CHAPTER 1

INTRODUCTION

1.1 NANOTECHNOLOGY IN DRUG DELIVERY AND PHARMACEUTICAL APPLICATIONS

Nanoparticles are solid colloidal particles or particulate dispersions with particle size ranging from 10-1000 nm. (Limayem et al 2004). In recent years, nanotechnology has attracted attention as it improves the effectiveness of drugs. Polymeric nanoparticulate systems appear to be feasible and promising tool for delivery of many bioactive. The development of polymeric nanoparticles for targeted delivery and controlled drug release may improve the therapeutic index of drugs (Gu et al 2008), as they are stable in blood, nontoxic, nonthrombogenic, nonimmunogenic, noninflammatory and biodegradable. They also act as carriers for proteins, peptides and nucleic acids (Kumari et al 2010).

There has been considerable interest in developing nanoparticles as effective drug carriers, wherein the drug or biologically active material is entrapped, encapsulated, adsorbed or attached (Liu et al 2007, Muthu & Singh 2009). Recently, a nanoparticle formulation of paclitaxel (nanometer sized albumin bound paclitaxel (Abraxane)), has been applied for the treatment of metastatic breast cancer (Cho et al 2008). Synthetic polymers have contributed to further interest in this area. Some of these polymers are known for their biocompatibility and resorbability through natural pathways. Research is now focused on the preparation of nanoparticles using hydrophilic polymers (Hans & Lowman 2002, Soppimath et al 2001). Their high degree of surface hydrophilicity will enhance the circulation time of nanoparticles,
water solubility and decrease the sensitivity to enzymatic degradation, thereby enhancing biocompatibility. When exposed to an aqueous environment, hydrophilic nanoparticles would degrade easily (Wang et al 2008, Feng & Chien 2003).

1.2 POLY (2-HYDROXYETHYL METHACRYLATE)

Poly (2-hydroxyethyl methacrylate) (PHEMA) is a hydrophilic synthetic polymer widely used as a drug carrier (Hong et al 2010, Chouhan & Bajpai 2009). PHEMA is successfully used in biomedical applications because of their distinctive biocompatibility like absence of toxicity and compatibility with blood (Ma et al 2012, Holly & Refojo 1975, Ayhan & Ozkan 2007, Hsiue et al 2001). Some of its applications include soft eye contact lenses, drug delivery scaffold materials in tissue engineering, hydrogels in biomedical engineering, artificial cornea, potential substrate for artificial skin, rhinoplast, drug delivery systems, tube shaped structured grafts and bone composite materials (Chouhan & Bajpai 2009a, Chouhan & Bajpai 2010, Slaughter et al 2009, Kiremitci et al 1991). The presence of polar (hydroxyl) and carbonyl groups on each repeat unit in PHEMA makes this polymer highly compatible in water. The presence of hydrophobic α-methyl groups of the backbone conveys hydrolytic stability to the polymer (Chouhan & Bajpai 2009a). There is wide interest with PHEMA nanoparticles in drug delivery systems. Several researchers have employed the PHEMA as a carrier matrix for delivering drugs such as doxorubicin, paclitaxel, quercitin, prednisolone, 5-fluoro-uracil, ciprofloxacin dexamethasone and gentamicin sulphate (Chouhan & Bajpai 2009, Ma et al 2012, Curcio et al 2011, Lou et al 2004, Chouhan & Bajpai 2009a, Chouhan & Bajpai 2010, Hong et al 2010a).
1.3 CURCUMIN

