by the pharmaceutical industries. In this study, activity of catalase in the presence and absence of lawsone studied by uv-visible spectroscopy and binding parameters estimated using molecular docking. Results showed that lawsone inhibits the activity of bovin liver catalase after two minutes of preincubation. The inhibition was non-competitive and decreasing of V_{max} was observed as a result of lawsone concentration rise while K_m was constant. Also the result of molecular docking demonstrated that hydrophobic interactions and one hydrogen bond play the main role in the binding of lawsone to catalase and the optimum binding energy obtained -5.54 kcal mol^{-1} which indicate that it has a good interaction with catalase. Therefore, binding of lawsone to catalase enzyme changes its catalytic activity that may play an important role in the antimicrobial activity of henna extract.

Keywords: Catalase activity, Lawsone, Non-competitive inhibition

A Biophysical Study on the Interaction Between Alginate and Fibrinogen

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ABSTRACT

Alginate is a natural polysaccharide present primarily in brown seaweed and also in bacteria. It is widely used in medicine and pharmacy because of its properties such as gelling, stabilization of dispersions, biocompatibility and antibacterial effects. These features have led us to investigate the interaction of alginate with fibrinogen for use in the wound healing. Fibrinogen has a coagulation property that plays a significant role in the wound healing. The objective of this study was to investigation on the structural change of fibrinogen in the absence and presence of alginate to determine the appropriate function of this protein and fundamental evidence for determining the sufficiency and safety of the alginate. We used fluorescence and circular dichroism (CD) spectroscopic techniques. We found that the addition of alginate to fibrinogen leads to formation of a complex, which reduced the fluorescence intensity, due to the fact that alginates are

located in the hydrophobic domain of the protein and coating of tryptophan in the hydrophobic domain of fibrinogen indicating the interaction of alginate with fibrinogen. The results of CD spectroscopy also showed that the second structures of the protein have been altered by the formation of the complex by alginate.

Keyword: Alginate, Fibrinogen, Circular Dichroism Spectroscopy, Fluorescence Spectroscopy

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Different Fibrillation Behaviors of Lipase and Its Mutants

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ABSTRACT

These fibrils have been the goal of many studies due to existence in neurodegenerative disease such as Parkinson's, Alzheimer's, and Huntington's and over expression of recombinant protein in bacteria host. The novel lipase that was isolated from a local Pseudomonas sp. was cloned and expressed in E.coli. This lipase forms fibrillar structure rapidly in physiological condition in the absence of urea or any denaturants. This feature of lipase makes it a good candidate for fibrils studies. Glutamic acid residues at position 171 and 28 of N-terminus of this protein were opted for investigation of fibril formation. The mutants with single point mutation in glutamic acid residue and deletion in the first 28 residue were cloned and express in E.coli. These proteins were purified using nickel agarose column and their fibrillation characterization were compared using Congo red assay and thioflavin T fluorescent assay with the native one. This comparison shows although none of the proteins have activity after purification in the absence of denaturants but the behavior of their fibrillation is really different. The mutant with single point mutation forms fibrillar structure with urea dilution but the amounts of it decreased considerably. In this work the mechanism of Lipase fibrillation is suggested.

Keywords: Fibrillation, Lipase, ThT assay, Recombinant protein production

Compactness of Plasmid DNA in complexation with Urethane Cationic Gemini Surfactants

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ABSTRACT

Gemini surfactants are a series of surfactants composed of two hydrophobic chains and two polar head groups linked by a spacer. They have lower CMC and krafft point values, higher solubilization power, better wetting and foaming abilities compared with conventional surfactants. Gemini surfactants can be categorized into different sub-classes depending on the variation of three structural elements *i.e.* hydrocarbon chains, head groups and the spacer. These surfactants have different applications in industries, such as synthesis of mesoporous materials, electrode surface modification in electrochemistry and so on. In addition, some research has been conducted on the use of these gemini surfactants as gene and/or drug carriers. In this research, nanoparticles are synthesized from cationic gemini surfactants having 6 methylene group (C_6) as a spacer. The binding of these surfactants with plasmid DNA (pDNA) is studied in various surfactant to pDNA molar ratios. Gel retardation assay is used to confirm the

formation of pDNA-gemini surfactant complexes. Results indicate that gemini surfactants neutralize the negative charge of pDNA even at low concentrations. Furthermore, stable complexes are formed at higher concentrations of surfactants which cause complete retardation in gel electrophoresis. In other words, these gemini surfactants are able to compact the pDNA and then can be considered as effective pDNA nano-carriers.

Keywords: Gemini surfactant, DNA binding, pDNA nano-carrier, Charge neutralization

Chicken Egg white Lysozyme Structural and Functional Changes in its Interaction with Different Size Super Paramagnetic Nanoparticles (SPION)

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ABSTRACT

Lysozyme is an antibacterial enzyme and a part of the innate immune system in animals. This protein includes on polypeptide chain with 14.3 kD weight and 129 amino acids and five helical structure, which have important roles in lysozyme activity. The amino acid sequence and 3D structure of Hen Egg Withe Lysozyme had known in 1965, so its structure, folding and stability have been well known and investigated till now. On the other hand, accessibility, small size and high solubility in aqueous environment makes HEW-Lysozyme a suitable model protein in structural and functional study of proteins. Superparamagnetic iron oxide nanoparticles (SPIONs) with Fe_3O_4 molecular composition are taken into consideration in protein interactions and drug delivery. In attention to importance of SPIONs in nanomedicine and drug delivery systems, investigation in interaction between SPION and a model protein and structural and functional changes could be helpful. SPIONs with 20, 50 and 100 nm size were chosen. UV-visible spectroscopy study showed a protein-nanoparticle interaction. Circular dichroism was used in order to measure changes in the secondary structure of lysozyme in interaction with SPIONs and showed a remarkable decrease in helical structure of the protein. Activity and enzymatic properties of SPION bonded lysozyme changed due to helical decrease.

Keywords: Lysozyme, protein structure, protein function, Nano-Bio interaction, SPION

Preparation and Crystallization of Amorphous Calcium Carbonate in Solution

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ABSTRACT

Calcium carbonate ($CaCO_3$) is one of the most abundant minerals. It precipitates from aqueous solution enabling three anhydrous polymorphs (calcite, aragonite, and vaterite), two hydrated forms (hex hydrate ikaite and monohydrate) and an amorphous phase. The non-crystalline forms of calcium carbonate are known as amorphous calcium carbonates (ACCs). Studies of ACCs show that ACCs play a crucial role as transient precursors to formation of calcite or aragonite (a crystalline form of

$CaCO_3$) [1,2]. The distribution of ACCs in biological systems as well as the role they play in calcium carbonate bio mineralization must not be underestimated. The synthesis of inorganic-organic layers by bio mineralization methods has become interest to scientists and researchers. In this work we have experienced solutions of amorphous calcium carbonates and Polyethylene glycol (PEG). Many parameters intervene in the precipitation of the calcium carbonate crystal which is reviewed in order to optimize of effective parameters required for specific types of product. Variables that affect the precipitation process have been experimented such as time and concentration. In this work, microscopic and spectroscopic figures have been obtained from control and sample. The main changes are regarding as particle sizes and cubic morphology of particles .It seems that time has more influence on the change of morphology.

Keywords: Amorphous calcium carbonate (ACC), Crystallization, Polyethylene glycol (PEG), Spectroscopy

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The Interaction of L-Cys with α-Chymotrypsin: Molecular Dynamic Simulation and Spectroscopic Methods

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ABSTRACT

L-Cysteine (L-Cys) is one of 20 α-amino acids, which connected by peptide and disulfide bonds in proteins and polypeptides. The L-Cys plays a very considerable role in stabilization of protein structure at a higher level due to disulfide bridges. The interaction of small chemical molecules, such as drugs to proteins has been very much considered in recent years. Distinguishing the thermodynamic and kinetic properties of α-Chymotrypsin (α-Chy) helps us for understanding this protein. For investigating the effect of L-Cys on structure and activity of α-Chy, spectroscopic and computational approaches were used. The UV–Vis results are revealed that the most absorption peaks were found at 260–300 nm due to Trp residues. Hyperchromism shift was seen in the presence of L-Cys. This was because of the forming of the ground state complex between α-Chy and L-Cys. Static quenching was seen by emission intensity changes. The more polar environment for Trp residue was recommended by the fluorescence quenching. The secondary structure alterations were slight. A reduce in the content of β-sheet structure and an increase in the a-helix were shown. Kinetic parameters display that L-Cys inhibited the activity of the enzyme by a mixed mod. Molecular docking results show a negative value for the Gibbs free energy of the binding of L-Cys to α-Chy with hydrophobic interactions. The molecular dynamic simulation revealed α-Chy becomes more stable in the presence of L-Cys.

Keywords: L-Cysteine, α-Chymotrypsin, Circular dichroism, Fluorescence quenching, Molecular dynamic simulation

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Effect of Chitosan on the Fibrinogen Structure and Conformation

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ABSTRACT

Chitosan is a beneficial biopolymer that recognized as functional biomaterials because of biocompatibility, biodegradability and non-toxicity. Chitosan that carry out *in vivo* would contact with blood components like fibrinogen. Fibrinogen is a crucial clotting protein that plays an important role in the anticoagulant control in controlling bleeding and accelerating the process of clotting and repairing the skin at the wound site. Also chitosan with antimicrobial and wound-healing effect is used for wound dressing for accelerating the wound healing. So the interaction study of chitosan with fibrinogen and investigation on the structural change of protein to determine the appropriate function of fibrinogen for wound healing applications can be important. The present study was aimed to investigate the structural change of fibrinogen by using fluorescence and circular dichroism spectroscopic techniques. As a result of CD spectroscopy, the conformation and secondary structure of fibrinogen are changed by chitosan. The fluorescence study shows that addition of chitosan to fibrinogen give rise to form complex and cause intensity reduction. This result is due to the coating of tryptophan in the hydrophobic part of the protein by chitosan. These conclusion provide an important vision into the determining the sufficiency and safety of the chitosan for biomedical applications.

Keywords: Chitosan, Fibrinogen, Conformation, Biopolymer

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Binding of Maltose to Human Serum Albumin: A Multi-Spectroscopic Approach and Molecular Dynamic Simulation

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ABSTRACT

Human Serum Albumin is the most vital carrierprotein which is responsible for 80% ofblood pressure in blood plasma that is synthesized inthe liver and the main role in the transport and constituents ofthe circulatory system.This protein is a mono polypeptide chain with 585 residues.Maltose is present in most herbal and food product. In the present study the interaction between maltose and HSA were investigated in vitro under simulative physiological conditions (pH = 7) using many approach such asFluorescence spectroscopy, UV-Vis absorption, Molecular dynamics simulations and Molecular docking. Fluorescence spectroscopy analysis showed thatquenching mechanism of HSA was dynamic. The result of UV-Vis absorption demonstrated that HSA has a maxima absorbance peak at 278 nm due to Trp and Tyr residues. Themaxima absorption was decreased with increasing the maltose concentration. The Far-UV CD data showed that the maltose induced a change in the proportion of secondary structure.Spectrofluorometric and docking study obviously showed that the binding between maltose and HSA was essentially driven by the non-covalent interaction consist of hydrophobic interactions.This binding has a negative value of the Gibbs free energy.

Keywords: Human Serum Albumin, Fluorescence spectroscopy, UV-Vis absorption, Molecular dynamics simulations and Molecular docking

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Stabilization of Alpha-lipoic Acid by Complex Formation with Human Serum Albumin

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ABSTRACT

Alpha lipoic acid (ALA) is produced in the human body and applied as an antioxidant drug in treatment of various diseases such as alcoholic liver disease, glaucoma, atherosclerosis, insulin resistance, neuropathy, neurodegenerative diseases and ischemia-reperfusion injury, diabetes and HIV. Currently, ALA is used as a dietary supplement. However, due to its poor water solubility and the instability against oxidation and thermal process, its application has been hampered. The distorted five membered ring of dithiolane may allow ALA to polymerize, especially during the thermal process at a temperature above its melting range (48-50 °C). The polymerization and oxidative degradation of ALA result in the loss of its bioactivity and formation of unpleasant sulfurous odor. The elimination rate of ALA with a biological half-life of less than 30 min hinders its clinical application. The thermal and oxidative stability, photochemical stability of alpha-lipoic acid (ALA) in aqueous dispersions were compared with complex human serum albumin. In the current study, the antioxidant activity of α-lipoic acid, human serum albumin (HSA), and the complex HSA-ALA were determined using ABTS* (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) radical reaction scavenging. The results indicated that ALA complex with HSA was most stable against digestion. The HSA was an effective protecting agent for ALA in aqueous media, as well as a suitable delivery carrier for ALA in digestive tract.

Keywords: Alpha lipoic acid (α-LA), Antioxidant, Scavenging test, Reduced

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Changes in the Molecular Weight and pH Isoelectric of Proteins in a Malignant Tumor of Human Glioma

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ABSTRACT

Proteins with known pH isoelectric points (pI) and native molecular weight (MW), but pI is more specific to MW. Isoelectric point is mean. The pH at which a particular molecule carries no net electrical charge or is electrically neutral in the statistical Previous research shows that even conserved proteins subjected to strong selection constraints follow the global trend in the pi distribution. Gliomas are the most frequent primary brain tumors and include a variety of different histological tumor type and malignancy grades. Glioma tumors are derived from glial cells and are sub-divided into astrocytoma, oligodendroglioma and oligoastrocytoma. We extracted proteins of tumor and normal brain tissues and then evaluated the protein purity by Bradford test. In this study, we separated proteins by Two-Dimensional Gel Electrophoresis method and the spots were then analyzed and compared using statistical data and specific software, SDS-PAGE gels were scanned using scanner Densitometer GS-800 (BioRad) scanner at 600 dpi in tagged image file format (TIFF). Spots were identified by pH isoelectric, molecular weights and data banks. The molecular weight and isoelectric pH of each of the proteins (G protein beta subunit, GBN1 protein, mutant beta-actin and alpha-tubulin) compared to control independently. Our results showed that the pI and MW of proteins in the tumor tissue has changed. The distribution

of the isoelectric point of proteins in a protein is universal for all organisms.

Keywords: pH Isoelectric, Molecular Weight, Protein, Glioma

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A Study on the Interaction of Fe_3O_4 Nano Particles with Bovine Beta-casein

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ABSTRACT

Beta-casein, a major milk protein, is amphiphilic and self-associates into micelles in aqueous solutions. In the present study, the effects of, Fe_3O_4 nano particles on the structure and function of β-casein were investigated using spectroscopic method of fluorescence at two temperatures of 25 and 37 °C. The resulted data from intrinsic fluorescence spectra of protein indicated that Fe_3O_4 nano particles can quench the fluorescence intensity of β-casein *via* a static mechanism of fluorescence quenching. Analysis of quenching data have represented that there is one binding site on β-casein for binding of Fe_3O_4 nano particles at both temperatures according to the Stern-Volmer curve of protein analyzing. As a result, it can be concluded that Fe_3O_4 nano particles can bind to the carrier protein of β-casein and change the structures of protein.

