2. REVIEW OF LITERATURE

Gelatin microspheres

Hashida et al. (1977) studied the efficiency of gelatin-microsphere-in-oil emulsions as drug delivery systems for achieving specificity into lymphatics in rats. Anticancer agents were used as model drug and the study revealed the ability of gelatin microspheres-in-oil emulsion to transfer the drug into regional lymph nodes than that of aqueous solution of same drug. In addition the delivery through gelatin microspheres improved the bioavailability and provided prolonged release of the entrapped drug.

Gelatin microspheres containing muramyl dipeptide (Tabata and Ikada, 1987) were prepared by cross-linking with glutaraldehyde and added to mouse peritoneal macrophages to potentiate the tumour growth inhibitory activity. Dose-response experiments revealed the ability of muramyl dipeptide encapsulated in gelatin microspheres, to inhibit growth of tumour cells at concentrations approximately 2000 times lower than that of free muramyl dipeptide. In addition, peritoneal macrophages were activated in shorter periods upon incubation with the microsphere than the free drug. The duration of activity could be controlled up to 7 days by changing the extent of crosslinking of microspheres.

Leucuta (1990) formulated gelatin microsphere loaded with nifedipine for controlled drug delivery. He also studied the effects of polymer/drug ratio, particle size, extent of cross-linking and ethylcellulose coating on the \textit{in vitro} release of the drug from the microspheres. His findings suggested that the desired controlled release could be obtained by modifying formulation parameters. The pharmacokineti c studies in human volunteers, after the administration of a single oral dose of drug loaded microspheres,
suggested that the preparation can be considered as a sustained release delivery system for nifedipine.

Yan *et al.* (1991) formulated gelatin microspheres containing mitomycin C with an average diameter of 70 μm by cross-linking with glutaraldehyde for hepatic intra-arterial infusion. After hepatic intra-arterial infusion, the drug loaded gelatin microspheres were accumulated in the specific site (liver) and remained within the hepatic arteries for one month. *In vitro* release of drugs from the microspheres was also quantified using a dynamic dialysis method.

Narayani and Rao (1994) reported the controlled release of anticancer drug methotrexate from biodegradable gelatin microspheres. The microspheres were prepared by polymer dispersion technique and cross-linked with glutaraldehyde. The *in vitro* release study showed zero order release of entrapped drug from microspheres. The rate of release was decreased with increase in the particle size of microspheres.

Lou *et al.* (1994) attempted to target an antitumor polysaccharide, glycan through gelatin microparticles. *In vitro* experiments demonstrated a sustained release behavior of microspheres and its ability of targeting drug to the tumour cells through fibronectin mediated mechanisms. *In vivo* experiments in mice showed enhanced activity of glycan in microspheres then the glycan alone in the initial stages, but later both became progressively less effective.

Nastruzzi *et al.* (1994) formulated and evaluated gelatin microspheres loaded with antitumor drug, tetra-amidine. The microspheres were prepared by coacervation technique with 95% drug loading and 16 μm size. The effect of various parameters on the production and chemical hardening of gelatin microspheres were also described. The
results obtained were suggested that gelatin-based microspheres could be used as carrier for the controlled release of poly amidines in anticancer chemotherapy.

Li et al. (1997) formulated gelatin nanoparticles for protein and peptide drug delivery. The nanoparticles were prepared by cooling the hot solution of gelatin, which is emulsified in oil. The thermo gelling property of gelatin has been utilized for the hardening of microparticles. Bovine serum albumin, as a model drug, was encapsulated into gelatin nanoparticles of 800 nm size and these nanoparticles were encapsulated in poly (lactic-co-glycolic acid) microspheres for sustained release characteristics.

