# TABLE OF CONTENTS

## CHAPTER I:
### INTRODUCTION

1.1 INTRODUCTION 1

1.2 AIMS AND OBJECTIVES 15

REFERENCES 18-29

## CHAPTER II:
### LITERATURE REVIEW

2.1 INTRODUCTION 30

2.2 STRATEGIES USED TO SYNTHESIZE NANOPARTICLES 31

2.2.1 Microemulsion mediated nanoparticle synthesis 33

2.2.1.1 Parameters influencing nanoparticle synthesis 35

2.2.2 Novel approaches for the extraction of nanoparticles from reaction Mixtures 37

2.3 RECENT ADVANCES IN THE USE OF MICROEMULSIONS AS CONFINED REACTION MEDIA FOR THE SYNTHESIS OF INORGANIC NANOPARTICLES 38

2.4 CHARACTERIZATION TECHNIQUES FOR MICROEMULSIONS 38

2.5 INORGANIC ANOPARTICLES 39

2.5.1 Magnetic nanoparticles 43

2.6 ENZYME ENCAPSULATION IN REVERSE MICELLAR SYSTEM 47

2.6.1 Advantages of using reverse micellar system 50

2.7 DETERMINANT FACTORS FOR BIOCATALYSIS IN REVERSED MICELLES 51
CHAPTER III
EXPERIMENTAL

3.1 MATERIALS AND THEIR PURIFICATION 90

3.1.1 Surfactants 90

3.1.2 Solvents 92

3.1.3 Enzymes and other chemicals 93

3.2 PREPARATION OF TERNARY SOLUTIONS OF WATER-AOT-HEXANE 94

3.3 CHARACTERIZATION OF THE NANO-PARTICLES 95

3.3.1 Quasi Elastic Light Scattering (QELS) 95

3.3.2 Transmission Electron Microscopy (TEM) 98

3.3.3 X-Ray Diffraction (XRD) 100

3.3.3.1 Crystal Lattice 100

3.3.3.2 Diffraction 100

3.3.3.3 How large are the crystallites? 101

3.3.4 Fourier Transform Infrared (FT-IR) Spectroscopy 102

3.3.5 Ultraviolet - Visible Spectrophotometric studies 103
3.3.6 Vibrating Sample Magnetometer (VSM) 104

3.3.7 Atomic Absorption Spectroscopy (AAS) 106

3.4 ENTRAPMENT EFFICIENCY STUDIES (SPECTROPHOTOMETRICALLY) 106

3.5 ENZYME KINETIC STUDIES (SPECTROPHOTOMETRICALLY) 107

3.6 ATTACHMENT OF NANO-PARTICLE TO POLYETHYLENE GLYCOL (PEGYATION) 107

3.7 In Vitro CELL VIABILITY/CYTOTOXICITY STUDIES 108

REFERENCES 109-110

CHAPTER – IV
COMPARATIVE STUDIES ON THE ENZYMATIC ACTIVITY OF HRP ENCAPSULATED IN REVERSE MICELLES OF ‘ANIONIC’, ‘CATIONIC’ AND ‘NON-IONIC SURFACTANT’ SYSTEMS

4.1 INTRODUCTION 111

4.1.1 Enzyme encapsulation in reverse micelles 115

4.2 APPLICATIONS AND PROSPECTS OF MICELLAR ENZYMEOLOGY 121

4.3 ENZYME KINETIC STUDIES OF ENCAPSULATED HRP IN MICELLER MEDIA 122

4.3.1 Determination of \( K_{\text{cat}} \) 123

4.4 ACTIVITY AND ENZYME KINETIC STUDIES OF HORSE RADISH PEROXIDASE ENTRAPPED IN TERNARY SYSTEMS OF REVERSE MICELLES 124

4.4.1 U.V. Spectrophotometric analysis 125

4.4.2 Effect of \( W_0 \) on the activity of HRP in reverse micelles 127

4.4.3 Effect of surfactant concentration on the activity of HRP 129
4.4.4 Estimation of $K_{\text{cat}}$ and $K_m$ in reverse micelles of anionic and non-ionic systems from activity data