Key words: Fe_3O_4 nano particles, β-casein, Fluorescence, Binding site

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Evaluation of Antioxidant Activity and Inhibition Capability of α-Glucosidase of Aqueous Extracts of *Malva Neglecta* and *Althaea Officinalis*

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ABSTRACT

Medicinal herbs are rich sources of bioactive compounds that can retard absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes and is one of the therapeutic approaches to decrease the postprandial hyperglycemia. The objective of this research was therefore to evaluate the antiradical activity and alpha-glucosidase inhibitory activity of different concentrations (6.25, 12.5, 25, 50 and 100 mg/ml) of the ethanolic extract of *Malva neglecta* and *Althaea officinalis***.** Results revealed that increasing the concentration of ethanolic extract of *Malva neglecta* and *Althaea officinalis* yielded a higher antiradical activity. For antiradical activity, IC50 values of the ethanolic extract of *Malva neglecta* and *Althaea officinalis* were calculated as 52.90 and 68.00 mg/ml, respectively, that represents the ethanolic extract of Malva neglecta has more antiradical activity. The results showed that increasing the concentration of ethanolic extract of Malva neglecta and Althaea officinalis caused significant increment in the alpha-glucosidase inhibitory activity. Data showed that alpha-glucosidase inhibitory activity by the ethanolic extract of Malva neglecta (IC50 = 76.17 mg ml^{-1}) was significantly higher than the ethanolic extract of Althaea officinalis (IC50 = 304.12 mg ml^{-1}). Generally the

results of this research showed that ethanolic extracts of Malva neglecta and Althaea officinalis due to its antioxidant properties can be used as substitutes for chemical food additives. Also remarkable inhibition of the alpha-glucosidase makes them appropriate candidates in treatment of diabetic patients.

Keywords: Antioxidant effect, Alpha-glucosidase, *Malva neglecta*, *Althaea officinalis*

Covalent Immobilization of Horseradish Peroxidase on Functionalized Reduced Graphene Oxide and Biodegradation of High Phenol Concentration

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ABSTRACT

Horseradish peroxidase (HRP) (EC 1.11.1.7) is an oxidoreductase enzyme that oxidases a variety of organic and inorganic compounds. The HRP is normally applied to catalyze the oxidation of substrates such as phenols and aromatic amines by H_2O_2. The HRP was immobilized onto modified reduced graphene oxide (RGO) nanoparticles (NPs) through covalent immobilization. In this study, we attempted to immobilize HRP on RGO functionalized NPs to be used in removing phenol from wastewater. The residual phenol compound in supernatants was measured using a colorimetric method with potassium ferricyanide and 4-aminoantipyrine, and for colorimetric assay, the absorbance was measured at 510 nm. The calibration curve was plotted based on the standard phenol concentration with the initial reaction catalytic rate. Absorbance values were converted to the concentrations of phenol compound by using the calibration curve provided ([phenol] = (Abs510 + 0.0952) /3139.1, R^2 = 0.98). The efficiency of the free/immobilized HRP and NPs were examined to remove phenol compound with the concentration 2500 mg l^{-1} in the aqueous solution. For comparison, the time for phenol degradation was set to 40 min. For free HRP, the removal efficiency lastly reached about 50%, the removal efficiency reached 100%, when immobilized HRP was applied. This result suggested that the immobilization through covalent bonding protected effectively the HRP against inactivation during the biodegradation reaction.
The graphs of removal efficiency for the alone NPs and the summation of the NPs and free HRP were also obtained separately. As a result, the immobilization through covalent bonding exhibited successfully a synergetic effect between the NPs and HRP. Additionally, it was significant to note that shorter degradation time needed, provided that HRP was immobilized on the NPs.

Keywords: Horseradish peroxidase, Covalent immobilization, Reduced graphene oxide, Phenol removal, Synergetic effect

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Investigation of Antibacterial Activity of Cathelicidin Using Molecular Dynamics Simulation

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ABSTRACT

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Antimicrobial peptides (AMPs) are essential components of innate immunity system that can be found in most living organisms. These small cationic peptides have indicated direct antimicrobial activity against various microorganisms such as bacteria, viruses, fungi and parasites. Nowadays AMPs are so important because they have been considered as potential replacement agents to conventional antibiotics due to their rarely antimicrobial resistance. One well-known AMP is Cathelicidin which is expressed in epithelial cells and in leukocytes such as monocytes, neutrophils, T cells, NK cells, and B cells. Cathelicidin is small, cationic peptide that possesses broad-spectrum antimicrobial activity. LL-37, the only human cathelicidin, is a 37-residue, amphipathic, helical and cationic (+6) peptide. Membrane insertion of small peptides plays important roles in antimicrobial defense. Molecular dynamics simulation technique is a useful tool to investigate this insertion. In this study, we tried to investigate the interactions between LL-37 and dipalmitoylphosphatidylcholine (DPPC) bilayer by use of Umbrella sampling (US) simulations. Our results showed that by pulling the peptide closer to the membrane, the PMF (potential of the mean force) and the free energy decreases with a remarkable value that is about $5K_BT$. Therefore comparing this energy difference with thermal flactuation (k_BT) we can conclude that LL-37 has a remarkable tendency to the membrane. In the following we are going to work on finding other parameters which are effective on antibacterial activity of cathelicidin like, the optimum direction and number of peptides.

Keywords: Antimicrobial peptides, Cathelicidin, Host defense peptide, DPPC, LL-37

Stabilizing Osmolytes' Effects on the Structure, Stability and Function of tc-Tenecteplase: A One Peptide bond Digested Form of Tenecteplase

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ABSTRACT

Organic osmolytes as chemical chaperones, are major cellular compounds that cause protein stabilization in the native form. In the present study, the possible chaperone effects of the three naturally occurring osmolytes (mannitol, sucrose, and trehalose) on the two-chain form of tenecteplase (tc-TNK), a recombinant, genetically engineered mutant tissue plasminogen activator, have been examined using circular dichroism, steady-state fluorescence, UV-Visible spectroscopy, and *in silico* experiments. The two-chain form is derived from the single-chain protein upon disruption of one peptide bond. We observed that the protein is stable up to a temperature of 64°C. Besides, it folds back to the native conformation from an unfolded state at the temperature as high as 80 °C. Monitoring change in absorption on unfolding and refolding gave a one-step thermal denaturation curve. Thermal denaturation experiments showed a slight more stabilizing effects of the three co-solvents on two-chain in comparison to that on single-chain form. Unlike single-chain tenecteplase, the two-chain form undergoes reversible denaturation which is somehow perturbed in some cases as the result of the presence of osmolytes. Very minor changes in the secondary structure and the tertiary structure were observed. The molecular dynamics simulations and comparative structural analysis of catalytic

domain of the protein in the single-chain and two-chain forms in pure water, mannitol/water, trehalose/water, and sucrose/water showed that while the stabilizing effect of the three osmolytes on tc-TNK is induced by preferential accumulation of these molecules around the nonpolar and aromatic residues (i.e., osmolytes decrease the interactions of water molecules with the hydrophobic residues of tc-TNK), the single-chain form is stabilized by preferential exclusion effect.

Keywords: Chemical chaperone, Two-chain tenecteplase (tc-TNK), Single-chain tenecteplase (sc-TNK), Tissue plasminogen activator (t-PA), Thermal unfolding, MD simulation

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Up Regulated Expression of β Actin Glioma Tumor Discovery by Tandem Mass Spectrometry

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ABSTRACT

MALDI-TOF-TOF Mass Spectrometry based analysis of the low molecular weight fraction of serum proteome allow identifying proteome profiles (signatures) that are potentially useful in detection an classification of cancer. The major objective of clinical proteomics is to identify differences between proteomes of healthy and sick persons, relations between the state of the proteome and the degree of development of the disease, and changes initiated in response to the treatment. The primary aim of clinical proteomics is identification characterization, and verification of protein biomarkers useful in the molecular diagnostics of the cancer. We extracted proteins of tumor and normal brain tissues and then evaluated the protein purity by Bradford test. In this study, we separated proteins by Two-Dimensional Gel Electrophoresis method and the spots were then analyzed and compared using statistical data and specific software, Spots were identified by pH isoelectric, molecular weights and data banks. We have determined their protein profiles using a 2D gel electrophoresis and MALDI-TOF-TOF Mass Spectrometry approach. β actin has been shown to be up-regulated in liver, gastric, colorectal, lung cancers and in melanomas. The overexpression of b actin in cancers suggests that it may have altered function in carcinogenesis, including of glioblastoma as lung cancers and melanomas are primary tumours that are responsible for the occurrence of secondary GBM. Recent studies have shown that β actin polymerization can promote cell motility, invasiveness and metastasis.

Keywords: β actin, Glioma, Proteome, MALDI-TOF-TOF

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Diabetes-induced Oxidative Stress and Cataract Development and Preventive Role of Antioxidant Defense Mechanism

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ABSTRACT

Diabetes-associated oxidative stress results in mass production of different oxidative agents in the lenticular

tissues, including hydrogen peroxide and peroxynitrite (PON), as well as the most potent glycating agent methylglyoxal (MGO). During chronic hyperglycemia, these highly reactive molecules and causative players in development of diabetic cataracts, induce important structural damages in a great variety of sensitive molecules of the lenticular tissues including crystallin proteins. On the other hand, eye lenses possess a powerful antioxidant defense mechanism, protecting various molecules against glycooxidation. The balance between oxidative/glycative agents and antioxidant defense mechanism is highly important for the protection against structural damages of eye lens proteins and other sensitive molecules of the lenticular tissues. However, during chronic hyperglycemia in diabetic patients this balance will alter in favor of the damaging molecules. The current investigation was aimed to study the effects of hydrogen peroxide, peroxynitrite and MGO on the structural and functional properties of lens crystallins using various spectroscopic methods and SDS-PAGE analysis. Modification of the lens proteins by PON and MGO led to the remarkable structural damages and crosslinking. Also, R12C and R54C mutant αA-crystallins indicated significant alteration in their structure, stability and chaperone activity upon incubation with H_2O_2 and PON, respectively. Additionally, ascorbic acid, glutathione and N-acetylcysteine revealed a significant preventive function against the structural damage of lens crystallins which induced by the reactive metabolites. Overall, our study highlights the protective functions of naturally occurring and therapeutic antioxidants against the structural damages of lens proteins which may occur during chronic hyperglycemia in diabetic patients.

Keywords: Lens crystallins, Cataract, Hyperglycemia, Chaperone activity, Antioxidants

Nanozymes as Alternative for Enzymes

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ABSTRACT

Some of nanoparticles display ‘enzyme-like’ activity and have been explored as alternatives to natural enzymes. These nanomaterials with enzyme-like characteristics are called as nanozymes. They were introduced a few decades ago. The overall benefit of artificial enzymes is being highly stable and bearing low-cost. In addition to these advantages, nanozymes in some cases even exhibit a higher catalytic property than the native enzyme. The intrinsic enzyme-like activity of nanoparticles has become a growing area of interest. They are stable against denaturing, and highly resistant to high concentrations of substrate. These advantages make them promising in various applications like biosensing, immunoassays, stem cell growth, pollutant removal, imaging equipment, targeted drug delivery, antimicrobial coatings and treatments. In this study, recent advances in the Laboratory of Bioanalysis for synthesis of two SOD mimetic nanozymes consisting of Cu-Cys complex, nano-albumin and gold nanoparticles are introduced and their analytical and environmental applications are discussed. The future of nanozyme is linked to the synthesis and application of new and effective enzyme-like nanomaterials.

Keywords: Enzyme mimic, Nanoparticles, Oxidase mimic, Peroxidase mimic, Nanozymes

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Probing the Interaction of a New Design Oxali-palladium Analogue with Human Serum Albumin: A Spectroscopic Study

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ABSTRACT

Protein-drug interactions are important in the pharmacokinetics of the drugs. Human serum albumin (HSA) is the most abundant protein fundamental of blood plasma and serves as a protein storage component. In the present study, the interaction of newly synthesized Pt(II) complex with HSA was studied using spectroscopic method of fluorescence at two temperatures of 25 and 37 °C. Analysis of the quenching mechanism via Stern-Volmer curve and determination of HSA binding parameters carried out using fluorescence data. Data analysis showed that dynamic mechanism has a main role in fluorescence quenching of has in the presence of complex. Also, the number of protein binding sites for complex indicated one binding site at two temperatures of 25 and 37 °C. According above results, we concluded that the new synthesized Pt(II) complex can bind to the blood carrier protein of HSA and change the structure of it which can be considered in design of new drugs.

Keywords: HSA, Pt complex, Fluorescence, Binding site

Curcumin Derivatives as Potential Antidiabetic Medicines: α-glucosidase and α-Amylase as the Target Enzymes

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ABSTRACT

Diabetes mellitus (DM) is a common metabolic disorder, characterizing by unusually high blood sugar level. One therapeutic strategy to reduce the postprandial hyperglycemia is delaying the absorption of glucose by inhibition of α-glucosidase (α-Gls) and α-amylase (α-Amy). The α-Gls inhibitors such as acarbose, miglitol, voglibose, and emiglitate are currently used as therapeutic medicines for the treatment of diabetic patients with postprandial hyperglycemia. However, the prolonged use of these medicines by the patients could result in development of different side effects such as stomach cramping, vomiting and diarrhea. Therefore, there is still an increased demand for the new and safer antidiabetic medicines. In this study, novel derivatives of curcumin were synthesized and their inhibitory activity against both α-Gls and α-Amy were examined spectroscopically. The curcumin derivatives indicated significant inhibitory properties against α-Gls enzyme. Also, these derivatives demonstrated a week inhibition against α-Amy which is also important in terms of their possibly reduced associated gastrointestinal side effects. The synthetic curcumin derivatives indicated significant antioxidant activity, increasing their therapeutic value when considering that oxidative stress is a pathogenic link between hyperglycemia and development of various diabetic

complications. These synthetic compounds were also revealed no significant effect against two bacterial target cells living as the microflora in human intestine. Our study suggests the synthetic curcumin derivatives as potential antidiabetic medicines.

Keywords: α-glucosidase, α-amylase, Inhibition, Antioxidant activity, Antibacterial activity

Comparison of Fluorescent and Electrochemical Techniques for Continuous Glucose Monitoring

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ABSTRACT

Continuous glucose monitoring (CGM) provides more information about shifting blood glucose levels throughout the day in comparison to the current methods and facilitates reaching optimal treatments for the diabetic patients. It has the potential for revolutionizing the care and treatment of individuals with type 1 diabetes. In continuous glucose monitoring devices, sensors are placed into the human body and measure the glucose in interstitial fluid. Most of the current CGM devices are working enzyme based on electrochemical sensing of the glucose concentration. Recently new methods had been developed which their sensing method is based on specific fluorescent indicators that measure the glucose concentrations optically. CGM devices can be used whether with insulin pumps or injections for insulin delivery. Usually, the sensor repeatedly reports glucose concentration every few minutes and transmitter wirelessly send the information to a receiver. The best part in the function of CGM devices is the continuous and real-time detection of the glucose concentration of diabetic patients, which results in better management of glucose concentration and lower amounts of HbA1c test. On the other hand, these devices can be programmed to warn patients in sleep, if their glucose concentration became very low. But these devices have some technical challenges such as the repetition of calibration for each sensor and demand on replacement of sensors after a short period of time, nearly every two weeks based on the product and producer. This problem results in the high price for usage of the devices. Continuous glucose monitoring devices have many important advantages that result in their usefulness in comparison with current devices being used for glucose monitoring. But they have some technical problems that more research and development solve them. Our previous results on electrochemical glucose detection and paper based colorimetric detection of glucose will be used to evaluate new continuous fluorometric method.