Morimoto et al. (2000) reported gelatin microspheres loaded with salmon calcitonin as effective vehicle for pulmonary delivery. The use of negatively and positively charged gelatin microspheres for pulmonary delivery of salmon calcitonin was studied in rats. The in vitro release rate of salmon calcitonin from the negatively charged gelatin microspheres was lower than that from positively charged gelatin microspheres. The cumulative release was 40% for negatively charged particle and 85% for positively charged particle. The pharmacological availability, after administration in rats, with positively charged gelatin microspheres was higher than negatively charged gelatin microspheres. Positively charged gelatin microsphere loaded with salmon calcitonin with smaller size produced high pharmacological availability than the larger size particles.

Brime et al. (2000) prepared and evaluated gelatin microspheres containing levo-dopa for nasal administration. Gelatin microspheres were prepared by the w/o emulsification and solvent extraction method. The influence of various preparation condition such as stirring speed, type and quantity of emulsifying agent, temperature and quantity of water used on the size of gelatin microspheres were studied. The release
of L-dopa from gelatin microsphere was prolonged with initial fast release, followed by slower release.

**Microspheres/magnetic microspheres used in controlled/targeted drug delivery**

Papisov *et al.* (1987) studied the *in vivo* kinetics of radio labeled magnetically targeted drug carriers. Biodistribution and capture intensity of the magnetic microparticles of different size have been compared. A mathematical model of carrier capture in animal tissues, based on capture mechanism and mass transfer process in circulating blood is proposed.

Gallo and Hassan (1988) reported about receptor-mediated magnetic carriers for targeting. They prepared cationic magnetic microspheres by using chitosan, which were designed to bind anionic glycosaminoglycan receptors on the surface of capillary endothelial cells. Heparin was used as a structural analogue for the glycosaminoglycans. Formation of complexes between chitosan/heparin and microspheres/heparin were also studied.

Langer *et al.* (1985) reviewed controlled release and magnetically modulated release systems for macromolecule up to 2,000,000 MW. The authors described various methods of preparing polymeric delivery systems, magnetic polymeric systems, magnetic triggering system for controlled and targeted drug delivery. They also discussed about *in vitro* release kinetics of these drug delivery systems.

Gupta and Hung (1989) reviewed about the magnetically controlled targeted micro carrier systems. Literature on this topic suggest that these delivery systems are capable of altering the distribution of chemotherapeutic agents in the body and enhancing therapeutic efficacy of the drugs by targeting them in to site of action. This paper reviewed the development and evaluation of biodegradable and non-
biodegradable magnetic targeted drug delivery systems and outlines their future prospects and limitations in cancer chemotherapy.

Kharkevich et al. (1989) formulated magnet-susceptible microspheres and liposomes containing neuromuscular blocking agents (dipyronium, pyrocurinum and diadonium). The authors demonstrated in cats that these magnet-susceptible carriers containing drugs caused a deeper inhibition of the neuromuscular transmission in the limb, where a magnetic field is applied, than in the control limb which located beyond the magnetic field. The microspheres containing a short-acting neuromuscular blocking agent diadonium appeared to have the highest selectivity of action and produced pronounced neuromuscular block in the targeted area.

Gupta and Hung (1990a) studied the efficacy of magnetic albumin microspheres in the targeted delivery of an anticancer agent, doxorubicin hydrochloride. The in vivo experiments were carried out in rats, free drug or microsphere loaded with drug was injected intra-arterially in the presence of 8000 Gauss magnet at the target site. Then the animals were sacrificed and various organs were analyzed for drug content. It was found that the drug concentration in target area was high and the study confirms the efficacy of magnetic albumin microspheres in the targeted delivery of chemotherapeutic agents.

Lalla and Ahuja (1991) prepared non-magnetic and magnetic microspheres of albumin–globulin mix containing mefenamic acid for targeted drug delivery. The formulated microsphere were characterized by various physiochemical methods such as appearance, drug content, encapsulation efficiency, density, particle size, magnetic content, IR spectroscopy, X-ray diffraction and in vitro release. An attempt has also been made to check the in vivo efficacy in rats.
Kim et al. (1993) studied the organ targetability of small (10 μm) and large (22.4 μm) size albumin microspheres containing radio labeled methotrexate. Injection of microspheres via the tail vein of mice produced immediate increase of radioactivity in lungs and then declined up to 3-4 weeks. However, the radioactivity in the liver, spleen and kidney increased slowly during the rapid decrease of radioactivity in lung. These observations suggested that the microsphere administered could be entrapped rapidly in lung through mechanical filtration, because of their larger size and then slowly redistributed to liver, spleen and kidney due to size reduction of microspheres by biodegradation or drug release.

