Chapter 2

LITERATURE REVIEW

2.1 Arthritis

2.1.1 Anatomy and physiology of the joints (Davidson, 1999a)

Joints are the part of the connective tissue of the body. Connective tissues as their name implies provide the structural framework of the body and all its organs. Connective tissues are composed of cells of mesenchymal origin that synthesize and secrete an extracellular matrix consisting of variable amounts of collagens, proteoglycans, and elastin as well as glycoproteins such as laminin and fibronectin. Fibronectin, laminin, collagen and some other glycoproteins act as ligands for transmembrane adhesion proteins called as integrins, which regulate cell-matrix interactions by linking the extra-cellular matrix proteins with the cytoskeleton. Early in embryonic development connective tissues differentiate to form the specialized tissues required for musculoskeletal function, such as muscles, tendons, ligaments, cartilage and bone.

Bones are joined to each other at joints. These may be fibrous, as in symphasis pubis; cartilaginous, as in ostochondral joints and intervertebral discs or synovial, as in the more complex joints of the limbs where greater movement is required.

Synovial joints

In the freely movable synovial joints the ends of the bones are covered with articular cartilage and separated by joint cavity filled with clear viscous synovial fluid. A fibrous joint capsule holds the bones together and encloses the joint cavity. The capsule is lined by synovial membrane which produces the synovial fluid.

Articular cartilage

Articular cartilage consists of chondrocytes embedded in a matrix which is calcified in its deeper layer adjacent to the underlying bone. The hyaline cartilage provides the joints with low friction surfaces. It is avascular and contains no nerves. The collagen fiber framework which gives the cartilage its tensile strength is composed principally of

type II collagen with small amounts of other collagens. The collagen meshwork entraps the proteoglycans to create a fiber-reinforced hydrated gel which resists compression. Aggrecan is the principal proteoglycan in articular cartilage. It consists of a protein core with side chains of chondroitin sulphate and keratin sulphate. Aggregan molecules are joined by link proteins to hyaluronic acid and cartilage also contains small amount of other glycoproteins. Articular cartilage matrix integrity and proteoglycan synthesis are critically dependent on movement and mechanical stimulation of chondrocytes. Aggrecan and collagen turnover depends on a balance between degradative enzymes (aggrecanase, collagenase, stromelysin and gelatinase) and a tissue inhibitor of metalloproteinases. Aggrecan turnover is much faster than that of collagen, and the collagen fibre network in cartilage has a very limited capacity of repair.

The synovial membrane

The synovial membrane lines the capsule of synovial joints, bursae and tendon sheaths. It consists of type-A macrophage-like phagocytic cells and type B fibroblast like cells which are responsible for the secretion of the synovial fluid. The intracellular matrix contains secreted hyaluronan, type VI collagen, chondroitin sulphate and tenascin. The synovial cells interdigitate without tight intercellular junctions and the lining is rich in fenestrated capillaries, allowing diffusion of fluid and plasma proteins between circulation and the joint.

The synovial fluid

The synovial fluid plays an important role in the nutrition of the avascular cartilage and in the lubrication of joints. It consists of an ultrafilterate of plasma, to which locally secreted hyaluronan and lubricin have been added. The hyaluronan gives the fluid a remarkable viscoelastic property which is important for lubrication during joint

movement, while the lubricin is a glycoprotein that acts as a lubricant during static loading of joints.

The capsule, ligaments and periosteum

These contain sensory afferent fast conducting myelinated nerve fibers which are responsible for proprioception. Slow conduction, unmyelinated c-fibres which transmit pain sensation are also found in these tissues and in the synovium, where they regulate microvascular function.

2.1.2 Introduction to arthritis

Arthritis is a disease of the musculoskeletal system and connective tissues in which various joints in the body are affected leading to swelling, pain, stiffness and the possible loss of function. It affects people of all ages and ethnic groups. The frequency of arthritis increases with age; as many as 40% of the people over the age of 65 years in the United Kingdom have some kind of rheumatic disorder and 20 million people experience a rheumatic complaint each year. The disease is three times more common in women than in men. Rheumatic diseases are the most common cause of physical impairment and about one-third of all people with physical disabilities have a rheumatic disease as the primary cause of their disability. Altogether there are more than 100 types of arthritis. Out of these, Osteoarthritis and Rheumatoid arthritis are the most prevalent types of arthritis.

Osteoarthritis, also called *degenerative joint disease*, is the most prevalent form of arthritis and second most common cause of long term disability (Hochberg et al, 1995). It occurs most often in older people. Nearly everyone has osteoarthritis-related radiographic changes in the knees or other joints by age 75, although most individuals have no symptoms (Fife, 1997). This disease affects *cartilage*, the tissue that cushions and protects the ends of bones in a joint. It is characterized by focal loss of cartilage as

well as hypertrophic bone spurs. There is an overgrowth of bone at the margins and subchondrol areas of the joint (Dieppe, 1999).

Rheumatoid arthritis is a chronic autoimmune disorder characterized by joint inflammation and cartilage destruction (Harris, 1990). In this disease, the body's immune system attacks the healthy joints, tissues and organs. Continuous inflammation of the synovium slowly destroys the cartilage, narrowing the joint space and eventually damaging bone. It can cause pain, stiffness, swelling, and loss of function in joints. When severe, rheumatoid arthritis can deform, or change a joint.

2.1.3 Pathogenesis of osteoarthritis

Osteoarthritis is primarily a disease of cartilage. At gross level, osteoarthritis is marked by loss of cartilage, mild at first but eventually leading to bone on bone contact. Only 5% of cartilage consists of living chondrocytes. It primarily consists of water and proteoglycans compressed in a tight collagen network. Proteoglycans are complex macromolecules that hold water osmotically and allow cartilage to absorb impact to joints. They are composed of a protein core with glycosaminoglycan side chains, predominantly chondroitin sulphate and keratan sulfate. Proteoglycans form aggregates with hyaluronic acid, another glycoaminoglycans and link proteins. Biochemically, osteoarthritis is associated with loss of glycoaminoglycans from cartilage. Osteoarthritic joints also have increased matrix metalloproteinase activity. MMP enzymes degrade the extracellular matrix of cartilage. Inflammation is not a major characteristic of osteoarthritis. It is uncertain what initiates degradation of cartilage but it may be mechanical stress that leads to microfractures of cartilage, increased stress on surrounding tissue and induction of altered chondrocyte metabolism to favor proteolytic enzymes such as matrix metalloproteinases. Once initiated, the process becomes self-perpetuating. The increased stress on subchondral bone leads to

formation of osteophytes, or spurs of new bone. These bones spur result in the hard, bony enlargement that is a clinical characteristic of osteoarthritis. The main symptoms of osteoarthritis are pain, limitation of movement, variable degrees of local inflammation and loss of function. The joints are affected asymmetrically and there are no systemic symptoms outside the joint.

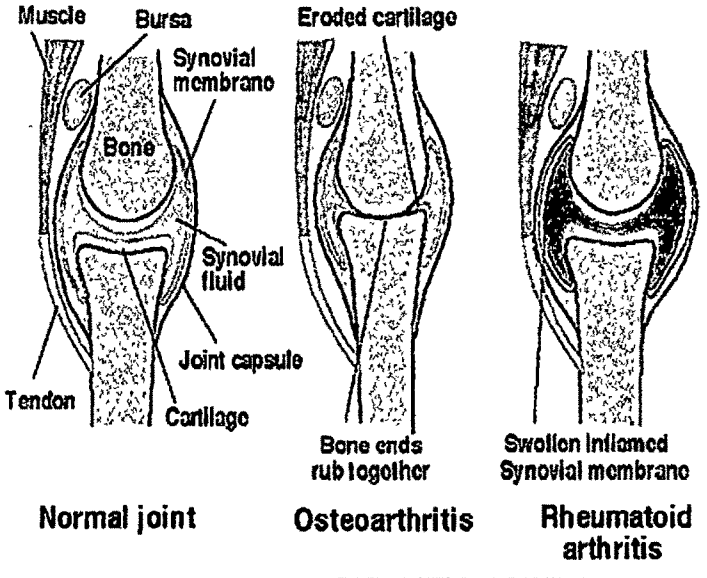
2.1.4 Pathogenesis of rheumatoid arthritis

Rheumatoid arthritis is a chronic disease, in which various joints in the body are inflamed leading to pain, stiffness, swelling and possible loss of function. Rheumatoid arthritis is an auto immune disease. It is unlikely to be due to a single cause but rather a combination of genetic and environmental factors that trigger an abnormal immune response. The inflammatory process is a byproduct of the body's immune system, which fights infection and heals wounds and injuries. Under the normal conditions, the inflammatory process is controlled and self-limited, but in people with chronic rheumatoid arthritis, certain defects, probably genetic, keeps this process going. The primary infection fighting cells are two types of white blood cells, lymphocytes and leucocytes. Lymphocytes include two subtypes known as T-cells and B-cells. Normally, when a foreign agent infects the body, helper T-cells recognize the invader and triggers a series of immune responses to destroy the imposter. In rheumatoid arthritis, the T-cells mistake the body's own collagen cells as foreign antigens and set off a series of events to rid the body of perceived threat, a process called as autoimmunity. Initial events include stimulation of lymphocyte B cells to produce antibodies, molecules designed for attack on a specific antigen. In rheumatoid arthritis, these auto antibodies also known as the rheumatoid factors, attack the body's own tissue. The leukocytes are the other major white blood cells that are spurred into action by the overactive T-cells. Leukocytes stimulate the production of two key players in the inflammatory process:

leukotrienes, which attract even more white blood cells to the area and prostaglandins which open blood vessels and increase blood flow. As a part of their activity leukocytes also produce cytokines, small proteins which are critical in the process which leads to joint damage and are responsible for inflammation beyond the joints. In small amounts, these powerful chemicals are indispensable for healing. If overproduced, these cytokines can cause serious damage, including fever, shock and even damage to organs such as the liver. Important cytokines in the process of rheumatoid arthritis are known as the tumor necrosis factor and interleukins. Some cytokines play a role in releasing enzymes such as collagenase and cathepsin L which destroys collagen. The continuous inflammation of the synovium and destruction of collagen leads to the narrowing joint space and eventually damaging bone. In progressive rheumatoid arthritis, destruction to the cartilage accelerates when the fluid and inflammatory cells accumulate in the synovium to produce pannus, a growth composed of thickened synovial tissue. The pannus produces more enzymes that destroy the cartilage, aggravating the area and attracting more inflammatory white cells thereby perpetuating the process. This inflammatory process not only affects cartilage but also harms organs in other parts of the body.

The knee joint, normal as well as that affected by osteoarthritis and rheumatoid arthritis is shown in figure 2.1

Figure 2.1: Structure of a normal synovial joint as well as joint affected by rheumatoid arthritis and osteoarthritis (Arguelles et al, 2003)



2.1.5 Drugs used in the treatment of arthritis (Davidson, 1999b)

Non-steroidal anti-inflammatory drugs and COX-2 inhibitors

These are the most widely used in relieving pain and stiffness of the arthritic joint but they do not alter the course of the disease and the margin between the effective dose and toxic dose is very small.

The most frequent side effects of NSAIDs are dyspepsia, ulceration and haemorrhage. Other side effects include fluid retention, rashes, interstitial nephritis, occasional hepatotoxicity and rarely asthma and anaphylaxis.

Specific cyclo-oxygenase-II inhibitors like celecoxib, rofecoxib and valdecoxib are associated with similar therapeutic effect as NSAIDs but significantly lower gastrointestinal toxicity. But since COX-II is constitutively present in some organs, including kidney and brain and can be induced in other tissues, COX-II inhibitors are not devoid of side effects. Thus celecoxib is associated with cardiovascular and renal

side effects, rofecoxib is associated with an increase in blood pressure and increased incidences of myocardial infarction and valdecoxib is associated with associated with a life threatening symptom called Steven Johnson syndrome.