Keywords: Continuous glucose monitoring, CGM, Artificial pancreas, Diabetes

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Bioinformatics Study of the Effect of Thymus Vulgaris on Alpha-Glucosidase Enzyme Inhibition for treating Diabetes

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ABSTRACT

Background and aims**:** The natural and synthetic α-glucosidase inhibitors are involved in controlling diabetes by interfering with carbohydrates digestion. Thymus vulgaris is a species of flowering plant in the mint family

Lamiaceae rich in essential oils and antioxidant phenolic compounds such as thymol, carvacrol, linalool. The aim of this study was to investigate the inhibitory effect of the main compounds found in the thyme extract on the activity of alpha-glucosidase by molecular docking (1,2,3,4,5). Method: In this study, investigation of the binding site of the four main compounds to the active site of the enzyme, drawing chemical structure of the compounds, energy optimization, docking studies and final analysis was performed by Lig plot, DS Visualizer, AutoDock 4.2, Hyperchem, ChemDraw softwares, respectively. IRCT: 7270149. Results: Among the main compounds in the plant extract, the best result was related to alpha-glucosidase docking with carvacrol ligand, with a binding energy level of -3.24Kcal / mol. This compound has the most negative binding energy and the best interaction with amino acids available in the active site of the enzyme. The results of docking studies in this study were compared with the result of docking of the co-crystal enzyme composition. Conclusion: carvacrol is well placed in the active site of the alpha-glucosidase enzyme and provides the best interaction with amino acids in the active site. By occupying the same position as the composition of the co-crystal, the composition forms the hydrogen bonds with the Asp69, Arg442 and inhibits the enzyme.

Keywords: Alpha-glucosidase, Molecular docking, Inhibitor, Thymus vulgaris, Thymol

Comparative Studies on the Interaction of Putrescine with Acid \hosphatase by Multispectroscopic

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ABSTRACT

The effect of putrescine on the Kinetics, conformation, and dynamics of native acid phosphatases was studied by thermal stability, intrinsic fluorescence, circular dichroism (CD), uitraviolet-visible (UV-Vis) spectroscopy, and kinetic measurement, at the temperatures of 298 and 308 K. The Stern-Volmer quenching constants (K_{sv}) for the acid phosphatases-putrescine complex were obtained at two temperatures, revealing that putrescine quenched the intensity of acid phosphatases through the static mode of the quenching mechanism. The corresponding themodynamic parameters, Gibbs free-energy, enthalpy, and entropy change, showed that the binding process was spontaneous. These values and the molecular docking technique revealed that the hydrogen bonding and van der Waals forces played a major role in stabilizing the complex. CD, absorption, and fluorescence results also indicated that putrescine binding had a partial effect on acid phosphatases structure. putrescine could also influence the activity of acid phosphatases. Upon putrescine binding, the V_{max} value of the enzyme was fixed and the K_{cat}/K_m values were enhanced slightly. The T_m of the putrescine - acid phosphatases complex was enhanced probably due to the higher H-bond formation and lower surface hydrophobicity after putrescine modification, as confirmed by UV-Vis spectroscopy and fluorescence spectra. UV absorption and CD studies also indicated that the binding of putrescine to acid phosphatases had induced microenvironmental changes around the enzyme, leading to changes in its secondary structure.

Keywords: Enzymes, Putrescine, Circular dichroism, Fluorescence analyzis, Thermal stability

Bioinformatics Study of the Effect of Thymol on Inhibiting Acetylcholinesterase Enzyme Compared to Donepezil and Galantamine

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ABSTRACT

Background and aims: Alzheimer's disease is the most common cause of dementia and its major pathological symptoms include synaptic loss and neurons, astrogliosis and accumulation of protein-containing sediment. Today, one method to control the progression of Alzheimer's disease is prescribing herbal medicine, which inhibits the cholinesterase enzyme (EC 3.1.1.7). Currently, four AChE inhibitors such as Tarystyn, donepezil, rivastigmine and galantamine are potentially approved by the FDA to treat Alzheimer's disease. Thymol is a phenolic chemical compound with antibacterial properties and it is an ingredient in the active ingredient of many plants. In the present study, the efficacy of thymol on the inhibition of acetylcholinesterase enzymes compared to donepezil and galantamine has been investigated. Methods: In order to investigate the connection method of this compound, two protein crystal structures of acetylcholinesterase (4EY7, 4EY6) and AutoDock 4.2 software were used and in the final stage, the results were analyzed using three AutoDockTools, DS Visualizer and Ligplot programs.
Results: Thymol was able to bind to aminoacids in the active site of the enzyme in a manner similar to donepezil and gelantamine. The binding energy for this compound was -5.89 Kcal mol^{-1} and -5.28 Kcal mol^{-1}, which is created hydrogen bond with amino acid PHE295 and PHE338. Conclusion: Considering the high efficacy of thymol in the bioinformatics study and the formation of similar hydrogen bonds with donepzil and gelatamine, for further studies, the effect of this compound can be studied *in vitro* and *in vivo*.

Keywords: The acetylcholinesterase enzyme, Molecular docking, Thymol and Alzheimer's

Encapsulation of Daidzein by Complex Coacervation of Casein and Arabic Gum

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ABSTRACT

The application of food additives especially antioxidant compounds in food industry has increased recently to improve the quality of products and to help the consumer health. In this context, the protection of antioxidants during food processing and storage is very vital due to their instability. Different strategies have been developed for this aim. Among them, encapsulation of food additives such as essential oils, vitamins, antioxidants, and sweeteners have greatly considered. Encapsulation not only enhances the storage stability of compounds but also increases their *in vivo* accessibility *via* their gradual delivery during digestion. In the present study, for the first time, we report the encapsulation of daidzein (DZ), as one of the most important antioxidants, by complex coacervation of casein (CA) and Arabic gum (AG), as two biodegradable and natural polymers. To this aim, the best condition for complex coacervation of casein/Arabic gum was first investigated using different concentrations and mixing ratios of biopolymers as well as various pH values. Based on the results, the highest stability and production yield of coacervate obtained at a 2:1 ratio of casein/Arabic gum with an optimum pH of 4.8. DLS and zeta potential measurements of the sample showed the formation of stable colloidal nanocapsules with an average hydrodynamic diameter of 110.8 nm and zeta potential of -37.1. The efficiency of coacervates for the entrapment of daidzein was studied using UV-Vis and fluorescence spectroscopy. The results indicated that more than 60% of the antioxidant was encapsulated by the coacervates. The

freeze-dried coacervates displayed effective and controlled release of daidzein (up to 81%) over 72 h. The overall results indicated the high potential of complex coacervation of casein and Arabic gum for encapsulation and controlled release of daidzein.

Keywords: Complex coacervation, Daidzein, Casein, Arabic gum

The Comparison of the Interaction between Epirubicin Hydrochloride and Mitoxantron Hydrochloride with Calf Thymus DNA by Fluorescence Spectroscopy and Thermodynamic Parameters

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ABSTRACT

The interaction of calf thymus DNA with the Epirubicin hydrochloride and Mitoxantron hydrochloride under physiological condition was investigated by fluorescence spectroscopy. Epirubicin hydrochloride and Mitoxantron hydrochloride were purchased from sigma chemical co. The fluorescence spectroscopy was recorded on a Hittachi model F-2500 spectrophotometer (Japan). Regular decrease in fluorescence intensity of Mitoxantron hydrochloride is observed with increasing concentration of DNA, which means that DNA quenching the intrinsic fluorescence of Mitoxantron hydrochloride and also regular decrease in fluorescence intensity of DNA is observed with increasing concentration of Epirubicin hydrochloride. The binding force between a biomacromolecule and a small molecule mainly contains van der waals forces, hydrogen bonds, electrostatic force, and hydrophobic interaction. When $\Delta H < 0$ or $\Delta H \approx 0$, $\Delta S > 0$, the main binding force is electrostatic force; when $\Delta H < 0$, $\Delta S < 0$, the main binding force is van der waals force or hydrogen bond; when $\Delta H > 0$, $\Delta S > 0$, the main acting force is hydrophobic force. thermodynamic parameters obtained from vant hoff plot showed that vander walls and hydrogen bonding plays a major role in binding between Epirubicin hydrochloride and Mitoxantron hydrochloride with ct-DNA. All these results suggested that the binding mode of Epirubicin hydrochloride and Mitoxantron hydrochloride should be intercalate with the base pairs of ct-DNA.

Keywords: Epirubicin hydrochloride, Fluorescence spectroscopy, Thermodynamic parameters, Mitoxantron hydrochloride, Calf thymus DNA

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Detection of HTLV-1 Genome Based on the Fluorescence Quenching of the Graphene Oxide in Proximity of Gold Nanoparticles

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ABSTRACT

Designing efficient biosensors for early detection of viruses are important. Although, the usual techniques comprising real-time polymerase chain reaction (RT-PCR) and Enzyme-linked immunosorbent assay (ELISA) are highly selective, they are time-consuming, expensive, and need to professional operators. Nanomaterial-based biosensors can be utilized to develop biosensors. Graphene oxide (GO) denotes a two-dimensional honeycomb lattice composed of single-layer carbon atoms, which can absorb the biomolecules on its surface. Moreover, gold nanoparticles (AuNPs) with inimitable optical properties

can quench the fluorescence of the carbon nanomaterials like graphene oxide and carbon nanotube. Herein, we developed a new biosensor for long segment detection of the human T cell-lymphotropic virus 1 genome via designing proper probes and tracing the fluorescence energy transfer between graphene oxide and gold nanoparticle. The fluorescence emission of GO was quenched when probes-AuNPs were adsorbed on its surface. In order to enhance the quenching efficiency, both two probes were functionalized with AuNPs, thus high number of AuNPs were placed close to the GO sheets. In the presence of target, the probes were desorbed from the GO surface and hybridized with the target. As a result, the fluorescence emission of GO nanosheets was recovered. The limit of detection of the biosensor was determined to be around 10 pg ml^{-1}. Our results proposed that the further development may be useful to detect other viruses.

Keywords: Biosensor, Graphene oxide, Gold nanoparticles, HTLV-1

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How Exposure to the Solar Radiation Increases the Risk of Cataract Development? Human Recombinant αA-crystallin as the Target Protein

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ABSTRACT

The cumulative exposure of eye lenses to ultraviolet and visible light (UV) has been indicated as the important causative player in development of the senile cataract disease. The eye lenses contain different types of UV filters which their levels ultimately decrease with human age. Moreover, upon direct or indirect exposure to the solar radiation, various photosensitizers including riboflavin (RF) actively participate in the photo-damages and opacification of human eye lenses. All of these events increase the rate of senile cataract development particularly in the outworkers. In the current study, the photo-oxidation of human recombinant αA-crystallin in the presence of RF and upon exposure to direct sunlight was analyzed using different spectroscopic assessments and SDS-PAGE mobility shift assay. The RF-mediated photo-oxidation of αA-crystallin led to the reduction in both Trp and Tyr intensities and formation of new chromophores which have been detected by UV-Visible and fluorescence spectroscopic assessments. It should be noted that eye lenses contain many antioxidant compounds protecting them against the destructive photo-damaging molecular events. Therefore, weakness of the powerful antioxidant defense mechanism of eye lenses in diabetic patients may increase the risk of solar radiation-associated cataract development. Our study, suggest that natural lens antioxidant molecules such as glutathione, cysteine and ascorbic acid were capable to significantly prevent the RF-mediated photo-oxidative damages of human αA-crystallin.

Keywords: Cataract, Photooxidation, Human αA-crystallin, Antioxidants

The Brain N-Acetyl-Aspartate Alterations due to Emotional Stress in Cuprizone-Induced Demyelination: An *in vivo* Proton Magnetic Resonance Spectroscopy Study at 3T

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ABSTRACT

Stress is considered as an important risk factor in progression and the onset of many disorders such as multiple sclerosis. However, the alterations in the concentration of N-Acetyl-Aspartate (NAA) as a neuronal marker which is uniquely synthesized in neurons by NAA synthase and degrade in oligodendrocyte by aspartoacylase, as a result of demyelination under detrimental effects of stress are not well understood. 24 female Wistar rats (i.e. Groups 1. No-cuprizone (control), 2. No-stress, 3. Emotional stress, 4. Physical stress) were studied. Following repetitive distress induction in stress groups, cuprizone treatment was carried out for 6 weeks in order to induce demyelination in all groups except control ones. Brain relative metabolite concentrations were investigated by single voxel proton magnetic resonance spectroscopy (reporting NAA relative to total creatine (tCr)). According to ^{1}H-MRS, rats in the no-stress group indicated a reduction in NAA/ tCr ($p < 0.001$) compared to control animals. In contrast, in both stress-exposed groups, NAA/tCr ratios remarkably increased versus the exclusively cuprizone fed animals ($p < 0.001$) and the control ones ($p < 0.001$ for emotionally stressed rats and $p < 0.05$ for physical ones). Findings show that brain susceptibility and changes underlying to emotional stress in the cuprizone model of multiple sclerosis are of greatest importance. It seems that decreased level of NAA is mainly relevant to neuronal mitochondria impairments, while as a result of stress oxidative enhancement due to emotional stress exposure, oligodendrocyte may become the main victim indicated by the increased level of NAA ratio.

Keywords: Emotional stress, Physical stress, Multiple Sclerosis, Cuprizone, ^{1}H-MRS

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Structural Studies of *Carboxypeptidase a* Thermal Stabilization in the Presence of Spermidine

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ABSTRACT

Carboxypeptidase A (CPA) (IUBMB: EC 3.4.17.1) is a zinc-containing proteolytic enzyme which removes the C-terminal amino acid from a peptide chain if the C-terminal carboxylate is free. Polyamines such as spermidine (NH2(CH2)3NH-(CH2)4NH2) are low-molecular-weight aliphatic polyamines that participate in cell growth, differentiation, proliferation processes and also are involved in the biology of protein aggregation and can be used to prevent thermal aggregation. In this study, the effect of spermidine on the structure and thermal stability of CPA were investigated by the method of UV-Vis, fluorescence spectroscopy and steady-state thermal denaturation at pH 7.5. It was found that by increasing of spermidine concentration, the thermal stability of CPA was increased in the range 303-353 K in the tris-HCl buffer and pH 7.5 and midpoint temperature of thermal unfolding (T_m) of CPA in the presence of spermidine, after heat treatment, was increased with increasing the concentration of this additive. The analysis of UV-Vis indicates that by increasing concentration of spermidine, absorption was decreased. In addition, Stern−Volmer quenching constants (Ksv) for the CPA−spermidine complex were obtained at two temperatures. The results of fluorescence spectroscopic measurements suggested that spermidine has a diametrical ability to quench the intrinsic fluorescence of CPA through the static quenching procedure. The thermodynamic parameters, Gibbs free-energy (ΔG°), enthalpy (ΔH°), and entropy (ΔS°) changes, shown that

the binding process was spontaneous. These values revealed that the hydrogen bonding and van der Waals forces played a major role in stabilizing of this complex.

