

Sr. No.	DETAILED CONTENTS	Page no.
<b>1.</b>	<b>INTRODUCTION</b>	<b>1</b>
1.1	Types of Magnetic particles	6
1.2	Molecular biology applications	7
1.2.1	DNA isolation	7
1.2.2	Magnetic separation of poly(A) mRNA	11
1.2.3	Isolation of nucleic acid binding biomolecules	14
1.2.4	Magnetocapture protein interaction assays	16
1.3	Cell biology applications	22
1.3.1	Immunomagnetic cell isolation and separation	22
1.4	Diagnostics applications	28
1.4.1	Immunomagnetic assays	28
1.5	Biomedical applications	30
1.5.1	Drug targeting using magnetic targeted carriers (MTCs)	30
1.5.2	Magnetic fluid hyperthermia	32
1.5.3	Magnetoliposomes as drug delivery vehicles	33
1.6	Objectives of the present study	40
<b>2.</b>	<b>MATERIALS AND METHODS</b>	<b>41</b>
2.1	Preparation of magnetic nanoparticles ( $\text{Fe}_3\text{O}_4$ )	42

2.2	Characterization of magnetic nanoparticles	44
2.2.1	Transmission electron microscopic (TEM) examination of magnetic nanoparticles for size determination	44
2.2.2	Determination of composition of magnetic nanoparticles (Fe <sub>3</sub> O <sub>4</sub> ) using Fourier transform infrared spectroscopy	45
2.3	Preparation of silica magnetite beads	46
2.4	Genomic DNA isolation using magnetite as solid-phase support	47
2.4.1	Determination of DNA binding capacity of the magnetite particles	47
2.4.2	Optimization of procedure for genomic DNA extraction from whole blood	49
2.4.3	Standardized procedure for DNA extraction from whole blood using magnetite as support	51
2.4.4	Optimized procedure of genomic DNA extraction from cultured cells and tissue homogenate	53
2.5	Isolation of genomic DNA from prokaryotic cells	53
2.5.1	Optimized procedure for genomic DNA extraction from gram positive bacteria ( <i>S. flaviscleroticus</i> )	54
2.5.2	Optimized procedure of genomic DNA extraction from gram negative bacteria ( <i>E. coli</i> )	55
2.5.3	Optimized procedure for genomic DNA extraction from yeast ( <i>S. cereviceae</i> )	56
2.5.4	Optimized procedure for genomic DNA extraction from <i>Dictyostelium discoideum</i>	57

2 5 5	Optimized procedure for Isolation of plasmid DNA from bacterial cells	57
2 5 6	Isolation of genomic DNA from plant tissue	58
2 5 7	Extraction of DNA from agarose gel using magnetite as solid-phase support	59
2 5.8	PCR amplification of genomic DNA from mammalian cells	60
2 5 9	Restriction endonuclease digestion of extracted DNA	61
2 5 10	Agarose gel electrophoresis of isolated DNA	61
2.6	Covalent immobilization of alkaline phosphatase and streptavidin onto naked magnetic particles	62
2 6 1	Determination of ALP activity	63
2 6 2	Procedure for performing streptavidin-biotin interaction	65
2 6.3	Procedure for stability study of immobilized preparation	65
2 6 4	Application of magnetic ALP particles for plasmid dephosphorylation	66
2 6 5	Restriction digestion of plasmid DNA (pcDNA3) with Hind III restriction enzyme	67
2 6 6	Protocol for dephosphorylation of 5'-termini of DNA	67
2 6 7	Bradford dye binding method for protein estimation	68
2.7	Magnetoliposomes as drug delivery carrier	70
2 7.1	Preparation of magnetoliposomes	70
2 7 2	Characterization of magnetoliposomes	71

2 7 3	Drug encapsulation in magnetoliposomes	71
2 7.4	Storage stability of magnetoliposomal formulation	73
2 7 5	<i>In vitro</i> study to evaluate drug leaching from liposomes/magnetoliposomes	73
2 7 6	Dilution induced drug leakage	73
2 7 7	Effect of serum component on drug loaded liposomes/magnetoliposomes	74
2 7 8	Study of flow characteristics of liposomal / magnetoliposomal formulation	74
2.7 9	<i>In vivo</i> biodistribution of DOX loaded liposomes / magnetoliposomes	76
2 7.10	Determination of DOX encapsulated liposome / magnetoliposome concentration in plasma	77
2 7 11	<i>In vivo</i> efficacy of DOX loaded liposomes / magnetoliposomes in tumor regression	77
<b>3.</b>	<b>RESULTS AND DISCUSSION</b>	<b>79</b>
3 1	Development of DNA extraction procedure using magnetite as solid support	80
3 1.1	Preparation of magnetite nanoparticles	80
3 1 2	Characterization of magnetic nanoparticles	81
3.2	Genomic DNA extraction using magnetite as solid-phase adsorbent	84
3.2.1	Standardization of adsorption/desorption conditions for DNA on magnetite	86

