Chapter 5

Summary

In the present work, nanoparticles are investigated as drug delivery system, with the aim to achieve desired drug release profiles from microparticles and to achieve high drug loading with retention of bioactivity of drug in nanoparticles.

Chapter 1 describes development and current status of biopolymers and their medical applications. Basic knowledge about drug delivery and colloidal drug delivery technology are crucial factors related to the release profiles of therapeutic agents.

Hence, polymeric nanoparticles as drug delivery systems are discussed with regard to the preparation methods, drug loading and release profiles. Concerning the preparation process, main problems are the instability of drug due to processing parameters during nanoparticle preparation, like high shear rate, temperature, and exposure to the organic solvent, and reproducibility of well defined nanoparticles. Solvent displacement with its narrow size distribution and without use of shear force was extensively employed for drug loaded nanoparticles preparation. However, a new strategy is still effectively needed because of the low encapsulation efficiency and exposure to organic solvent especially for drug loaded nanoparticles. Polymeric nanoparticles for pharmaceutical applications are in use since 1960's. Since then, various polymerization methods as well as methods such as emulsion crosslinking, ionotropic gelation, emulsification/solvent evaporation, spray drying, and coacervation / precipitation involving preformed polymers and in-situ polymerization methods involving monomer have been developed to prepare polymeric nanoparticles. They have been used as carriers for cytostatics, antibodies, antiparasitic compounds, anticancer drugs, hormones, antiviral drugs and many more.

The present work has been conducted with the practical end in mind of preparing nano delivery structures for the delivery of bioactive agents. In the undertaken research activity, polymers with different origin (natural and synthetic) and characteristics (hydrophobic, hydrophilic, amphiphilic) are prepared, characterized and used for the encapsulation of hydrophobic and hydrophilic model drugs for better understanding of

the suitability and versatility of the nano drug delivery systems. Nanoparticles morphology, size, surface charge and encapsulation efficiency were determined, as well as the evaluation of drug release profiles were studied at different conditions. The activity of the encapsulated drug was also evaluated to verify the influence of the loading and purification processes.

Chapter 2 reports copolymers based on 2-hydroxy ethylmethacrylate and ethyl methacrylate, which have wide applications in contact lenses, surgery and clinical medicine because of their ability to form biocompatible hydrogels with excellent tolerance and good stability. Copolymerization of 2-hydroxy ethylmethacrylate with alkyl acrylate may be of practical interest considering that linear and cross linked copolymers based on 2-hydroxy ethyl methacrylate are widely utilized in ophthalmic industry, as a controlled drug release matrix and as non-thrombogenic materials and surgical prostheses.

Copolymerization of ethylmethacrylate, a hydrophobic monomer and hydroxyl ethyl methacrylate, a hydrophilic monomer using redox initiator pair in oil-in-water micro emulsion polymerization technique at nearly room temperature has been carried out. Copolymeric nanoparticles of various compositions comprising EMA-HEMA were prepared using monomer mixtures, with monomer weight ratios varying from 90:10 to 50:50 EMA: HEMA, SDS and water. The reaction mixture was continuously stirred at 400 rpm with nitrogen purging for a period of 15 minutes. The reaction temperature was maintained at 40 ± 2 °C and reaction was carried out for 4 h. The bluish transparent stable latex confirmed the formation of nanoparticles. Attempts were made to calculate the reactivity ratios by using linear and non linear least square methods. The reactivity values of r_{EMA} and r_{HEMA} by linear least square methods were ***0.42, 0.40*** and ***0.58, 0.54*** and through nonlinear least square method, they were ***0.40*** and ***0.36*** respectively. "Lamotrigine" as a model antiepileptic drug was selected for entrapment and successive in-vitro release of it from synthesized nanoparticles. Placebo copolymeric nanoparticles and lamotrigine loaded copolymeric nanoparticles were successfully synthesized through a microemulsion polymerization technique. TEM and DLS analysis of nanolatex

confirmed the formation of well defined reasonably well monodispersed below 100 nm size particles with nearly spherical morphology. The addition of a model drug lamotrigine, to polymeric nanoparticles synthesized through microemulsion polymerization proved to be successful with a 26-62 % drug entrapment with respect to copolymer compositions and in–vitro release profile of lamotrigine from copolymeric nanoparticles showed initial burst effect followed by continuous slow release for a period of 15 hours indicating a sign of controlled release of lamotrigine from nanoparticles.