Keywords: Spermidine, Carboxypeptidase A, UV-Vis spectroscopy, Fluorescence spectroscopy, Thermal stability, Thermal denaturation

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A Molecular Biophysics Study of Interaction between Berberine and ct-DNA in Absence and Presence of Linker Histone

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ABSTRACT

Small molecules by binding to the histone-DNA complex, interfere with important cellular processes such as cell division and growth in cancerous cells, resulting in apoptosis. The study of the interaction of small molecules with histone-DNA complex is important for better understanding their mechanism of action and to design newer and more effective drug compounds. This treatise, the interaction of Berberine chloride (Ber) with a histone-DNA complex was investigated using different spectroscopic and molecular modeling techniques. Fluorecense quenching of ct-DNA upon interaction with Ber determine the binding of Ber to ct-DNA with Ksv = 9.46×10^7 M^{-1}. Ksv value of ct-DNA-Ber in the presence of H_1 is 3.10×10^7 M^{-1} that show the H_1 caused the reduction of binding affinity of Ber to ct-DNA. The ΔH^0 and ΔS^0 values of ct-DNA-Ber complex formation in absence and presence of H_1 determine two different behaviors in binary and ternary systems from the view point of interaction forces. In competitive emission spectrum, ethidium bromide (EB) and acridine orange (AO) as intercalator probes, by adding up Ber to ct-DNA complexes, ctDNA-EB and ctDNA-AO, the change in the emission spectra showed competition, but in the presence of histone H_1, competition between ligands and probes was not observed. Melting temperature (Tm) curve results, show that the binding mode of Ber to ctDNA in absence and presence of H1 were intercalative and groove binding respectively. The viscosity results confirm the different behavior of interaction between ctDNA and Ber in binary and ternary systems.

Keywords: DNA Interaction, Berberine chloride, Spectroscopy, UV-Visible, Ct-DNA, Intercalation, Groove binding, Linker Histone

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An Insight into the Effects of Point Mutations on Stability and Catalytic Mechanism of *Pseudomonas Aeruginosa* Exotoxin A for Immunotoxin Therapy

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ABSTRACT

Recombinant immunotoxins are a class of medicines which consist of an antibody or fragments of it fused to a toxic moiety. The most famous toxic moieties are based on Pseudomonas exotoxin A (PE), Diphtheria toxin (DT) and ricin. Denileukin diftitox is the first immunotoxin which was initially approved in US in 1999 and targets interleukin 2 (IL2) receptors and redirects its cytotoxic effects by part

of DT. Today's studies are focused mainly on PE toxins. Truncated PE has shown a significant decrease on tumor sizes. However, one of the major complications of immunotoxins in cancer therapy is immunogenicity of the toxic moiety. Different tactics have been deployed to reduce immunogenicity of PE. These tactics mainly aim to reduce the number of B-cell epitopes in the 3D structure of domain III. For this purpose, different point mutations have been designed and studies to reduce immunogenicity of the toxin. The successfully modified PE toxin had 7point mutations, R505A, R427A, R490A, R467A, D463A, R538A and R456A (the numbering is based on the residual number in secreted PE with 613 amino acids) and the toxin containing these mutations was named PEX7. Here in this study, we have applied a molecular dynamics approach to investigate the effects of point mutations on overall structure of shortened PE. Also, we have compared the results of MD simulations with CD spectroscopy and cell culture assay to get a deep insight into the catalytic mechanism of PE. For this purpose, we ran a 100ns MD simulations in Gromacs® platform and compared the catalytic activities of PE and PEX7 in an interaction with their natural target, NAD. Protein stability and secondary structure analysis were done using CD spectroscopy and MD simulations. Toxic effects (antitumor activities) on 2 cancerous cell lines (SKOV3 and MCF-7) were also examined and finally the results were confirmed by flowcytometry. The results showed that protein stability increases a bit in PEX7 rather than PE. It shows that as a medicine expressing PEX7 will be more beneficial. When NAD attaches the toxin active sites, a dramatic increase of stability happens in the active sites of both proteins. Cell culture studies showed that both toxins have maintained their catalytic activity well with a little increased IC50 for PEX7. So, PE and PEX7 both showed great toxic effects on turmeric cell lines and can have anticancer effects; but, PE is more potent because its active site is intact. On the other side, because of reduced immunogenicity of PEX7 in comparison to PE, PEX7 can have a longer time of exposure to cancerous cells. So, applying point mutations on PE will not affect its antitumor activities because PEX7 show an increased half life in comparison to intact PE.

Keywords: Immunotoxin, *Pseudomonas exotoxin A*, MD simulation, Gromacs, CD spectroscopy, Point mutation

Evaluation of Anticancer Potential of α, ά-Me_2-salen, N, N´-Ethylenebis (α methylsalicylideneiminate) Shiff Base Derivatives Based on Histone Deacetylases-ligand Docking

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ABSTRACT

In last decades evaluation of the Schiff base complex as synthetic compounds with several different applications in food chemistry, dye industry, agro chemical and pharmaceuticals has received much interest in these fields. Also, Based on the literature, metal complexes of Schiff base ligands possess a variety of applications in the biological, analytical, clinical, and industrial areas In our effort towards the development of metal-based antioxidant agents, α,ά-Me_2-salen, N,N´-ethylenebis(α-methylsalicylideneiminate) Schiff base and its metal (Cu(II) ,Ni(II) ,Co(II) and Mn(II)) complexes were designed, synthesized and evaluated as histone deacetylases (HDACs) inhibitors. The in silico protein–ligand docking using AUTODOCK 4.1 was successfully performed on these compounds. Among the studied compounds, the best docking result was obtained for Ligand that showed a high inhibitory potency compared to other derivatives. In fact, this compound had the most negative ΔGbind that indicated favorable interactions and tight binding with the key amino acid residues at active site

of HDACs. The His142, His143 of HDACs were the sites for hydrogen bonding interactions with this compound.

Keywords: Synthetic compounds, Docking, Anticancer

Biosensor for Detection of Livestock Pregnancy

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ABSTRACT

More than billion dollars' turnover of livestock industry has made that very attractive from economical point of view. One of the prominent issues in livestock industry is growing the birth rate to increase daily demands for meat and milk in the society. Therefore, on time detection of pregnancy of livestock is a facilitative element for this purpose. Traditional methods for detecting the pregnancy of livestock are essentially costly and late return, for instance, ultrasonography and uterus palpitation can have potential dangers for the fetus. Progesterone is an important hormone which can be used as a biomarker to monitor the pregnancy in mammalian. In the present research we proposed a biosensor for detection of progesterone at low concentrations. We used gold nanoparticles and progesterone specific aptamer to develop a newly designed biosensor. Gold nanoparticles have strong SPR and their color changes at dispersed or aggregated conditions can report the specific interaction of aptamer-progesterone. Based on this dual behavior of gold nanoparticles an aptasensor was introduced to monitor the concentration of progesterone at nanomolar ranges.

Keywords: Biosensor, GNP, Aptamer, Pregnancy, Livestock, Progesterone

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Discovery of Natural Products Acting on Inhibition of Quorum Sensing in Bacteria by Molecular Docking Study

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ABSTRACT

Quorum sensing (QS) is a cell-cell communication system that regulates gene expression at threshold cell density and allows bacteria to response to extracellular signaling molecules (AIS). This system (QS) controls virulence in many human and plant pathogens. Antibiotics are one of the most effective treatments for bacterial infection. Because of the emergence of antibiotic-resistant bacteria, developing a novel antimicrobial agent is an important subject in scientific research. In this study, structure-based molecular docking was used in search and discovery of natural compounds acting on inhibition of QS system in bacteria. The database of more than 100 natural phenolic compounds selected based on previously reported QS inhibitor. Six receptors involved in QS system, such as: Lux R, Las I, Las R, Las A, Tra R, Pil T, were used for virtual screening, using Glide platform with using force field optimized the potential for liquid simulation (OPLS3). The Qikprop performed the calculation of ADME properties. For all molecular modeling, the Small-Molecular Drug Discovery Suite 2015-2(Schrodinger, LLC, New York, NY, 2016) was used. As results, several compounds showed good binding affinity to the targets. At least, five compounds of each target with the highest binding affinities, much more than Vitexin as positive controls, were selected as potent compounds. The computed docking score suggested that these compounds with the docking score range of -6.3 to -12.8 KJ mol^{-1} have a high tendency to inhibit QS and it seems that they can be

good candidates for the *in vitro* inhibition tests of quorum sensing.

Keywords: Natural products, Antimicrobial agent, Quorum sensing, Vitexi

The Study of Structure and Stability of *Carboxypeptidase A* in the Presence of Spermine by Spectroscopy Methods

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ABSTRACT

Carboxypeptidase A (CPA) (EC 3.4.17.1) is a zinc-containing proteolytic enzyme which removes the C-terminal amino acid from a peptide chain with the free carboxylate-terminal. Polyamines such as spermine are low-molecular-weight aliphatic polyamines, that are involved in the biology of protein aggregation. In this study, the effect of spermine on the structure and thermal stability of CPA was investigated by ultraviolet-visible (UV-vis) spectroscopy, intrinsic fluorescence and steady-state thermal stability techniques at the pH of 7.5. Forming a complex between CPA and spermine caused the decrease in the absorbance intensity of the CPA. In addition, the thermal stability (T_m) of CPA in the presence of spermine was enhanced with increasing the concentration of spermine. Spectrofluorometric results proved that with the addition of spermine to the protein solution, the emission intensity of CPA was extremely reduced. The results of fluorescence intensity changes, at two temperatures of 308 and 318 K, also suggested that spermine had a great ability to quench the intrinsic fluorescence of CPA through the quenching procedure. The thermodynamic parameters such as ΔH and ΔS were calculated to be negative, indicating that the interactions between spermine and CPA were mainly due to van der Waals forces or hydrogen bonding. The results also showed that binding of spermine to CPA could cause conformational changes in CPA.

Keywords: Spermine, Carboxypeptidase A, UV-Vis spectroscopy, Fluorescence spectroscopy, Thermal stability

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Electrochemical Detection of Serum miRNA-155 for Breast Cancer Screening

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ABSTRACT

MicroRNAs (miRNAs) are a class of short, approximately 19–25 nucleotides that regulate the expression of a wide variety of genes. miRNAs has garnered considerable attention in the field of disease biomarkers and exploited as new kinds of molecular targets for early detection and diagnosing of cancers because of their remarkable stability in blood, low cost, minimal invasiveness and their characteristic expression in different diseases. Recently, in studies that focused on the association between miRNAs and tumors, abnormal expression of miRNA-155 in patients with breast cancer has *have attracted considerable attentions*. As an oncogene, high expression of miRNA-155 was considered as a breast cancer risk factor. As a result, label-free, rapid, and sensitive detection for miRNA-155 is of great

significance. In this study, we designed a sensitive and label-free assay for detection of miRNA-155 using positively charged silver nanoparticles. Briefly, thiolated probe was immobilized through self-assembly onto a gold electrode. Then, target (miRNA-155) was hybridized to the probe. In the final step, silver nanoparticles were casted onto the modified gold electrode. Due to the oxidation of silver nanoparticles, significant electrochemical signal is produced. The designed electrochemical biosensor provided an ultrasensitive detection of miRNA-155 at very low concentrations with the detection limit of 20 zeptomoles and a wide linear range from 20-2 × 10^9 zeptomoles. Moreover, such biosensor detected miRNA-155 in serum samples with satisfactory results.

Keywords: Silver nanoparticles, miRNA-155, Breast cancer, Biosensor

Fabrication of Rapid Diagnostic Strips for Detection of Influenza Virus

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ABSTRACT

Influenza is unique among viral infections because of its propensity for seasonal epidemics and occasional pandemics, and because of the morbidity and mortality that result from its pulmonary complications. The need for a timely diagnosis, which allows for optimal use of these treatments, led to the introduction of numerous rapid diagnostic tests. Herein, we report on a lateral flow immunoassay (LFIA) for influenza A antigen using colloidal gold nanoparticles (AuNPs) as reporters. The matrix protein of influenza A virion (one of its most abundant structural proteins) was used as a model to demonstrate a performance of the LFIA. The spectroscopic properties of colloidal AuNPs showed the typical surface plasmon resonance band at 525 nm in UV-visible spectrum. Using dynamic light scattering (DLS) spectroscopy, the binding and homogeneity of golds were confirmed. The optimum pH, antibody concentration for conjugation and optimum concentration of antibodies on strips were 9, 60 µg/ml and 100 µg/ml, respectively. Under optimized conditions, the nanogold-based LFIA is capable of detecting virus as low as 256 pfu·mL-1 using a sample volume of 20 µL, within 20 min, and without interference by other proteins.

Keywords: Antibody, Gold nanoparticles, Virus, Influenza, Immunochromatography, Rapid Diagnosis

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Investigation of the Interaction between DNA and a Sample of Textile Colors by Spectroscopic Methods

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ABSTRACT

Many of the synthesized compounds in the industry, despite their various applications, have side effects which can lead to diseases in the body. DNA plays a major role in all biological processes. DNA interaction with small molecules such as pigments is important. Some of these compounds can be connected to DNA and impair its conformation and prevent the replication. In this study, in order to provide a clear mechanism for the binding of a chemical color with the physiological macromolecules, the effects of different concentrations of a textile dye, Direct yellow 42, on the calf thymus DNA was investigated using fluorescence, UV-Vis and FT-IR methods. In this study it was determined that DNA has

interaction even at very low concentrations of dye; but at high concentrations, sudden changes in the intensity of the absorption spectrum of DNA was observed at the region of 260 nm that shows the denaturation of this biomacromolecule. Calculation of thermodynamic parameters showed that the type of interaction between DNA and Direct yellow 42 is groove and the binding process is spontaneous. The results of this study can provide a new insight into the application of dyes in the industry and the risks associated with their use.

Keywords: DNA, spectroscopic methods, thermodynamic parameters, Direct yellow 42

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Study of the Simultaneous Effect of Silver Nanoparticles and 3-β-Hydroxybutyrate on DNA Glycation Process

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ABSTRACT

The unique properties and performance of nanoparticles come from a variety of characteristics, including the same size as nanoparticles and biological macromolecules. Glycation of nucleotides leads to DNA damage and genetic disorders. In recent years, the antibiotic and pharmaceutical roles of silver nanoparticles have been identified in several studies. In this study, the diabetic DNA of calf thymus was treated during 28 days in a like-physiological condition along with 3-β-hydroxybutyrate keton bodies (3BHB) in presence and absence of silver nanoparticles. Then structural changes of DNA were investigated using different spectroscopic methods. The CD results show that the structural changes of glycated DNA in the presence of 3-b-hydroxybutyrate along with silver nanoparticles is less than that of glycated DNA in the presence of only 3-beta-hydroxybutyrate. Also, the results of fluorescence spectroscopy at λex = 290 nm showed a decrease in emission fluorescence intencity (λmax 450 nm) in presence 3BHB along with silver nanoparticles. Therefore, silver nanoparticles with decrease AGEs formations can decrease the structure changes of DNA and can prevent from cleavage of DNA in diabetic conditions.