Ciftci et al. (1994) formulated microspheres of 5-flourouracil using poly (dl-lactic acid) (PLA). The microspheres were prepared by a solvent evaporation technique using three different molecular weight polymers. The microspheres prepared with higher molecular weight (118 000 DA, PLA1) showed slow release rate in dissolution studies than the microspheres prepared with low molecular weight polymer (PLA2 109 000 DA and PLA3 33,300 DA). After intravenous injection of microspheres into mice, PLA1 accumulated in lung due to comparatively larger particle size and PLA2/PLA3 were accumulated in the liver due to smaller size.

Mehta et al. (1994) formulated lactide/glycolide microspheres loaded with salmon calcitonin by solvent evaporation technique. The prepared microspheres were injected subcutaneously in female Sparge-Dawley rats and blood samples were collected at different time intervals to analyze the drug content. The data showed sustained release of entrapped drug for a period of 3-9 days.
Boisdron-Celle et al. (1995) prepared and characterized 5-fluorouracil loaded poly(lactide-co-glycolide) microparticles for sustained drug delivery. The *in vitro* release studies revealed the ability of microspheres to sustain the drug release over 18 days.

Lübbe et al. (1996) performed preclinical studies with magnetic drug targeting with regard to its efficacy and tolerance. In the first part of study, various concentrations of magnetic fluid were tested in rats and immuno suppressed nude mice to understand the tolerance. In the second part, the same parameters were evaluated after administration of the magnetic fluid to which epirubicin was chemically bound. The magnetic fluid did not cause major abnormalities in behavior and there was no mortality. With very high concentrations of the magnetic fluid, animals showed lethargy for 1-2 days. There was no intolerance with the epirubicin-bound magnetic fluid as well. After administration, the magnetic fluid was concentrated in tumour cells and showed complete tumour responses in an experimental human kidney as well as in a xenotransplantated colon carcinoma model. The authors concluded that the magnetic fluid could be used in cancer treatment safely and effectively.

Kobayashi et al. (1998) studied the *in vivo* characteristics of injectable poly(DL-lactic acid) microspheres loaded with testosterone for prolonged drug delivery. The *in vivo* studies in rat showed sustain release of testosterone over a 6 weeks. The histopathological findings showed that devices used were completely degraded 10 weeks after injection.

Wang et al. (1999) prepared heterogeneously structure composite based on poly(lactic-co-glycolic acid) microspheres and poly (vinyl alcohol) hydrogel nanoparticles for long term protein drug delivery. A model protein drug bovine serum albumin was first encapsulated in polyvinyl alcohol nanoparticles, which is then loaded in poly
(lactic-co-glycolic acid) microspheres. The microsphere were characterized by size
distribution, loading efficiency, in vitro release and stability. The microspheres of 180
μm size has released the protein for two months.

Ravikumar (2000) reviewed the applications of nano and microparticles as
controlled drug delivery devices. The author described various materials and methods
used for the nano and microparticles, their applications in drug delivery.

Corvo et al. (2000) formulated liposomes loaded with superoxide dismutase for
targeting into inflamed cells. The liposomes (110 and 450 nm) were injected
subcutaneously in rats with chronic arthritis inflammation. Liposomes of 110 nm size
left the site of injection and localized in inflamed foot whereas liposomes of 450 nm
size retained at the site of injection. Intravenous administration of liposomes yielded
higher localization in inflamed foot irrespective of their sizes.