Curcumin (Diferuloylmethane), a low molecular weight, lipophilic, natural yellow polyphenolic compound of Indian spice turmeric (Curcuma longa), has the chemical structure of (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione). It has been used in traditional medicine for many centuries in India and China (Mukerjee & Vishwanatha 2009, Shaikh et al 2009, Cartiera et al 2010, Duan et al 2010, Manju & Sreenivasan 2011, Suwannateep et al 2011). It possesses a wide range of biological and pharmacological properties, and is used for the treatment of neurodegenerative, cardiovascular, pulmonary, autoimmune and neoplastic diseases (Shaikh et al 2009), biliary disorders, hepatic disorders, diabetic wounds, rheumatism (Duan et al 2010), skin cancer treatment (Mangalathillam et al 2012) and is an antioxidant, antiviral, anticancer agent (Rejinold et al 2011, Anitha et al 2011), showing anti-inflammatory drug properties (Mukerjee & Vishwanatha 2009, Zheng et al 2010, Kim et al 2011). Though curcumin has good therapeutic efficacy (topical and oral), the problem is its low solubility in aqueous solution, degradation at alkaline pH, photodegradation and poor oral bioavailability (Duan et al 2010, Song et al 2011, Rejinold et al 2011a). Several methods have been undertaken to enhance the bioavailability of curcumin.

Carrier mediated curcumin delivery is the potential way to overcome these problems without affecting its efficacy (Rejinold et al 2011b). Yallapu et al (2010) have synthesized the poly (lactic acid-co-glycolic acid) Nanoparticles of Curcumin (Nano-CUR) and this formulation was found to inhibit the cell proliferation in chemo/radio-resistant cancer cells. Mohanty & Sahoo (2010) studied Glycerol Mono Oleate (GMO) nanoparticles loaded curcumin that improved the systemic bioavailability. They found that nanoparticulate curcumin was more effective than native curcumin in
different cancer cell lines. Recent research has concentrated on delivering curcumin (Maya et al 2013) through different carrier molecules such as polymeric micelles, polymeric nanoparticles, liposomes, lipid based nanoparticles, hydrophilic polymers or hydrogels, cyclodextrin etc. Hence an attempt has been made in this study to increase the bioavailability of curcumin by incorporating it in the PHEMA nano particulate system.

1.4 STEARIC ACID

Stearic Acid (SA), an endogenous long-chain fatty acid, biocompatible with human tissues and neutral with relevance to physiological fluids. Lipids in combination with a hydrophilic polymer (in the assembly of nanoparticles) are found to possess excellent physical and chemical stability and hence provide protection against degradation of drugs (Hu et al 2013). Lipid mixtures have the ability to load hydrophobic, hydrophilic and alternative biomacromolecules like peptides and proteins (Severino et al 2011, Hu et al 2005). Stearic acid has potential and is a well-documented pharmaceutical excipient for anticancer drug delivery due to its ability to internalize in cancer cells and accumulate in cytoplasm (Severino et al 2011, Miao et al 2012). The polymer/lipid based nanoparticles have not yet been explored as potential systems for the delivery of curcumin.

Generally, free radical initiators are employed to initiate HEMA polymerization in contrast to the present method wherein toxic initiators are not used to produce the polymer/hydrogels. Here choline based ionic liquid was employed as an initiator to produce polymeric gels. Jensen et al (2009) reported the ability of choline based ionic liquid (choline formate) to make hydrogels and copolymers. The main objective here is to replace the traditional solvents with choline based ionic liquid and investigate the potential of CF as an initiator for the polymerization of HEMA. The IL
method provides an alternate route to produce polymers and offers an easy method to recycle the ionic liquid employed in the process.

In comparison to hydrophobic polymer matrix for drug loading, hydrophilic matrix has a distinct advantage of biocompatibility to the aqueous body environment and does not require surface modifications. Furthermore, the biocompatible polymers prevent monocytes from adhering to their surface (Yamit et al 2009).

There is no report now on the use of P (2-Hydroxyethyl methacrylate) (PHEMA) nanoparticles for the delivery of curcumin. PHEMA gels were synthesized and curcumin loaded PHEMA nanoparticles (C-PHEMA-NPs) prepared from gelled ionic liquid using nanoprecipitation technique. The C-PHEMA-NPs were characterized by different physicochemical techniques and an in vitro anticancer study was carried out to evaluate their potential as drug carriers for the sustained delivery of curcumin on cancer cell lines.

1.5 SCOPE AND OBJECTIVES

The major objectives are

- Studies on the role of choline formate ionic liquid in free radical polymerization in the preparation of PHEMA.


