# CHAPTER 12

## SUMMARY AND CONCLUSION

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The major aim of the thesis was to develop a safe technology for the preparation of drug loaded micropellets in a completely non toxic, aqueous environment entirely avoiding the use of organic solvents. As it has become mandatory to implement Safety, Health and Environment (SHE) norms in pharmaceutical manufacturing technology, uses of organic solvents are now discouraged by good corporate governance and good manufacturing practices. The aim of this study was to design, formulate and evaluate oral controlled release micropellets containing frusemide, a potent loop diuretic, as the model drug by adopting ionotropic gelation technique, which completely eliminates the involvement of organic solvent in any stages of preparation and finally optimizing the formulation statistically by employing Factorial analysis and Response Surface methodology (RSM). The author aspires to have prepared micropellets using a novel, economical and ecological method which can be feasibly reproduced in a large scale by the pharmaceutical industry.
The thesis, being the documentation of the entire research work, comprises of twelve (12) chapters placed in accordance with the chronological sequence of the work plan. Each preceding and subsequent chapters are interwoven to maintain the continuity.

In **Chapter 1**, a review of conventional and controlled release drug delivery systems have been presented. Properties of a drug that influences the design of controlled release dosage form have been discussed in details in this chapter. The different techniques of micropelletization have been listed and particularly ionotropic gelation technique, the adopted technique in the entire study, has adequately been discussed.

In **Chapter 2**, a detailed survey of the literature related to ionotropic gelation technique has been documented. The current updated literature survey shows a wide spectrum application of ionotropic gelation method in the field of controlled release drug delivery system.

Subsequently in **Chapter 3**, physicochemical, pharmacokinetic and pharmacological aspects of the model drug, Frusemide, has been covered. The clinical information of the drug available from authentic sources has been given more importance. The rationale behind the selection of Frusemide has also been accounted in this chapter.

Similarly in **Chapter 4**, all the excipients which the author dealt with during this research work has been enlisted. In the development of novel drug delivery systems, the excipients have great influence on the biopharmaceutical and physiological availability and the stability of the drug in the formulation. Hence, the physicochemical properties of the chief excipients, including all the polymers used in the formulations, have been thoroughly discussed here.

In **Chapter 5**, the drug sample used in the entire work was subjected to preformulation studies. This study is the initiation point in the development of rationality behind selecting Frusemide as a suitable molecule to be designed for controlled delivery. In this investigation, author determined the $\lambda$ – max of Frusemide sample and also prepared its standard curve equation in the Phosphate buffer medium of pH 6.8 and 7.4. The generated standard curve equation was later utilized for the study of the drug release profile during the dissolution study to determine the amount of drug released in the dissolution medium. Subsequently, the supplied drug was assayed following the official methods given in Indian Pharmacopoeia. The assay value was observed to be well within the standard limit. The purity of the drug sample was determined by infrared (IR) spectroscopy by comparing the IR spectrum with that of the official compendia.

**Chapter 6** further continues with the preformulation study which was directed towards the optimization of the process parameters for the consequent development of alginate micropellets. The effect of the process parameters such as bore diameter of the needle, height of dropping of the dispersion from the needle tip, drying time and temperature, contact time of the micropellets in the calcium chloride solution were investigated and finalized. Also the effect of the variation in the concentration of sodium alginate, calcium chloride and different concentration of loaded drug were also studied. The copolymers used were also subjected to several concentrations and their effect on the physicochemical properties of the resultant micropellets were recorded and utilized in the optimization of the copolymer for the final set of batches. For all the formulations mean particle size by Sieve analysis, surface morphology by Scanning Electron Microscopy (SEM), Drug Entrapment Efficiency (DEE), Disintegration time (DT) and *in vitro* dissolution studies were determined. 17G needle size was optimized, as smaller bore makes the viscous dispersion of the drug-polymer difficult to extrude. Height of 2 cm was maintained further which resulted more spherical pellets. Curing time of the wet micropellets in the calcium chloride solution

was optimized at 30 minutes as it gave maximum gelation time and formation of hard and firm micropellets. Drying time was optimized for 6 hour and at a temperature of 60°C in hot air oven. It was observed that at a particular concentration of sodium alginate, 5% w/v calcium chloride gave nearly spherical and uniform pellets with retarded release upto 1.5 hour. Sodium alginate at a concentration of 5.0 w/v was found to produce moderately retarded micropellets but of much bigger size. Considering these points, author has finally chosen 1 to 4% w/v concentration of sodium alginate for the final design of the envisaged formulation of Frusemide. Since concentration of 5% w/v or higher was found to be too viscous to squeeze through the nozzle of the 17G needle, therefore concentration on and above 5% w/v was not considered for final formulation. To produce more prolonged actions of frusemide, the author incorporated several copolymers, both water soluble and insoluble, in the form of aqueous dispersions of acrylic polymers (Acrycoat E30D, L30D and S100), aqueous dispersions of Ethyl cellulose (Surelease) and powder form (Methocel K15M) of Hydroxy propyl methyl cellulose (HPMC). Acrylic based colloidal polymer dispersions (Acrycoat E30D) showed high encapsulation efficiency and maximum prolongation of drug release among all. Hence, further study was extended taking Acrycoat E30D in a concentration range of 0-4% w/w as the optimized release controlling copolymer. Higher concentrations could not be tested due to viscosity and corresponding syringability factor. Though at higher drug load, the release of the drug was more sustained, a moderate concentration of 30%w/w of drug load was maintained for all the formulations.