Keywords: DNA, Silver nanoparticles, 3-B-hydroxybutyrate, Glycation

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Signal Amplification Methods for Biosensing

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ABSTRACT

Highly sensitive detection of biomaterials in various areas such as clinical diagnosis and environmental protection are of importance. Many efforts have been made with the use of signal amplification techniques to very sensitive detection .Two common strategies based on using nanomaterials have been employed. The first one can be achieved using optical- and electrochemical- enhanced nanomaterials, and also employing nanomaterials as carriers of the signal-producing molecules. The second one includes older techniques such as PCR amplification, and enzyme catalysis .In this report, two electrochemical and optical biosensors based on using nanomaterials for signal

amplification performed in Bio-Analysis Lab are introduced. In the first study, the biotinylated hepatitis B surface antibody was immobilized on the streptavidin magnetic nanoparticles and used for targeting the HBsAg .By the addition of HRP conjugated with secondary antibody (HRP–HBsAb), an immunoassay sandwich was formed. Therefore, aminophenol as substrate for conjugated HRP was enzymatically changed into 3-aminophenoxazone (3-APZ), which was proportional to the HBsAg concentration and was monitored by cyclic voltammetry technique. In another work, a novel method was developed to identify human T-lymphotropic virus-1 (HTLV-1) using cadmium–tellurium quantum dots. Two probes including the biotin-labeled acceptor and NH2-reporter probes with target DNA were hybridized and a sandwich complex was constituted. The quantum dot solution was added to the sandwich complex, conjugated with the amine group of reporter probe, and emission spectra of the quantum dots were recorded .The use of new nanomaterials and molecular biology technologies is a promising method to develop new biosensors.

Keywords: Signal amplification, Sensitive detection, Nanomaterials

The Effect of Emotional Suffering on Protein-related Cognition by Influence on Intrinsic Cardiac Ganglia in Female Rats

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ABSTRACT

The fine intrinsic cardiac nervous system (ICNS) including intrinsic cardiac ganglia (ICG) has provided a complex network described as a "heart's little brain" by which many neural peptides and proteins are produced [1]. One of the ICG's main proteins produced in large quantities is M2 muscarinic receptors (M2MR) [2]. Considering the key role of M2MR [3,4]and ICG in heart performance [5,6], persistent effects of emotional suffering on ICG structures and M2MR in ICNS was investigated. 21 female Wistar rats were divided into three groups: (1) control, (2) electric foot-shocks and (3) emotional suffering (witnessing electric foot-shocks). After stress exposure in 5 successive days, serum corticosterone measurement verified the proper stress induction. Rats were then returned to their home cage for 6 weeks. In their adulthood, before animal sacrifice, the behavioral tasks were performed to assess cognitive performances of each group. Then, M2MR variation in ICNS were evaluated in emotionally suffered rats by immunohistochemistry, qPCR and western blot. According to the results of behavioral tasks, the experimental group exhibited a significant reduction in cognitive performance 6 weeks post-stress. Immunohistochemistry, qPCR and Western blot analysis for M2MR expression level represented a significant increase in ICNS of the experimental group compared to control. Moreover, heart sections of emotionally suffered animals not only displayed increased density of M2MR, but also showed a much larger cardiac ganglion. Considering major cognitive impairment in emotionally suffered rats, adolescent emotional healthcare would be an effective approach for cognitive damages prevention at older ages.

Keywords: Emotional suffering, Protein-related cognition, M2 muscarinic receptors, Cognitive impairment, Intrinsic cardiac ganglia

Lateral Flow Biosensors: Nanoparticles as Labels

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ABSTRACT

Lateral Flow Biosensors (LFBS) can be used as analytical platform with the advantages such as friendly user format, short assay time, low cost, and easy operation. However, these biosensors often are face with key challenges: low-signal intensity and poor sensitivity. Efficiency of these systems has been considerably improved by using different kinds of labels such as colored nanoparticles (NPs), luminescent material, and magnetic NPs. Two types of NPs have been designed in Bioanalysis Lab which are applicable for signal amplification in LFBSs. Once, we have developed an ultrasensitive optical biosensor for microRNA-155 (miR-155) detection. At first, the DNA probe covalently binds to the negatively charged AuNPs. Then, the miR-155 electrostatically is adsorbed onto the positively charged AuNPs surface. Finally, by mixing these AuNPs, hybridization occurs and the optical signal of the mixture give a measure to quantify the miR-155 content. So, miR-155 was monitored at detection limit of 100 aM and a wide linear range from 100 aM to 100 fM. In another experiment, a chemiluminescence label based biosensor was developed by co-immobilization of secondary antibody and luminol on AuNPs. Hepatitis B surface antigen was targeted by a primary antibody, immobilized in polystyrene wells and the secondary antibody, co-immobilized on luminol-AuNPs. After formation of the immune sandwich, the luminescence intensity was recorded in the presence of hydrogen peroxide as oxidant agent and Au^{3+} as an efficient catalyst for luminol oxidation. Hepatitis B surface antigen was detected in the linear concentration range from 0.12 to 30 ng ml^{-1} and the detection limit of 14 pg ml^{-1}.

Keywords: Lateral flow, Biosensors, Gold Nanoparticle, Labels, Luminescent

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Effect of Oxidative Stress and Free Fatty Acid on Protein Fibrillation: a Model for Metabolic Syndrome

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ABSTRACT

Metabolic diseases (MetS) including type two diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) are among the most important health threats in western societies and countries with western life style. Currently about 30% of the population in our country is affected with NAFLD which can lead to T2DM. During these diseases the free fatty acid (FFA) content of plasma is elevated which along with oxidative stress play important roles in pathogenesis of MetS. Furthermore, MetS is known to increase the chance for development of Alzheimer's disease which is largely due to the accumulation of amyloid-beta plaques in the brain. Therefore investigating the effects of metabolic syndrome conditions specially NAFLD, due to its high prevalence, on protein fibrillation is very important to understand the pathogenesis of MetS. Here we use serum albumin (SA) along with spectroscopic methods to investigate the effect of oxidative stress (H_2O_2) and FFA (palmitic acid; PA) on thermal fibrillation of SA. Our results indicated that FFA protected SA from fibrillation; this effect increased in higher concentrations of PA. Furthermore oxidative stress further decreased fibrillation of SA. These data indicates that the contribution of other factors may be necessary for pathogenic effects of FFA and oxidative stress during MetS.

Keywords: Protein fibrillation, Petabolic syndrome, Non-alcoholic fatty liver disease, Alzheimer's disease

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Application of Statistical Methods and Artificial Neural Network for Prediction of Inhibition Ability of Tyrosinase

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ABSTRACT

Tyrosinase is responsible for many biologically essential functions such as enzymatic browning in fruits, fungies, vegetables, and hyperpigmentation in human skin. Therefore, the inhibition of the tyrosinase is important in relation of agriculture. Cosmetic industry and medication due to decreasing the excessive accumulation of pigmentation resulting from the enzyme action. Nowadays, quantitative structure-activity relationship (QSAR) methods are applied in rational drug design or computer-assisted drug design to obtain essential information for designing more effective drugs. Using QSAR techniques, molecular descriptors that are important in relation to a specific activity of the molecule are selected. Then the relation between these descriptors and molecular activity is described by suitable quantitative models. Different kinds of linear and non-linear classifying models are used in QSAR studies. Also, recently artificial neural networks (ANNs) have been extensively used in QSAR studies and have been applied in numerous application areas of chemistry and pharmacy. Accordingly, first the numerous of structural descriptors associated with physico-chemical properties of 35 inhibitors derived from the thiosemicarbarzide, was created. After initial filtration of these descriptors, 39 of them remained for the QSAR study. In the present study, using non-linear BLR four structural descriptors selected and introduced as factors that affect on the function of biological inhibitory of the tyrosinase. These descriptors were 1 -nCaR2- R3e^{+} 3- H3u 4- E1s. For more examine on accuracy of results obtaining by the BLR, also check the power of ANN in the structure and function relationships, of this model (ANN) was used. For this propose, total of seven different inputs in the ANN model created. The best model and the network is created as 3-11-1. in addition, evaluating index value of this network, mean FC, FAR and POD are 84.75%, 13.45% and 93.15% respectively. At a glance, the results conclusion of the ANN model is consistent with the previous two mentioned. The results of studies in the first stage of the research shows that the number and position of the operating groups, hydrophobicity rate, and size of molecules, are the main factors on the efficacy of tyrosinase inhibitors derived from thiosemicarbarzide.

Keywords: Tyrosinase, QSAR, MLR, BLR, ANN

Probing the Effects of Some Physicochemical Factors on Hemolytic Activity of Bee Venom

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ABSTRACT

Bee Venom (BV) as a biotoxin which composed of mixture of biologically active complex and have a wide variety of pharmaceutical properties. Melittin as a most important component of BV, is a water soluble and hemolytic peptide. In the present study, we have investigated the BV functional and structural changes by using hemolysis test and spectrophotometric method

against a number of physico-chemical variables. These variables are effective in laboratory conditions for medicine manufacturing of BV. So, the cell hemolysis (CH50) of BV was determined in the normal conditions. Then, thermal resistance of BV was evaluated by CH50 against heat (up to 100 °C), pH, Ultera-Violet light (UV) and Ultra-Sound Waves (US) as physico-chemical laboratory variables.The results of Uv-Visible showed that BV has a absorption peak at 280 nm and the melting temperature (Tm) of it was calculated 70 °C by changes in maximum absorbance of BV at 280 nm. Heat changes in CH50 was started above 70 °C and it obtained with a slight increasing 1.37 µg/ml for boiling temperature than to control 0.94 µg/ml. The values of CH50 of BV was determined 0.94 µg/ml at pH 2 to 13, but slightly different 3 µg/ml at pH 1 and 1.56 µg/ml at pH 14. It is notable that the values of CH50 for control and treated BV samples with UV and US exposure hasn`t shown any changes. According above results, it can be concluded that melittin as an important component of BV, is a resistant peptide to some of physico-chemical variables such as temperature, pH, UV and US which makes it useful for therapeutic applications in a variety of laboratory manufacturing methods.

Keywords: Bee venom, Melittin, Hemolytic activity, Temperature, PH, Ultera-Violet, Ultra-Sound

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Computational Study on Natural Flavonoids Compounds of H1N1 Influenza Virus Neuraminidase

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ABSTRACT

Type A influenza is considered as a serious threat to public health. The mechanism of drugs applied for the control of this virus depends on two two surface glycoproteins with antigenic namely hemagglutinin (HA) and neuraminidase (NA) are responsible for binding and entry of the virus into the target cell. Neuraminidase inhibitors (NAI) interfere with the release of progeny influenza virus from infected host cells and thereby halt the spread of infection. Oseltamivir is an antiviral medication that blocks the actions of influenza virus types A and B in body. Naringenin and Hispidulin are natural flavonoids compounds with medical function in the mentioned active site. in the present study, molecular analysis of binding affinity in some flavonoids was investigated by docking method to determine the potential of these compounds as drug candidates for the control and treatment of type A influenza. from the docking simulation the magnitudes of binding free energy of oseltamivir, Naringenin and hispidulin were obtained: -5.8, -6.9 and -6.8 Kcal mol^{-1}, respectively. The obtained docking results confirmed capability of the selected compounds in terms of effective molecular interaction with the investigated protein, when compared to Oseltamivir.

Keywords: Type A Influenza, Oseltamivir, Naringenin, Hispidulin, Molecular docking

A Thermostable Amylase from Halophilic Bacterial Strains Isolated from Lut Desert, Iran

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ABSTRACT

The Lut Desert, located in the southeast of Iran with very dry and hot climate. The hottest temperature on the Earth was registered from this desert. A variety of microorganisms live in this desert and therefore, it would be a proper source for important industrial and biotechnological enzymes such as amylase. A total of 250 halophilic bacterial strains isolated from different soils and waters of Lut Desert were screened for their amylase activity. The α-amylase activity was measured qualitatively using starch agar and quantitatively based on DNS (3,5-dinitrosalicylic acid) methods, using maltose as a standard. Among all strains, 70 strains exhibited the amylolytic activity. The halo zone of 40 positive strains extended and selected for determining the effect of tempereture on their amylolytic activity. One isolates named SD33, showed stability for 30 minutes at 70°C. The amplified 16SrRNA gene sequence was compared with the sequence in EZbiocloud sequence database. The bacterial strain was identified as *Bacillus atrophaeus* strain JCM 9070(T). *Bacillus atrophaeus* is a gram negetive, rod shaped bacteria which forms white raised irregular colony. SD33 optimum production was achieved at pH 8.0, 0.5% NaCl and 37°C in starch medium. The influence of various carbon sources on alpha-amylase production was also quantified. Lactose was observed to be the ideal carbon source. In future, the selected amylase (SD33) will be purified for industrial purposes.

Keywords: Lut desert, Thermostable amylase, Halophiles, Bacteria

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Evaluation of Serum miR-155 and TNF-α Expression Among Rheumatoid Arthritis Patients (RA) with Positive RF and Anti-CCP

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ABSTRACT

MicroRNAs play a key role in the regulation of immune response as well as in rheumatoid arthritis (RA) pathology as a potential biomarker for disease control. We hypothesis some roles as a marker for serum miR-155 in the prognosis and the diagnosis of RA and its correlation with including rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) and tumour necrosis factor-alpha (TNF-α). In our cross-sectional study, serum samples among participations were collected and miR-155 expression was measured by real-time polymerase chain reaction (PCR). TNF-α levels were detected by enzyme-linked immunosorbent assay among 40 patients with RA and 12 individuals as healthy controls. Data analysis was performed by Stata statistical software. MiR-155 expression was higher in RA patients compared to controls

although there was no significance difference between the groups [mean 26.9 (4.4, 49.4) *vs*. 8.2 (0.8, 15.5), p < 0.185]. Based on ROC curve analysis, miR-155 had AUC = 0.456. Our results showed that miR-155 expression correlated with RF with p-value = 0.109, Anti-CCP with p-value = 0.119, also TNF-a with the correlation 0.1 and p-value = 0.465. As a result, there was no correlation between TNF-a, RF and Anti-CCP with gene expression miR-155. We concluded that miR-155 might not be a better diagnostic marker in RA patients and also there was no significant correlation between TNF-a and miR-155 gene expressions.

