to be about 0.19mM and 0.09mM, respectively. The enzyme was purified after ammonium sulfate precipitation, DEAE cellulose and G100 gel filtration chromatography. SDS-PAGE electrophoresis showed only one bond with molecular weight of 6.5 kDa. The fluorescence emission of pure enzyme showed that tramadol could bind to both free enzyme and enzyme-substrate complex which was accompanied with reduction of emission intensity and conformational changes.

**Keywords:** Alkaline Phosphatase, Drug, Inhibition, Tramadol, Kidney.

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**Abstract No.140**

### Structure-function Studies of Three Types of Enzymes Involved in Disease, a Pectate Lyase, an E3 ubiquitin Ligase and Three Methyltransferases

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In the current world of research on agents of disease, proteins (enzymes in particular) are of great importance as they play a pivotal role in infection. Structural and functional studies of enzymes can be carried out through the use of biophysical and biochemical methods, in order to answer the many yet unanswered questions about their important roles.In this talk the results from the structural and functional studies carried out on two bacterial enzymes causing disease in plants and humans will be presented. These bacterial enzymes are pectate lyase1 from Bacillus subtilis and IpaH9.8 E3 ubiquitin ligase2 from Shigella flexneri which cause infection in plants and humans, respectively. Pectate lyase is involved in crop spoilage while IpaH9.8 E3 ubiquitin ligase is involved in the colonization of human intestinal cells causing Shigellosis, a severe bloody diarrhea. In addition, structural data on methyltransferases3 essential for the very important Vitamin B12 biosynthesis in Rhodobacter Capsulatus will be presented. It is important to mention that these methyltransferases play an important role in decorating tetrapyrroles in the Vitamin B12 biosynthetic pathway. Vitamin B12 produced by bacteria is an essential dietary requirement for humans and its deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system and is therefore of great medical importance.The biochemical techniques used in this study include protein over-production, protein purification, SDS-PAGE analysis, affinity chromatography, gel filtration chromatography, ubiquitination assays and western blot analysis. The biophysical techniques used include dynamic light scattering (DLS), isothermal titration calorimetry (ITC), crystallisation screening, and X-ray crystallography leading to structure determination.These techniques have come hand in hand in answering many questions relating to the structure and function of these enzymes of interest and have opened up new avenues in furthering our knowledge about these agents of disease.

**Keywords:** Disease, Pectate Lyase, E3 Ubiquitin Ligase, Methyltransferases, Crystallography.

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**Abstract No.141**

### Micro-/ Nanoparticles from Polyethylene Glycol and Poly(L-lactic acid) Triblock Copolymer in Aqueous Solution as Drug Delivery Systems

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Block copolymers are linear macromolecules that consist of two or more different blocks of different types of monomers. Amphiphilic block copolymers self-assemble in suitable solvents to form various supramolecular structures, like spherical and/or worm micelles, flower-like micelles, vesicles, microspheres, etc. Over the past decade, the effectiveness of such self-assembled structures as drug delivery vehicles has been demonstrated. In this research, different PLA-PEG-PLA (PLA is poly(L-lactic acid) and PEG is polyethylene glycol) triblock copolymers varying in block lengths are studied. The formation of micro-/ or nanoparticles made of these copolymers is investigated. The effect of hydrophilic / lipophilic balance (HLB) on the critical aggregation concentration (C.A.C.) and size distribution of the particles is reported. On the basis of obtained results, PLA37-PEG136-PLA37 was selected for other studies. A comparative study on the interaction of this copolymer with human and bovine insulins was carried out using CD, fluorescence and UV-Vis spectroscopy. Binding affinities of copolymer to both proteins are comparable: $K_d$(Bovine)= $29.17\times10^{-6}$ M and $K_d$(Human)=$36.63\times10^{-6}$ M. CD results show that, copolymer interaction with proteins affects the secondary structure of both proteins. Fluorescence emission intensity of complex was decreased by increasing copolymer concentration.

**Keywords:** Copolymer, Insulin, Binding Affinity, Critical Aggregation Concentration (C.A.C), Nanoparticles.

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