In **Chapter 7**, the formulation design was framed using $3^2$ factorial analyses. Two variables (concentration of sodium alginate and Acrycoat E30D) at three levels [high (+1), medium (0) and low (-1)] was considered for the study resulting in nine (9) different formulations (F1 – F9). The following concentrations (4%, 2% and 1% w/v) of sodium alginate and (4%, 2% and 0% w/w) of Acrycoat E30D was investigated. From the preformulation studies the optimized parameters obtained were employed in the ionotropic gelation method adopted in the final formulation study. The method of preparation and its flow sheet diagram were lucidly represented.

Subsequently in **Chapter 8,** the final nine formulations prepared were subjected to several physicochemical studies to judge the efficiency, reliability and uniformity of the micropellets. Parameters such as general appearance, rheological behavior, moisture content, particle size, drug content and drug entrapment efficiency, loose surface crystal

(LSC) study and drug – polymer incompatibility study by infrared (IR) spectroscopy and topographical study by scanning electron microscopy (SEM) were all investigated for each formulations. On analyzing the results obtained, formulation F9 (4 % w/v and 4% w/w sodium alginate and AcrycoatE30D respectively) was found to give best results in all respects.

**Chapter 9** was separately kept to document the results obtained from *in vitro* release studies of prepared micropellets. The *in vitro* dissolution test is a good index of bioavailability if it meets two conditions, namely,

1. The dissolved drug remains free and intact and does not decompose or form a complex in the gastrointestinal tract, and,
2. Dissolution and not absorption, is the rate-limiting step in the availability of the drug in the systemic circulation.

In this context at the beginning of this chapter 9, theories of dissolution, factors affecting dissolution rate of drugs from solid dosage form, dissolution rate testing methodology and *in vitro* dissolution testing of controlled release dosage forms have been reviewed. Interpretation of several *in vitro* drug release kinetic models and equations e.g. Noyes – Whitney's equation, Weibull equation, Logarithmic – Logistic equation, Hixon – Crowell's cube-root equation, Zero-order, First-order and Second-order kinetic model, Exponential release kinetics, Square-root of time equations (Higuchi equation), Korsmeyer – Peppas equation and Baker – Lonsdale model have been described and discussed in details.
The data obtained from the dissolution of the formulations were fitted in Zero order, First order, Higuchi and Hixon – Crowell model and the results were established from Korsmeyer - Peppas equation. The correlation coefficient ($R^2$) was used to compare the model equations and to determine the 'best fit' model that explains the release kinetics of the drug from the prepared micropellets. Micropellets prepared with low level of sodium alginate (F1, F2 and F3) acted as reservoir devices of frusemide which released its content depending on the concentration gradient. In spite of incorporation of Acrycoat E30D as copolymer, there could not form any drug-polymer matrix. Their release profile followed first order kinetics. For the rest six batches (F4 – F9), the phase-I (0-2 hr) release was fitting with first order kinetics followed by zero order kinetics in the phase-II (2-9 hr). Since the release mechanism involved more than one type of release kinetics, the investigator used the semi-empirical formula of Korsmeyer-Peppas equation to justify the primary outcome of the

analysis of release mechanism. For all the batches, the release exponent (n) of the Peppas equation, in an overall span of 0-9 hr was found to be > 1 signifying super Case-II non-Fickian anomalous diffusion transport mechanism. On segregating the study in two phases, Phase-I and Phase-II, this phenomenon of drug release was very much in proximity with the theory as it is seen that in the initial phase –I, n >>1, reflecting non-Fickian release. With time, water penetrated into the hydrogel matrix containing the dispersed drug. The polymer chains take up a finite amount of time to rearrange to an equilibrium state in order to accommodate the penetrating solvent. On significant hydration drug release tends to be linear with time (n~1) signifying zero-order transport mechanism.

From this chapter, the conclusion was drawn that by increasing the polymer mass in the micropellets such drug delivery device could be generated which can retain a constant geometry with a constant release rate of drug following zero order kinetic model. The reproducibility of the polymers to produce micropellets of similar release mechanism was optimized statistically in the **Chapter 10** of this thesis.