Keywords: MiR-155, TNF-a, Anti-CCP, RF

An *in Silico* Structural Analysis of Pathogenic Mutation Effects with Three Dimensional Protein Modeling of Human Medium Chain Acyl-CoA Dehydrogenase

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ABSTRACT

The most frequently diagnosed mitochondrial β-oxidation defect is medium chain acyl-CoA dehydrogenase (MCAD) deficiency. The influences of pathogenic missense mutations on MCAD structure were evaluated in this study. We constructed wild type and 16 mutant models of MCAD by homology modeling using YASARA.16.4.6. FAD dimethylbenzene (DMB) solvent accessibility, root-mean-square deviation of residues, secondary structure, radius of gyration and potential energy were studied in each model. Important contacts and interactions between substrate and protein were investigated as well. To further explore the effects of mutations, 120 ns molecular dynamics simulations were performed on native and two mutants (K329Q and R206H) proteins with temperatures of 299 K, 310 K, and 314 K. The mutations have diverse effects on the structural and dynamics properties of MCAD. DMB solvent accessibility increment in G195R, M149I, S245L, C244R, R206H and R281T are indicative of a loosened structure. R206H mutation does not alter the native secondary structure while radius of gyration and energy level of R206H are close to native protein at 310 K and 314 K. Despite the lower initial potential energy, total energy levels of K329Q mutant are higher than native model at all used simulation temperatures. In conclusion, our present study indicates that MCAD pathogenic point mutations may exert their effects by different mechanisms and atomistic simulations can provide insights into disease-associated mutations effects on MCAD dynamics and stability, which can ultimately increase our understanding of mutation effects.

Keywords: Beta-oxidation, Missense mutation, Acyl-CoA dehydrogenase, ACADD

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Investigation of Vitamin B9 Effect on Tau Protein Aggregation *In Vitro*

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ABSTRACT

Tau protein is one of the most important proteins which attaches to the axonal microtubule. aggregated tau is observed in all neurodegenerative diseases including Alzheimer's disease. Vitamin B9 as a single-carbon unit

donator could prevent tau aggregations by methylation PP2A resulting protein activity. We assumed that vitamin B9 through methylation of tau protein may inhibit or disturb tau aggregation. For this purpose, the tau protein was extracted from E. coli BL21(DE3) carrying recombinant pET23expression vector containing 1N/4R tau gene and purified by using metal chelate affinity chromatography (MCAC). Afterwards, the interaction between various concentration of vitamin B9 (0, 5, 10, 20, 40, and 50 µM) with tau protein (10µM) was investigated by using spectrofluorometer for fluorescence quantification as well as process of *tau* aggregation. The data showed that the quenching phenomenon resulting from the binding of vitamin B9 to the tau was a static type, the number of binding sites of the vitamin on tau was one at 25 and 37 °C. Furthermore, 50 µM of vitamin B9 caused the most inhibitory affection on tau aggregation. Our study showed that *increasing concentration dependence* of vitamin B9 inhibits the aggregation of tau protein significantly *and could be assumed as an effective compound to reduce the risk of Alzheimer's* disease.

Keywords: Tau, Vitamin *B9, Aggregation, Alzheimer*

Study the Effect of Vitamin A on Memory by Interaction with Microtubule Protein *In vitro*

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ABSTRACT

Microtubules are one of the major cytoskelatal proteins of neurons which are highly dynamic. Many neuronal processes including neuronal migration and neuroplasticity which underlie cellular and molecular mechanisms of memory, are dependent on dynamic instability of microtubules (1). Therefore effects of many drugs such as Beta-boswellic acid has been demonstrated on the dynamic instability of microtubules with the aim of improving memory (2). Following previous researches that had been done on the dynamic instability of microtubules, we decided to determine the role of another terpenoid, Vitamin A on the dynamic instability of microtubules to realize whether this natural nutrient enhances memory through interaction with microtubules. For this purpose Microtubules extracted from rasts brain through temperature dependent assembly-disassembly cycle (3). Microtubules (22.8 µM) were polymerized in presence of 1mM GTP at 37 °C with UV-visible spectrophotometer. To discover the effect of Vitamin A (All-trans Retinal) on dynamic instability of microtubules, three different concentrations of vitamin A (11.4, 22.8 and 34.2 µM) were added to polymerized microtubules and microtubule assembly examined in presence of these three concentration. Turbidity measurements showed that the rate of assembly increased as a result of vitamin A addition. However the rate of this assembly is independent on vitamin A concentration. Microtubules were assembled faster in presence of 11.4µM vitamin A in comparison with 22.8 and 32.4 µM vitamin A. According to our data, it is suggested that vitamin A can improve memory by increasing the rate of microtubule assembly.

Keywords: Microtubule, Vitamin A, Dynamic instability, microtubule assembly

Anti-cancer Properties of Green Synthesized Nano Oxali-palladium Using Turmeric Extract Coating in Comparison of Free Turmeric Extract Against Human Colon Cancer Cell Line HCT116

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ABSTRACT

Previous reports showed the anti-cancer activity of oxali-Palladium and turmeric extract in colon cancer treatment. In this research, the cytotoxic effects of green synthesized nano-oxali palladium using turmeric extract by green chemistry method in comparison with turmeric extract against human colon cancer cell line of HCT116 were investigated. At first, the nano-oxali-palladium has synthesized by green chemistry method, then, the physical and chemical properties of nano particles such as size, shape and zeta potential were studied. Finally, the cytotoxicity and anti-proliferative activities of nano-oxali palladium, and turmeric extract were examined by MTT assay after 24 and 48 h incubation times. The 50% cytotoxic concentration (Cc50) of the nano-oxali palladium was calculated 78 and 57 (µM) after 24 and 48 h incubation times, whereas45 and 32 (mg/ml) of turmeric extract after 24 and 48 h incubation times were induced 50% death in of HCT116 cell line. The results show that green synthesized nano-oxali palladium using turmeric extract has more cytotoxic effect on HCT116 colon cancer cell line to induce death in 50% of cells after different incubation times of 24 and 48 hours in comparison with free turmeric extract. So, the anti-cancer activity of turmeric extract which is used as cover of nano-oxali Palladium, is amplifying in nano form and increases the cytotoxicity of this compound.

Keywords: Green chemistry, Turmeric, Colon cancer, Oxali-palladium

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Comparing the Superoxide Dismutase Mimetic Activity of Gold and Silver Nanoparticles

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ABSTRACT

Superoxide anion radical ($O_2^{\bullet-}$) is generated by normal cellular metabolism. $O_2^{\bullet-}$ would be scavenged by superoxide dismutase (SOD). However, excess $O_2^{\bullet-}$ generated by environmental stress, such as ionizing radiation, redox and heavy metals, smoking and pollution will not be scavenged and induced oxidative stress. Consequently, quantitative analysis of $O_2^{\bullet-}$ is very important. Conventional methods to detect $O_2^{\bullet-}$ by SOD modified electrodes suffer from disadvantages of the high cost and difficult handling of natural SOD. Recently, gold and silver nanoparticles (GNPs and SNPs) have been found to exhibit SOD mimetic activity. In this research, an electrochemical sensor was fabricated based on GNPs or SNPs, as SOD mimetic compound, for detection of $O_2^{\bullet-}$. The sensor was fabricated by immobilizing GNPs or SNPs *via* 1,6-hexanedithiol (HDT) modified Au electrode. HDT forms a monolayer on Au surface through one of the two SH groups and the other SH group is covalently attach colloidal nanoparticles. The amperometric response of $O_2^{\bullet-}$ on Au/HDT/GNPs and Au/HDT/SNPs electrodes in potential 250 mV (*vs*. Ag/AgCl) and pH 7.4 phosphate buffer 0.1 M was monitored. The linear detection ranges of $O_2^{\bullet-}$ for GNPs and SNPs silver were 5 to 216 and 5 to 71 µM respectively. The sensitivity of these $O_2^{\bullet-}$ sensor by GNPs comparing to SNPs was 0.017 nA/µM against 0.011 nA/µM. The results show that both gold and silver nanoparticles presented SOD mimetic activity in decomposing $O_2^{\bullet-}$. But gold nanoparticles show greater activity than silver nanoparticles.

Keywords: Superoxide dismutase, Gold nanoparticles, Silver nanoparticles, Electrochemistry

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Polyethylene Glycol as Semi-permeable Ppartition at Pseudo-Chloroperoxidase Nanozyme

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ABSTRACT

Chloroperoxidase (CLP) is a versatile heme-based enzyme that behaves as chlorinating substrate. Here, the system consisted of the metal-TPP (tetrakis pyridyl porphine)-cysteine complex and polyethylene glycol (PEG as pocket) has used to model the native chloroperoxidase enzyme (CLP) via biomimetic approach. TEM images show a cross-linked vesicular Iron-TPP/Cysteine/PEG nanozyme. The high surface area of nanozyme causes high activity and 28% native CLP enzyme efficiency at pH 3 for thionine and hydrogen peroxide substrates. It seems that hydrogen peroxide substrate could walk on PEG structure. So far, we have looked at the vesicle and micelle as a hydrophobic envelope that attracts the inorganic-organic substrate near to active site but it can be a selective semi-permeable partition that displays the role of the nano-reactor.

Keywords: polyethylene glycol (PEG), Iron-Tetrakis pyridyl porphine, Cysteine, Chloroperoxidase nanozyme

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Stability Study and *In-vitro* Release of Chondroitinase ABCI Immobilized on Fe_3O_4 Nanoparticles

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ABSTRACT

In this research, the chondroitinase ABCI (cABCI) enzyme was immobilized on magnetite nanoparticle using physical adsorption method. The Fe_3O_4 nanoparticles were synthesized using chemical co-precipitation method of Fe^{2+} and Fe^{3+} ions. The analyses of XRD, VSM, and SEM were employed to characterize the structure, magnetic property, size and morphology of magnetite nanoparticles. The immobilization of cABCI enzyme onto magnetic nanoparticles was verified by FTIR spectra. The study of the factors affecting immobilization process showed that the optimum conditions for pH, temperature, mass ratio of cABCI to Fe_3O_4, and incubation time of cABCI and Fe_3O_4 were 6.5, 15 °C, 0.75 and 4.5 hr, respectively, and 0.037 mg cABCI was approximately bound to 1 mg of Fe_3O_4 at optimum conditions. It was found that the maximum activity of free cABCI was obtained at 25 °C, whereas the activity of immobilized cABCI was nearly constant at 10-25 °C. The V_{max} value was the same for both the free and immobilized cABCI, but the value of K_m for immobilized cABCI was 1.6 times higher than that for free one. The storage stability of immobilized enzyme was significantly improved at low temperatures, *e.g.*, free cABCI retained 19% of its activity after 6 days at -20 ˚C, whereas the immobilized one retained 96% of its activity in the same conditions. *In vitro* release of cABCI from Fe_3O_4 indicated that about 94% of the enzyme was released after 6 hr.

Keywords: Chondroitinase ABCI, Fe_3O_4 nanoparticles, Thermal stability, Immobilization, *In-vitro* release

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Concentration Dependency Effect of L-Arginine Modified Magnetic Nanoparticles (Fe_3O_4@Arg) on Lysozyme

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ABSTRACT

L-Arginine (Arg) is one of the most metabolically versatile amino acids that have not been found to display toxigenic properties in nature. Kosmotropic-like or chaotropic-like impacts of Arg on bulk and hydration shell water dynamics in a lysozyme solution, stability, enzymatic activity, and denaturation are reported. Modification of magnetic nanoparticles with Arg as the functional group (Fe_3O_4@Arg) can confer many advantages to their utilization in biological applications since it provides them with Arg and nanoparticles advantages. In this study, Fe_3O_4@Arg was synthesized and then characterized using a set of complementary methods including spectrometry, magnetometry, diffraction, scattering, and microscopy. The investigation of the effect of Fe_3O_4@Arg on lysozyme structure and activity as a model protein on the structure and stability using fluorescence, circular dichroism (CD) and UV/Vis spectroscopies is explored. Moreover, functional stability analysis was performed by monitoring the remaining enzymatic activity of lysozyme. Here, we are reporting, Fe_3O_4@Arg NPs confer an ordering (kosmotropic-like) or disordering (chaotropic-like) effects on lysozyme structure at Fe_3O_4@Arg:lysozyme ratios lower or higher than the concentration ratio of threshold (CRT) at 0.296, respectively. The protein was rendered more ordered in the structure at ratios lower than the CRT (<CRT) by improvements in lysozyme folding and helicity. Conversely, at ratios higher than the CRT (>CRT), lysozyme became unfolded, and the helicity was reduced, leaving the lysozyme more disordered consequent to interact with Fe_3O_4@Arg. The protein folding, helicity, and half-life were improved at a low concentration of Fe_3O_4@Arg. The Fe_3O_4@Arg act on lysozyme structure and stability most presumably through electrostatic adsorption on Fe_3O_4@Arg which in turn has an impact on the structure and dynamics of the hydration water shell that surrounds the protein molecules in the aqueous solution of lysozyme. Results from this research show that Fe_3O_4@Arg either act as stabilizers or destabilizers depending on their CRT on lysozyme. This has led to a proposed mechanism about the concentration dependency effects of Fe_3O_4@Arg on proteins. In conclusion, application of Fe_3O_4@Arg at <CRT is recommended to be used to preserve lysozyme structure and function.

Keywords: Fe_3O_4@Arg nanoparticles, Stability, Lysozyme, Kosmotropic-like, Chaotropic-like

Green Synthesis of Iron Oxide Nanoparticles Using *Carum carvi* L. and Modified with Chitosan in Order to Optimize the Anti-cancer Drug Adsorption

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ABSTRACT

Magnetic iron oxide nanoparticles have gained a lot of attention in drug delivery systems because they can control a drug pathway to deliver it to the specific site under a magnetic field which is related to their magnetic core and surface coating. Chitosan-coated FeNPs, have prominent antimicrobial and biological properties that make chitosan a promising biopolymer for drug delivery application, especially in cancer treatment. In this research, FeNPs were green synthesized using the aqueous extract of *Carum carvi* L. and under optimum conditions. Formation of FeNPs was confirmed by UV-Vis spectroscopy, XRD analysis, and SEM. Also, chitosan-coated FeNPs were synthesized to increased biocompatibility and the absorption capacity of nanoparticles. Chitosan coating on FeNPs was detected by FTIR. After the production of nano-absorbent, the maximum absorbance of different concentrations of doxorubicin was determined. The effect of pH was investigated on the absorption of doxorubicin in maximum absorbance at pH 3-10 by UV-Vis spectroscopy. The results obtained from the characterization of FeNPs showed they are spherical particles with less than 300 nm in size. The maximum absorbance of different concentrations of doxorubicin was in 280 nm. Doxorubicin showed maximum absorption at pH 7. This green biosynthesis method has been found to be eco-friendly, cost-effective and promising for different applications. The seeds extract of *Carum carvi* L. have a great ability to reduce Fe ions to FeNPs. Also, doxorubicin loaded chitosan-coated FeNPs can successfully use in drug delivery systems.