Simple analgesics

Drugs without appreciable anti-inflammatory action include peripherally acting agents such as paracetamol and centrally acting narcotic analgesics such as dextropropoxyphene, dihydrocodeine, nefopam and tramadol. Although centrally acting narcotic analgesics should generally be avoided in the management of rheumatic diseases, simple analgesics are frequently used as additions to therapy when pain relief is inadequate. Combination drugs such as co-proxamol (Paracetamol and dextropropoxyphene) can be safe and effective when used in moderate doses.

Slow acting anti-rheumatic drugs (Disease modifying anti-rheumatic drugs)

The addition of a disease-modifying anti-rheumatic drug should be considered in all patients with symptoms and signs of active inflammatory arthritis. Drugs of this type do not possess immediate anti-inflammatory effects but will improve joint pain, stiffness and swelling and reduce systemic symptoms, acute phase proteins over a period of months. They are usually introduced in a pyradimal fashion, starting with the safest agent but the threshold for ascending the pyramid is determined by the severity of the disease.

1) Antimalarials

Hydroxychloroquine sulphate can be used as an adjunct to basic therapy. Clinical benefit is noted in about half the patients in 4-12 weeks and the drug should be discontinued if there is no effect within 6 months. Occasional side effects include nausea, diarrhea, rashes, haemolytic anemia, ototoxicity and neuromyopathy.

2) Sulphasalazine

This has a good benefit to risk ratio and is frequently used as the first choice of DMARD. Approximately 50% of the patients respond in 3-6 months. The side effects associated with its use are nausea, vomiting, depression, rashes, megaloblastic anemia and hepatitis.

3) Methotrexate, d-penicillamine and parenteral gold.

These are slow acting, suppressive antirheumatic drugs which have been shown to decrease the progression of erosive changes as well as reduce the activity of the disease in 50-60% of the patients. Due to high incidence of toxic effects, treatment with these agents should only be considered as an addition to basic therapy when there are clear indications for the use of disease-modifying drug and the patient has failed to respond to antimalarials or sulphasalazine.

Other disease modifying anti-rheumatic drugs

Dapsone is associated with slow clinical improvement and reduction of acute phase proteins but haemolytic anemia can be a troublesome side effect, particularly in slow acetylators. Other compounds which have been shown to have slow acting anti-rheumatic activity include the anti-bacterial agent rifampicin, the anti-hypertensive agnet captopril, some d-penicillamine analogues, the pentapeptide thymosin and the isoxazole derivative leflunamide.

Corticosteroids

These have very potent anti-inflammatory activity but doses required to maintain adequate symptomatic relief on a long term basis are accompanied by an unacceptable level of side effects. The main indications for the use of systemic corticosteroids are:

a) In exceptionally sever exacerbations which are not remitting with rest, intra-articular injection of corticosteroids, NSAIDs and DMARDs.

b) When other measures fail to control persistently disabling symptoms in breadwinners or young mothers who have to return to work.

c) In some elderly patients when acute disease is threatening to render them bed-bound. In life or sight threatening visceral disease such as severe pericarditis or scleritis.

Immunomodulation

A number of cytotoxic and immunostimulant agents have been found to have both symptomatic and slow acting disease modifying activity in rheumatoid arthritis. The immunomodulators used are:

a) Azathioprine

This has been shown to be effective in both high (2.5mg/kg) oral doses. Adverse effects include vomiting, stomatitis, diarrhoea, hepatitis and particularly bone marrow suppression and susceptibility to infection.

b) Cyclophosphamide

This has a narrow therapeutic range but is effective in a daily oral dose of 1-2 mg/kg. Adverse effects include alopecia, azoospermia, anovulation,cystitis, nausea and vomiting, susceptibility to infection, bone marrow suppression and teratogenesis.

c) Cyclosporin A

This is effective in doses of 2.5-4 mg/Kg daily given in divided oral doses 12 hourly. Adverse effects include anorexia, nausea, hepatotoxicity, hypertension and dose related impairment of glomerular filtration.

Topical analgesics

In osteoarthritic patients who do not wish to take oral drugs, or who want adjunctive relief, topical analgesics such as methyl salicylate or capsaicin cream are appropriate. Capsaicin, available as a topical cream in various concentrations, is a derivative of red chilly peppers that depletes peripheral sensory nerves of the neurotransmitter called substance P. Drawbacks of capsaicin is the need for frequent application and local burning sensation that will occur for first few days.

Hyaluronic acid

Hyaluronic acid is a substance in joint fluid that gives it viscosity. In osteoarthritis, the molecular weight and concentration of hyaluronic acid are diminished. Two new medical products have been approved for viscosupplementation in osteoarthritis. Both are believed to work as lubricants by substituting for hyaluronic acid in the joint. Because of the mechanism of action, they were approved by FDA for osteoarthritis of the knee as medical devices rather than as drugs. The two supplements are similar, but not the same. Hyalgan is sodium hyaluronate and Syncvisc is hylan G-F- 20, a longer acting polymer of hyaluronate. Even though the supplements are cleared from the joint within days, pain relieving effects of these agents last for approximately six months. The effectiveness of these supplements is no better than the NSAIDs but the adverse effects of the NSAIDs are avoided. Systemic adverse reactions with hyaluronic acid injections are rare and local pain and swelling from the injections is usually mild and transient.

Glucosamine and chondroitin

These dietery supplements have received much public attention. Glucosamine is a precursor to glycosaminoglycans in cartilage and chondroitin is a component of glycosaminoglycans. A number of small, double blind controlled studies have shown

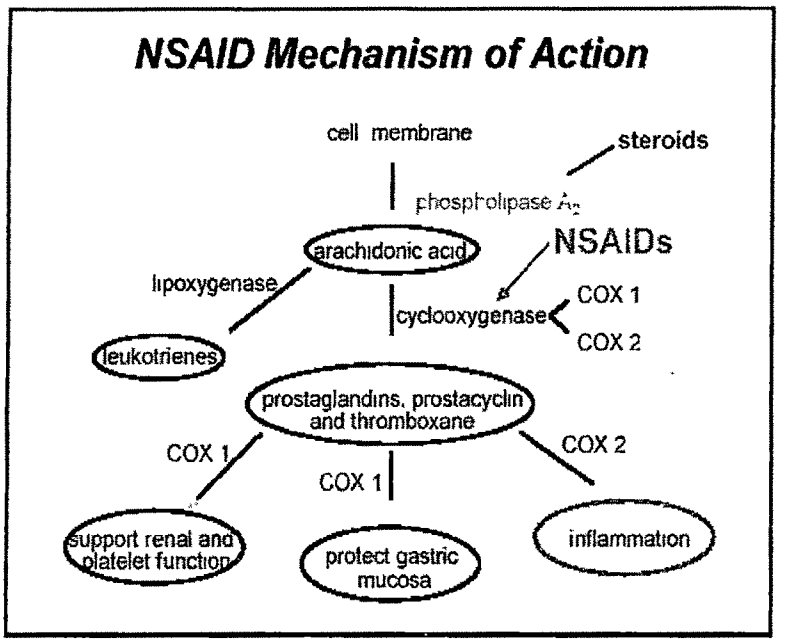
that glucosamine can reduce osteoarthritic related pain. The supplements have few side effects like nausea or mild gas.

The most widely used drugs in the treatment of osteoarthritis and rheumatoid arthritis are the Non-steroidal anti-inflammatory drugs and most recently the selective COX-2 inhibitors. Hence in the present study selective COX-2 inhibitors Celecoxib, Rofecoxib and Valdecoxib were chosen for preparing the targeted drug delivery formulations and much attention has been given to explain the Mechanism of action of NSAIDs and COX-II inhibitors in depth.

2.1. 6 Mechanism of action of NSAIDs and COX-2 inhibitors

Non-steroidal anti-inflammatory drugs act by inhibiting the enzyme cyclo-oxygenase which is the first enzyme in the pathway that converts arachadonic acid into various prostaglandins. Prostaglandins are locally synthesized chemicals that have a role in inflammation as well as numerous other body processes. While their individual physiological roles may vary, prostaglandins are clearly implicated in the inflammatory response to injury and in sensitizing pain receptors to other tissue mediators of the inflammatory response. Many physiological actions other than inflammation such as maintenance of gastric mucosal integrity and modulation of renal microvascular hemodynamics, renin release, and tubular salt and water reabsorption are also mediated by the prostaglandins. Positive role of prostaglandins in maintaining the GFR in the face of adverse circumstances has been known for sometime. NSAIDs inhibit the prostaglandin synthesis through out the body which leads to a wide range of side effects. The mechanism of action of NSAIDs is shown in figure 2.2.

Figure 2.2: Mechanism of action of NSAIDs



Recent research has revealed that there are two isoforms of Cyclo-oxygenase, COX-1 and COX-2. These enzymes have similar actions in the prostaglandin synthetic pathway, with both catalyzing the first step and both inhibited by NSAIDs. COX-1 is present as part of everyday physiological function, and is involved in the homeostasis of the entire organism. For example, it acts to protect the stomach by limiting acid secretion and improves the distribution of blood flow in the kidney. COX-1 also helps platelets limit bleeding by increasing their adhesiveness in certain situations (patients receiving NSAIDs preoperatively may, therefore, have more bleeding because of the inhibition of COX-1 and, hence, platelet function). In contrast, COX-2 appears to be variably expressed in the normal person, and its expression is induced by various stimuli. COX-2 is significantly more important in producing large amounts of prostaglandins to mediate pain and the inflammatory response to injury. Thus the therapeutic benefit of NSAIDs is due to inhibition of COX-2 but the adverse effects are due to COX-2 inhibition. This led to the development of selective COX-2 inhibitors

like rofecoxib and celecoxib. Both the COX-1 and COX-2 enzymes are very similar enzymes that consist of long, narrow tunnel and a hairpin bend at the end. Although the two forms of COX are very similar, COX-II has a smaller valine amino acid substituted for an isoleucine in COX-I. This leaves an opening to a side pocket in the COX-2 enzyme. Consequently, if NSAIDs are synthesized with a bulkier side group (typically a sulfone, sulfonyl or sulphonamide group) they can fit into and block the COX-2 enzyme but not COX-1. Thus as shown in figure 2.4, celecoxib which contains a sulphonamide side group can fit into the COX-2 enzyme but not the COX-1 enzyme. Thus COX-II inhibitors have the same therapeutic efficacy as the traditional NSAIDs but are associated with decreased side effects.