3 2 2	Mechanism of DNA binding to magnetite	87
3 2 3	Genomic DNA extraction from whole blood	88
3 2 4	Genomic DNA isolation from PBMC's	91
3 2 5	Genomic DNA isolation from buffy-coat	92
3 2 6	Genomic DNA isolation from cultured cells (HCT116)	95
3 2 7	Genomic DNA isolation from tissue homogenate	95
3.3	Comparative study of present method with conventional and coated magnetic beads	98
3.4	Performance comparison of Magnetic DNA extraction method with commercial kit (Qiagen)	101
3.5	Genomic DNA isolation from bacteria	106
3 5 1	Genomic DNA isolation from gram-positive bacteria ( <i>S. flaviscleroticus</i> )	106
3 5 2	Genomic DNA isolation from gram-negative bacteria ( <i>E. coli</i> , <i>S. typhi</i> )	110
3 5 3	Plasmid DNA isolation from bacteria	113
3.6	Genomic DNA isolation from yeast ( <i>S. cereviceae</i> )	118
3.7	Genomic DNA isolation from <i>Dictyostelium discoideum</i>	121
3.8	Genomic DNA isolation from plant tissue	123
3.9	DNA extraction from agarose gel	127
3.10	Suitability of the extracted DNA with the present method for downstream application	133

3.10.1	Restriction endonuclease digestion of extracted genomic DNA from blood and cultured cells	133
3.10.2	Restriction endonuclease digestion of extracted genomic DNA from mammalian tissue	137
3.10.3	Restriction endonuclease digestion of extracted genomic DNA from bacterial cells	138
3.10.4	Restriction endonuclease digestion of extracted genomic DNA from yeast and <i>Dictyostelium</i>	140
3.11	Polymerase Chain Reaction (PCR) amplification	142
3.11.1	PCR amplification of 226-bp fragment of GAPDH gene	142
3.12	SUMMARY	145
4.1	Covalent immobilization of calf alkaline phosphatase on magnetic nanoparticles	153
4.1.1	Preparation of magnetic particles	155
4.1.2	Immobilization of ALP onto magnetic particles	157
4.1.3	Confirmation of ALP binding with FTIR spectroscopy	167
4.1.4	Stability of immobilized ALP	168
4.1.5	Use of immobilized ALP for plasmid dephosphorylation	172
4.2	Covalent immobilization of streptavidin on magnetic nanoparticles	176
4.2.1	Immobilization of streptavidin on magnetic particle	177
4.2.2	Biotin binding capacity of magnetic streptavidin nanoparticles	180

4 2.3	Confirmation of streptavidin binding with FTIR spectroscopy	185
4 2.4	Stability of immobilized streptavidin	186
4 3	<b>SUMMARY</b>	187
5.1	<b>Magnetic liposomes as drug targeting carriers</b>	189
5 1 1	Preparation of Magnetoliposomes (MLs)	190
5 1 2	Physical characterization of the Magnetoliposomes	191
5 1 3	TEM characterization of the Magnetoliposomes	191
5 1 4	Biochemical characterization of the Magnetoliposomes	193
5 1 5	Drug encapsulation efficiency of magnetoliposomes	193
5 1 6	Storage stability of liposomes / magnetoliposomes-encapsulated doxorubicin	195
5 2	<i>In vitro</i> drug release characteristics of liposomes / magnetoliposomes	197
5 2 1	Dilution induced drug release from liposomes/magnetoliposomes	197
5 2 2	Effect of serum component on the stability of liposome/ magnetoliposomes -encapsulated doxorubicin	198
5 2 3	Stability of liposomes/ magnetoliposomes in closed circulatory system ( <i>in vitro</i> study)	201
5.3	<i>In vivo</i> biodistribution of DOX	205
5 3.1	<i>In vivo</i> efficacy of DOX loaded liposomes / magnetoliposomes in tumor regression	208

5.4	SUMMARY	212
6	CONCLUSIONS	217
7	BIBLIOGRAPHY	234
8	List of publications and presentations	256