# Chapter 11

# SUMMARY AND CONCLUSIONS

## 11.1 Summary

The severity of the side effects associated with the use of anti-arthritic drugs is of a major concern. With an ever increasing number of arthritic patients, the number of patients using these anti-arthritic drugs is dramatically increasing. It has thus become absolutely necessary to develop a drug delivery system which could reduce these side effects. Targeted drug delivery is one of the approaches receiving considerable attention in the recent years. The ideal requirement of targeted drug delivery system is that the distribution to the other organs is reduced and high concentration of the drug is maintained at the diseased site for prolonged periods of time. Thus, in the present study, an attempt was made to develop a targeted drug delivery system for delivering the drug to the arthritic joints. One way of achieving high concentrations of the drug at the arthritic joints is to inject the drug intra-articularly in the arthritic joint. The second way used was to target the drug to the arthritic joint after intra-venous injection. The intra-articular injection of the drug loaded microspheres or solid lipid nanoparticles is expected to enhance the retention of the drug in the joint cavity for prolonged periods of time compared to the drug solution which rapidly escapes from the joint into the blood. It is also important that the formulation should be non-toxic and non-inflammatory to the synovium. So biodegradable and biocompatible polymers such as gelatin, chitosan, bovine serum albumin were used for preparation of the microspheres and the lipid compritol (glycerol behenate) was used for preparation of solid lipid nanoparticles. The biodistribution of celecoxib solution as well as its solid lipid nanoparticles and albumin microspheres was studied after intravenous injection.

The formulations were optimized by varying the process as well as formulation variables. For selecting optimized batch, the entrapment efficiency, particle size and

drug release was taken into consideration. The results of the characterization of the different formulations can be summarized as follows:

1. **Gelatin microspheres**

The microspheres prepared using emulsification chemical cross-linking method gave microspheres with better entrapment efficiency than those prepared using emulsification solvent-extraction method.

For preparation of microspheres by emulsification chemical crosslinking method, it was necessary to add polyethylene glycol as a deaggregating agent.

The different factors affecting the entrapment efficiency, particle size and the release rate from the microspheres were identified by varying the factor in consideration and studying its effect on the characteristics of the microspheres. The main factors affecting the entrapment efficiency of the celecoxib loaded gelatin microspheres are the concentration of gelatin and concentration of span-85. The volume of the crosslinking agent had no significant influence on the entrapment efficiency of the microspheres.

The main factors affecting the particle size of the microspheres are the concentration of gelatin, concentration of span-85 and stirring speed. The volume of the crosslinking agent had no significant influence on the particle size of the microspheres. However, the particle size of the formaldehyde crosslinked microspheres was found to be higher than that of the glutaraldehyde crosslinked microspheres.

The factors affecting the release of celecoxib from the gelatin microspheres were the crosslinking agent used, its volume, duration of crosslinking and concentration of gelatin. The release rate from glutaraldehyde crosslinked microspheres is slower than that from formaldehyde crosslinked microspheres. The release rate of the celecoxib from the gelatin microspheres increases in presence of collagenase added in the release medium due to the enzyme mediated degradation of the microspheres.

In case of celecoxib loaded gelatin microspheres, Batch G-4, formulated using 25% gelatin, which had an entrapment efficiency of 86.82% and a particle size of 20.51μm and which gave the slowest release was selected as an optimum batch and was used for in-vivo studies.

In case of rofecoxib loaded gelatin microspheres, the concentration of span-85 had no significant influence on the entrapment efficiency since rofecoxib is not soluble in the external phase of the emulsion even at high concentration of span-85. Thus, the main factor affecting the entrapment efficiency of the rofecoxib loaded gelatin microspheres was the concentration of gelatin. The particle size of the microspheres was affected by the concentration of gelatin and the concentration of span-85. The release rate of rofecoxib is affected by the volume of glutaraldehyde and the concentration of gelatin. The duration of crosslinking had no significant influence on the release rate. Hence, batch R-GM8 prepared using 25% gelatin, 5% span-85, 1.0 ml glutaraldehyde and crosslinked for 1 hour was selected as an optimum batch.

In case of the valdecoxib loaded gelatin microspheres, the main factors affecting the entrapment efficiency and particle size were the concentration of gelatin and concentration of span-85. The batch V-GM6 prepared using 25% gelatin, 2% span-85 and 1.0 ml glutaraldehyde had highest entrapment efficiency of around 95% and particle size of around 22μm. The release rate studies indicated that the main factors affecting the release of valdecoxib from gelatin microspheres were the concentration of gelatin and volume of glutaraldehyde. The optimum duration of crosslinking was 1 hour since an increase in the duration of crosslinking to 3 hours caused an increase in the drug release due to formation of fractures on the microsphere surface. Hence batch V-GM6 crosslinked for 1 hour was considered optimum.