Figure 2.3: COX-1 and COX-2 effects

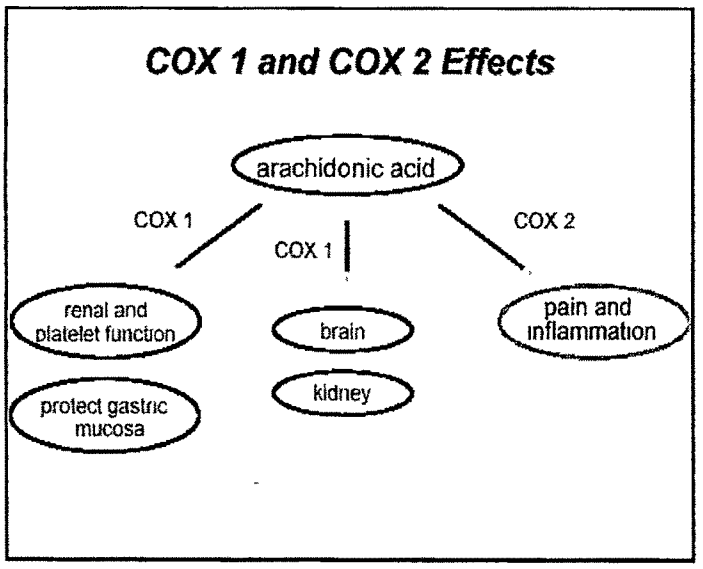
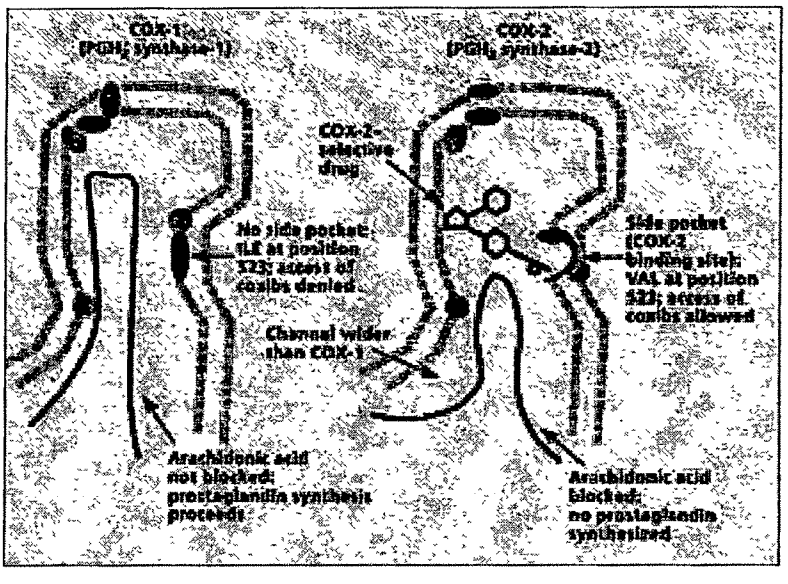


Figure 2.4: Structure of COX-I and COX-2 enzymes



Schematic showing active site similarities and differences.
ILE = isoleucine; VAL - valine.

2.1.7 Side effects of NSAIDs and COX-II inhibitors

Due to non-specific inhibition of prostaglandin synthesis throughout the body, NSAIDs are associated with a variety of side effects like gastrointestinal bleeding and ulceration, inhibition of platelet aggregation. NSAIDs-associated upper gastrointestinal haemorrhage is the most frequent serious adverse drug-related event to be reported to the committee on safety of medicines. Elderly women are particularly susceptible and case control studies suggest that one-fifth of all admissions to hospital in patients over the age of 60 years with bleeding gastric or duodenal ulcers are directly attributable to taking NSAIDs. About 1% of the Rheumatoid arthritis patients receiving NSAIDs are admitted to hospital each year with a major bleed. Endoscopic evidence of ulcers is found in 20% of NSAID-treated patients even in the absence of symptoms Even selective COX-II inhibitors like celecoxib and rofecoxib are associated with side effects because COX-II is constitutively present in some organs and can be induced in others.

In particular, safety of these drugs in patients with renal impairment needs to be established. COX-II is induced in the kidney in response to sodium depletion. COX-II is also induced in brain under stress and in the ovary during ovulation and implantation and in colon adenoma and carcinoma cells. COX-II is inducible in H-pylori related gastritis and other gastric injury. Thus even if COX-II specific inhibitors cause fewer ulcers themselves, they may still inhibit healing of existing ulcers. Moreover, since celecoxib contains a sulphonamide group, it should be used with caution in patients with sulpha allergies. COX-2 inhibitors are also associated with increased cardio-vascular risk. Mechanisms by which cyclooxygenase-2 inhibitors may increase cardiovascular risk are selective inhibition of prostaglandin I(2) over thromboxane A(2) within the eicosanoid pathway, which promotes thrombosis, and inhibition of prostaglandins E(2) and I(2) within the kidney, which leads to sodium and water retention and blood pressure elevation.

Table 2.1: Prevalence of COX-1 and COX-2 in different organs

Location	COX-1	COX-2
Chondrocyte	X	X
Gastrointestinal tract	X	
Platelets	X	
Endothelial cells	X	
Renal medulla	X	
Renal cortex		X
Brain		X
Synovial tissue		X
Colorectal tumors		X
Breast cancer		X
Lung	X	
Liver	X	
Spleen	X	

2.1.8 Need for targeted drug delivery to the joint

The side effects associated with the use of an anti-arthritic drug can be avoided if the drug is selectively targeted to the diseased site and its distribution to the other organs is minimized. Thus developing a targeted drug delivery system in which high concentrations of the drug are maintained in the joint for prolonged periods of time with minimum concentrations in organs like kidney, liver and heart would reduce the side effects associated with the use of these drugs.

2.1.9 References

Argüelles MG, Molina VP, Solá GR, Cerizola MV, Berlanga IL, Bottazzini DL Preparation and Evaluation of Holmium-166 Ferric Hydroxide Macroaggregates for Radiation Synovectomy. Alasbimn Journal, 2003 year 5, No 19.

Dieppe P. Osteoarthritis: Time to shift the paradigm. Br Med J. 1999; 318: 1229-1230.

Diseases of the connective tissues, joints and bones in "Davidson's principles and practice of medicine", 1999a, Haslett C, Chilvers ER, Hunter AA, Boon NA, eds., Churchill Livingstone, London, pp-802-804.

Diseases of the connective tissues, joints and bones in "Davidson's principles and practice of medicine", 1999b, Haslett C, Chilvers ER, Hunter AA, Boon NA, eds., Churchill Livingstone, London, pp-842-846.

Fife RS. Osteoarthritis: Epidemiology, pathology and pathogenesis in "Primer on the Rheumatic Diseases", 1997, Arthritis Foundation, Atlanta, pp- 216-218.

Harris ED. Rheumatoid arthritis: pathophysiology and implications for therapy.N.Eng.J.Med. 1990; 322: 1277-1289.

Hochberg MC, Altman RD, Brandt KD. Guidelines for the medical management of osteoarthritis Part 1. Osteoarthritis of the hip. Arthritis Rheum. 1995; 38: 1535-1540.

2.2 Targeted drug delivery systems

2.2.1 Introduction

Targeted drug delivery is the most important goal of pharmaceutical research and development. The problems associated with the conventional drug delivery are:

a) Even biodistribution of pharmaceuticals throughout the body.

b) The lack of drug specific affinity toward a pathological site.

c) The necessity of a large total dose of a drug.

d) Non-specific toxicity and other adverse side-effects.

Drug targeting may eliminate these problems associated with conventional drug delivery. In this context drug targeting is defined in the broadest sense, that is, to optimize a drug's therapeutic index by strictly localizing its pharmacological activity to the site or organ of action.

In principle, drug targeting can be achieved by physical, biological, or molecular systems that result in high concentrations of the pharmacologically active agent at the pathophysiologically relevant site. If successful, the result of the targeting would be a significant reduction in drug toxicity, reduction of the drug dose, and increased treatment efficacy. All in all, it is evident that with a biologically active agent of reasonable activity at hand, targeting to the site of action should be superior to molecular manipulations aimed at refining the receptor substrate interactions. There are two types of targeting: Passive targeting which refers to the natural distribution pattern of the carrier and active targeting which refers to an alteration in the natural distribution of the carrier. Successful drug targeting is a very complicated problem. It involves affecting the various distributional and rate processes, as well as sometimes the drug metabolism and disposition. There are a number of important parameters to be considered in designing drug targeting of any kind. These include the nature of

biological and cellular membranes, distribution and presence of drug receptors, as well as the enzymes responsible for drug metabolism, time-plasma concentration profiles, and local blood flow.

2.2.2 Advantages of targeted drug delivery systems

1. Prevention of side effects of drugs on healthy tissues and enhancement of drug uptake by targeted cells.
2. Drug administration protocols may be simplified
3. Drug quantity as well as the cost of therapy may be greatly reduced.
4. Drug concentration in the required sites can be sharply increased without negative effects on non-target compartments.

2.2.3 The ideal characteristics of a drug carrier for targeted drug delivery

1. It should be selective and only deliver the drug to the target site.
2. It should be able to carry many different types of drugs at a high payload and release the appropriate drug at a controllable rate.
3. It should be non-immunogenic and not produce new antigenic determinants when combined with the drug.
4. It should be biodegradable and produce harmless metabolites.

The side effects associated with the use of the anti-arthritic drugs can be minimized by targeting the anti-arthritic drug to the joint and minimizing its distribution to the other organs. In the present investigation, it was aimed to prepare targeted drug delivery systems containing the anti-arthritic drugs celecoxib, rofecoxib and valdecoxib. One way of achieving high concentrations of the drug in the joint for prolonged periods of time is to inject the drug intra-articularly. But the intra-articular administration is complicated by the fact that the very efficient lymphatic system clears the drug from the joints very rapidly (Noble et al, 1983, Wallis et al, 1987). Encapsulation of the drug

in polymeric particles has shown to reduce the extra-articular distribution of the drug (Ratcliffe et al, 1987). Colloidal particles introduced into the circulation are able to concentrate in the inflammatory lesions (Alpar et al, 1989). Thus in the present investigation two different routes of administration viz. intra-articular as well as intra-venous routes have been studied to study the targeting of the drugs to the arthritic joints.

2.2.4 Drug targeting to the joints

Lewis et al, 1992 investigated the use of albumin microspheres in the treatment of carrageenan induced inflammation in the rat. Free hydrocortisone, hydrocortisone incorporated into microspheres and empty microspheres were administered orally to the rats with carrageenan-induced hindpaw inflammation. The inhibition of inflammation in all the groups was measured by plethysmometer. The microspheres were labeled with fluorescein in order to trace the passage of the particles from the gastrointestinal tract to the other tissues and fluids in the rat. The fluids and the tissues were examined under the microscope for the presence of the particles. They concluded that steroid administered in microspheres is more effective than free steroid against carageenan induced inflammation in the rat. Around 0.2% of the particles administered orally were able to accumulate in the inflammatory exudates and tissues.

Corvo et al, 1999 explored the utility of PEGylated liposomes (PEG-liposomes) for targeting Superoxide dismutase(SOD) to arthritic sites. Superoxide dismutase (SOD) is a free radical scavenger enzyme which may be used as a therapeutic agent in rheumatoid arthritis, but its rapid elimination from the circulation is a major limitation. Targeted delivery of SOD may overcome this limitation. The targeting of SOD to arthritic sites following intravenous administration of both PEG-liposomes and positively charged liposomes lacking PEG but containing stearylamine (SA-liposomes)

in rats with adjuvant arthritis was studied. At 24 h post injection, the blood level of long circulating liposomes was significantly higher than that of the SA-liposomes. The highest target uptake was observed with PEG-liposomes with a mean size of 0.11 micrometer and the lowest uptake with the SA-liposomes. These results demonstrate that SOD can be targeted to inflamed sites most efficiently via small-sized PEG-liposomes. Small-sized PEG-coated liposomes are to be preferred if prolonged circulation and enhanced localization of SOD at arthritic sites are desired.

Richards et al, 1999 investigated the efficacy of a single i.v. dose of clodronate encapsulated within small unilamellar vesicles in suppressing joint inflammation and the histological progression of rat antigen-induced arthritis (AIA). Rats with AIA received a single i.v. injection of 20 mg of clodronate encapsulated within small unilamellar vesicles (SUVc) or larger multilamellar vesicles (MLVc) 7 days post-arthritis induction. Free clodronate or saline were used as negative controls. SUVc was shown to be more effective than MLVc, sustaining a significant reduction in knee swelling for up to 7 days after the initial systemic administration. Knee swelling in free clodronate-treated animals was not significantly affected. The increased efficacy of SUVc in reducing inflammation and joint destruction was associated with a significant depletion of resident ED1+, ED2+ and ED3+ macrophages from the synovial membrane (SM). They concluded that SUVc is more efficient than MLVc in reducing the severity of inflammation and joint destruction in rat AIA, and is associated with the specific elimination of macrophage subpopulations from the SM.