- Synthesis of curcumin loaded poly (2-hydroxyethyl methacrylate) nanoparticles from gelled ionic liquid and in vitro evaluation of anticancer activity in SKOV-3 cells.
- Synthesis and characterization of curcumin loaded polymer/lipid based nanoparticles and evaluation of their antitumor effects on MCF-7 cells.

The steps involved are as follows:

**Studies on the role of choline formate ionic liquid in free radical polymerization.**

- The main objective here is to replace the traditional toxic solvents and initiators with choline based ionic liquid and investigate its potential as an initiator for the polymerization of styrene, MMA and HEMA.
- The ionic liquid method provides an alternate route to produce polymers and offers an easy method to recycle the ionic liquid employed in the process.
- To optimize the reaction parameters and investigate the mechanistic aspects of polymerization.

**Evaluation of in vitro biocompatibility of synthesized nanoparticles – hemotoxicity, cytotoxicity and zebra fish toxicity studies.**

- To study the biocompatibility of synthesized nanoparticles (PHEMA-NPs & PSA NPs) using hemolysis method.
- To study the cytocompatibility of synthesized nanoparticles using various VERO, chang liver and 3T3-L1 cell lines.
- To find out the in vivo toxicity of nanoparticle, zebra fish studies also employed in terms of hatching rate (%), mortality rate (%) and morphological changes.
Synthesis of Curcumin loaded poly (2-hydroxyethyl methacrylate) nanoparticles (C-PHEMA-NPs) from gelled ionic liquid and in vitro evaluation of anti-cancer activity in SKOV-3 cells

- To synthesize PHEMA nanoparticles and C-PHEMA-NPs prepared from gelled ionic liquid using nanoprecipitation method.

- To characterize C-PHEMA-NPs using different physicochemical techniques such as SEM, AFM, FTIR, XRD, TGA, DSC.

- To demonstrate the potential of C-PHEMA-NPs as drug carrier using SKOV-3 cells in vitro.

Synthesis and characterization of curcumin loaded polymer/lipid based nanoparticles and evaluation of their antitumor effects on MCF-7 cells

- To prepare and characterize the curcumin loaded PHEMA/Stearic Acid (C-PSA-NPs) based nanoparticles, through environmentally benign approach.

- The use of amphiphilic pluronic F-68 as an emulsifier and a stabilizer is investigated to understand the means to provide an apt drug delivery system for a hydrophobic drug like curcumin.

- To study the anti cancer efficacy of synthesized nanoparticles with MCF-7 cell line in vitro.
1.6 PRESENTATION OF THESIS

General introduction, scope and objectives of the present investigation and format of the thesis are presented in Chapter 1.

In Chapter 2, the current status of literature on the nanoparticles in drug delivery, preparation methodology for polymeric nanoparticles, polymer/hybrid nanoparticles, physicochemical characterization techniques, release studies in phosphate buffer saline (PBS), biocompatibility of nanoparticles using \textit{in vitro} cell line studies, evaluation of the anticancer activity of nanoparticles with specific reference to PHEMA polymer and drug curcumin are discussed. The gaps in literature are summarized.

In Chapter 3, studies on the role of choline formate ionic liquid in free radical polymerization and the use of electron paramagnetic resonance studies to investigate the mechanistic aspects of polymerization process are discussed.

In Chapter 4, evaluation of \textit{in vitro} biocompatibility of synthesized nanoparticles – hemolysis assay using blood cells, cytotoxicity studies performed using different cell lines and zebra fish are discussed.

In chapter 5, synthesis of curcumin loaded poly (2-hydroxyethyl methacrylate) nanoparticles from gelled ionic liquid \textit{in vitro} cytotoxicity and anti-cancer activity in SKOV-3 cells are presented.

In Chapter 6, synthesis, characterization of curcumin loaded polymer/lipid based nanoparticles and evaluation of their antitumor effects on MCF-7 cells are discussed.

Chapter 7, summary, major findings and recommendations for future work are presented.
Each chapter carries self-contained information with introductory remarks, detailed experimental techniques procedures adopted, and results and discussion with major findings are reported in sequence.