Keywords: Doxorubicin, Surface modification, Drug delivery, Green synthesis, Iron nanoparticles

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Applications of Microfluidics in Biosensors and Nanoparticle Synthesis

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ABSTRACT

In microfluidics, very small flow of fluids is directed and controlled. This knowledge is used in various fields of biology and nanomaterial synthesis. Also, the identification of biomarkers is important and todays, biosensors are vastly used to facilitate this approach. Fabrication and integration of biosensor to form a diagnostic tool can be facilitated by microfluidic technology in different parts including nanoparticle synthesis. Different materials have been used to fabricate microfluidic devices for application in biosensors. In one of our recent work, polymethylmethacrylate as a polymeric substrate was used for fabrication of microfluidic device. These devices can be fabricated by using either photolithography or etching or thin film deposition. We designed a microfluidic chip with several micro channels by Auto-CAD program. The fabrication procedure followed by etching technique *via* laser apparatus was used to provide a hydrodynamic flow focusing region to form high stable nanoparticles. In this work for fluid control, a programmable syringe pump was used. We successfully prepared nanoparticles via microfluidic techniques with uniform morphology. These microfluidic-assisted nanoparticles have potential to be applied in the field of biosensor due to their engineered average size, high stability and appropriate physical and chemical properties. As conclusion, microfluidic based fabrication of nanoparticles and application of them in microfluidic based platforms have potential to be applied in functional diagnostic tools.

Keywords: Microfluidics, Biosensors, Nanoparticles, Photolithography

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Study of BCL2L12 Isoforms Various Functions

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ABSTRACT

Bcl2-like12 (BCL2L12) protein is a novel multi-functional and member of apoptosis-related Bcl-2 family. Currently, 13 distinct transcripts, six nonsense-mediated mRNA decay and seven protein isoforms resulting from BCL2L12 gene are known. Although BCL2L12 is certainly involved in apoptosis process, the pro- or anti-apoptotic role of BCL2L12 remains somewhat controversial. BCL2L12 has been detected in most human tissues. The main BCL2L12 transcript consists of seven coding exons and its translation produces the "classical" BCL2L12 protein isoform (BCL2L12 isoform 1), a 334-amino acid polypeptide, containing a highly conserved BH2 domain, a BH3-like motif and and a proline-rich region with both nuclear and cytosolic localization. Classical BCL2L12 isoform exerts its anti-apoptotic role via multiple protein–protein interactions. In the cytoplasm, BCL2L12 inhibits effectors caspases 3 and 7. In the nucleus, BCL2L12 forms a complex with the tumor protein p53 and prevents its binding to promoters of apoptosis-related genes. Other isoforms (2-7) are shorter, lack some internal segments and sometimes have distinct C-terminuses compared to isoform 1. These differences can affect their 3D structures, and functions. In this study we reviwed predicted models of BCL2L12 isoforms, which are analayzed with I-TASSER server. Predicted 3D structure models of BCL2L12 isoforms are very different, some of which are BH3-only proteins. This structural difference could have a major impact on the functionality of BCL2L12 and enhances its pro-apoptotic

activity as it has shown previously about other BCL2 family members.

Keywords: BCL2L12, Isoform, Pro-apoptotic, Anti-apoptotic

Enhancement of Cellulase Enzyme in Activity and Thermal Stability on Core-shell Magnetic Nanoparticles Functionalized by Aspartic Acid

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ABSTRACT

A new support was fabricated to immobilize cellulase enzyme (CEL) to improve enzyme activity. Magnetic gold core shells (mAu@PSNs) were synthesized as a support and their characteristics were further investigated by X-Ray diffraction (XRD), vibrating sample magnetometer (VSM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), CEL was immobilized on mAu@PSNs via covalent bonding and the successful binding of enzyme to support confirmed chemically by fourier transform infrared (FTIR) spectroscopy. The binding efficiency was about 80% which was calculated by Bradford assay. FPase method was applied to measure enzyme activity at different temperatures (35-70 ºC) and pHs (2-8). The immobilized

enzyme maintained 70% of its initial catalytic activity after nine hours.

Keywords: Cellulase, Core shell nanoparticles, Immobilization, Thermal stability, Aspartic acid

Title: An Inductive Immunobiosensor Based on Modified Magnetic Nanoparticles for Detection of Hepatitis B Surface Antigen

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ABSTRACT

Early diagnosis of diseases is an important factor in their treatment. So far the hepatitis B virus was detected using different methods such as ELISA, chemiluminescence, amperometry, voltammetry and capacitance changing. The main objective of this research is to design an immunosensor with high accuracy and fast detection rate. In the present research at first, we will design and make a new kind of electrodes that consist of a coil on a glass base in very small scale. The coil will be 250 turns of a flat gold wire (the thickness is approximately 200nm, the width is 2µm and the length is1256mm) in a circle area with 5mm in diameter. Then, the primary antibody (anti-hepatitis B) will be stabilized on the coil. Thus, the inductive immunosensor is formed. Afterwards, by exposing the hepatitis B surface antigen (HBsAg), antibody-antigen interactions is occurred. In the next stage, by adding the secondary antibody conjugated with magnetic nanoparticles, the immuno-sandwich was formed. Due to high permeability (electromagnetism) of magnetic nanoparticles and their existence near the coil, influence on coil inductance. Therefore, changes in inductance will increase the sensitivity of the system significantly. All of the factors affecting immunosensor function such as temperature, pH, number of turns, the dimensions of coil wire and the electrode surface area will be optimized.

Keywords: Inductive immunosensor, Modified magnetic nanoparticles, Hepatitis B surface antigen, Immunosandwich

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Syntheses, crystal, molecular structures and Antimicrobial activity of Ni(II) and Cu(II) complexes with pyridine-2,6-dicarboxylic acid

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ABSTRACT

Two proton-transfer metal–organic compounds that formulated as, [Cu(pydc)2].3H2o (1), [Ni(pydc)2].3H2o (2) synthesized and structurally characterized by elemental analysis single-crystal diffraction, 1H NMR spectroscopies, infrared spectroscopy, TGA/DTA and antimicrobial studies where pydc is pridine-2,6-dicarboxylic. Compounds are crystallized in the monoclinic space group p21/c with four molecules per unit cell in which the central Ni(II) and Cu(II) atoms are six coordinated in a distorted octahedral coordination geometry by two tridentate pydc ligands via their O and N atoms with The asymmetric units of complexes contain three water molecules. In the crystal structures of compounds, extensive O-H…O and N-H…O hydrogen bonds as well as electrostatic forces, C-H… π and π-π stacking play important roles in stabilizing structures

which are assembled into 3D-supramolecular arrays. Bacterial activity complexes were studied against gram-positive bacteria: Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis and gram-negative bacteria: Pseudomonas aeruginosa, Escherichia coli, Serratia marcescens, also anti-fungal effect was investigated on Aspergillus niger and Saccharomyces cerevisiae in vitro by using minimum inhibitory concentrations (MIC) and disk diffusion method, the antimicrobial values of the complexes were determined to be in range 64–1024 µg/ml. These two compound exhibited nearly equal to gentamicin as a standard drug toward S. marcescens with IZD (inhibition zone) of 18 mm and exhibited nearly equal to Amphotericin B as a standard drug toward Saccharomyces cerevisiae with IZD of 18 mm.

Keywords: Proton-transfer metal-organic compounds, Pidine-2,6-dicarboxylic, Bacterial activity, Anti-fungal activity, Minimum inhibitory concentrations (MIC), Inhibition zone(IZ)

Introducing a New Model of Sweet Taste Receptor Heterodimer: A Validated Model for Drug Design Studies

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ABSTRACT

Sweet taste receptor (STR), a heterodimer of T1R2 and T1R3 C GPCRs, has no experimentally observed structure, and improvement of its models is important for ligand design studies. Here, a new model of the receptor, based on the most recent templates, is introduced. In particular, heterodimer structure of the orthosteric ligand binding domain (Venus Flytrap Module; VFTM) has been validated for drug design studies. Each domain of the protein, VFTM, CR and TMD, were separately constructed by hybrid-model construction methods and then assembled to build whole monomers or heterodimer structure. Stereochemical features were found satisfying for each domain as 99.4% of T1R2 and 97.7% of T1R3 residues were located in favored regions. Domains and whole monomers of T1R2 and T1R3 were found reliable and stable through sixty nanoseconds of MD simulations, as well as further quality analyses including accordance of local three-dimensional structures with the template structures and secondary structure predictions, satisfaction of experimental data *via* docking with known ligands binding, and examination of conserved characteristics of C GPCR family. VFTM domain heterodimer underwent MD simulation for 200-nanoseconds (two iterations of 100 ns) and the average structure was docked with aspartame. Despite close energies of binding for separate T1R2 and T1R3 domains interactions with aspartame, in the VFTM heterodimer, T1R2 was preferred for binding by aspartame in accordance with experimental data. In conclusion, this study introduces new hybrid models of STR components that, based on several validations, are found reliable for further molecular modeling studies on STR and C GPCR family.

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Improving the Anti-cancer Properties of Green Synthesized Oxali-palladium Nanoparticles in Comparision with

Free Oxali-Palladium Using *Carumcapticum* Extract

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ABSTRACT

Metal compounds, including oxali- platinum, are the best candidates for cancer treatment that particularly effective against colorectal cancer. Due to limitation of the use of platinum comples, including higher toxicity, the metal- based Palladium complexes were synthesized that have lower toxicity and higher anticancer effects. The synthesis of metal nanoparticles using green chemistry is one of the safe production methods. In the present study, green chemistry was applied to produce oxali-pallladium nanoparticles using aqueous extract of plant seeds of *Carumcapticum*. The anti-tumor activity of nanoparticles was investigated against human colon cancer cell line ofHCT116 and compared with the free oxali- pallladium and routine chemotherapeutic drug of oxali- platinum using MTT after different incubation times of 24 and 48 h. MTT results have shown that the anti-proliferative and growth inhibitory effects of green synthesized oxali-palladium nanoparticles have increased by increasing its concentration at both of the incubation times of 24 and 48 hours. Then, the Cc_{50} value (50% cytotoxicity concentration) of the oxali-palladium nanoparticles after 24 and 48 hours incubation times was calculated and represented significantly decreasing compared with free oxali- platinum and free oxali-palladium drug.According to above results, it can be concluded that that synthesized oxali-palladium nanoparticles via green chemistry method using extract of plant seeds of *carumcapticum* might be considered as a new drug for cancer treatment in the future.

Keywords: *Carumcapticum* extract, Green chemistry, Oxali-palladium

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The Application of Born Nitride Nano-Cages as Aromasin Anticancer Drug Delivery by DFT Computations

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ABSTRACT

Breast cancer is cancer that develops from breast tissue. Aromasin (Exemestane) an oral steroidal-type aromatase inhibitor, which irreversibly blocks aromatase, is very effective in the treatment of metastatic breast cancer. In this study, we considered density functional theory (DFT) calculations upon the adsorption of Aromasin as a antitumor agent on a series of pure $B_{12}N_{12}$ and carbon-doped boron nitride including $B_{12}N_6C_6$ and $B_6N_6C_{12}$ fullerenes for both vacuum and solvent (water) environment conditions by means of PBE-1 and M06-2X functionals and 6-311+G (d,p) basis set. Our study successfully demonstrated that the adsorption of antitumor agent upon the fullerenes will increase the polarizability of boron nitride fullerenes in comparison with C_{60} fullerene, but on the other hand, the energy of the interaction of this anticancer drug with $B_6N_6C_{12}$ is greater, which can reduce its release rate from the system.

Keywords: Breast Cancer, Aromasin, Density Functional Theory, (DFT),

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Investigation of Fluoxymesterone and Human Serum Albumin Interaction at

the Presence and Absence of Beta-Lactoglobulin and Cell Phone Electromagnetic Field by Quenching Fluorescence Method

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ABSTRACT

Stern-Volmer equation was performed to distinguish binding affinities and binding quantities. Static quenching and dynamic quenching are two way of fluorescence quenching which the second one can be demonstrated by Stern-Volmer equation. K_{sv} and K_q of human serum albumin (HSA)-fluoxymesterone complex were calculated at the presence and absence of beta-lactoglobulin (BLG) and cell phone electromagnetic field. Slop of each systems curve was differ from the others that this differences were effect of diversity in affinity of drug to protein. In all of systems, value of K_q is greater than 2×10^{10} l mol^{-1} s^{-1} and K_q of polymer which can illustrate static nature of reactions. Static quenching is effect of flourophore and quencher (drug) complex formation. Increasing and decreasing in affinity of drug to protein can be conducted than Ksv value. Based on present study, affinity of HSA-fluoxymesterone and HSA-fluoxymesterone-BLG interactions was increased at the presence of cell phone electromagnetic field.

Keywords: Fluoxymesterone, Human serum albumin, Beta-lactoglobulin, Electromagnetic field

Insensol Acetat as a New Histone Deacetylase Inhibitor

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ABSTRACT

Histone acetylation plays a key role in epigenetic regulation of gene expression. Alterations in acetylation levels and overexpression of various genes by histone dacetylases can lead to several diseases including cancer. Therefore, HDAC inhibitors seem to be promising anti-cancer drugs by interfering with histone dacetylases activity and regulating biological events, such as cell cycle, differentiation and apoptosis in cancer cells. Insencol acetate, a natural molecule with a chemical formula $C_{22}H_{36}O_3$, is a resin of the *Boswellia carterii* species, which has the most anti-inflammatory effect and shows its effects through inhibition of protein cytokinins such as TNFa, IL1b, TGFb, Cox2 and NFkb. Here, we studied the effects of Insencol acetate on histone deacetylase by the analysis of computational simulations. Our results revealed that insencol acetate is potentially able to interact with amino acid residues of the enzyme in the active site. Our docking study showed the this compund occupied the same space as SAHA, a famous histone deacetylase inhibitor, with a similar binding mode. Binding energy of insencol acetate was -4.93 kcal mol^{-1} for AutoDock4.2. These results suggested that this compund interacts mostly with Phe208 and Gly206.

Keywords: Epigenetic, Histone acetylation, Simulation

In Silico Investigation of ENPP2 Inhibition by some of Plant Metabolites

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ABSTRACT

Ectonucleotide pyrophosphatase (ENPP2) is a secreted enzyme which hydrolysis lysophosphatidylcholine (LPC) to the bioactive lysophosphatidate(LPA), the many phospholipids in the blood plasma. LPA act on a series of G-protein coupled receptors. LPA can promote many physiological and pathological processes which include cell proliferation, survival, migration and differentiation. The axis of the ENPP2/LPA signal transmits many of the chronic inflammatory conditions from fibrosis to colitis, asthma, atherosclerosis, neuropathic pain and cancer and increases cancer and resistance to chemotherapy and radiotherapy with increased angiogenesis and metastasis. By blocking the ENPP2 in the production by reducing metastases, a new treatment for cancer can be found. Despite these efforts, there has been a widespread success in the preclinical development of therapeutic agents that target ENPP2. Many of the natural compounds in plants have therapeutic effects. Plant hydrophobic compounds have a chemical structure similar to that of ENPP2 inhibitors. Among the plant's natural compounds, an appropriate inhibitor for ENPP2 with the least adverse effects can be identified. In ENPP2 to find the appropriate inhibitor, the structure of enzyme was extracted from PDB. In the next step, a collection of chemical compounds containing some of the derivatives of plant metabolites was docked to the ENPP2. Then, the interactions between these compounds with ENPP2 were studied and one of the most effective compounds was selected as a potent inhibitor.

