CHAPTER VIII

CONCLUSION
 CHAPTER VIII

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This study is an attempt to investigate the effect of chemical structure of surfactant on the enzymatic activity and modulation of parameters used for the enhancement of enzyme activity and formation of nanoparticles. For this purpose, the interactions between three types of surfactants with enzyme HRP were studied. The surfactants used were anionic Aerosol-OT (AOT), cationic CTAB and non-ionic TX-100. The aqueous core of the water in oil microemulsion was used as nanoreactor for the enzymatic reactions and formation of nano-particles. The size of the reverse micellar droplets of the water-in-oil microemulsion is in nanometer range, so the particles formed in the aqueous core are also in the same scale. Moreover, the conformation and activity of the biomolecules like enzymes depends upon the size of the aqueous core. Therefore by tuning the size of the host aqueous core, biomolecules of varying activities can be immobilized. The conformation and activity of bio-molecules depends upon various parameters like surfactant type, surfactant concentration, etc. Micro-emulsion route is used to synthesize enzyme encapsulated nano-materials. These bio-molecules doped nano-carriers can be used in many areas like immunochemistry, drug delivery, enzyme therapy, bio-sensors, electro-chemistry etc.

This thesis work was started with three main objectives, the first one is to find out which surfactant is appropriate for bio-molecules encapsulation and the formation of nanoparticles specially enzyme encapsulated nano-particles.

The second was to develop a reproducible methodology for the synthesis of magnetic nanoparticles encapsulating enzyme HRP with smaller size and monodispersity.

The third objective was to find out the activity of encapsulated enzyme and these enzyme encapsulated nanomaterials was applied as “in vitro” prodrug activation. That is to explore the potentials of these carriers for cancer therapy.
Chapter VIII

We have started our experiments oriented at optimizing different micellar conditions at which the enzyme would show maximum catalytic activity. We used three surfactant systems anionic (AOT), cationic (CTAB) and non-ionic (TX-100) to encapsulate the enzyme HRP in the aqueous core of reverse micellar system and to study immobilized enzyme kinetics. The enzyme encapsulate in anionic and non-ionic surfactant systems retain its catalytic activity. In TX-100 reverse micellar system enzyme showed higher activity and in AOT reverse micelle enzyme showed moderate activity. But enzyme entrapped in CTAB surfactant system alone did not show any activity but CTAB in mixed state with anionic and non-ionic surfactant systems show appreciable enhancement in activity.

In the present investigation, we describe the enzymatic activity of the enzyme in aqueous as well as in micellar system at constant temperature (25°C) and at constant pH (7.2). The entrapped enzyme in the aqueous core of reverse micelles shows significant activity. It is found that enzyme shows a bell-shaped dependence of activity with the $W_0$ and surfactant concentration. AOT and TX-100 shows maximum activity at $W_0$ 21 and 10 respectively, higher than that of free enzyme in aqueous buffer. This may be attributed to the phenomenon of ‘superactivity’ of enzyme where the most favourable conformation of the enzyme gets rigidified. This situation is observed when the size of the host aqueous core becomes identical to the size of the enzymes.

The above observation has prompted us to use AOT reverse micellar system for the formation of enzyme encapsulated magnetic nanoparticles. We used AOT/ hexane reverse micellar system for the rest of our studies because this system does not require any co-surfactant and because of the ease of their removal from the nanoparticles. In this system the hexane was used as a solvent. The hexane is a volatile solvent and it can be evaporated on a vacuum evaporator, and surfactant AOT can easily be removed by washing with ethanol 2-3 times.

Towards the next goal, we prepared HRP encapsulated iron-oxide nanoparticles via co-precipitation method in the aqueous core of AOT reverse micellar system. As in
case of nano-materials the most important thing is to prepare nanoparticles with optimum size and monodispersity so we have maintained parameters like temperature, $W_0$, surfactant concentration and reactant concentrations. The interdroplet interaction of the reverse micellar droplet has a strong influence on the size of the nano-particles prepared. To minimize this effect the iron-oxide nanoparticles were prepared at low temperature $\sim$5-6 °C. Characterization was done with TEM, XRD, VSM, FT-IR and DLS. The magnetization studies revealed the superparamagnetic behaviour of the nanoparticles.

We found that the iron-oxide nanoparticles, because of magnetic interaction, have high tendency to agglomerate. We need to control various parameters like temperature, pH, concentration etc to reduce the agglomeration. Surface modification with PEG also controls agglomeration. We also found that bare iron-oxide nanoparticles, having higher tendency to agglomerate are cytotoxic, but PEG modified nanoparticles are non-toxic to cells.

We have also prepared enzyme encapsulated microemulsion mediated bio-compatible ultrafine manganese phosphate nanoparticles and have extensively characterized them. TEM image showed the monodispersity of the particles. The prepared particles are very small (<100nm) and are highly monodispersed with considerable uniformity of biomaterial within the matrices. VSM study showed the ferromagnetic nature of the particles. Comparative in vitro cytotoxicity analysis on cell-lines showed that manganese phosphate nanoparticles without any surface modification showed 60% cell viability and bare iron-oxide nanoparticles showed 30% cell viability.