Masuda et al. (2001) evaluated carboxy methylpullulan (CMPul) as a novel
carrier for targeting immune tissues. The biodistribution of carboxy methyl pullulan was
investigated to evaluate its potency as a carrier for targeting immune tissues.
Furthermore, an immunosuppressant–CMPul conjugate was prepared and its
suppressive effect on rat adjuvant arthritis was examined. The results indicated the
ability of CMPul as carrier to target immune tissues with an immunosuppressant to
enable treatment of autoimmune diseases.

**Intra-articular drug delivery**

Pitt and Lewis (1984) studied the effect of intra-articular administration of
cortisol and alpha-I-proteinase inhibitor in monoarthritic rabbits. Both the drugs were
administered separately and in combination. All treatments improved parameters
associated with arthritis and the greatest effect was found in the combination of drugs.
Ratcliffe et al. (1984) formulated colloidal suspensions of four biodegradable polymers, polylactic acid (PLA), poly-butylcyanoacrylate (PBCA), gelatin and albumin for intra-articular injection. *In vivo* biocompatibility tests with synovial tissues were carried out to assess the irritancy of the polymers following intra-articular injection into rabbit knee joint. PLA and PBCA were found to cause joint inflammation, whereas gelatin produced mild and albumin produced no inflammation.

Ratcliffe et al. (1987) investigated the retention of albumin microspheres in normal and arthritic knee joints of rabbits, after intra-articular administration. Albumin microspheres were cleared slowly from the joint cavity and no significant difference was observed between normal and inflamed joints. About 80% of injected albumin microspheres were retained in the knee joint 10 day post injection.

Green and Foong (1993) treated antigen-induced arthritis in rabbits by the intra-articular injection of methyl prednisolone, $^{90}$Y or chlorambucil. The severity of arthritis was determined by joint swelling and skin surface temperature, macroscopic and histological changes in the joint. Treatment with chlorambucil was comparatively more effective than the other drugs at the doses employed.

Foong and Green (1993) treated antigen-induced arthritis in rabbits by intra-articular injection of free and liposome-entrapped methotrexate. Liposome methotrexate (with lesser dose) and free drug, injected at the time of antigen challenge, suppressed the development of joint swelling and the rise in skin temperature. Neither free nor liposomal methotrexate was effective in suppressing an established arthritis.

Pavanetto et al. (1994) formulated spray-dried albumin microspheres for the intra-articular delivery of dexamethasone. Microspheres of less than 10 $\mu$m were produced by spray drying method. The effects of polymer/drug ratio and heat
stabilization conditions on morphology, size, drug loading and in vitro drug release were evaluated.

Brown et al. (1998) formulated gelatin/chondroitin-6-sulfate microspheres for the delivery of therapeutic proteins to the joint. Microspheres were produced by complex coacervation method. Radio labeled protein release and microsphere degradation was performed in human synovial fluids. Microsphere degradation was confirmed by scanning electron microscopy. Microsphere biocompatibility was evaluated in vitro by incubating the microsphere with human synoviocytes and in vivo by injecting into mouse joints. The microspheres showed optimum release in synovial fluid and they were found to be noncytotoxic in vitro and noninflammatory in vivo.

Tuncay et al. (2000a) formulated diclofenac sodium loaded albumin microspheres for intra-articular delivery. The formulated microspheres were characterized by particle size, drug loading, surface morphology and in vitro drug release. The microspheres prolonged the drug release upto 8 h (in vitro). In vivo experiments revealed the efficacy of microspheres in reducing antigen-induced arthritis in rabbits.

Bozdag et al. (2001) evaluated biodegradable microspheres containing naproxen sodium for intra-articular administration. The microspheres were prepared with bovine serum albumin (BSA) and poly (lactide-co-glycolic acid) (PLGA) as biodegradable carrier. The microspheres were evaluated for physicochemical properties and drug release. The in vivo experiments in rabbits indicated that PLGA, a synthetic polymer is more promising than the BSA microspheres for an effective cure of arthritis in rabbits.
Delivery systems of diclofenac sodium and other drugs used in the treatment of arthritis

Gohel and Amin (1998) formulated alginate microspheres loaded with diclofenac sodium for controlled drug delivery. The formulation was optimized by using factorial design to get optimum release profile.