Shrinath et al, 2000 prepared long circulating liposomes of indomethacin in order to improve the targeting efficiency of liposomes to the arthritic joints. The circulation half-life of the liposomes was increased by grafting amphipathic polyethylene glycol-2000 to the bilayer surface. A comparative biodistribution study was performed

between the conventional liposomes and long-circulating liposomes in arthritic rats. Pharmacokinetics of the drug changed significantly when administered in liposomal form. Significant difference in pharmacokinetics was observed between the conventional liposomes and long-circulating liposomes. The increased AUC0-t and reduced clearance of the drug with long-circulating liposomes, increased the availability of the drug by reducing RES uptake, in turn localization in arthritic paw tissue was also increased. They concluded that the targeting efficiency of the long-circulating liposomes was about four times more than the conventional liposomes.

Metselaar et al, 2003 studied the effect of encapsulating glucocorticosteroids in long circulating liposomes on its therapeutic activity in experimental arthritis. Rats with adjuvant-induced arthritis (AIA) were treated intravenously with liposomal and free prednisolone phosphate (PLP) a few days after the first signs of disease. The effect on paw inflammation scores during the weeks after treatment was evaluated. Liposome biodistribution and joint localization were investigated by labelling the preparation with radioactive (111)In-oxine. They observed that a single injection of Liposomal PLP resulted in complete remission of the inflammatory response for almost a week. In contrast, the same dose of unencapsulated PLP did not reduce inflammation, and only a slight effect was observed after repeated daily injections. It was evidenced that there is a preferential glucocorticoid delivery to the inflamed joint resulting in a strong therapeutic benefit obtained with the liposomal formulation. The other possible mechanisms, such as splenic accumulation or prolonged release of prednisolone in the circulation, were excluded.

Wunder et al, 2003 studied the use of albumin as a carrier for targeted delivery of methotrexate to rheumatoid arthritic joints. Albumin has been reported as a suitable drug carrier for targeted drug delivery to tumors. Because synovium of patients with

rheumatoid arthritis (RA) shares various features observed also in tumors, albumin-based delivery systems can be used to target the drug to the inflamed joints. In this study, the pharmacokinetics of albumin and MTX in a mouse model of arthritis was examined. Uptake of albumin by synovial fibroblasts of RA patients and the efficacy of MTX and MTX-HSA in arthritic mice were also studied. The results show that when compared with MTX, significantly higher amounts of albumin accumulate in inflamed paws, and significantly lower amounts of albumin are found in the liver and the kidneys. The protein is metabolized by human synovial fibroblasts in vitro and in vivo. MTX-HSA was significantly more effective in suppression of the onset of arthritis in mice than was MTX. In conclusion, albumin appears to be a suitable drug carrier in RA, most likely due to effects on synovial fibroblasts, which might increase therapeutic efficacy and reduce side effects of MTX.

2.2.5 Intra-articular drug delivery systems

Ratcliffe et al, 1984 investigated the biocompatibility of the microspheres prepared from various polymers with the synovial tissues. The polymers used were polylactic acid, poly butyl cynoacrylate, gelatin and albumin. Microspheres were prepared using these polymers and colloidal suspensions of these microspheres were injected intra-articularly in rabbit knee joints. Evaluation of biocompatibility was done by histopathology studies of the joints. The studies indicated that poly-lactic acid, poly butyl cynoacrylate and gelatin were found to cause inflammation while albumin microspheres were well tolerated by the tissues.

Bonanomi et al, 1987 studied the fate of oligolamellar and multilamellar vesicles containing dexamethasone palmitate after intra-articular injection into healthy rabbit joints. The defined negatively charged oligolamellar vesicles of a mean diameter of 0.75 micron gave better results than multilamellar vesicles used for the same purpose

by several other authors. The positive charge carrier stearylamine does not induce any improvement.

Ratcliffe et al, 1987 studied the retention of ^{131}I-labelled albumin microspheres and microsphere-entrapped ^{131}I rose bengal in normal and experimentally arthritic knee joints of rabbits. Albumin microspheres were cleared slowly from the joint cavity and no significant difference was observed between normal and inflamed joints. Entrapment of rose bengal within albumin microspheres was found to delay the clearance of the drug from the joint when compared with a solution of rose bengal. In addition, the retention time for entrapped rose bengal was dependent on the degree of inflammation present.

Foong et al, 1993 prepared methotrexate incorporated liposomes for intra-articular administration. The anti-arthritic effect of the free methotrexate with that of the liposomal methotrexate was compared by injecting free as well as liposomal methotrexate into joints of arthritic rabbits and measuring the joint swelling and skin surface temperature. Free methotrexate suppressed the development of joint swelling and the rise in skin surface temperature of treated joints by 20-30% compared with contralateral control arthritic joints. The beneficial effect of methotrexate occurred within 24 h of injection and was maintained for at least 56 days. However, methotrexate was ineffective in suppressing arthritis when injected 7 days after antigen challenge. Liposomal methotrexate suppressed the development of arthritis at a dose one-tenth that of the free drug and it was also effective in suppressing arthritis of 7 days duration, although significant beneficial effects of liposomal methotrexate were delayed for 10 to 14 days after injection. Neither free nor liposomal methotrexate was effective in suppressing an established arthritis, when injected 21 and 35 days after antigen challenge. The histological examination of the joints at the end of the study

indicated that free methotrexate generally had no significant effect on joint pathology but liposomal methotrexate suppressed the development of synovial hyperplasia, cellular infiltration and the erosion of cartilage and bone when injected at the time of antigen challenge or 7 days later, but affected none of these parameters in an established arthritis of 3 weeks duration.

Lopez et al, 1993 synthesized triamcinolone acetonide-21-palmitate and incorporated into liposomes for intra-articular treatment of an experimentally-induced arthritis in the knee joints of rabbits. The liposomal formulation was more efficient than free triamcinolone acetonide in solution in suppressing the arthritis. Using radioactive tracers, it was found that triamcinolone acetonide-21-palmitate incorporated into liposomes was retained in the articular cavity, together with the liposomal lipids, for a much longer period than free triamcinolone acetonide, and this correlated with its anti-inflammatory effect.

Hou and Yu, 1997 prepared lidocaine incorporated liposomes for intra-articular delivery. Both aqueous lidocaine and liposomal lidocaine were injected into knee joints of adult rabbits and the pharmacokinetic changes were studied. The peak serum level of lidocaine from the liposomal preparation was significantly lower than that from the aqueous preparation. The amount of lidocaine absorbed in 4 hours was also significantly lower in the liposomal group. This phenomenon may have been due to local accumulation of liposomal lidocaine and the slow release of lidocaine from liposomes. They concluded that intra-articular use of liposomal lidocaine may have advantages over the aqueous form because of its lower systemic serum concentration.

Tuncay et al, 2000a prepared diclofenac sodium loaded albumin microsphere for intra-articular delivery in order to extend the residence time of the drug in the joint. The microspheres were evaluated in vitro for particle size, yield value, encapsulation

efficiency, surface morphology, and in vitro drug release. For the in vivo studies, Technetium-99m labelled polyclonal human immunogammaglobulin (99mTc-HIG) was used as the radiopharmaceutical to demonstrate arthritic lesions by gamma scintigraphy. After the induction of arthritis in knee joints of rabbits, the microspheres loaded with Diclofenac sodium were injected intra-articularly and at specific time points gamma scintigrams were obtained in order to compare the duration of anti-inflammatory effect of the drug in solution form with that of the drug encapsulated inmicrospheres. They concluded that the Diclofenac sodium loaded in the BSA microspheres gave a prolonged anti-inflammatory effect compared to the Diclofenac sodium in solution form.

Tuncay et al, 2000b prepared diclofenac sodium incorporated PLGA (50:50) microsphere and performed in-vitro as well as in-vivo evaluation. The microspheres were prepared by solvent evaporation process. The prepared microspheres were evaluated in-vitro for particle size, yield, drug loading, surface morphology and release characteristics. For in-vivo studies, Technetium-99m labeled polyclonal human immuno gammaglobulin was used as radiopharmaceutical to demonstrate arthritic lesions by gamma scintigraphy. Evaluation of arthritis post therapy in rabbits showed no significant difference in the group treated with PLGA (50:50) diclofenac sodium microspheres compared to the control group. The authors concluded that a natural polymer, Bovine-serum albumin is more promising for intra-articular drug delivery than the synthetic polymer PLGA.

Bozdag et al, 2001 prepared naproxen sodium loaded microsphere formulation using a natural polymer Bovine serum albumin and a synthetic biodegradable polymer PLGA for intra-articular administration and studied the retention of the drug at the site of injection in the knee joint. The microspheres were characterized in-vitro for particle

size, yield value, drug loading and release characteristics. The evaluation of arthritis was done by the use of gamma scintigraphy before and after therapy. The results indicated that the synthetic polymer PLGA is more promising than the natural polymer BSA.

Trif et al, 2001 investigated the possibility of using liposome-entrapped human lactoferrin (hlf) as a delivery system to prolong its retention at sites of local inflammation such as the rheumatoid joint. Entrapment of hLf in negatively charged liposomes enhanced its accumulation in cultured human synovial fibroblasts from rheumatoid arthritis (RA) patients, compared with positively charged formulations or free protein. However, in the presence of synovial fluid, positively charged liposomes with entrapped hLf were more stable than the negatively charged formulations. In vivo experiments in mice with collagen-induced arthritis showed that the positive liposomes were more efficient in prolonging the residence time of hLf in the inflamed joint as compared with other liposomes. The results suggest that entrapment of hLf in positively charged liposomes may modify its pharmacodynamic profile and be of therapeutic benefit in the treatment of Rheumatoid arthritis and other local inflammatory conditions.

Horisawa et al, 2002a prepared Betamethasone sodium loaded DL-lactide/glycolide copolymer (PLGA) nanosphere for producing a prolonged anti-inflammatory action after intra-articular injection. They also demonstrated the biocompatibility and biologic efficacy of these BSP-loaded nanospheres directly administered into ovalbumin-induced chronic synovitis in the rabbit. For in-vivo studies, the BSP-loaded nanosphere suspensions were administered into the joint cavity in a model of antigen-induced arthritic rabbit and evaluated by measuring the joint swelling, and the biocompatibility was appraised by histologic microscopy. The in vitro release study demonstrated that

sustained drug release occurred for over three weeks. In the antigen-induced arthritic rabbit, the joint swelling decreased significantly by administering BSP-loaded nanospheres during a 21-day period after intra-articular challenge. With regards to the prolonged anti-inflammatory efficacy, serum antibody to ovalbumin showed a sustained reduction during the period, and the steroidal effect appeared by the degradation of the polymer in the synovium. The BSP-nanosphere administered was phagocytosed by the synovial activated-cells and the cartilage degradation was almost prevented.

Horisawa et al, 2002b studied the size dependency of DL-lactide/glycolide copolymer particulates for intra-articular delivery system on phagocytosis in rat synovium Fluoresceinamine bound PLGA (FA-PLGA) nanospheres and microspheres were prepared by the modified emulsion solvent diffusion method. The suspension of these particulate systems was administered into the rat-joint cavity and the biological action of the synovium was evaluated by histological inspection and fluorescence microscopy. They found that the FA-PLGA nanospheres, with a mean diameter of 265 nm, were phagocytosed in the synovium by the macrophages infiltrated through the synovial tissues. The phagocytosed nanospheres were delivered to the deep underlying tissues. An aqueous suspension of the FA-PLGA microspheres, with a mean diameter of 26.5 microm, was not phagocytosed in the macrophages. The macrophages slightly proliferated in the epithelial lining synovial-cells and the microspheres were covered with a granulation of multinucleated giant cells. The molecular weights of the polymer in these particulate systems were slowly reduced in the synovium. Localize inflammatory responses were almost undetected. They concluded that PLGA nanospheres should be more suitable for delivery to inflamed synovial tissue than microspheres due to their ability to penetrate the synovium.

