

Abstract No.23

**Neohesperidin Dihydrochalcon Activator of Alpha- amylase:
a Mechanistic Study**

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Alpha Amylase digest starch in a wide variety of organisms. Because of its important role in controlling blood sugar its inhibitors and activators are of interest. Previously, we found that neohesperidin dihydrochalcone (NDC) is an activator of porcine pancreatic alpha amylase (PPA). This study was aimed to determine the kinetic parameters of this activator. We applied Bernfeld method to assay the enzyme, using different concentrations of substrate against NDC. NDC behavior was also assessed in the presence of acarbose (strong enzyme inhibitor). Lineweaver-Burk plots were drawn to obtain kinetic parameters of NDC. To get an insight on the putative NDC activation site, we docked NDC on PPA with the use of Autodock Vina. Lineweaver-Burk plot and replots were drawn to obtain α , β and K_a values. Two intercepts were obtained, affinity to substrate (α) and k_{cat} (β) in both intercepts followed a $\beta > 1$ and $\alpha < 1$ pattern. While under 1400 ($\mu\text{mole/lit}$) of NDC, intercept is above X-axis and above 1400 ($\mu\text{mole.lit}^{-1}$) intercept is under X-axis, it's assumed that NDC follows both increasing cooperativity (between activator and substrate) and increasing catalytic constant pattern to raise enzyme activity. Some competition is also observed between acarbose and NDC. NDC is proposed to interact with PPA in a pocket near Asp402 and Arg 252 which was previously suggested to become occupied by caffeine and theobromine (other weaker activators of PPA that we have reported). NDC may bind in this pocket via hydrogen bonds which are not observed in the weaker activators, a fact that could be related to the higher effectiveness of NDC. In conclusion, NDC is a highly activator of PPA which may play its role through boosting intramolecular hydrogen bonding in a putative activating site.

Keywords: Neohesperidin Dihydrochalcon, Alpha-Amylase, Kinetic Parameters, Activator.

Abstract No.24

**Novel Hydrogen Peroxide Biosensor Using Functionalized-
carbon Nanotubes and Nanoclay**

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Phenolic compounds are important starting materials in a broad range of chemical manufacturing materials. Due to drastic environmental legislation, analytical determination in the subnanomolar range is one of the challenges of all analytical devices. Among these different analytical devices, biosensors play an important role due to some generally claimed advantages: intrinsic specificity, low costs and fast analyses. In this study, we demonstrate the synthesis of carbon nanotubes (CNTs) on clay minerals, and the development of biosensors based on COOH-MWCNT/Clay-HRP and composite films for the detection of hydrogen peroxide (H_2O_2). The mixed hybrid film of CNT/Clay, HRP is coated on the glassy carbon (GC) electrode to detect H_2O_2 . This film exhibits a detection limit of 5.0×10^{-5} M for H_2O_2 with a sensitivity of $280 \mu\text{A mM}^{-1}$. A higher sensitivity and more stability of enzyme are observed with increasing H_2O_2 content in the composite matrix film. Consequently, the CNT/Clay medium can probably be a useful electrode for the development of sensors due to its high sensitivity and applicability

Keywords: Clay, Biosensor, Carbon Nanotube, HRP.

Abstract No.25

**Spectroscopic Investigation of Gold Nanorice – Enzyme
Nanobioconjugates for Drug Delivery Applications**

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The remarkable feature of plasmonic-anisotropic nanoparticles arises from their structural, electronic, magnetic and catalytic properties. Tunability of the size, morphology and surface of nanoparticles in controlled synthesis strategies has raised great interest of nanobiotechnologists. A combination of optical-thermal properties of plasmonic nanoparticles and biomolecules could be utilized for development of non-destructive therapeutic and diagnostic agents. Although applicabilities are undeniable, a number of undesirable

