Gold nanoparticles are extensively used in diverse fields as matrix for immobilization or functionalization due to their unique optical and physical properties, such as surface plasmon oscillations for labeling, imaging, and sensing. Due to better biocompatibility and least toxicity, in the last two decades, its biomedical applications have gained much attention in disease diagnosis and therapeutics. These nanomaterials can be easily conjugated to different functionalizing agents such as polymers, surfactants, ligand, dendrimers, drugs, DNA, RNA, proteins, peptides and oligonucleotides depending upon area of application. Here, we have chosen two biomolecules namely lipase and sophorolipid for bio-functionalization of gold nanoparticles for different applications. Lipase and sophorolipid functionalization were performed to enhance the enzyme activity and antimicrobial respectively. To achieve these goals, objectives were framed accordingly and experiments were performed.

Although various methods of immobilization have already been employed to conjugate lipase to different matrices, among them EDC/NHS cross linking is simple and single step process for covalent coupling between enzyme and nanoparticles. To achieve proper orientation, the contribution of different functional groups on enzyme's (lipase) surface was analyzed by computational method (PyMOL) to find the exposed acidic amino acid residues that can be exploited for conjugation. Various techniques such as agarose gel electrophoresis, zeta measurement, FTIR-spectroscopy and TEM were performed to confirm of conjugation and these studies assured the lipase immobilization to nanoparticles. Catalytic (V_{max}, $K_{M,app}$, K_{cat}, and $K_{cat}/K_{M,app}$) and thermodynamic parameters for the deactivation of the enzymes (ΔH°_{D}, ΔS°_{D} and ΔG°_{D}) were evaluated to compare the differences in catalytic and stability properties of the free enzyme and immobilized enzyme. The higher specificity constant (80%) and 1.8-fold higher value of $V_{max}/ K_{M,app}$ for AuNP-NH_2-lipase conjugate over free lipase indicated easy substrate binding to the conjugated lipase and therefore significantly increase in catalytic efficiency. The T_m value of conjugated enzyme was higher than free enzyme by 10 units. These data suggested improved stability and activity upon immobilization of lipase to gold nanoparticles. The gain of activity was correlated with the structural studies using CD and fluorescence spectroscopic techniques that revealed minor structural rearrangements in the enzyme upon conjugation. Thus, it can be said that the significance of the study lies in the fact that the information obtained through structural studies helped in carrying out conjugation reaction and obtaining such immobilized lipase having minimum loss of structure with higher catalytic efficiency and improved stability

parameters. Therefore, it can be concluded that covalent conjugation in this way is reliable in obtaining nanozyme composite having enhanced activity, stability with the minor secondary changes. Such nanozyme composite formulations will be useful for various applications in industries due to their above mentioned qualities.

Sophorolipid; a glycolipid is another biomolecules that was used for the bio-fabrication of gold nanoparticles. In this study the synthesis of water-soluble gold nanoparticles was done using sophorolipid (SL) and sodium borohydride that was tested for antimicrobial activity. Characterization of nanoparticles was done by UV-Visible spectroscopy, DLS, zeta potential measurement and TEM that confirms the functionalization of sophorolipid to the gold nanoparticles (AuNPs-SL). Further antimicrobial studies such as agar plate assay, XTT and growth kinetics, revealed the potency of AuNPs-SL against metabolically active state of cells of Gram-positive *Staphylococcus aureus* and Gram-negative *Vibrio cholerae* and MIC was found to be 25µg/ml of AuNPs-SL. The activity of AuNPs-SL against these microbes also signifies to least metabolically active state; nondividing cells and biofilm of these microbes. AuNPs-SL induced morphological changes were studied by SEM that revealed AuNPs-SL led to disruption of the cell wall and leakage of intracellular fluid to the surroundings. Inhibition of respiratory enzymes activity also plays a crucial role in bactericidal action as indicated by LDH assay. Synergy of AuNPs-SL with different antibiotics ampicillin, kanamycin and polymyxin B was also analysed using checker board assay. Kanamycin and polymyxin B have shown synergy with AuNPs-SL for both of the microbes used in the study. In conclusion, it can be said that AuNPs-SL has potent antimicrobial activity against the microbes in their different phases of life cycle (either metabolically active cells or non dividing cells). The synergy of AuNPs-SL with antimicrobial agents can help in reduction of the need for high dosages and minimize side effects. These results suggested the possible use of AuNPs-SL as an antimicrobial therapy in the field of nanomedicine. Altogether it can be said that sophorolipid capped gold nanoparticles can be the promising agent for antimicrobial therapy (especially for Gram negative bacteria) in the field of nanomedicine.

Since AuNPs-SL is effective against both Gram-negative and Gram-positive microorganisms with higher efficacy toward Gram-negative bacteria therefore, we wanted to elucidate its mechanism of action against *V. cholerae*. The effect of cell wall and cell membrane was already observed that leads to release of DNA and protein content out of cells. Apart from this physical and mechanical exersion, physiological phenomenon was studied in detail.

AuNPs-SL stress affects the overall metabolism that leads to the formation of reactive oxygen species (ROS) and ultimately generates oxidative stress mediated responses. Changes in cell envelop integrity induce generation of ROS and alter the iron homeostasis. A concentration dependent increase in generation of ROS was also observed. In order to know the types of ROS species generated, different ROS quenchers (NAC, Tiron, Thiourea, ascorbate and SP) were used and that suggested the major ROS species were hydrogen peroxide, superoxide and hydroxyl radicals (H_2O_2, O_2^-, •OH). In presence of these quenchers, ROS generation was minimised and cells were rescued from AuNPs-SL mediated stress. Generation of ROS induces a cascade of events such as alteration of membrane potential, ATP depletion, DNA fragmentation and up-regulation of expression of genes encoding ROS scavenging enzymes and cellular repair mechanism. Here, we observed loss of membrane potential that leads to 50% ATP depletion of the cells at AuNPs-SL-10 µg/ml. DNA fragmentation is another outcome of oxidative stress and was measured by TUNEL assay. Cells undergo apoptotic mediated cell death as shown by Annexin affinity assay. Another interesting finding was related to supplementation of iron and DTT in AuNPs-SL stress cells as these two molecules rescue the cells in the stress condition. Concluding the study it can be said that AuNPs-SL generates the ROS and leads to oxidative stress mediated cell death.