## 2. Chitosan microspheres

Chitosan microspheres were prepared by emulsification crosslinking method. Three different crosslinking agents used were heat, formaldehyde and glutaraldehyde. The % entrapment efficiencies of microspheres crosslinked using formaldehyde or glutaraldehyde was significantly higher than that of the heat crosslinked microspheres. There was no significant difference between the entrapment efficiencies of formaldehyde or glutaraldehyde crosslinked microspheres. The particle size of the heat crosslinked microspheres was found to be significantly lower than that of the microspheres prepared using formaldehyde or glutaraldehyde as crosslinking agents. The particle size of the formaldehyde crosslinked microspheres was significantly higher than that of the glutaraldehyde crosslinked microspheres.

The in-vitro drug release studies indicated that the release rate is highest in case of heat crosslinked microspheres, followed by formaldehyde crosslinked microspheres and slowest release is observed in glutaraldehyde crosslinked microspheres. There is a burst release of celecoxib from all the formulations which is due to the drug crystals present on the surface of the microspheres.

In case of celecoxib loaded chitosan microspheres, the batch D prepared using 3% chitosan, 5% span-85, 1.0 ml glutaraldehyde and crosslinked for 3 hours was considered as an optimum batch due to the high entrapment efficiency, low particle size and slow drug release. The in-vitro release study of the optimized batch in presence of collagenase indicated that the release is significantly higher in presence of collagenase indicating the enzyme mediated degradation of chitosan microspheres.

In case of rofecoxib loaded chitosan microspheres, the entrapment efficiency was not affected by the concentration of span-85 due to insolubility of rofecoxib in the external phase of the emulsion. The formation of microspheres in the form of fine

powder was dependent on the volume of glutaraldehyde. At higher volumes of glutaraldehyde, microspheres could not be formed because of the extensive crosslinking between the chitosan microdrops. Hence the volume of glutaraldehyde had to be carefully selected in order to achieve discrete microspheres. There was no significant difference in the release rates of the batches R-CM7 and R-CM8 prepared using 0.1 and 0.25 ml of glutaraldehyde respectively. Hence batch R-CM7 was selected as an optimum batch which gave high entrapment efficiency and low particle size.

In case of valdecoxib loaded chitosan microspheres, batch V-CM7 which was formulated using 3% chitosan, 5% span-85 and 1.0 ml glutaraldehyde was considered as an optimum batch owing to high entrapment efficiency, low particle size and slow drug release rates.

**3. Albumin microspheres**

Albumin microspheres were prepared using two different methods viz. Thermal denaturation method and emulsification chemical crosslinking method. The microspheres prepared using thermal denaturation method had significantly lower entrapment efficiency than the microspheres prepared using emulsification chemical crosslinking method. The particle size of the microspheres prepared using thermal denaturation of albumin was significantly lower than the microspheres prepared using emulsification chemical crosslinking. There was no significant difference in the entrapment efficiencies of the microspheres crosslinked using formaldehyde or glutaraldehyde. However, the particle size of the formaldehyde crosslinked microspheres was significantly higher than that of the glutaraldehyde crosslinked microspheres. The release rate of celecoxib from albumin microspheres prepared by thermal denaturation is highest while that of glutaraldehyde crosslinked microspheres

was lowest. Formaldehyde crosslinked microspheres exhibit intermediate release rates, higher than glutaraldehyde crosslinked microspheres and slower than the microspheres prepared by thermal denaturation. Thus, out of the two chemical crosslinking agents used viz. glutaraldehyde and formaldehyde, glutaraldehyde proved to be more promising because of the low particle size and slower drug release.

The entrapment efficiency of the microspheres was mainly dependent on the concentration of albumin, concentration of span-85 and stirring speed. The batches C-GA5, C-GA6 and C-GA9 prepared using 30% albumin, 2% span-85 were studied for in-vitro drug release. The in-vitro release studies of celecoxib loaded albumin microspheres indicated that with an increase in the volume of glutaraldehyde, there is a decrease in the drug release. The release rates of celecoxib from microspheres crosslinked using 1.5 ml glutaraldehyde was found to be too slow and hence the microspheres crosslinked using 1.0 ml glutaraldehyde was considered to be optimum. The release studies of batch C-GA6 conducted in presence of collagenase indicated that the release rates increases in presence of collagenase.