changes might occur in the structure and function of biomolecules upon interaction with nanoparticles. Therefore, conducting a series of fundamental investigations before designing any nano based system would be of high significance. Herein, structure of lysozyme has been studied upon interaction with a specific type of gold nanorods (gold nanorice). Gold nanorices were synthesized by modification of conventional seed-mediated growth protocol, by reduction of surfactant concentration up to 5 mM. The nanorices were characterized by UV- Vis Spectroscopy, Transmission Electron Microscopy (TEM) and Atomic Absorption Spectroscopy (AAS). Lysozyme solution ($300 \mu\text{g} \cdot \text{mL}^{-1}$) was then interacted with nanoparticles of 25 nM concentration. Conformation of lysozyme treated gold nanorice was studied by circular dichroism spectroscopy under different pH conditions, and quenching of the intrinsic fluorescence was traced in varying concentrations of gold nanorices (0-200 nM). Circular dichroism revealed mild conformational change in the secondary structure of lysozyme, with a slight increase of compactness upon binding to gold nanorices. Intrinsic fluorescence spectra showed a steady reduction in the fluorescence intensity of protein, indicating the proximity of gold nanorices' interacting surface to the location of tryptophan residues in lysozyme, and possibility of complex formation. Maintaining high fraction of native structure and internal quenching, the enzyme could perform its catalytic activity in the presence of gold nanorices. Findings of this investigation might encourage utilization of gold nanorices as a new generation of nanocarrier within the domain of drug delivery applications.

Keywords: Nanocarrier, Plasmonic Nanoparticles, Circular Dichroism, Internal Quenching.

Abstract No.26

β -Casein Nanoparticles As An Efficient Drug Carrier System For Curcumin And Its Derivatives

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In milk β -casein micelles exists a natural nanostructure, which can be used as a Nancarrier of hydrophobic drugs. Curcumin, diacetylcurcumin and bis-demethoxy curcumin, are the active curcuminoids of turmeric (*Curcuma longa* L.) with many physiological, biochemical, and pharmacological properties. Here, we investigated the

complex formation of curcuminoids and some of its derivatives with β -casein micelles and its use as a vehicle for drug delivery to cancer cells. Steady-state fluorescence spectroscopy of the β -Casein micelle-curcuminoids complex formation revealed that at pH 7, curcuminoids molecule binds to β -Casein micelle and formed complexes through hydrophobic interactions. The binding parameters including number of substantive binding sites and the binding constants have been evaluated by fluorescence quenching method. Results showed that curcuminoids molecules quench the intrinsic fluorescence of β -Casein upon binding. The distance (r) between donor (β -Casein) and acceptor (CUR, DAC and BDMC) was obtained according to Förster theory of non-radiative energy transfer. The utility of β -Casein micelle as carriers of curcuminoids was evaluated in vitro, using cultured MCF7 cells. Cytotoxicity studies revealed that, the β -Casein micelle-curcuminoids complex exhibited similar cytotoxic effects on MCF7 cells compared to an equal dose of free curcuminoids. Because β -Casein is an edible protein, the β -Casein micelle-curcuminoids complex has a potential to be administered orally

Keywords: Curcumin, Diacetylcurcumin, Bis-demethoxy curcumin, β -Casein micelle, Fluorescence, Förster's theory.

Abstract No.27

DNA Binding of Cyclopentadienyliron Substituted Polyoxotungstate CoW11O39(CpFe)]7-

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The ctDNA (calf thymus DNA) binding properties of water soluble keggin type polyoxometalate(POM): [CoW11O39CpFe]7-, (CoWCpFe) were carried out with electronic absorption, fluorescence spectrophotometry and cyclic voltammetry. Absorption spectrum trace revealed 12.08%, hypochromism, indicative of moderate interaction to ctDNA. The binding constant has been estimated $3.10 \times 10^3 \text{ M}^{-1}$, by analysis of UV- Vis titration experiments. In addition, number of substantive binding sites and the binding constants parameters were obtained from fluorescence spectroscopy. The estimated parameters from two spectroscopic methods are consistent. Also, voltammetric method was used to probe the interaction binding mode through shift in potential and change in limiting current of POM due to addition of ctDNA. The results of voltammetric study represent the binding of POM to ctDNA is via of phosphate groups of nucleic acid. It is noticeable,