We found that, the enzyme, HRP was successfully entrapped, with high entrapment efficiency, inside the SPION and manganese phosphate nano-particles. The activity of entrapped HRP was determined by using it for the oxidation of o-dianisidine. The activity of entrapped enzyme was found to be less as compared to the activity of free enzyme in aqueous buffer. The reduction in activity may be accounted for various factors viz constrained diffusion of the substrate across the pores of nanoparticles, some less active conformation taken by the enzyme when it is entrapped inside.
nanomatrix. The temperature and pH dependent enzyme kinetic studies indicate that the entrapped enzyme in manganese phosphate nano-particles showed an increase in thermal stability and are stable towards pH changes. These observations can be attributed to the rigid nano-matrix providing the protective environment to the enzyme, by reducing the freedom of peptide chain refolding molecular motions, which occurs in the thermal denaturation process but at the same time local mobility of the enzymes is sufficiently high to preserve an enzymatic activity.

The exciting results obtained above, prompted us to use the same carrier as efficient and safer biomedical tool. The third objective of this work is in vitro prodrug activation by enzyme HRP encapsulated in iron-oxide and manganese phosphate nanoparticles. Cytotoxicity studies were done with these particles on mammalian cell-lines using MTT-assay. PEG modified iron-oxide nanoparticles were used which are non-toxic and biocompatible at the level it is used. In addition PEGylation also help in the aqueous dispersion of these nanoparticles.

**Based on the above observations the following conclusions are drawn:**

1. The aqueous core of reverse micelles can be used as host for enzymes.
2. Enzyme HRP was encapsulated in AOT, TX-100 and CTAB and mixed surfactant systems to find out the appropriate surfactant system for HRP encapsulation.
3. There is no need of co-surfactant in AOT reverse micelle system but non-ionic and cationic surfactant systems need co-surfactant for reverse micelle formation.
4. The activity of HRP encapsulated in reverse micelles depends on various parameters like $W_0$, surfactant concentration and enzyme shows bell-shaped dependence of activity with these parameters.
5. The enzyme shows Michaelis-Menton behaviour encapsulated in reverse micelles as in aqueous buffer.
Chapter VIII

6. The enzyme HRP shows more activity in reverse micelles than in aqueous buffer, this may be attributed to the phenomenon of ‘superactivity’ of enzyme although the enzyme is less stable in reverse micellar system.

7. The enzymatic reaction rate can be modulated by modulating the interdroplet interaction in reverse micelles.

8. The enzyme HRP shows higher activity in TX-100 reverse micelles, moderate activity in AOT reverse micellar system, HRP is inactive in CTAB reverse micellar system and in case of CTAB surfactant mixed with anionic and non-ionic surfactant, enzyme HRP shows moderate activity.

9. The aqueous core of reverse micelles can also be used as nano-reactor for the preparation of different kinds of particles in the nanometer range.

10. The size and shape of nanoparticles prepared by water in oil microemulsion method can be varied by modulating the water-to-surfactant ratio.

11. The ultra-fine and mono-disperse magnetic iron-oxide nanoparticles encapsulating enzyme HRP can be prepared via co-precipitation method in reverse micelles.

12. TEM and VSM analysis showed iron-oxide nanoparticles are spherical in shape having 30nm diameter and showed superparamagnetic nature.

13. The Michaelis-Menton kinetics of entrapped HRP was determined by using the entrapped enzyme for the oxidation of o-dianisidine with H₂O₂. The kinetic parameters were determined by varying the concentration of H₂O₂.

14. Enzyme entrapped within the iron-oxide matrix are able to retain their activities and the enzyme substrate reaction takes place by the diffusion of smaller substrate molecules through the pores of the iron-oxide matrix.

15. A high value of Km for the enzyme entrapped in iron-oxide nanoparticles as compared to free enzyme in aqueous buffer indicates low affinity of the enzyme towards the substrate.

16. Iron-oxide nanoparticles have high tendency for agglomeration, followed by sedimentation. The sedimentation rate of nanoparticles depends on various parameters.

17. PEGylation controls the agglomeration of nanoparticles.
18. Agglomeration of iron-oxide nanoparticles also causes cyto-toxicity. But PEGylated iron-oxide nanoparticles are non-toxic.

19. Biocompatible enzyme encapsulated manganese phosphate nanoparticles can be prepared by reverse micellar route.

20. TEM and VSM analysis showed that the particles are monodispesee, spherical in shape with 50nm diameter and having ferromagnetic nature.

21. The enzyme HRP entrapped in manganese phosphate nanoparticles is protected from the extreme pH and temperature conditions and is not denatured by these extreme conditions.

22. The free enzyme shows maximum activity at a temperature of about 40°C and at pH 7.2, but entrapped enzyme shows maximum activity at temperature of about 55°C and at a pH 7.4.

23. The Michaelis-Menten kinetics of the HRP entrapped in manganese phosphate nanoparticles was determined for a reaction in which a dye o-dianisidine was oxidized by HRP in the presence of H₂O₂ into a coloured product having maximum absorption at 445nm. The kinetics of entrapped HRP was compared with that of free enzyme in aqueous buffer.

24. The kinetic parameters showed that the activity of enzyme entrapped in manganese phosphate is lower as compared to the free enzyme in aqueous buffer.

25. The comparative cytotoxicity studies of bare iron-oxide nanoparticles and manganese phosphate nanoparticles showed that manganese phosphate nanoparticles are less toxic.

26. These enzyme encapsulated magnetic nano-particles can be used as in-vitro prodrug activation, having the potential to become promising system for cancer treatment.