The *in vitro* release data obtained from chapter 9 was treated statistically in the **Chapter 10.** At the outset of this chapter, introductory concept of experimental design and method of statistical analysis by factorial analysis has been explained. Analysis of Variance (ANOVA), Regression analysis and Response Surface Methodology (RSM) have also been discussed. Factorial design was used as a tool for the optimization of dependent parameters to achieve the predetermined goal of the research work.

The following dissolution parameters were considered as responses of the variables to get an estimate of the performance of the drug delivery system: Zero order release rate constant [Ko (mg / hr)]; amount of drug released in 2 hours (Burst effect) [$X_{120}$ (mg)]; time required for 80% drug release [$t_{80}$ (hr)] and Peppas Diffusion coefficient [n]. On performing linear regression analysis of Ko, sufficiently high degree of correlation ($R^2 = 0.8856$) among the predicted and actual value was observed. On performing Two-way ANOVA, the effect of sodium alginate was found to be significant ($p < 0.05$) whereas Acrycoat E30D was found to be statistically insignificant.

The experimental design was done using the software 'Design Expert version 7.1.2'. A full $3^2$ factorial analysis using 2FI design model and 9 runs were performed. Apart from numerical optimization, graphical optimization by response surface methodology (RSM) had also had been adequately illustrated. For all the dissolution parameters, sodium alginate was found to have significant effect as compared to Acrycoat E30D. Non-additivity or the

interaction was observed not to play any significant role. By applying multiple regression analysis on the experimental data, the response variable Ko and the test variables A (concentration of Sodium alginate % w/w) and B (concentration of Acrycoat E30D % w/w) were found to be related by the following second order polynomial equation:

$$Ko = 11.11 - 0.72 \times A - 0.28\ B - 0.23\ \times A \times B - 0.80 \times A^2 + 9.683E\text{-}003 \times B^2$$

The predictive results obtained from this equation for a certain set of values for factor A and B were validated by preparing new set of formulations and performing actual *in vitro* release study and determining correlation among the two. Similarly for other parameters, high degree of correlation was obtained. From the trial and error method or serendipity of the development of Microparticulate Drug Delivery System (MDDS), an optimized and validated formulation design was successfully framed.

In the last chapter (**Chapter 11**), assessment of the pharmacological (diuretic) activity through *in vivo* performance of the prepared sustained release Calcium alginate micropellets were performed and recorded. To achieve this objective, modified LIPSCHITZ test model was employed. Male Wistar rat species were administered with test samples and standard Frusemide tablets orally with normal saline as a control. Along with the diuretic activity, natriuretic activities of the micropellets were calculated by estimating the excretion of Sodium (Na+) and Potassium (K+) ion from the collected urine samples of the test animals. After comparing the Lipschitz quotient with that of the standard tablet, micropellets containing frusemide were found to produce a much greater diuretic effect over an extended period of 24 hour. They also showed significantly high natriuretic activity with less hypokalemic effect, reducing all the possible side effects of a standard tablet of frusemide, associated with excessive loss of K+ ion and sudden loss of water from the body.

The author **concludes** with the satisfaction that, though small, but some contribution to the field of pharmaceutical sciences could be made through this research work. The controlled release microparticulate dosage form of Frusemide can be prepared in a completely aqueous environment without using any organic solvents. Calcium alginate micropellets are insoluble in gastric pH making the process advantageous for incorporating acid sensitive drugs in the formulation as well as minimizing the gastric irritation of some drugs. Solubility of Acrycoat E30D, the release controlling copolymer, being pH independent, ensures uniform release of the drug in the entire pH spectrum of the intestine. The polymers

employed, can be safely used along with the drug without any possibility of interactions. The resultant micropellets obtained can be administered as such or can act as an Active Pharmaceutical Ingredient (API) for the preparation of capsules or sustained release tablets. People from pharmaceutical industry can consider this economical and ecological method for scaling up into medium and large capacity, through a proper and detailed pilot project.

The goal of the research work was achieved through well designed plan of experimentation using factorial design and well-thought of physicochemical concept with support from statistical software and sophisticated instrumentation and at the same time without causing any environmental hazard. This research work comply with all the standards of manufacturing practice following Safety, Health and Environment (SHE) norms for the international requirements in all the categories of bulk pharmaceuticals, conventional and controlled dosage forms.

Some inadequacy in the entire work cannot be ruled out. Elaborate animal experimentation to estimate the effect of the formulation on the bioavailability of the drug and clinical trials to get the data of actual therapeutic effect along with the informations on the toxicity of the formulation was left out. Development of micropellets with more sphericity and high output using proper machinery is still open to be worked upon. The author welcomes any constructive suggestions and criticism from research workers and industrial pharmacists which will help in the enrichment of the work done.