9. SUMMARY AND CONCLUSION

Arthritis is inflammatory disorders that affect one or more joints and cause pain. This results in immobility of affected joint and makes the patients dependable on others for their day to day activity. The two main types of arthritis are osteoarthritis and rheumatoid arthritis. The detailed description about the disease and its treatment are given in Chapter 1. Aging is one of the factors that cause arthritis and most of the arthritic patients are old people. At present the diseases is treated with two types of agents. One is nonsteroidal anti-inflammatory drug, which is used as first line agents to reduce the pain and inflammation. Though, this reduces pain and inflammation but fails to modify the disease status and provide only temporary relief. The other type of category is called as disease-modifying agent, also called as second line agent. Disease modifying agent is known to posses severe toxic effects and should be given under careful monitoring for any possible side effects. Detailed information about the drugs used in the treatment of arthritis is given in the introduction part.

Diclofenac sodium is the one of the nonsteroidal anti-inflammatory drug widely used to treat the arthritis. Because of shorter half-life (less than 2 h), it has to be given frequently to maintain effective plasma concentration which leads to fluctuation in plasma drug concentration and results in ineffective therapy. One of the major side effects of the diclofenac sodium is ulceration in GIT. To maintain effective drug plasma concentration for prolonged period extended/sustained release tablet of diclofenac sodium was formulated and available in the market. Though these formulations reduced the dosage frequency but still produced gastric ulceration.

To avoid the drug toxicity, diclofenac sodium was targeted to its site of action that is to the inflamed joint by intra-articular injection of polymeric microspheres made
upof albumin and poly (lactide-co-glycolide). Magnetic microspheres loaded with mefenamic acid also formulated to localize the drug at its site of action by magnetic means. Though these microspheres prolonged and sustained the drug release, failed to control the drug release depending upon the disease condition. Moreover, the presence of proteolytic enzyme in the inflamed joint may digest these microspheres, which are protein in nature.

In the present study, the gelatin microspheres were formulated in such a way that it could control the drug release based on disease conditions or by using external stimuli. The diclofenac sodium loaded gelatin microspheres were formulated for intra-articular injection, thereby localizing the drug in the arthritic knee. The diclofenac sodium loaded gelatin magnetic microspheres were formulated for intra-arterial and intravenous administration and were localized at the site of action by keeping a magnet near target site. The microspheres were formulated by using emulsification/cross-linking technique. Glutaraldehyde in toluene was used as cross-linking agent and the unreacted glutaraldehyde was removed by the treatment with sodium metabisulphite. The formulated microspheres were characterized by drug loading, percentage of entrapment/encapsulation and by various analytical techniques such as optical microscopy, scanning electron microscopy, particle size analysis, FT-IR spectroscopy, differential scanning calorimetry, X-ray diffraction and atomic absorption spectroscopy.

Gelatin microspheres meant for intra-articular injection and gelatin magnetic microspheres meant for intra-arterial injection were formulated with theoretical loading of 10, 20 and 30% w/w. Gelatin magnetic microspheres meant for intravenous injection were formulated with a theoretical loading of 10% w/w. All formulated
microspheres with different percentage of loading and magnetite content showed good entrapment (above 81%) and encapsulation efficiency (above 75%). The magnetite content of magnetic microspheres was 27-29% w/w. The average particle sizes of formulated gelatin microspheres for intra-articular and intra-arterial administration were about 29-34 and 22-30 µm, respectively. The average particle size of magnetic microspheres meant for intravenous administration was 2.4 µm. The size distribution of gelatin microspheres meant for intra-articular injection and intra-arterial injection were narrow and within a range of 1-60 µm. The size range of gelatin magnetic microspheres for intravenous administration was between 0.1 and 5 µm. The particle sizes of microspheres were well within the injectable range through desired routes with 20-27 gauge needle.