Stimuli responsive polymers and hydrogels which are becoming increasingly attractive in biotechnology and medicinal uses, such as basic components of *biosensors*, *"switch on/off" drug release, drug delivery systems, affinity precipitation, immobilization of* enzymes, *cells, tissue engineering* and *artificial muscles* are examined in **Chapter 3**. Among temperature sensitive polymers, poly (N-isopropylacrylamide) (PNIPAM) and its copolymers have been studied extensively. Recently, poly(N-vinylcaprolactum) (PVCL), water soluble, biodegradable, temperature responsive polymer having lower critical solution temperature (LCST) near to body temperature (around 32 °C) has attracted much attention of researchers and technologist. So far, only a few studies related to its properties and applications have been reported and much of the published work is concentrated on the PVCL based hydrogels as they have potential applications in controlled drug delivery system, separation science, immobilization of enzymes and in oil-recovery technology. Similarly, colloidal systems can be equally attractive for such applications, which could be easily synthesized through free radical micro emulsion polymerizations.

The present chapter is dedicated to the synthesis and characterization of thermoresponsive poly (MMA-co-VCL) copolymeric nanoparticles and further attempts are made to explore these nanoparticles as a delivery vehicle for model anticancer drug "etoposide". Homogeneous thermally responsive copolymeric nanolatex was synthesized through a continuous gradual feed microemulsion polymerization technique. Attempts were made to calculate the reactivity ratios by using linear and non linear least square methods. The reactivity values of r_{MMA} and r_{VCL} by linear and nonlinear least square methods were observed to be ***2.68, 2.63*** and ***0.19, 0.25*** respectively. Monomer reactivity

ratios determined showed good correlation between the linear and nonlinear least square methods. TEM and DLS analysis of nanolatex confirmed the formation of well defined reasonably well mono-dispersed below 100 nm size particles with nearly spherical morphology. Latex remained stable for a period of more than six months at room temperature. Cloud point temperature values of the copolymers were observed to vary with copolymer composition and copolymers with more than 40 % VCL content showed cloud temperature near to body temperature. The addition of a model high molecular weight drug etoposide to polymeric nanoparticles synthesized through micro emulsion polymerization proved to be successful with a 35-67 % drug entrapment with respect to copolymer compositions and release profile of etoposide from copolymeric nanoparticles showed initial burst effect followed by continuous slow release for a period of 30 hours indicating a sign of controlled release of etoposide from nanoparticles. The preliminary evaluation of cell-viability data showed that, IC_{50} value of etoposide is observed in the range of 0.00001-0.0001 mg/ml, while IC_{50} value of placebo nanoparticles and etoposide loaded polymeric nanoparticles was in the range of 0.01-0.1 mg/ml respectively. The observed results show that, the technique has a possible technological importance for etoposide based nanoparticles formulation.

In Chapter 4 chitosan nanoparticles prepared by ionic gelation methods have been studied as carriers for model drug venlafaxine hydrochloride. Subsequent study of in-vitro release of venlafaxine hydrochloride from the nanoparticles was also undertaken. Optimization of the experimental conditions was done in order to achieve maximum encapsulation efficiency and controlled release of venlafaxine hydrochloride from CS/TPP nanoparticles. From the results obtained in encapsulation of model drug venlafaxine hydrochloride in CS based nanoparticles, we can say that the CS/TPP nanoparticles and PEG coated CS/TPP nanoparticles can offer some attractive features which favor them as a promising carriers for the further study of charged therapeutic agents. The results are summarized here;

- Formation of nanoparticles can be achieved under extremely mild conditions, without affecting structure and properties of drug encapsulated.

