Chapter 5

Summary and Conclusions

The summary of the results obtained and the conclusions drawn are presented in this chapter with future potential developments in the area.
5.1 Summary of the work

The major objectives of the thesis were to explore the biotransformation of arachidonic acid for production of 19-HETE and 20-HETE using Candida bombicola. A brief summary of the thesis is given below.

Chapter one presents a general introduction about eicosanoids and biotransformation of arachidonic acid metabolites. 20-hydroxyeicosatetraenoic acid (20-HETE) and 19-hydroxyeicosatetraenoic acid (19-HETE) are omega and omega–1 hydroxylated products of arachidonic acid which are important in autoregulation of blood pressure, vascular tone and other physiological roles. Different methods were discussed for the production of 19-HETE and 20-HETE. The intermediate sophorolipid was discussed in brief. Based on these review the scope and objective of the present work have been outlined.

Chapter two describes the fermentation parameters of Candida bombicola (ATCC 22214) for the production of arachidonic acid derived sophorolipids. The derived sophorolipids on acid hydrolysis liberated 19-HETE and 20-HETE. The different experimental techniques and analytical tools were used during the course of the present work are discussed in detail.

Chapter three deals with purification of the mono-, di- acetate forms of lactonic and acidic sophorolipids produced by Candida bombicola grown on glucose and long chain fatty acid i.e. arachidonic acid. The derived sophorolipids were isolated by silicagel chromatography using dialysis tubing. Different analytical tools were used to characterized the purified sophorolipids and their derived products This chapter also describes the detailed study of purified sophorolipids structure and presence of 19-
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hydroxyeicosatetraenoic acid and 20- hydroxyeicosatetraenoic acid in the sophorolipid form.

Chapter four focuses on the continuous synthesis of 19-HETE and 20-HETE by immobilization of Candida bombicola on different biocompatible materials. The patterned thermally evaporated octadecylamine (ODA) lipid films and free-standing organic–gold nanoparticles embedded in a polymeric membrane provide a biocompatible surface for the immobilization of whole cells. The presence of gold nanoparticles in the membrane enables facile modification of the surface properties of the membrane and has been used as enzyme sources for the transformation of arachidonic acid to 19-HETE and 20-HETE. The attachment of the cells to the ODA film surface occurs possibly through nonspecific interactions such as hydrophobic interactions between the cell walls and the ODA molecules. The enzyme cytochrome P450 present in the immobilized yeast cell membrane was used to catalyze the biotransformation of the arachidonic acid to sophorolipids and thereafter, acid hydrolysis to yield 19-hydroxyecosatetraenoic acid (19-HETE) and 20-hydroxyecosatetraenoic acid (20-HETE). These biocomposite materials could be easily separated from the reaction mixture and exhibit excellent reusability.

5.2. Conclusions of the work

Our approach here is to address the problem, the difficulties associated in eicosanoids (oxylipins) chemical synthesis, which expensive as well as hazardous in nature. The chemical synthesis of 19-HETE and 20-HETE involve more than 10 steps starting from arachidonic acid. These chemical methods are not commercially feasible. Presented work is aiming to reduce the price for the production of these HETE’s through biotransformation method.
Biotransformation of arachidonic acid by yeasts *Candida bombicola* (ATCC 22214) and *Candida apicola* (ATCC 96134) produced vasoactive molecules 19-hydroxyeicosatetraenoic acid (19-HETE) and 20-hydroxyeicosatetraenoic acid (20-HETE). These are omega and omega–1 hydroxylated products of arachidonic acid. 20-HETE and 19-HETE which play an important role in autoregulation of renal blood flow, tubuloglomerular feedback, renal sodium transport, pulmonary function and vasoconstrictor responses to numerous vasoactive hormones. This thesis describes the innovated methods for the continuous production of 19-HETE and 20-HETE. Different biocompatible materials were synthesized like patterned lipid films, and free-standing organic-gold nanoparticle poly membranes for the immobilization of *Candida bombicola* whole cells.