Keywords: Autotaxin, Cancer, Plant metabolite, Enzyme inhibitor

Probing the Binding Site of a Newly Synthesized Palladium Complex on Human Serum Albumin *via* Competitive Ligand Binding Method

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ABSTRACT

In order to investigate the pharmacokinetics and pharmacodynamics of any drug it is important to study its interaction with blood carrier protein of human serum albumin (HSA). So, in the present study, the interaction between newly synthesized Pd(II) complex, as an anti-tumor component, with this protein was studied at different temperatures by spectroscopic techniques of UV-visible, fluorescenceand circular dichroism (CD) at two temperatures of 25 and 37 °C. Fluorescence spectra was observed that the Pd(II) complx has an abillity to quench the intrinsic fluorescence of HSAS throuh a static quenching procedure. Also, site marker competitive binding tests using warfarin and ibuprofen were carried out in order to identify the binding location of a new designed Pd(II) complex on HAS, Then, the number of binding sites and the association binding constants as well as the thermodynamic parameters of Pd(II) complex on protein were calculated at both temperatures. The quantitative CD spectra analysis represented that Pd(II) complex induced alterations in the secondary structure of the protein *via* decreasing in the content of α helical structure of protein. According above results, we concluded that the new synthesized Pd(II) complex can bind to the blood carrier protein of HSA and change the

structure of it which can be considered in design of new drugs.

Keywords: Warfarin, HSA-competitive binding method, Palladium complex

Investigating the Enzymatic Profile of Productive Halophytes in Salty Water Ecosystems

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ABSTRACT

Salty lakes and some saline environments with salinity approximately seawater are extreme environments, but often have a wide range of microbial community. The germs in these habitats include Archeae, Bacteria and Eukarya. To adapt to salt conditions, microorganisms have broad strategies to maintain cellular and functional structure. Therefore, studying on these microbes is important due to their ability to produce various compounds used in the industry, such as hydrolytic enzymes with wide potential for biomedical and chemical industries. Most industrial processes are carried out under special physicochemical conditions that regulate the desired conditions for the activity of the enzymes present. Therefore, this issue is of great importance for enzymes that show optimal activity in a wide range of salt concentrations, pH and temperature. Halophiles are a great source of these enzymes that not only tolerate salt but are also active at high temperatures and variable pH rates. Hence isolation of halophilic microorganisms generating hydrolysis enzymes those have optimum activity in different salt concentrations can be useful in some industrial processes. Therefore, the objectives of this project are in line with halophilic enzyme kinetics.

Keywords: Halophiles, Enzymatic kinetics, Saline water ecosystem

Antioxidant and Protective Effect of Selenium Nanoparticles against Isoniazid Induced Oxidative Stress in Rat's Brain and Blood

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ABSTRACT

Overdose of Isoniazid (INH), a famous drug for tuberculosis treatment, leads to oxidative stress due to production of reactive oxygen species. Our study aimed to assess the protective activity of Selenium Nanoparticule (SeNP) against INH-induced oxidative stress in the brain and blood. For this purpose, forty rats male rats were used in the study and arbitrarily assigned to five equal groups (n = 8 per group) including: control, sham (saline-treated), INH treatment alone (50 mg kg^{-1}, intraperitoneal [i.p.]), and SeNP (0.1 and 0.2 mg kg^{-1}) administered orally as pretreatment. Animals were treated for 14 days and euthanized 1 h after the last drug administration. In the brain, INH induced significant elevations in malondialdehyde content, Catalase and GST activities compared to control rats, while Superoxide Dismutase activity remained unchanged. Elevated activities of Catalase and GST and elevated concentration of GSH and MDA following INH administration were significantly lowered due to pretreatment with SeNP. Also, INH induced significant reduction in the total antioxidant capacity and elevation in nitric oxide level, in the blood. While,

selenium nanoparticles by increasing GPx activity and decreasing SOD activity, lead in reduction of these damages produced by NO. The results of this study indicated that the protective effect of SeNP might be attributed to its antioxidant property due to increasing activities of antioxidant enzymes.

Keywords: Isoniazid, Selenium nanoparticle, Antioxidants, Oxidative stress

Study the Biochemical Inhibitory Effects of Caffeine and Theine Alkaloids against Antibiotic Resistant Bacterial Isolates

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ABSTRACT

Due to fact that bacteria are increasingly resistant to current antibiotics and may not respond to treatment processes, there will be serious requirements for creating new forms of medication. Caffeine and theine, along with other alkaloids that are considered as herbal metabolites and included in phytoanticipins, are natural source of high consumption medications, so they are suitable candidate to confront to microbial infections, because they can reduce their harmful effects in the body by inhibiting pump and drug resistance mechanisms in microorganisms. According to these characteristics, the antimicrobial activity of aqueous extracts from two types of coffee including Arabica and Robusta, as well as two types of black and green tea against antibiotic resistant hospital isolates was carried out. The results showed that *E. coli* and *P. aeruginosa*, were the most sensitive and resistant microorganisms respectively. Robusta coffee had a relatively higher inhibitory effect than Arabica and green tea than black. Quantitative analysis of the amount of caffeine and theine of the extracts also indicate a high dose of caffeine in the Robusta coffee extract, which shows the direct effects of this agent on the antibacterial properties. Therefore, since drug resistance in various infections has been monitored and confirmed, the use of different antimicrobial compounds such as caffeine (coffee) and theine (tea) is recommended to prevent or control such resistance.

Keywords: Caffeine, Theine, Plant alkaloids, Antibacterial effect, Hospital-acquired resistant isolates

The Investigation of the T30695 Aptamer Selectivity toward Pb^{2+} Ion: A Molecular Dynamic Simulation Study

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ABSTRACT

Today, the need for biosensors with improved detection limits has been enhanced to detect specific targets with a high selectivity. Aptamers, as a group of nucleic acid sequences, are introduced to meet this demand. The T30695 aptamer, with the ability to form the G-quadruplex

structure, is selectively bonded to Pb^{2+}. Therefore, it is significantly utilized to design the Pb^{2+} biosensors. The investigation of the aptamer interactions with different ions can be an effective strategy to determine the aptamer selectivity toward a specific ion. Molecular dynamic (MD) simulations provide information about the type and strength of interactions between the aptamer and its target. Hence, MD simulations were applied in this article to study the selectivity of the T30695 aptamer towards Pb^{2+} in comparison with the other ions. The Free Energy Landscape (FEL) analysis illustrates that Pb^{2+} ion remains within the aptamer during the MD simulation while the others leave it. The Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) binding Gibbs energies demonstrate the greatest conformational stability of the aptamer in the presence of Pb^{2+}. The ion-aptamer complex with the most compaction is induced in the presence of Pb^{2+}, which confirms the highest aptamer interaction with the ion. These results verify the selectivity of the aptamer toward Pb^{2+}. The contact maps clarify the appearance of the new contacts in the presence of Pb^{2+}, which is another confirmation for the aptamer selectivity toward Pb^{2+}.

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Keywords: MD simulation, Aptamer, G-quadruplex; Aptasensor, FEL analysis, MM-PBSA

Comparison of Inhibitory Effect of Retinol and Hydroquinone as Antioxidants on Tyrosinase Enzyme by Molecular Docking Computations

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ABSTRACT

Tyrosinase is an enzyme containing copper ion that catalyzes the two reactions of melanin production. Tyrosinase is considered as an effective enzyme in different levels of life. It is important in terms of performance and structure and stability of the enzyme, diversity of the enzyme substrates makes it possible to analyze the effect of different ligands on the enzyme kinetics in both cresolase and catecholase activities of tyrosinase. Hydroquinone and other cutaneous depigmenting agents are widely used by dermatologists to treat pigmentary disorders.

Mushroom tyrosinase, which is very similar to human tyrosinase, has been used in this study conducted by molecular docking which in fact has become an important tool for drug discovery. In this method, one hundred binding mode predictions of the protein-ligand complexes are compared and the lowest energy form is predicted as the best structure. Molecular docking was carried out using *AutoDockTools-1/5.6rc3* software for prediction of binding structures of the protein by *pdb 2y9w* file with hydroquinone and retinol as ligands. The results of docking computations show that, hydroquinone the free energy of binding and inhibition constant are -5.30 kcal mol^{-1} and 131.35 um *(micromolar)* and for retinol are -8.16 kcal mol^{-1} and 1.04 um (*micromolar*), respectively. These show that retinol is winner in this competition in compare with hydroquinone and probably both of these antioxidants are included in drugs or cosmetic combinations, retinol can inhibit the actual effect of hydroquinone

Keywords: Hydroquinone, Molecular docking, Tyrosinase, Retinol

Serological Study of Enterotoxin Production by Coagulase-Positive *Staphylococcal* Strains Isolated from Restaurant Staff in Golestan Province

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ABSTRACT

Staphylococcus.aureus inducing various types of enterotoxin, is one of the most important causings of food poisoning. The aim of this study was to determine the frequency of enterotoxigenic *S.aureus* strains isolated from restaurant staff in Golestan province. In this descriptive study the samples were taken from the anterior nasal part and hand of resturant staff (n = 91), using a sterile swab. Being cultured on Mannitol salt agar, the suspected colonies were identified by gram staining as well as catalase, coagulase and Dnase tests. Staphylococcal coagulase-positive strains with RPLA (Reversa passive latex agglutination), a semi-quantitative method, also using the SET-RPLA kit were evaluated. The frequency of *S.aureus* isolates from resturant staff was reported 31.9%. The most abundance by gender was in male (76%) and in terms of staff position at the restaurant was found in crew (51.7%). ($P < 0.05$) In the serological study, 24.1% of the isolates were able to produce enterotoxin, the most common type belonged to enterotoxin B (SEB) (57.1%). Based on our findings, it has been found that in carriers related to food, there is a possibility of enterotoxigenic coagulase-positive *Staphylococci*, which is considered as a risk factor for public health. Therefore, it is recommended that the health Individuals should be emphasized when preparing food at all stages to reduce the risk in the food chain.

Keywords: *Staphylococcus.aureus*, Restaurant staff, Enterotoxin

Interaction of β-Cyclodextrin with Molten Globule state of Hen Egg-White Lysozyme

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ABSTRACT

The molten globule state of hen egg-white lysozyme (HEWL) is the major intermediate of a protein folding. Chaperones prevent aggregation and precipitation of proteins under conditions of stress. The present study aims to investigate the conformational state of the hen egg-white lysozyme when it in forming a high molecular weight complex with β-cyclodextrin (β-CyD) as a molecular chaperone using UV spectroscopy, fluorescence spectrophotometery, circular dichroism (CD) spectropolarimetry, Iso thermal titration (ITC) as well as the measurement of viscosity and Stokes radius of protein. The results showed that β-CyD reduces and delays the aggregation of lysozyme and increases the ANS (8-anilino-1-naphthalenesulfonic acid) fluorescence intensity of the spectra. Therefore, a hydrophobic interaction occurred between β-CyD and lysozyme. In the range of 204-250 nm CD spectra of lysozyme did not change at the presence of β-CyD in ellipticity relative to native lysozyme. Measuring Stokes radius of lysozyme in the presence and absence of β-CyD and comparing its value with denatured state showed that molten globule was an intermediate state in protein folding process.

Keywords: Lysozyme, β-Cyclodextrin, Molten globule, Circular dichroism spetropolarimetry, Fluorescence Spectrophotometery, Isothermal titration

Effect of temperature on activity and stability of cellulose of termite's gut bacteria

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ABSTRACT

Introduction and aims: Cellulases have attracted much interest because of the diversity of their applications in industry. However, the major bottleneck of comprehensive application of cellulase in industry is the high cost of the enzyme production. It is therefore imperative to look for microorganisms that have a high rate of cellulase production. It has been suggested that termite gut microbiota has a significant impact on cellulose degradation. Therefore isolation of cellulase enzyme from the bacteria of the termite gut, which has high ability to produce cellulase enzymes, is economical and can be used in the industry as well. In order to better utilize the enzymes in the industry, enzymes are monitored for activity and thermal stability. This study investigates the effect of temperature on activity and stability of cellulase enzymes isolated from the gut bacteria of termite.

Material and methods: First, the cellulase enzyme was extracted from the gut bacteria of the termite and then purified by column chromatography. The optimum temperature for cellulase activity was examined by incubation of the purified enzymes with 0.75% (w/v) CMC at temperatures ranging from 20 to 80°C under standard assay conditions. Thermal stability was evaluated by pre-incubation of the enzymes without substrates for 60 min at temperatures of 20–70°C. The enzyme activity was measured under standard conditions. Results and Discussion: Based on research results, the cellulose enzyme showed the highest activity at 50°C. The enzyme maintained more than 60% of its activity in a broad range of temperature from 20 to 70 °C and demonstrated its highest thermal stability at 40°C. Due to the wide thermal stability, this enzyme is suitable for use in various industries.

Keywords: Cellulose, Termite, Thermal stability.

Precise Method Based on Primers Designed for Cytochrome b Gene for Detecting Fraud in Meat Products

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ABSTRACT

The Today, mixing in meat products, especially processed products, is considered as one of the most important problems of some production units in the food industry. Therefore, the meat type test is very important, and the identification of it leads to confidence in consumers and also the factories that use animal protein sources in their products. In recent decades, new biotechnology techniques have allowed for health control and preventing fraud by producers in foods, and in some ways it encourages quality control and consumer health. Among the new biological methods used to identify the type of meat used, the genetic methods are highly accurate. In addition to being quick and accurate, it allows the content of processed meats, such as hamburgers, sausages, Salami, kebab, and so on. One of the proposed methods in this project is to determine the type of meat using the

mitochondrial cytochrome b gene using the PCR method. In this method, after extraction of DNA from the tissue studied, using the primers designed for cytochrome b gene, the PCR reaction is performed to amplify the desired gene, this way it is possible to understand the content of mixed meat. Due to the speed, simplicity, sensitivity and specificity of this technique, the method has a high potential for detecting meat, and also does not limit the use of processed meat.

Keywors: Polymerase chain reaction (PCR), Cytochrome b, Mitochondrion sequences, Meats detection

The inhibitory effect of gingerol on oxidizing of human serum albumin

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ABSTRACT

Oxidative stress and its derived products have been considered as the main components of all pathogenic processes. Proteins are the major target of intrinsic transformation by free radicals and non-radical compounds directly or indirectly. This deep transformation changes the physicochemical and functional properties of native proteins, peptides and amino acids which lead to toxic compounds formation. Nowadays, natural phenolic components have been paid attention for diseases' upheaval. Zingiber officinale Roscoe is one of the well-known herbal medicines in the world, which has always been used historically for its beneficial effects on human health. Gingerol, shogaol and some phenolic derivatives are the main phenolic antioxidants in ginger. In this research we traced the effect of gingerol on oxidized human serum albumin and its fibrillation by preservatives via the array of biophysical techniques include, fluorescence and UV-Visible spectroscopy and AFM. The results showed the significant inhibitory effect of gingerol on HSA glycotoxins formation. All glycoxidation products (amadori products, carbonyl contents and AGEs) were decreased by gingerol which lead to the benefits of its application in food, cosmetic and health care industries.

Keywors: Gingerol, human serum albumin, glycotoxins, oxidative stress, anti-fibrillation.