2.2.6 References

Alpar HO, Field WN, Hyde R, Lewis DA. The transport of microspheres from the gastrointestinal tract to inflammatory air pouches in the rat. J.Pharm.Pharmacol 1989; 41:194-196.

Bonanomi MH, Velvart M, Weder HG. Fate of different kinds of liposomes containing dexamethasone palmitate after intra-articular injection into rabbit joints. J Microencapsul. 1987; 4:189-200.

Bozdag S, Calis S, Kas HS, Ercan MT, Peksoy L, Hincal A. In-vitro evaluation and intra-articular administration of bio-degradable microspheres containing naproxen sodium. J. Microencapsul. 2001; 18: 443–456.

Corvo ML, Boerman OC, Oyen WJ, Van Bloois L, Cruz ME, Crommelin DJ, Storm G. Intravenous administration of superoxide dismutase entrapped in long circulating liposomes. II. In vivo fate in a rat model of adjuvant arthritis. Biochim Biophys Acta. 1999; 1419:325-34.

Foong WC, Green KL. Treatment of antigen-induced arthritis in rabbits with liposome-entrapped methotrexate injected intra-articularly. J Pharm Pharmacol. 1993; 45:204-9.

Horisawa E, Hirota T, Kawazoe S, Yamada J, Yamamoto H, Takeuchi H, Kawashima Y. Prolonged anti-inflammatory action of DL-lactide/glycolide copolymer nanospheres containing betamethasone sodium phosphate for an intra-articular delivery system in antigen-induced arthritic rabbit.Pharm Res. 2002a; 19:403-10.

Horisawa E, Kubota K, Tuboi I, Sato K, Yamamoto H, Takeuchi H, Kawashima Y. Size-dependency of DL-lactide/glycolide copolymer particulates for intra-articular delivery system on phagocytosis in rat synovium. Pharm Res. 2002b; 19:132-9.

Hou SM, Yu HY. Comparison of systemic absorption of aqueous and liposomal lidocaine following intra-articular injection in rabbits.J Formos Med Assoc. 1997; 96:141-3.

Lewis DA, Field, WN, Hayes, K., Alpar HO. The use of albumin microspheres in the treatment of carageenan-induced inflammation in the rat. J.Pharm.Pharmacol.1992; 44: 271-274.

Lopez-GF, Vazquez JM, Gil F, Latoore R, Moreno F, Villalain J, Gomez-Fernandez JC. Intra-articular therapy of experimental arthritis with a derivative of triamcinolone acetonide incorporated in liposomes.J Pharm Pharmacol. 1993; 45:576-8.

Metselaar JM, Wauben MH, Wagenaar-Hilbers JP, Boerman OC, Storm G. Complete remission of experimental arthritis by joint targeting of glucocorticoids with long-circulating liposomes. Arthritis Rheum. 2003; 48:2059-66.

Noble J, Jones AG, Davies MA, Sledge CB, Kramer RI, Livni E. Leakage of radioactive particle systems from synovial joint studied with a gamma camera. J. Bone Joint Surg.Am.1983; 65-A: 381–389.

Ratcliffe JH, Hunneyball IM, Smith A, Wilson CG, Davis SS. Preparation and evaluation of biodegradable polymeric systems for the intra-articular delivery of drugs. J. Pharm. Pharmacol. 1984; 36: 431–436.

Ratcliffe JH, Hunneyball IM, Smith A, Wilson CG, Davis SS Albumin microspheres for intra-articular drug delivery:investigation of their retention in normal and arthritic knee joints in rabbits. J. Pharm. Pharmacol. 1987; 28: 290–295.

Richards PJ, Williams AS, Goodfellow RM, Williams BD. Liposomal clodronate eliminates synovial macrophages, reduces inflammation and ameliorates joint destruction in antigen-induced arthritis. Rheumatology (Oxford).1999; 38:818-25

Srinath P, Chary MG, Vyas SP, Diwan PV. Long-circulating liposomes of indomethacin in arthritic rats-a biodisposition study. Pharm Acta Helv. 2000; 74:399-404.

Trif M, Guillen C, Vaughan D, Telfer JM, Brewer JM, Roseanu A Brock JH. Liposomes as Possible Carriers for Lactoferrin in the Local Treatment of Inflammatory Diseases Exp Biol Med. 2001; 226:559–564.

Tuncay M, Calis S, Kas HS, Ercan MT, Peksoy I, Hincal AA. In vitro and in vivo evaluation of diclofenac sodium loaded albumin microspheres. J Microencapsul. 2000a; 17:145-55.

Tuncay M, Calis S, Kas HS, Ercan MT, Peksoy I, Hincal AA. Diclofenac sodium incorporated PLGA (50:50) microspheres: formulation considerations and in vitro/in vivo evaluation. Int J Pharm. 2000b;195:179-88.

Wunder A, Muller-Ladner U, Stelzer EH, Funk J, Neumann E, Stehle G, Pap T, Sinn H, Gay S, Fiehn C. Albumin-based drug delivery as novel therapeutic approach for rheumatoid arthritis. J Immunol. 2003; 170:4793-801.

Wallis WJ, Simkin PA, Nelp WB. Protein traffic in human synovial effusions. Arthritis Rheum. 1987; 30: 57–63.

2.3 Microspheres

2.3.1 Introduction

Microspheres can be defined as solid spherical particles containing dispersed drug in either solution or microcrystalline form. They range from 1 to 100 μm in size. They are made of polymeric wax or other protective material like biodegradable, biocompatible synthetic polymers and modified natural products like starch, gum, proteins, fats and peptides. There are two types of microspheres: (Chein, 1982)

Microcapsules: Where the entrapped substance is completely surrounded by a distinct capsule wall.

Micromatrices: Where the entrapped substance is dispersed throughout the polymer matrix.

Microspheres of biodegradable and non-biodegradable polymers have been investigated for sustained release depending on their application. In case of non-biodegradable drug carriers, when administered parenterally, the carrier remaining in the body after the drug is completely released poses the possibility of carrier toxicity over a long period of time. Biodegradable, biocompatible carriers which degrade in the body to non-toxic degradation products do not pose a problem of carrier toxicity and are more suited for parenteral application (Jaykrishnan 1997).

2.3.2 Ideal characteristics of the colloidal system intended for use as a drug carrier (Mills and Davis, 1987)

1. Biocompatible with the environment at the injection site.
2. Biodegradable, with non-toxic degradation products.
3. Injectable, with good syringeability.
4. Able to be sterilized.
5. Pharmaceutically stable, with an adequate shelf life.
6. Compatible with diluents, should the need arise.

7. Able to incorporate drugs with good efficiency and structurally versatile to enable the desired drug release profile to be achieved.

2.3.3 Advantages of microspheres

1. Taste and odour masking: eg. Butobarbitone, citric acid, cod-liver oil, dicloxacillin, disulfiram, doxycycline hydrochlonde.
2. Conversion of liquids to solids. eg. castor oil, clofibrate, dimethicone fluid etc.
3. Protection of drugs against environment. eg. Ferrous citrate, levodopa,meclofenoxate HCl.
4. Have controlled release. E.g. chlorpheniramine maleate, codeine phosphate
5. Targeting of drugs. 5-fluorouracil.
6. Reduced side effects.

2.3.4 Disadvantages of microspheres

1. Burst effect
2. Inadequate shelf life of sensitive pharmaceuticals.
3. Non-reproducible
4. Costly
5. Difficult to scale-up.

2.3.5 Desirable criteria for preparation of microspheres

1. The ability to incorporate reasonably high concentrations of the drug.
2. Stability of the preparation after synthesis with clinically acceptable shelf life.
3. Controllable particle size and dispersibility in aqueous vehicle for injection.
4. Release of active agent with good control over a wide time scale.
5. Bio-compatibility with a controllable bio-degradability
6. Susceptibility to chemical modification.

2.3.6 References

Mills SN and Davis SS, Controlled drug delivery in "Polymers in controlled drug delivery" 1987, Lisbeth IL, Davis SS,eds., IOP publishing limited, Bristol, pp-10.

Chein YW, Novel drug delivery systems: Fundamentals, Development concepts, biomedical assessments in "Drugs and Pharmaceutical sciences", 1982, Swarbrick J. Ed., Marcel Dekker, New York, Vol. 14 pp-1-11.

Jayakrishnan A and Latha MS. Biodegradable polymeric microspheres as drug carriers in "Controlled and Novel drug delivery", 1997, CBS publishers, New-Delhi, pp-236-255.

2.4 Solid Lipid nanoparticles

2.4.1 Introduction

Solid lipid nanoparticles consist of spherical solid particles in nanometer range, which are dispersed in water or in aqueous surfactant solution. Generally they are made of solid hydrophobic core having a monolayer of phospholipids coating. The solid core contains the drug dissolved or dispersed in the solid high melting fat matrix. The hydrophobic chains of phospholipids are embedded in the fat matrix. They have the potential to carry lipophilic or hydrophilic drugs or diagnostics (Utreja and Jain, 2001).

2.4.2 Advantages of solid lipid nanoparticles

1. They are bio-degradable and non-toxic, stable against coalescence, drug leakage, hydrolysis, particle growth often observed in lipid emulsions and liposomes.
2. They possess a solid matrix, which has the potential for allowing drug release over a prolonged period.
3. The cost of ingredients is low
4. The methods of preparation and scale up are easy.
5. It has a high dispersibility in aqueous medium.
6. High entrapment efficiency for hydrophobic drugs
7. Extended release of the entrapped drug after single injection from few hours to several days.

2.4.3 Methods of preparation

High pressure homogenization

Two basic production techniques for the preparation of Solid lipid nanoparticles using this method are hot homogenization technique and cold homogenization technique (Muller et al, 1993). For both the techniques, the drug is dissolved or solubilized in the lipid being melted at approximately 5-10 above its melting point.

For the hot homogenization technique the drug containing melt is dispersed under stirring in a hot aqueous surfactant solution of identical temperature. Then the obtained pre-emulsion is homogenized using a piston-pump homogenizer. The hot O/W nanoemulsion is cooled down to room temperature; the lipid recrystallizes and leads to solid lipid nanoparticles.

For the cold homogenization technique the drug containing lipid melt is cooled, the solid lipid ground to lipid microparticles (approximately 50-100μ) and these lipid microparticles are dispersed in a cold surfactant solution yielding a pre-suspension. This pre-suspension is homogenized at or below room temperature. The cavitation forces are strong enough to break the lipid microparticles directly to solid lipid nanoparticles.

Microemulsification technique

In this method, first a microemulsion is obtained which is subsequently dispersed in an aqueous solution which leads to the precipitiation of the lipid. For preparation of the microemulsion, the lipid (fatty acids and/or glycerides) are melted, a mixture of water, co-surfactants is heated to the same temperature as the lipid and added under mild stirring to the lipid melt. A transparent, thermodynamically stable system is formed when the compounds are mixed in the correct ratio for the microemulsion formation. This microemulsion is then dispersed in cold aqueous medium under mild mechanical stirring ensuring that the small size of the particles is due to the precipitation and not mechanically induced by a stirring process. This dispersion is then washed twice with distilled water by ultracentrifugation and then to obtain a dry product, the suspension is freeze dried.

Precipitation method

In this method, the lipid is dissolved in an organic solvent (eg. Chloroform) and this solution is emulsified in an aqueous phase. The evaporation of the solvent from the

internal phase leads to the precipitation of the lipid in the form of nanoparticles (Sekmann and Westesen, 1996).