In case of rofecoxib loaded albumin microspheres, the main factors affecting the entrapment efficiency was the concentration of albumin. There was no significant effect of the concentration of span-85 and volume of glutaraldehyde on the entrapment efficiency. The particle size of the microspheres was affected by the concentration of albumin and concentration of span-85. There was no significant effect of the volume of glutaraldehyde on the particle size of the microspheres. The drug release studies indicated that with an increase in glutaraldehyde volume from 0.5 ml to 1.0 ml there was a decrease in the drug release rates. With further increase in the volume to 1.5 ml there was no significant decrease in the drug release. Hence for

rofecoxib loaded albumin microspheres, the batch R-AMS11 formulated using 30% albumin, 5% span-85 and 1.0 ml glutaraldehyde was considered as an optimum batch. For valdecoxib loaded albumin microspheres, the batches V-AMS10, V-AMS11 and V-AMS12 prepared using 30% albumin and 5% span-85 had high entrapment efficiency and low particle size. The in-vitro release studies of these batches indicted that there is a steady decrease in the release rates of valdecoxib from the microsphere as the glutaraldehyde volume is increased from 0.5 ml to 1.0 ml to1.5ml. Hence, batch V-AMS12 crosslinked using 1.5 ml glutaraldehyde was considered as an optimum batch due to high entrapment efficiency, low particle size and slow drug release.

**4. Solid lipid nanoparticles**

Solid lipid nanoparticles of celecoxib were prepared by hot melt homogenization technique. Nanoparticles with high entrapment efficiency (>95%) were obtained and the particle size of the optimized nanoparticles was around 250nm. The main factors affecting the entrapment efficiency were found to be the concentration of the lipid and concentration of celecoxib. The poloxamer concentration, homogenization pressure and the number of homogenization cycles had no significant influence on the entrapment efficiency. The particle size of the nanoparticles was affected by the concentration of the lipid, concentration of celecoxib, poloxamer concentration, homogenization pressure and the number of homogenization cycles. The optimized nanoparticles formulation was prepared using 5%w/v lipid, 4%w/w celecoxib, 3%w/v surfactant (poloxamer) at a homogenization pressure of 10000 psi/ 3 cycles.

*Radiolabelling studies*

The anti-arthritic drug celecoxib and its formulations were radiolabelled with $^{99m}$Tc prior to in-vivo studies. The various factors that affected the radiolabelling efficiency were pH, concentration of stannous chloride and incubation time. The

effect of changing these factors on the labelling efficiency of celecoxib and its formulations was studied. The optimum stannous chloride concentration for celecoxib, solid lipid nanoparticles and albumin microspheres was found to be 60μg whereas for chitosan microspheres and gelatin microspheres it was found to be 100 μg. The optimum pH for labelling of celecoxib, chitosan microspheres, albumin microspheres and gelatin microspheres was 6.5. For solid lipid nanoparticles, the optimum pH for radiolabelling is 7.0. The optimum incubation time for radiolabelling of celecoxib, chitosan microspheres, gelatin microspheres and albumin microspheres was 10 minutes. For solid lipid nanoparticles, the optimum incubation time was 5 minutes. Transchelation of the radiolabelled complexes with DTPA indicates that the radiolabeled complexes are highly stable in-vitro. The saline stability and serum stability studies indicated that the radiolabeled complexes are highly stable.

*In-vivo studies*

Two different routes of administration were chosen for targeting celecoxib to the arthritic joints. The first was the intra-articular injection and the second was the intravenous injection.

Two different approaches were used for studying the retention of the radiolabelled complexes in the joint after intra-articular injection.

The first one was the short term study for 24 hours in which the radiolabelled complexes were injected in the arthritic joints of the rats and the radioactivity present in the joint at various time intervals is determined.

The second approach was a long term study for 18 days in which the duration of anti-inflammatory effect of celecoxib and its formulations was studied by γ-scintigraphy. Evaluation of arthritis was carried out by injecting a marker ($^{99m}$Tc- glutathione) which specifically accumulates in the arthritic joints after intravenous injection. This

accumulation of the marker is then imaged using a gamma camera. Higher accumulation of the marker in the arthritic joint is an indication of higher degree of inflammation. Celecoxib and its formulations were injected intra-articularly in arthritic joints of the rats and then the gamma images of the rats were taken at specific time intervals upto 18 days.

The pharmacokinetic and biodistribution study of celecoxib and its albumin microspheres and solid lipid nanoparticles after intravenous injection was also carried out in arthritic rats.

The evaluation of the biocompatibility of the formulations of celecoxib was done by histopathology studies.