The optical microscopy and SEM analysis revealed the spherical geometry of the microspheres. The SEM photographs showed the presence of magnetite particles on the surface of magnetic microspheres. The microspheres were compact, discrete and free flowing in nature. The FT-IR spectrum of microspheres loaded with drug showed many characteristics peaks of diclofenac sodium and revealed the absence of drug-carrier interaction. To confirm the physical nature of the entrapped drug in the gelatin microspheres, DSC and X-ray diffraction studies were performed. The absence of diclofenac sodium peaks in the thermogram and X-ray diffraction pattern of gelatin microspheres loaded with diclofenac sodium indicated the amorphous nature of drug. The SEM photographs of microsphere surfaces, which showed no crystalline drug particles, further supported the amorphous nature of diclofenac sodium present in the microspheres. The formulated microspheres were tested for the presence of residual glutaraldehyde by gas chromatography technique and presence of any such residue may
produce inflammation or irritation at the site of injection. All formulated batches were
free from residual glutaraldehyde and the method by which the microspheres were
formulated was completely eliminated the glutaraldehyde residue. Sodium
metabisulphite was used to terminate glutaraldehyde cross-linking and to remove
unreacted glutaraldehyde.

In vitro studies indicated that the formulated microspheres could
modulate/regulate according to the disease state. In severe arthritis presence of more
proteolytic enzyme in the synovium breakups gelatin microspheres for faster drug
release (internal stimuli) for immediate therapeutic effect. In absence of proteolytic
enzyme, the drug release is slow for a prolonged period. Apart from that, if required
ultra sonic waves can be used as external stimulii to induce faster drug release. The data
obtained from in vitro release study were applied to various kinetic equations to
determine the release rate and mechanism. The release of diclofenac sodium from
gelatin microsphere was found to be diffusion controlled, since high correlation
coefficient was observed in Higuchi (diffusion controlled) rather than first order and
zero order release kinetics. The data were well fit in to Korsmeyer-Peppas equation and
the release exponent \( n \) was found to be between 0.27 and 0.5 indicating the Fickian
model. Magnetic microspheres formulated for intravenous administration showed non-
Fickian diffusion principle (anomalous transport, since the \( n \) value is between 0.5 and
1). Based on physiochemical characters and in vitro release kinetics gelatin
microspheres meant for intra-articular and intra-arterial administration with 20%
theoretical drug loading were selected for in vivo studies. The gelatin magnetic
microspheres formulated with 10% theoretical loading for intravenous administration
was also tested in rabbits in vivo for its targeting efficiency.
The targeting efficiency of formulated microspheres was tested in rabbits. The appropriate formulations were injected intra-articularly, intra-arterially and intravenously and after 1 day of post injection, the drug available at the target area was determined using HPLC technique. Administration of gelatin microspheres by intra-articular route showed higher percentage of targeting. Alternatively, to avoid pain and physical breakup in synovium during intra-articular injection, magnetic microspheres were formulated for intra-arterial administration and showed good and comparatively less percentage of targeting than intra-articular route. Further to simplify the route of administration, magnetic microspheres were formulated for intravenous administration, but the targeting efficiency of this route was poor. The efficacy of the diclofenac sodium loaded gelatin microspheres for intra-articular and intra-arterial administration was tested in rabbits with mono arthritic joint. The effect produced by the microsphere formulation was compared with the effect produced by the conventional oral dose of diclofenac sodium. Though, the daily oral administration of diclofenac sodium reduced the joint swelling, comparatively in higher extent than the microspheres, it produced severe ulcerations in the GIT. Single administration of microspheres by intra-articular and intra-arterial route reduced the joint swelling, though in lesser extent, in antigen induced mono arthritic rabbits, without producing ulceration in the GIT.

In conclusion, the gelatin microspheres loaded with diclofenac sodium showed promising results in reducing joint swelling in arthritic knee without drug induced toxicity. The results obtained from this study indicated the scope of gelatin microspheres in the effective treatment of arthritis.