- Easy modulation of particle size and surface charge through control of experimental conditions such as chitosan, PEG concentration and CS/TPP mass ratio, which can have critical role in their applications.
- Molecular level dispersion of venlafaxine hydrochloride within the nanoparticles was confirmed from XRD and DSC analysis.
- Encapsulation efficiency upto 70 ± 5 % was observed for CS/TPP nanoparticles at 0.6 mg/ml drug concentration.
- Better encapsulation efficiency of drug and superior physicochemical properties of nanoparticles were observed at lower chitosan concentration and lower chitosan to TPP mass ratio at specific drug concentration.
- Addition of PEG during nanoparticle formation has no effect on encapsulation efficiency of drug but shows delayed release of entrapped drug molecules due to increased bulk density and coating as observed in TEM on the nanoparticles.
- PEG coating on the surface of CS/TPP nanoparticles confirmed through XPS and TEM investigation.

All these observations make the system as interesting carrier for drug delivery applications.

Publications

"Lamotrigine loaded polyacrylate nanoparticles synthesized through emulsion polymerization" **Sunil Shah,** A. Pal. T. Rajyaguru, R.S.R. Murthy and S. Devi. **J. Appl. Polym. Sci.** 107 (2008) 3221-3229.

"Preparation and characterization of venlafaxine hydrochloride loaded chitosan nanoparticles and in vitro release of drug"
Sunil Shah, A. Pal, V.K.Kaushik and S. Devi. **J. Appl. Polym. Sci.** (Article in Press)

"Synthesis of Au, Ag and Au-Ag alloy nanoparticles in aqueous polymer solution"
A.Pal, **Sunil Shah** and S. Devi. **Colloids and Surface A: Physicochemical and Engineering Aspects.** 302 (2007) 51-57.

"Preparation of silver, gold and silver-gold bimetallic nanoparticles in w/o microemulsion containing TritonX-100"
A Pal, **Sunil Shah** and S. Devi. **Colloids and Surface A: Physicochemical & Engineering Aspects** 302 (2007) 483-487.

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A Pal, **Sunil Shah**, S. Belochapkine, D. Tanner, E. Magner and S. Devi. **Colloid Surface A: Physicochemical & Engineering Aspects** 337 (2009) 205-207.

"Preparation of silver–gold alloy nanoparticles at higher concentration using sodium dodecyl sulfate"A.Pal, **Sunil Shah** and S. Devi. **Aust. J. Chem.** 61 (2008) 66–71.

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A Pal, **Sunil Shah,** D. Chakraborty and S. Devi **Aust. J. Chem.** 61 (2008) 833-836.

"Template free synthesis of silver-gold alloy nanoparticles and cellular uptake of gold nanoparticles in Chinese Hamster Ovary cell"
A.Pal, **Sunil Shah**, V. Kulkarni, R. S. R. Murthy and S. Devi. **Materials Chemistry and Physics** 113 (2009) 276-282.

"Microwave-assisted synthesis of Silver nanoparticles using ethanol as a reducing agent"
A Pal, **Sunil Shah,** and S. Devi **Materials Chemistry and Physics** 114 (2009) 530-532.

Papers Communicated

"Synthesis and characterization of thermo responsive co polymeric nanoparticles of poly (MMA-co-VCL)"
Sunil Shah, A Pal, R.P.Gude and S Devi. **European Polymer J.**

"Etoposide loaded Poly (MMA-co-VCL) nanoparticles synthesized through continuous feed Emulsion polymerization"
Sunil Shah, A Pal, R.P.Gude and S Devi. **Reactive and Functional Polymer.**

Presentation

Poster Presentation in ***RSC-West India Sections Student Symposium – 2006*** held on 13th and 14th Oct. 2006 at the Department of Chemistry, Faculty of Science, The M.S. University of Baroda, Vadodara.

Best Oral Presentation in ***Vth All Gujarat Research Scholar Meet-2008*** held on 17th Feb.2008 at the Department of Applied Chemistry, Faculty of Technology and Engineering, The M.S. University of Baroda, Vadodara.

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In

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