The results of the in-vivo studies can be summarized as follows:

a) The pharmacokinetic and extra-articular distribution studies of celecoxib and its formulations (celecoxib loaded gelatin microspheres, chitosan microspheres, albumin microspheres and solid lipid nanoparticles) after intra-articular injection revealed that the formulations of celecoxib exhibit enhanced retention in the joint compared to the celecoxib. Celecoxib is rapidly cleared from the joint to the circulation and is distributed to the organs like liver, spleen and kidney, whereas the extra-articular distribution of the all the formulations studied was significantly lower ($p<0.05$) than that of celecoxib. Out of all the formulations studied, gelatin microspheres exhibit highest retention in the joint while lowest extra-articular distribution was observed with albumin microspheres.

b) The γ-scintigraphy studies indicated that the formulations of celecoxib (Gelatin microspheres, chitosan microspheres, albumin microspheres and solid lipid nanoparticles) had a prolonged duration of anti-inflammatory effect compared to celecoxib. It was concluded that compared to albumin microspheres

and solid lipid nanoparticles, gelatin microspheres and chitosan microspheres exhibited a prolonged duration of anti-inflammatory effect on the inflamed synovium of rats.

c) The pharmacokinetic and biodistribution study of radiolabelled solid lipid nanoparticles and albumin microspheres after intravenous injection was conducted because of the low particle size of these formulations. Both albumin microspheres and solid lipid nanoparticles exhibited a prolonged circulation in blood compared to celecoxib. Albumin microspheres were able to reach the inflamed synovium of the rats after intravenous injection and the radioactivity present in the inflamed joint was 2.5 folds higher than the non-inflamed joint. The distribution of albumin microspheres to organs like liver and spleen was significantly lower than that of celecoxib. However a major portion of albumin microspheres were found accumulated in lungs indicating its use for lung targeting.

   In case of solid lipid nanoparticles, there was no significant difference in the radioactivity present in the inflamed as well as non-inflamed joint indicating that solid lipid nanoparticles are not able to reach the inflamed joint. However the distribution of solid lipid nanoparticles to the organs like liver, spleen and kidney is significantly lower than that of celecoxib. Thus it can be used to reduce the side effects associated with the use of celecoxib.

d) The histopathology study of the joints was done after intra-articular injection of the plain formulations (gelatin microspheres, chitosan microspheres, albumin microspheres and solid lipid nanoparticles) to study the potential toxicity of the formulations to the joints. The study indicated that the chitosan microspheres, albumin microspheres and solid lipid nanoparticles were free from toxicity to the

synovium whereas albumin microspheres did cause some inflammation to the synovium.

## 11.2 Conclusions

An attempt was made to prepare a targeted drug delivery system for the anti-arthritic drugs. Three different drugs studied were celecoxib, rofecoxib and valdecoxib. Microspheres loaded with these drugs were prepared using biodegradable polymers gelatin, chitosan and bovine serum albumin. Microspheres with high drug entrapment efficiency, the desired particle size range and controlled release rate were successfully prepared. The microspheres degraded in presence of the enzyme collagenase to render drug release at an optimum rate. This indicates that the microspheres are expected to degrade in the arthritic joints, where the collagenase activity is higher. Solid lipid nanoparticles of celecoxib with high entrapment efficiency were also prepared for the study. The optimized formulations of celecoxib were subjected to in-vivo studies. Pharmacokinetic studies were carried out using white New-Zealand rabbits after intra-articular as well as intra-venous administration. The biodistribution studies were carried out using male Sprague-Dawley rats. The in-vivo studies indicated that celecoxib loaded microspheres and the solid lipid nanoparticles exhibit an enhanced retention in the arthritic joint of the rat following intra-articular injection. Moreover, the duration of the anti-inflammatory effect was also significantly higher for the prepared formulations compared to celecoxib solution. Out of the four formulations studied, gelatin microspheres and chitosan microspheres had significantly prolonged duration of anti-inflammatory effect. The results of the pharmacokinetic studies of celecoxib and its formulations (albumin microspheres and solid lipid nanoparticles) after intravenous injection indicated that the formulations exhibit a prolonged circulation in the blood. The biodistribution studies indicated that albumin microspheres are able to reach the

inflamed joint whereas solid lipid nanoparticles failed to accumulate in the arthritic joints. However, both the formulations have a potential to reduce the side effects associated with the use of celecoxib by reducing its distribution to the organs like liver spleen and kidney. The formulations were found to be non-toxic and biocompatible with the rat synovium. The present study led to a development of a targeted drug delivery system which could reduce the distribution of the anti-arthritic drug to the organs other than the inflamed joint to a significant extent. This is expected to reduce the side effects associated with the use of anti-arthritic drug. Thus, the present study led to some interesting findings which may be exploited in improving the therapeutic efficacy of the anti-arthritic drugs.