Veronese et al. (1998) developed polyorganophosphazene microspheres loaded with naproxen. The microspheres were prepared by spray drying showed different in vitro release rates depending on imidazole content of polymer. In vivo experiments were carried out in rats by injecting the microspheres subcutaneously. The constant level of naproxen in plasma was maintained up to 400 h, at a suitable concentration for anti-inflammatory activity, by the formulated microspheres.

D'Souza and DeSouza (1998) tested efficacy of poly (lactic-glycolic acid) microspheres loaded with cyclosporine in the treatment of poly arthritis in rats. Uptake studies were carried out using radio labeled microspheres and macrophages obtained from normal and polyarthritic rats. The arthritic macrophages comparatively showed higher uptake of cyclosporine microspheres and also released high quantity of cyclosporin, which in turn increased the inhibition of lymphocyte culture proliferation than the normal macrophages. The in vivo studies performed by the authors suggested that cyclosporine microspheres, even at low dose levels, were highly effective in inhibiting polyarthritis in rats.

Gohel and Amin (1999) formulated modified release microspheres of diclofenac sodium by using polyvinyl alcohol as carrier. The authors suggested a two level factorial design to formulate microspheres with required drug release property.

Williams et al. (1999) developed methotrexate-loaded liposomes and tested its efficacy in reducing inflammation, by using collagen induced arthritis model. The
authors suggested that methotrexate loaded liposomes could be used at low doses for the effective treatment of arthritis.

Tuncay et al. (2000b) was formulated poly (lactic-co-glycolic acid) microspheres loaded with diclofenac sodium for intra-articular administration. The microspheres were evaluated for particle size, yield, drug loading, surface morphology and release characters. The microspheres released the drug slowly for more than 120 h. The efficacy of microspheres was tested in arthritic rabbits after intra-articular injection. Evaluation of arthritic lesions post-therapy in rabbits showed no significant difference in the group tested with diclofenac sodium loaded microspheres compared to control groups.

Srinath et al. (2000) prepared and performed pharmacodynamic evaluation of liposomes of indomethacin with an intention to reduce the drug toxicity. A series of liposomal formulations of indomethacin were prepared and characterized for physicochemical parameters. Pharmacodynamic evaluation of the liposomes was performed in carrageenan-induced rat paw edema (acute) and adjuvant arthritis (chronic) models. Liposomal formulation of indomethacin showed greater anti-inflammatory activity in both the models than the free drug.

Hirabayashi et al. (2001) synthesized osteotropic diclofenac with bisphosphonic moiety (DIC-BP) based on the concept of osteotropic drug delivery. After intravenous (i.v.) injection into rats, DIC-BP was incorporated into bone and the diclofenac was gradually released over 28 days. The efficiency of DIC-BP (iv) was compared with diclofenac sodium (oral) in adjuvant–induced arthritic rats. DIC-BP produced anti-inflammatory effect with lesser doses when compared to diclofenac sodium. Moreover, DIC-BP exhibited no side effects of gastrointestinal damage.
3. OBJECTIVES OF THE STUDY

1) To develop gelatin microspheres loaded with diclofenac sodium, which can control/prolong the drug release depending on disease condition or by external stimuli.

2) To formulate gelatin microspheres loaded with diclofenac sodium for intra-articular delivery, thereby localizing and targeting diclofenac sodium at the site of action.

3) To formulate gelatin magnetic microspheres loaded with diclofenac sodium for intra-arterial/intravenous administration and to localize/target the magnetic microspheres near the joint as depot by keeping a suitable magnet near the site of action.

4) To characterize the formulated gelatin microspheres 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.

5) To perform in vitro release studies to determine release rate and release mechanism of the drug from the gelatin microspheres.

6) To evaluate targeting ability of formulated gelatin microspheres in normal rabbits.

7) To evaluate efficacy of microspheres in arthritic rabbits against conventional oral therapy.