Biomolecules may easily get denatured and loose their biocatalytic activity after adsorbing on solid surfaces during immobilization. This was primary reason to design completely new class of material for immobilization. These provide a biocompatible environment and can readily conserve the native structure of biomolecules. This thesis describes the use of patterned lipid films, gold nanoparticles embedded polymeric membrane for the immobilization of *Candida bombicola* whole cells. The immobilized cells show enhanced temporal and indicating protective nature offered to whole cells on biocompatible materials. The enzyme cytochrome P450 present in the yeast cells was used to catalyze in situ ω and ω-1 hydroxylation of arachidonic acid. Cytochrome P450, the enzymes of interest is unstable outside the cellular environment and in such cases, immobilization of the whole cells would be important to catalyze reactions that are dependent on the cofactors. These biocompatible materials were easily separated from
the reaction mixture and reused by simple distilled water washing. They showed excellent reuse characteristics for the biotransformation of arachidonic acid.

The yeasts *Candida bombicola* (ATCC 22214) and *Candida apicola* (ATCC 96134) when grown on primary carbon source glucose and secondary carbon source arachidonic acid produced mixture of sophorolipids of 19-HETE and 20-HETE. The derived sophorolipids are stable than liberated hydroxylated fatty acids. These sophorolipids on acid hydrolysis yielded 19-HETE and 20-HETE. We have shown separation of sophorolipids to give increase stability to these heat and light labile biomolecules.

Patterned thermally evaporated octadecylamine (ODA) films are used for the immobilization of *Candida bombicola* cells. The attachment of the cells to the ODA film surface occurs possibly through the nonspecific interactions such as hydrophobic interactions between the cell walls and the ODA molecules. The presence of gold nanoparticles in the polymeric membrane enables facile modification of the properties of the membrane. Cytochrome P450 is a membrane bound protein and is known to be highly unstable outside the cells environment. Since the yeast cells are used, the cofactors such as nicotinamide adenine dinucleotide phosphate (NADPH) is not required and is readily supplied by the cells along with the primary enzyme. This is a major advantage in using the whole cells rather than using enzyme. Hence it was of paramount interest to used biocompatible materials for yeast cells. Biocompatible environment for the immobilization of *Candida bombicola* have done the hydroxylation of arachidonic acid to form sophorolipids and thereafter, acid hydrolysis gives 19-hydroxyeicosatetraenoic acid (19-HETE) and 20-hydroxyeicosatetraenoic acid (20-HETE).
This thesis also discusses about the intermediate arachidonic acid derived sophorolipids. These are extracellular surface active glycolipids produced by the yeasts *Candida bombicola*. The crude product was a heterogeneous mixture of sophorolipids, which are glycolipids of sophorose linked to the fatty acid through glycosidic bond between $\omega$ and $\omega$-1 carbon of arachidonic acid. The derived arachidonic acid sophorolipids were isolated by silicagel chromatography using dialysis tubing. Acid hydrolysis of the resolved sophorolipids yielded 20-hydroxyeicosatetraenoic acid (20-HETE) and 19-hydroxyeicosatetraenoic acid (19-HETE), the compounds of pronounced pharmaceutical importance.

5.3. Scope for future work

The present study examined the importance of oxylipins 19-hydroxyeicosatetraenoic acid and 20-hydroxyeicosatetraenoic acid derivatives of arachidonic acid. Arachidonic acid is primarily metabolized by a cytochrome P450 to these HETE’s. The importance of HETE’s in the regulation of renal function, vascular tone, airway resistance and many other physiological significance. The chemical synthesis of 19-HETE and 20-HETE methods are commercially cumbersome. It is known that terminal carbon center hydroxylation (omega) by chemical method is difficult due to lack of reactivity at this carbon. In this context the most useful characteristic of microbial transformation is that offers unusual activation at normally unreactive carbon centers where no conventional chemistry is applicable. These microbes not only help to permit simpler methods but also safer and economical ways of producing these biologically active compounds.
Immobilization of whole cells on biocompatible materials enhances the production of HETE’s. Thermally evaporated lipid films and gold nanoparticle polymeric membranes can act as scaffolds for growth of different cells in tissue engineering. These materials can be used for the growth of different microorganisms and thus suggests potential biomedical applications as biocompatible implants, grafting in bone surgery, drug delivery, etc. The facility of patterning biomaterials can be extended towards the patterned immobilization of DNA, and can be used for screening genomic libraries. Immobilizing various proteins on a single chip can easily perform multi-step biocatalytic reactions requiring many enzymes, wherein each enzyme would specifically react with its substrate giving desired final product. The replacing the chemical approaches for eicosanoids with microbial route will reduce the cost coupled with the possibility of obtaining other novel compounds. From these studies it is expected to obtain hitherto unknown but structurally relevant novel products which may posses biological activation far superior to the known product.