SUMMARY
The present investigation has been undertaken to develop the most rational drug delivery system for anti HIV drugs by the method of o/w emulsion in-situ cross linking method. Preparations and study of nanoparticles of lopinavir and ritonavir by the standardized method with variable drug/polymer ratio and variable encapsulating solvents have been adopted in this investigation. A comparative study has been made among the physicochemical properties, pharmaceutical properties and biological properties of these prepared nanoparticles. As the drugs were excreted unchanged in the urine, nanoparticulation has been adopted to retard its release and thereby dose of administration to get sustained action. In view of optimization of drug action including improved drug safety may be achieved by controlling the rate of drug delivery from the controlled dosage form. This study presented a compact form of method of preparation and characterization of anti retroviral drugs with immense importance in pharmaceutical field.

**Standardization of process parameter**

Polymer nanoparticles were prepared using a novel method based on emulsion-solvent-evaporation cross-linking technique and the properties of the nanoparticles were studied. The nanoparticles were prepared from various drugs and pharmaceutically acceptable biodegradable polymers. Such nanosized particles could be used for oral, ocular, or parenteral administration of drug materials. This method could be used to prepare spherical, amorphous, homogeneous matrix-type drug-polymer nanoparticles. The size distributions of the nanoparticles prepared were found to be uniform, reflecting the droplet size distribution produced by the emulsion-solvent-evaporation cross-linking technique. The concentration of the starting solution determined the resulting particle size. The morphology of the nanoparticles was affected by the solvent properties and the solvent composition. Particularly, the solute solubility and the solvent vapour pressure influenced the morphology of the particles. It was found that the polymeric component determined the
morphology of the nanoparticles. The nanoparticles, when prepared, were amorphous, but depending on the thermal properties of the drug and on the interactions between the drug and the polymer. The nanoparticles containing lopinavir and ritonavir were found to be amorphous at room temperature regardless of the amounts of drug and polymer in the nanoparticles. Smooth, spherical lopinavir and ritonavir alginate nanoparticles were successfully synthesized in size of 134 nm for lopinavir and 214 nm for ritonavir using the suspension cross-linking method. The pilot study identified polymer concentration, stabilizer concentration, D/P ratio, stirring rate and process time as the important variables controlling particle size. The investigated variables were polymer concentration, stabilizer concentration, cross linker concentration, stirring rate during cross-linking reaction, temperature of DD water and D/P ratio. Based on the results of this study predicted that the particle size increased as the alginate concentration increased and/or as the concentration of stabilizer concentration decreased. The smallest particle could be produced by using sodium alginate concentration at 0.1 % (m/v), stabilizer (glycerol) amount 10 ml, surfactant amount 0.1 mg, crosslinker amount 4 ml of 2M L⁻¹, stirring rate 1200 rpm and process time 1.5 hour. In addition to these main effects, the model predicted synergy between the alginate and stabilizer concentration. These results are consistent with published reports, some of which were multi-factor experiments analyzed using multivariate analyses. However, to our knowledge, this is the only report investigating the effects of alginate concentration, surfactant concentration, crosslinker concentration, and D/P ratio using a statistical analysis. The effects of alginate concentration and stabilizer concentration on particle size of lopinavir-loaded alginate nanoparticles were studied using an experimental design. A linear regression model that fitted the data was generated. As predicted in the pilot study particle size increased as the alginate concentration increased. However, the crosslinker concentration did not have a significant effect on particle size of the in situ-loaded nanoparticles. in situ-loaded nanoparticles had a distinct morphology
that varied from smooth to pitted and rough. The porosity and roughness appeared most severe at the higher drug-polymer ratio, but it also depended on the stirring rate. It is unclear at this time how these factors influence the nanoparticles morphology; however, it is reasoned that prior to emulsification, the alginate molecules in solution undergo morphological transformations to maximize the binding of lopinavir/ritonavir. After emulsification, the alginate molecules must undergo further morphological changes to minimize the interfacial energy between the aqueous and organic phases. The tween 80, a steric hindrance agent, stabilizes the nanodroplets in suspension. The crosslinker locks in this morphology by cross-linking the alginate molecules. Upon hardening, this morphology may give rise to residual stresses within the matrix of the synthesized nanoparticles. It was concluded that this coupling was successful because a good number of intermolecular peptide bonds were formed.

**Characterization of nanoparticles**

**Surface topography**
Scanning electron micrograph and FE-SEM showed that the nanoparticles were almost spherical and discrete and free flowing.
Transmission electron micrograph showed that the nanoparticles have spherical morphology with dense core materials.

**DTA and FT-IR evaluation**
Differential thermal analysis and Fourier transform infra red spectroscopy showed that the lopinavir and ritonavir have been encapsulated without complex formation and the crystallinity of the drugs after encapsulation have been retained, preserving the desired biological activity of the drugs.
Release kinetics of lopinavir/ritonavir nanoparticles

The time dependent cumulative percentage release profile of different formulations varying in drug/polymer ratio, nature of encapsulating solvents, release upto 50% and release of final 50% of LP-NP and RT-NP were studied in the light of different kinetic models. The present investigation was undertaken to fabricate and evaluation the sustained release formulation of lopinavir and ritonavir nanoparticle using nanoparticulation technique. The linear regression of the drug release profiles and the corresponding correlation coefficients are illustrated based on the different kinetic mechanism of zero order, first order, Higuchi matrix and Korsmayer and Peppas model with a ambition to find out the best fit model. It can be concluded that the in-vitro release kinetic follows overall the Higuchi Matrix model and also satisfied the Korsmayer and Peppas model (power law) of release upto 50% of the drug release.

Stability study

Physical stability of the nanoparticle dry powder was studied at three different conditions to evaluate the effects of temperature and relative humidity. It can be observed that, there were no significant changes in the total cumulative percentage release, from nanoparticles stored at 25°C up to 3 months. Whereas there was a significant decrease in the extent of release from nanoparticles stored at 37°C up to 3 months. There was no significant difference in the total cumulative percentage release, even after 3 months of storage at 25°C.

Bio-distribution of LP-NP

Lopinavir nanoparticles showed longer half-life than free Lopinavir in blood. In particular, the Lopinavir nanoparticles showed significantly greater concentrations and extended residence time in blood compared
with the free Lopinavir all throughout the study. It is evident that the
Lopinavir nanoparticles exhibited relatively higher concentrations in
blood compared with free Lopinavir. This finding might be the reason
for the observed low concentrations of Lopinavir in, RES organs
compared with free Lopinavir. In vitro cytotoxicity of lopinavir
nanoparticle revealed, the results derived through in vitro cytotoxicity
study revealed the enhanced cell-killing efficacy of nanoparticle bound
drug over free drug. For sterile preparation of Colloidal polymer
Dispersions of Lopinavir and Ritonavir nanoparticles, autoclave was
not the best instrument due to heating effect; in that case gamma
irradiation was the right technique to prepare sterile preparation of LP-
NP/RT-NP.