

cross linking in the network of chitosan in the film and microsphere forms, it seems that the macromolecules probably become more rigid and thus decrease the release of drug. Therefore increase on degree of cross linking affected the release rate. Although fast release of drug from chitosan film, as explained above, was attributed to low degree of cross linking in its network than that of chitosan microsphere (Figure). In vitro release from chitosan carriers in film and microsphere forms as a function of time at pH 7.4. The films and microspheres prepared at 12% w/w degree of cross-linking have shown better features in comparison to those prepared with low and high degree of cross-linking (6 and 18% w/w, respectively). The films and microspheres prepared with all degree of cross-linking (6, 12 and 18% w/w) showed burst-release of mezalazine within a first step of drug release. Then mezalazine was released in a controlled manner in second stage of drug release during 9 h.

**Keywords:** Release Kinetics, Enzyme Delivery, Bioactive Model.

#### Abstract No.147

##### Evaluating of Tyrosinase Mechanism by it's Inhibition and Activation

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The mechanism of mushroom tyrosinase (MT) is complicated because of occurrence of two different enzyme forms (Em and Eo) simultaneously. In this study the inhibition kinetics of a new synthesized ligand (derivative of aniline) in two different forms of the enzyme (Em & Eo) were investigated separately by using Double reciprocal Lineweaver-Burk plots. In both forms the ligand inhibits the enzyme competitively but the results revealed that the oxidation of diphenol by Eo is about 1.3 times faster than oxidation by Em and the tendency of the ligand to bind Em is about 1.57 times more than binding to Eo. These results describe why the low concentration of ligand ( $2.5 \times 10^{-5}$  M) activates creolase activity and decreases lag phase and also activates and inhibits the catecholase activity at below and at saturated concentration of substrate, respectively. Also circular dichroism (CD) and fluorescence experiments confirm the binding of ligand to the enzyme. Docking results show that ligand makes 3

hydrogen bonds, 2 with one histidine residue in the active site and one with proline residue.

**Keywords:** Mushroom Tyrosinase, Inhibition, Activation, Mechanism.

#### Abstract No.148

##### Site-directed Mutagenesis of Mnemiopsis: Implication of one of Conserved Residues in Bioluminescence

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Bioluminescence helps for communication, attraction, repulsion, or camouflage in marine life. Photoproteins are the primary reactants of the light-emitting reactions of various bioluminescent organisms and mostly found in marine organisms including coelenterates and ctenophores. Although many photoproteins in nature were discovered but have been very few studied in detail, except for coelenterate photoproteins. In this group, biochemical aspects of photoproteins aequorin and obelin have been well investigated and their crystal structures have been solved. With the help of protein engineering, they have found variety of analytical and biological applications. In contrast, little efforts have been made on ctenophore photoproteins such as mnemiopsin. Recently, cDNA encoding mnemiopsin photoprotein of mnemiopsis leidyi was cloned in our lab. In this study, site directed mutagenesis using the quick change method was used to make variants of mnemiopsin displaying different functional and structural properties. Since our goal was determination of the role of one conserved residue in coelenterazine binding cavity in mnemiopsin bioluminescence, mutations were introduced at this position (position 39). This residue is among highly conserved residues in coelenterazine binding cavity in each type of photoproteins. For investigation of side chain size and charge effect on luminescence activity, we replaced Arg 39 by Lys and neutral and negatively charged residues, Met and Glu, respectively. The results indicate that except for R39K mutant, that increases luminescence intensity more than eight fold, compared to wild type, R39M and R39E mutants lost luminescence activity completely. The most notable properties of mutant R39K was its higher activity, slow rate of luminescence decay and broad pH activity profile compared to wild type. These properties make it highly attractive for both basic studies and applications. The results of structural studies