Multiple microemulsification solidification

This method is particularly suitable for preparing solid lipid nanoparticles containing hydrophilic drug. For this purpose, first a multiple microemulsion (w/o/w) is prepared. For the preparation of the multiple w/o/w microemulsion, an aqueous solution containing drug is added to a mixture of melted lipid, surfactant and co-surfactant at a temperature above the melting point of the lipid to obtain a clear system. The formed w/o microemulsion is then added to a mixture of water, surfactant and co-surfactant to obtain a clear w/o/w system. Solid lipid nanoparticles are then obtained by dispersing the warm micromultiple emulsion to a cold aqueous medium in a fixed ratio, under mechanical stirring. The suspension of lipid particles is then washed with dispersion medium by ultrafilteration system.

2.4. 4 Solid lipid nanoparticles in drug delivery

Muller et al, 1996 compared the phagocytic uptake of solid lipid nanoparticles with that of the polystyrene particles. A comparison of the cytotoxity of the solid lipid nanoparticles with that of the polylactide and butylcyanoacrylate nanoparticles was also done. The solid lipid nanoparticles were produced by high pressure homogenization of melted lipids (glycerolbehenate, cetylpalmitate). Their surface was modified by using hydrophilic poloxamine 908 and poloxamer 407 block copolymers in order to reduce the phagocytic uptake by the reticuloendothelial system (RES) after intravenous injection. The phagocytosis reducing effect of the polymers was investigated in vitro in cultures of human granulocytes, uptake was quantified by chemiluminescence. Modification of the SLN with poloxamine 908 and poloxamer 407 reduced the phagocytic uptake to approximately 8-15% compared to the phagocytosis of

hydrophobic polystyrene particles. The modified SLN proved more efficient in avoiding phagocytic uptake than polystyrene particles surface-modified with these block copolymers (48% and 38%, respectively). Viability determinations revealed the SLN to be 10 fold less cytotoxic than polylactide nanoparticles and 100 fold less than butylcyanoacrylate particles.

Freitas et al, 1998 converted the aqueous dispersions of solid lipid nanoparticles (SLNTM) by spray-drying into dry, reconstitutable powders which could be stored over a long period. After redispersion, the resulting granulates were still acceptable for intravenous administration with respect to the particle size distribution and toxicity. The particle size was influenced by the applied spraying parameters and by the chemical nature of the lipid phase, the type of carbohydrate and the spraying, and the redispersion medium. An identical size distribution before and after the spraying process, followed by subsequent redispersion was achieved by reducing the temperature by spraying alcoholic dispersions, reducing the lipid concentration while increasing the sugar concentration, and by redispersion in a poloxamer 188 solution.

Zimmerman et al, 2000 investigated the influence of different lyophilization parameters like the protective effect of cryoprotectants, freezing velocity, and thermal treatment on the Drug-loaded solid lipid nanoparticles (SLN) suitable for parenteral administration. The lipid matrix Imwitor 900 (concentration, 2.5%) was stabilized with Lipoid E 80 and sodium glycocholate. The results of this study demonstrate that, by optimizing critical process parameters, i.v.-injectable SLN-dispersions can be freeze-dried, preserving their small particle size.

Cavalli et al, 2002 evaluated solid lipid nanoparticles (SLN) as carriers for topical ocular delivery of tobramycin (TOB). The preocular retention of SLN in rabbit eyes was tested using drug-free, fluorescent SLN (F-SLN). The results indicated that the

SLNs were retained for longer times on the corneal surface and in the conjunctival sac when compared with an aqueous fluorescent solution. A suspension of TOB-loaded SLN (TOB-SLN) containing 0.3% w/v TOB was administered topically to rabbits, and the aqueous humour concentration of TOB were determined up to six hours. When compared with an equal dose of TOB administered by standard commercial eyedrops, TOB-SLN produced a significantly higher TOB bioavailability in the aqueous humour.

Zara et al, 2002a studied the pharmacokinetics and tissue distribution of doxorubicin incorporated in non-stealth solid lipid nanoparticles (SLN) and in stealth solid lipid nanoparticles (SSLN) (three formulations at increasing concentrations of stearic acid-PEG 2000 as stealth agent) after intravenous administration to conscious rabbits. The control was the commercial doxorubicin solution. Doxorubicin AUC increased as a function of the amount of stealth agent present in the SLN. In case of the intravenous injection of doxorubicin in SLN, doxorubicin levels were detected even after 6 hours of the injection where as in case of drug solution, no doxorubicin was detected at the same time. Tissue distribution of doxorubicin determined at 30 min, 2h and 6 h post intra-venous injection indicated that Doxorubicin was present in the brain only after the SLN administration. The increase in the stealth agent affected the doxorubicin transported into the brain; 6 h after injection, doxorubicin was detectable in the brain only with the SSLN at the highest amount of stealth agent. In the other rabbit tissues (liver, lungs, spleen, heart and kidneys) the amount of doxorubicin present was always lower after the injection of any of the four types of SLN than after the commercial solution. In particular, all SLN formulations significantly decreased heart and liver concentrations of doxorubicin.

Zara et al, 2002b investigated the use of Idarubicin-loaded solid lipid nanoparticles (IDA-SLN) for improving the bioavaoilability of idarubicin. Idarubicin loaded solid

lipid nanoparticles and idarubicin in solution were prepared and the two formulations were administered to rats, either by the duodenal route or intravenously (iv). Idarubicin and its main metabolite idarubicinol were determined in plasma and tissues by reverse-phase high-performance liquid chromatography. The pharmacokinetic parameters of idarubicin found after duodenal administration of the two formulations were different: area under the curve of concentration versus time (AUC) and elimination half-life were approximately 21 times and 30 times, respectively, higher after IDA-SLN administration than after the solution administration. Tissue distribution also differed: idarubicin and idarubicinol concentrations were lower in heart, lung, spleen, and kidneys after IDA-SLN administration than after solution administration. The drug and its metabolite were detected in the brain only after IDA-SLN administration, indicating that SLN were able to pass the blood-brain barrier. After iv IDA-SLN administration, the AUC of idarubicin was lower than after duodenal administration of the same formulation. Duodenal administration of IDA-SLN modifies the pharmacokinetics and tissue distribution of idarubicin. The IDA-SLN act as a prolonged release system for the drug.

Hu et al, 2004 investigated the use of solid lipid nanoparticles (SLN) as an alternative colloidal carrier system for controlled drug delivery of peptides. The peptide loaded solid lipid nanoparticles were prepared by a novel solvent diffusion method in an aqueous system. The SLN were characterized for entrapment efficiency, size, zeta potential (charge) and drug delivery characterization. The entrapment efficiency was found to be 69.4%, the average volume diameter of gonadorelin-loaded SLN was found to be 421.7 nm and the zeta potential was -21.1 mV dispersed in distilled water. The in-vitro release studies were conducted in the test solution of a 0.1N hydrochloric acid for 2h and then transferred in a pH 6.8 phosphate buffer (simulative gastrointestinal fluid).

The results indicated that the drug-release behavior from SLN suspension exhibited a biphasic pattern. After burst drug-release at the first 6h at a percentage of 24.4% of loaded gonadorelin, a distinctly prolonged release over a monitored period of 12 days was observed and nearly 3.81% of drug was released in each day. In the test solution of a pH 6.8 phosphate buffer (simulative intestinal fluid), the drug-release rate from SLN was similar to that in the simulative gastrointestinal fluid. The results also demonstrate the principle suitability of SLN as a prolonged release formulation for hydrophilic peptide drugs.

Souto et al, 2004 investigated the use of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for providing controlled release of clotrimazole. Clotrimazole-loaded SLN and NLC were prepared by the hot high pressure homogenization technique and evaluated for the physical stability of these particles, entrapment efficiency and its in-vitro drug release. The particles remained in their colloidal state during 3 months of storage at 4, 20 and 40 degrees C. For all tested formulations the entrapment efficiency was higher than 50%. The obtained results also demonstrate the use of these lipid nanoparticles as modified release formulations for lipophilic drugs over a period of 10 h.

Uner et al, 2004 prepared the solid lipid nanoparticles using different surfactants used for improving the stability of the solid lipid nanoparticles. In this study, it was found that 1.5% TegoCare 450 was the most effective stabilizer for the Witepsol E85 SLN dispersion compared to Tween 80, Tyloxapol and Pluronic F68 according to the data obtained from differential scanning calorimetry (DSC), zeta potential (ZP) measurements and particle size analysis.

2.4.5 References

Cavalli R, Gasco MR, Chetoni P, Burgalassi S, Saettone MF. Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. Int J Pharm. 2002; 238:241-5.

Freitas C, Mullera RH. Spray-drying of solid lipid nanoparticles (SLN TM). Eur J Pharm Biopharm. 1998; 46:145-51.

Hu FQ, Hong Y, Yuan H. Preparation and characterization of solid lipid nanoparticles containing peptide. Int J Pharm. 2004; 273:29-35.

Muller RH, Schwarz C, Mehnert W, Lucks JS. Production of solid lipid nanoparticles for controlled drug delivery. Proc. Int. Symp. Control. Release Bioact. Mater. 1993; 20: 480-481.

Muller RH, Maassen S, Weyhers H, Mehnert W. Phagocytic uptake and cytotoxicity of solid lipid nanoparticles (SLN) sterically stabilized with poloxamine 908 and poloxamer 407. J Drug Target. 1996; 4:161-70.

Siekmann B and Westesen K. Investigations on solid lipid nanoparticles prepared by precipitation in o/w emulsions, Eur. J. Pharm. Biopharm. 43(1996) 104-109.

Souto EB, Wissing SA, Barbosa CM, Muller RH. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int J Pharm. 2004; 278:71-7.

Uner M, Wissing SA, Yener G, Muller RH. Influence of surfactants on the physical stability of solid lipid nanoparticle (SLN) formulations. Pharmazie. 2004; 59:331-2.

Utreja S and Jain NK, Solid lipid nanoparticles in "Advances in controlled and novel drug delivery", Jain NK ed., 2001, CBS publishers and distributors, New-Delhi, pp-408.

Zara GP, Cavalli R, Bargoni A, Fundaro A, Vighetto D, Gasco MR. Intravenous administration to rabbits of non-stealth and stealth doxorubicin-loaded solid lipid nanoparticles at increasing concentrations of stealth agent: pharmacokinetics and distribution of doxorubicin in brain and other tissues. J Drug Target. 2002a; 10:327-35.

Zara GP, Bargoni A, Cavalli R, Fundaro A, Vighetto D, Gasco MR. Pharmacokinetics and tissue distribution of idarubicin-loaded solid lipid nanoparticles after duodenal administration to rats. J Pharm Sci. 2002b; 91:1324-33.

Zimmermann E, Muller RH, Mader K. Influence of different parameters on reconstitution of lyophilized SLN. Int J Pharm. 2000; 196:211-3.

2.5 Profile of Celecoxib

2.5.1 Introduction

Celecoxib is a selective Cyclo-oxygenase-II inhibitor which is used in the treatment of rheumatoid arthritis and osteoarthritis. At therapeutic concentrations it does not inhibit the Cyclo-oxygenase-I iso-enzyme, the inhibition of which is responsible for gastro-intestinal bleeding and ulcers.

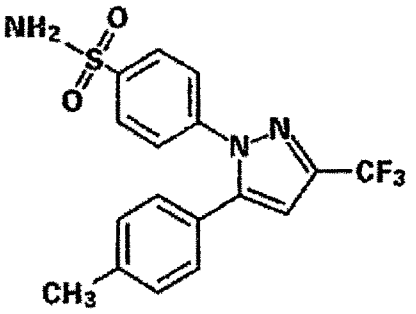
2.5.2 Description

Systematic name: 4-[5-(4 methylphenyl)-3-(trifluoromethyl) 1H-pyrazol-1-yl] Benzenesulfonamide.

Molecular formula: $C_{17}H_{14}F_3N_3O_2S$

Molecular weight: 381.38.