4.5 ACTIVITY MEASUREMENT OF HRP IN CATIONIC AND MIXED REVERSE MICELLAR SYSTEM

4.5.1 Measurement of peroxidase activity

4.6 CONCLUSION

REFERENCES

CHAPTER V
SUPERPARAMAGNETIC IRON-OXIDE NANO-PARTICLES ENCAPSULATING ENZYME HRP, IT’S STABILITY STUDIES AND EFFECT OF AGGLOMERATION ON CYTOTOXICITY

5.1 INTRODUCTION

5.2 MAGNETIC NANO-PARTICLES

5.2.1 Iron-oxide nano-particles

5.3 TARGETED DRUG/ENZYME DELIVERY USING SUPERPARAMAGNETIC IRON-OXIDE NANO-PARTICLES

5.4 PREPARATION OF VOID AND HRP DOPED IRON-OXIDE NANO-PARTICLES IN MICROEMULSION MEDIA

5.5 STUDY OF PARTICLE SIZE AND SHAPE BY QELS AND TEM

5.6 X-RAY DIFFRACTION STUDIES OF IRON-OXIDE NANOPARTICLES

5.7 MAGNETIC PROPERTIES OF IRON-OXIDE NANOPARTICLES

5.8 TOTAL IRON CONCENTRATION

5.9 KINETICS OF HORSEBADISH PEROXIDASE (HRP) IN AQUEOUS BUFFER AND HRP ENCAPSULATED IN IRON-OXIDE NANOPARTICLES

5.9.1 Determination of catalytic constant ($K_{\text{cat}}$)
CHAPTER VI
PHYSICO-CHEMICAL STUDIES OF ENZYME HRP ENCAPSULATED IN MANGANESE PHOSPHATE NANO-PARTICLES AND COMPARATIVE CYTOTOXIC STUDIES OF MANGANESE PHOSPHATE WITH IRON-OXIDE NPs

6.1 INTRODUCTION 213

6.2 INORGANIC NANO-PARTICLES 214

6.3 MANGANESE PHOSPHATE NANO-PARTICLES 216

6.4 INORGANIC NANO-PARTICLES AS DRUG DELIVERY VEHICLES 217

6.5 PREPARATION OF HRP ENCAPSULATED MANGANESE PHOSPHATE NANO-PARTICLES 220
6.6 CHARACTERIZATION

6.6.1 Transmission Electron Microscopy (TEM)

6.6.2 X-RAY Diffractogram (XRD) of manganese phosphate Nanoparticles

6.6.3 FT-IR spectra of manganese phosphate nanoparticles

6.6.4 VSM analysis of manganese phosphate nanoparticles

6.7 ENZYME KINETIC STUDIES OF HRP ENCAPSULATED IN MANGANESE PHOSPHATE NANOPARTICLES

6.7.1 Determination of catalytic constant ($K_{cat}$)

6.8 EFFECT OF pH ON THE ACTIVITY OF FREE HRP IN AQUEOUS BUFFER AND HRP ENCAPSULATED INSIDE MANGANESE PHOSPHATE NANOPARTICLES

6.9 EFFECT OF TEMPERATURE ON THE ACTIVITY OF FREE HRP IN AQUEOUS BUFFER AND HRP ENCAPSULATED IN MANGANESE PHOSPHATE NANO-PARTICLES

6.10 CYTOTOXICITY STUDIES

6.10.1 COMPARATIVE IN VITRO CYTOTOXICITY STUDIES OF MANGANESE PHOSPHATE NANOPARTICLES WITH IRON-OXIDE NANO-PARTICLES

6.10.1.1 Maintenance of cell culture and assessment of the cytotoxicity of nanoparticles to MCF-7 cell-line

6.10.1.2 Survival assay

6.11 CONCLUSION

REFERENCES
CHAPTER VII
ENZYME ‘HRP’ ENCAPSULATED MAGNETIC NANOPARTICLES APPLIED AS IN VITRO PRODRUG ACTIVATION BY ENZYME HRP

7.1 INTRODUCTION 253

7.2 PRODRUG ACTIVATION BY ENZYME, A PROMISING STRATEGY FOR CHEMOTHERAPY 257

7.3 MAINTENANCE OF CELL CULTURE 261

7.3.1 Survival assay 262

7.4 CONCLUSION 264

REFERENCES 266-270

CHAPTER VIII

CONCLUSION 271-276