Chemical structure:



2.5.3 Physical properties

Description: A white crystalline powder, odorless or almost odorless.

Solubility: Practically insoluble in water. Soluble in methanol, isopropyl alcohol and acetone. Insoluble in petroleum ether. Soluble in 0.1 N sodium hydroxide.

2.5.4 Pharmacokinetics

Absorption

Peak plasma levels of celecoxib occur approximately 3 hours after an oral dose. Both peak plasma levels Cmax and area under the curve (AUC) are roughly dose proportional across the clinical dose range of 100-200 mg studied. At higher dose, under fasting conditions, there is a less than proportional increase in Cmax and AUC which is thought to be due to the low solubility of the drug in aqueous media. Because of low solubility, absolute bioavailability studies have not been conducted. With multiple dosing 3 steady state conditions are reached on or before day 5. Intake of high fat meal delayed the peak plasma levels for about 1 to 2 hours with an increase in total absorption (AUC) of 10% to 20%.

Distribution

In healthy subjects celecoxib is highly protein bound (97%) within the clinical dose range. The apparent volume of distribution at steady state (Vss/F) is approximately 400 L suggesting extensive distribution to the tissues.

Metabolism

Celecoxib metabolism is primarily mediated via cytochrome P450 2C9. Three metabolites, a primary alcohol, the corresponding carboxylic acid and its glucuronide conjugate have been identified in human plasma. These metabolites are inactive as COX-I or COX-II inhibitors.

Excretion

Celecoxib is eliminated predominantly by hepatic metabolism with little (<3%) unchanged drug recovered in the urine and feces. Following a single oral dose of radiolabelled drug, approximately 57% of the dose was excreted in the faeces and 27% was excreted in urine. The primary metabolite in both urine and feces was the

carboxylic acid metabolite (73% of the dose) with low amounts of the glucuronide also appearing in the urine. It appears that the low solubility of the drug prolongs the absorption process making terminal half life determinations more variable. The effective half life is approximately 11 hours under fasted conditions. The apparent plasma clearance (Cl/F) is about 500 ml/min.

2.5.5. Indications

Celecoxib is indicated for

1. Osteoarthritis
2. Rheumatoid arthritis

2.5.6 Dosage and administration

Osteoarthritis: For relief of the signs and symptoms of osteoarthritis the recommended oral dose is 200 mg per day administered as a single dose or as 100 mg twice per day.

Rheumatoid arthritis: For relief of the signs and symptoms of rheumatoid arthritis the recommended oral dose is 100 to 200 mg twice per day.

2.5.7 Methods of analysis

Rose et al, 2000 developed a normal phase HPLC method for the determination of celecoxib in human plasma. Samples were extracted using 3M Empore membrane extraction cartridges and separated under normal-phase HPLC conditions using a Nucleosil-NO2 (150x4.6 mm, 5) column. Detection was accomplished using UV absorbance at 260 nm. The assay was linear in the concentration range of 25-2000 ng/ml when 1-ml aliquots of plasma were extracted. Recoveries of celecoxib were greater than 91% over the calibration curve range. Intraday precision and accuracy for this assay were 5.7% C.V. or better and within 2.3% of nominal, respectively. The assay was used to analyze samples collected during human clinical studies.

Bebawy et al, 2002 developed two stability-indicating methods for the determination of celecoxib in the presence of their degradation products. The first method depends on the use of first derivative spectrophotometry (D(1)) 269 nm. This method determines celecoxib range of 1-20 microg ml(-1) with mean percentage accuracy of 99.59+/-1.67%. The second method depends on the quantitative densitometric evaluation of thin-layer chromatography of celecoxib in the presence of its degradation products without any interference. Cyclohexane-dichloromethane-diethyleamine (50:40:10) was used as the mobile phase and the chromatograms were scanned at 253 nm. This method determines celecoxib in concentration range of l-4 microg per spot for both drugs with mean percentage accuracies of 99.91+/-1.95%. The suggested methods were used to determine celecoxib in bulk powder, laboratory-prepared mixtures and pharmaceutical dosage forms (celebrex capsule). The results obtained by applying the proposed methods were statistically analyzed and compared with those obtained by the reported methods.

Dhabu et al, 2002 developed a simple and accurate high-performance liquid chromatographic (HPLC) method to determine Celecoxib in capsule formulations. The drug was chromatographed on a reversed-phase C-18 column. Eluents were monitored at a wavelength of 251 nm using a mixture (85:15) of methanol and water. Solution concentrations were measured on a weight basis to avoid the use of an internal standard. The method was statistically validated for linearity, accuracy, precision, and selectivity.

Jayasagar et al, 2002 developed a simple high performance liquid chromatographic method using UV detection for the determination of celecoxib in serum. The internal standard was tolbutamide and the column used was a C18, Wakosil column. After extracting with dichloromethane, the eluent was monitored at 250 nm. The mobile

phase comprised of 10 mM potassium dihydrogen ortho phosphate (pH 3.2) and acetonitrile (50:50 v/v) with a flow rate of 1 ml/min. The mean absolute recovery value was about 70-80%, while the intra day and inter day coefficient of variation and percent error values of the assay method were less than 10%. The calibration curve was linear over a concentration range of 10-1000 ng/ml.

Saha et al, 2002 developed a new UV spectrophotometric method (UV method) and a reversed phase liquid chromatographic method for the quantitative estimation of celecoxib in pure form and in solid dosage form. The linear regression equations obtained by least square regression method, were Abs=4.949 x 10(-2).Conc. (in microg/ml)+1.110 x 10(-2) for the UV method and Area under the curve=5.340 x 10(1).Conc. (in ng/ml)+3.144 x 10(2) for the LC method, respectively. The detection limit, as per the error propagation theory, was found to be 0.26 microg/ml and 25 ng/ml, respectively, for the UV and LC methods. The developed methods were employed with a high degree of precision and accuracy for the estimation of total drug content in three commercial capsule formulations of celecoxib. The results of analysis were treated statistically, as per International Conference on Harmonisation (ICH) guidelines for validation of analytical procedures, and by recovery studies. The results were found to be accurate, reproducible and free from interference and better than the earlier reported methods.

Schonberger et al, 2002 developed a method for the determination of celecoxib in human serum by HPLC using the demethylated analogue as internal standard. After protein precipitation with acetonitrile, samples were extracted with chloroform. The column used was a Prontosil C18 AQ column (150x3 mm I.D., 3-microm particle size) at a flow-rate of 0.35 ml/min using water-acetonitrile (40:60, v/v) as the mobile phase. Using fluorescence detection with excitation at 240 nm and emission at 380 nm, the

limit of quantification was 12.5 ng/ml for a sample size of 0.5 ml of serum. The assay was linear in the concentration range of 12.5-1500 ng/ml and showed good accuracy and reproducibility. At all concentrations intra- and inter-assay variability was below 11% with less than 9% error. The method was applied to the determination of celecoxib for pharmacokinetic studies in man.

Srinivasu et al, 2002 developed a micellar electrokinetic chromatographic (MEKC) method for the quantification of celecoxib in pharmaceutical dosage forms within the total analysis time of 7 min. The method was validated and proven to be rugged. The quantification was carried out at 35 degrees C and 25 kV, using a 25 mM borate buffer (pH 9.3), 25 mM sodium dodecyl sulphate with an extended light path capillary (48.5 cm x 50 micro I.D., 40 cm to detector). Calibration curves were constructed for celecoxib (0.2-0.6 mg/ml) by the internal standard method with 2-nitro aniline as an internal standard (coefficient of correlation greater than 0.999). The intermediate precision (between day precision) of migration times and peak area ratios of celecoxib to internal standard were 1.44 and 1.58% R.S.D., demonstrates good reproducibility of the method. The method was applied to a commercial celecoxib formulation (Revibra, 100 mg) and the percentage recoveries were ranged from 93.0 to 98.4%.

Damiani et al, 2003 developed a rapid, selective, sensitive and simple fluorescence method for the direct determination of celecoxib in capsules. The capsules were emptied, pulverized and dissolved in either ethanol or acetonitrile, sonicated and filtered. Direct fluorescence emission was measured at 355+/-5 nm (exciting at 272 nm). The method was fully validated and the recoveries were excellent, even in presence of excipients.

2.5.8 References

Bebawy LI, Moustafa AA, Abo-Talib NF. Stability-indicating methods for the determination of doxazosin mezylate and celecoxib. J Pharm Biomed Anal. 2002; 27:779-93.

Damiani P, Bearzotti M, Cabezon MA. A validated spectrofluorometric method for the determination of celecoxib in capsules. Anal Bioanal Chem. 2003;376:1141-6.

Dhabu PM, Akamanchi KG. A stability-indicating HPLC method to determine Celecoxib in capsule formulations. Drug Dev Ind Pharm. 2002; 28:815-21.

Jayasagar G, Kumar MK, Chandrasekhar K, Prasad PS, Rao YM. Validated HPLC method for the determination of celecoxib in human serum and its application in a clinical pharmacokinetic study. Pharmazie. 2002; 57:619-21.

Rose MJ, Woolf EJ, Matuszewski BK Determination of celecoxib in human plasma by normal-phase high-performance liquid chromatography with column switching and ultraviolet absorbance detection. J Chromatogr B Biomed Sci Appl. 2000; 738:377-85.

Saha RN, Sajeev C, Jadhav PR, Patil SP, Srinivasan N. Determination of celecoxib in pharmaceutical formulations using UV spectrophotometry and liquid chromatography. J Pharm Biomed Anal. 2002; 28:741-51.

Schonberger F, Heinkele G, Murdter TE, Brenner S, Klotz U, Hofmann U. Simple and sensitive method for the determination of celecoxib in human serum by high-performance liquid chromatography with fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci. 2002; 768:255-60.

Srinivasu MK, Sreenivas Rao D, Reddy GO. Determination of celecoxib, a COX-2 inhibitor, in pharmaceutical dosage forms by MEKC. J Pharm Biomed Anal. 2002; 28: 493-500.

2.6 Profile of Rofecoxib

2.6.1 Introduction

Rofecoxib is a non-steroidal anti- inflammatory drug that exhibits anti- inflammatory, analgesic, and antipyretic activities in animal models. The mechanism of action of VIOXX is believed to be due to inhibition of prostaglandin synthesis, via inhibition of cyclooxygenase- 2 (COX- 2). At therapeutic concentrations in humans, VIOXX does not inhibit the cyclooxygenase-1 (COX-1) isoenzyme.

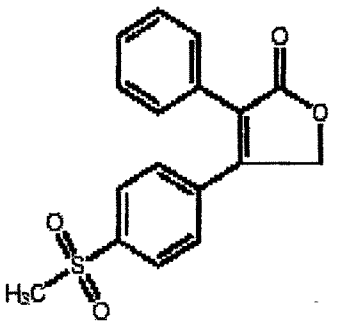
2.6.2 Description

Systematic name: 4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5*H*)-furanone

Molecular formula: $C_{17}H_{14}O_4S$

Molceular weight: 314.36

Chemical structure:



2.6.3 Physical properties

Description: Rofecoxib is a white to off-white to light yellow powder.

Solubility: It is sparingly soluble in acetone, slightly soluble in methanol and isopropyl acetate, very slightly soluble in ethanol, practically insoluble in octanol, and insoluble in water.

2.6.4 Pharmacokinetics

Absorption

The mean oral bioavailability of Rofecoxib at therapeutically recommended doses of 12.5, 25, and 50 mg is approximately 93%. The area under the curve (AUC) and peak plasma level (C_{max}) following a single 25- mg dose were 3286 (± 843) ng·hr/ mL and 207 (± 111) ng/ mL, respectively. Both C_{max} and AUC are roughly dose proportional across the clinical dose range. At doses greater than 50 mg, there is a less than proportional increase in C_{max} and AUC which is thought to be due to the low solubility of the drug in aqueous media. The plasma concentration- time profile exhibited multiple peaks. The median time to maximal concentration (T_{max}), as assessed in nine pharmacokinetic studies, is 2 to 3 hours. Individual T_{max} values in these studies ranged between 2 to 9 hours. This may not reflect rate of absorption as T_{max} may occur as a secondary peak in some individuals. With multiple dosing, steady- state conditions are reached by Day 4. The AUC 0-24hr and C_{max} at steady state after multiple doses of 25 mg rofecoxib was 4018 (± 1140) ng·hr/ mL and 321 (± 104) ng/ mL, respectively. The accumulation factor based on geometric means was 1.67.

Distribution

Rofecoxib is approximately 87% bound to human plasma protein over the range of concentrations of 0.05 to 25 g/mL. The apparent volume of distribution at steady state (V dss) is approximately 91 L following a 12.5- mg dose and 86 L following a 25- mg dose. Rofecoxib has been shown to cross the placenta in rats and rabbits, and the blood-brain barrier in rats.

Metabolism

Metabolism of rofecoxib is primarily mediated through reduction by cytosolic enzymes. The principal metabolic products are the dihydro derivatives of rofecoxib, which

account for nearly 56% of recovered radioactivity in the urine. An additional 8.8% of the dose was recovered as the glucuronide of the hydroxy derivative, a product of oxidative metabolism. The biotransformation of rofecoxib and this metabolite is reversible in humans to a limited extent (< 5%). These metabolites are inactive as COX-1 or COX- 2 inhibitors.

Cytochrome P450 plays a minor role in metabolism of rofecoxib. Inhibition of CYP 3A activity by administration of ketoconazole 400 mg daily does not affect rofecoxib disposition. However, induction of general hepatic metabolic activity by administration of the non- specific inducer rifampin 600 mg daily produces a 50% decrease in rofecoxib plasma concentrations.

Excretion

Rofecoxib is eliminated predominantly by hepatic metabolism with little (<1%) unchanged drug recovered in the urine. Following a single radiolabeled dose of 125 mg, approximately 72% of the dose was excreted into the urine as metabolites and 14% in the feces as unchanged drug. The plasma clearance after 12.5- and 25- mg dose was approximately 141 and 120 mL/ min, respectively. Higher plasma clearance was observed at doses below the therapeutic range, suggesting the presence of a saturable route of metabolism (i. e., non- linear elimination). The effective half- life (based on steady- state levels) was approximately 17 hours.

2.6.5 Indications

Rofecoxib is indicated for:

- Relief of the signs and symptoms of osteoarthritis.
- For the management of acute pain in adults.
- For the treatment of primary dysmenorrhea.

2.6.6 Dosage and administration

Osteoarthritis

The recommended starting dose of VIOXX is 12.5 mg once daily. Some patients may receive additional benefit by increasing the dose to 25 mg once daily. The maximum recommended daily dose is 25 mg.

Management of Acute Pain and Treatment of Primary Dysmenorrhea

The recommended initial dose of VIOXX is 50 mg once daily. Subsequent doses should be 50 mg once daily as needed.

2.6.7 Methods of analysis

Radhakrishnan et al, 2001 developed a simple, rapid and selective isocratic reversed phase-liquid chromatographic (RP-LC) method for the determination and purity evaluation of rofecoxib in bulk and pharmaceutical dosage forms using photodiode array detection set at 225 nm. The method is capable of detecting all process intermediates and other related compounds, which may be present at trace levels in finished products. Hence the method is very useful for process monitoring during the production of rofecoxib. The method is linear in the range of 125-500 microg. The method was found to be precise and accurate and the percentage recoveries from the tablet ranged from 98.2% to 102.6%.

Werner et al, 2001 developed an easy, rapid and selective method for the determination of rofecoxib in human plasma. The analytical technique is based on reversed-phase high-performance liquid chromatography coupled to atmospheric pressure chemical ionisation mass spectrometry. The method was validated over a linear range from 1 to 500 microg/l using celecoxib as internal standard. After validation, the method was used to study the pharmacokinetic profile of rofecoxib in 12 healthy volunteers after administration of a single oral dose (12.5 mg). The presented

method was sufficient to cover more than 95% of the area under the curve. The pharmacokinetic characteristics (mean+/-SD) were tmax: 2.4+/-1.0 h, Cmax: 147+/-34 microg/l, AUCinfinity: 2038+/-581 microg h/l and t 1/2: 11.3+/-2.1 h.

Aravind et al, 2002 developed a sensitive and rapid high-performance liquid chromatographic (HPLC) method of determining rofecoxib in human serum. Alkalinized plasma samples were extracted into an organic solvent containing an internal standard and evaporated under nitrogen. The dried sample residues were reconstituted with mobile phase and analyzed by HPLC. The method uses 100 microL of the sample and is linear from 20 to 2000 ng/mL of rofecoxib. Precision and accuracy studies were performed and the stability of the drug in serum over four weeks was documented. The method was found to be simple, sensitive, precise, and accurate. The small sample volume required (100 microL) enables this method to be used for pediatric patients.

Mao et al, 2002 developed a stability-indicating method for the assay of rofecoxib using reverse-phase high-performance liquid chromatography (HPLC). Stress testing of rofecoxib was conducted during the method development and validation. HPLC analysis of rofecoxib solutions stressed under alkaline and photolytic conditions revealed the presence of several degradates. Two main degradates were determined to be the cyclization product formed by photo-cyclization and the dicarboxylate formed by ring opening in the presence of base and oxygen. The identities of these degradates were confirmed by comparison of UV spectra and HPLC retention time with the independently synthesized products. The mechanistic pathways for the formation of these degradates were discussed.

Vallano et al, 2002 developed a method for determination of rofecoxib and isopropoxy-4-(4-methanesulfonylphenyl)-5,5'-dimethyl-5H-furan-2-one (DFP, III) in human plasma

employing monolithic HPLC columns. Each analyte, together with an internal standard was extracted from the plasma matrix using solid-phase extraction in the 96-well format. The analytes were chromatographed on a Chromolith Speed Rod monolithic HPLC column (4.6 x 50 mm). Analyte detection for rofecoxib was via fluorescence following post-column photochemical derivatization. Detection for III was based on the native fluorescence of the compound. The precision, accuracy, and linearity of the methods were found to be comparable to those obtained using methods employing conventional packed HPLC columns. Use of the monolithic column permitted mobile phase flow-rates of up to 6.5 ml/min to be employed in the assays. The use of elevated flow-rates enabled the per sample analysis time to be reduced by up to a factor of 5 compared with assays based on packed HPLC columns.

Mandal et al, 2003 developed a convenient, sensitive and simple method for the determination of rofecoxib in human plasma. The analytical technique is based on reversed phase high performance liquid chromatography coupled with UV detector (Knauer, Germany) set at 272 nm. The retention time of rofecoxib after recovery from plasma, was 8.9 minutes. The method has been validated over a linear range of 50-450 ng/ml from plasma. After validation the method was used to study the pharmacokinetic profile of rofecoxib in 6 healthy volunteers as per DCGI guidelines after administration of a single oral dose (50 mg). The extraction efficiency from plasma varied from 93.95-99.58%. The minimum quantifiable concentration was set at 50 ng/ml (% CV < 10%). The pharmacokinetic parameters were Cmax = 318.58 +/- 30.65 ng/ml at tmax = 2.66 +/- 0.25 hours, AUC0-t = 4007.88 +/- 438.32 ng hour/ml, AUC0-yen = 5454.66 +/- 822.29 ng hour/ml, Kel = 0.0433 +/- 0.0067/hour, and t1/2 = 16.36 +/- 2.89 hours.

Duran et al, 2004 developed a method for assay of rofecoxib by UV spectrophotometry and HPLC in the concentration ranges of 2-30 µg/ml and 5-50 µg/ml, respectively. The

results, obtained by the two methods in pharmaceutical preparations were compared of each other. There were no significant differences between the mean values and the precisions.

Erk et al, 2004 developed two different UV spectrophotometric methods for the determination of rofecoxib in bulk form and in pharmaceutical formulations. The first method was a UV spectrophotometric method and the second was the first derivative spectrophotometry method. For UV spectrophotometric method, the absorbance of the rofecoxib solutions was measured at 279 nm and for the first derivative spectrophotometry, amplitude at 228, 256 and 308 nm. Calibration curves were linear in the concentration range using peak to zero 1.5-35.0 μg/ml. These methods were compared with a HPLC method, which was carried out at 225 nm with a partisil 5 ODS (3) column and a mobile phase constituted of acetonitrile and water (50 :50 v/v). A linear range was found to be 0.05-35.0 μg/ml. The results obtained by first derivative spectrophotometry were compared with HPLC and no significant difference was found.

Shehata et al, 2004 determined rofecoxib in the presence of its photo-degradation product using first derivative spectrophotometry and first derivative of the ratio spectra by measuring the amplitude at 316.3 and 284 nm respectively. Rofecoxib can be determined in the presence of up to 70% and 80% of its photodegradation product by the first derivative spectrophotometry and first derivative of the ratio spectra, respectively. The linearity range of both the methods was the same (5.8-26.2 μg/ml) with mean percentage recoveries around 100% for both the methods. The first derivative method was used to study kinetics of rofecoxib photo-degradation that was found to follow a first-order reaction. The t(1/2) was 20.2 min while K (reaction rate constant) was 0.0336 mol min(-1). Both methods were applied to the analysis of rofecoxib in bulk powder and in pharmaceutical formulations. Also a

spectrofluorimetric method was described to determine rofecoxib at very low concentrations (25-540 ng/ml) where rofecoxib is converted to its photo-degradate, which possesses a native fluorescence that could be measured. The proposed method was applied for the analysis of tablets containing rofecoxib as well as to rofecoxib-spiked human plasma.

Zhang et al, 2004 developed a rapid and simple HPLC assay was developed for the determination of rofecoxib in human plasma and breast milk. After solid-phase extraction, rofecoxib was resolved on a C18 column and detected by UV detection at 272 nm. Standard curves were linear over the concentration range 10-2000 microg/L (r2 >0.99). Intra- and inter-day coefficients of variation for both matrices were <10% and the limit of quantification was around 10 microg/L.

2.6.8 References

Aravind MK, Prescilla R, Ofenstein JP. A rapid and sensitive high-performance liquid chromatography assay for rofecoxib in human serum. J Chromatogr Sci. 2002; 40:26-8

Duran A, Bekce B, Dogan HN. Quantitative determination of rofecoxib in pharmaceutical preparations. Pharmazie. 2004;59:71-2.

Erk N, Altuntas TG. Comparison of derivative spectrophotometric and liquid chromatographic methods for the determination of rofecoxib. Pharmazie. 2004; 59:453-6.

Mandal U, Ganesan M, Jayakumar M, Pal TK, Chattaraj TK, Ray K, Banerjee SN. High performance liquid chromatographic determination of cox-2 inhibitor rofecoxib in human plasma. J Indian Med Assoc. 2003; 101:486-8.

Mao B, Abrahim A, Ge Z, Ellison DK, Hartman R, Prabhu SV, Reamer RA, Wyvratt J. Examination of rofecoxib solution decomposition under alkaline and photolytic stress conditions. J Pharm Biomed Anal. 2002; 28:1101-13

Radhakrishna T, Sreenivas Rao D, Om Reddy G. LC determination of rofecoxib in bulk and pharmaceutical formulations. J Pharm Biomed Anal 2001; 26:617-28.

Shehata MA, Hassan NY, Fayed AS, El-Zeany BA. Derivative spectrophotometric and fluorimetric methods for determination of rofecoxib in tablets and in human plasma in presence of its photo-degradation product. Farmaco. 2004; 59:139-45.

Vallano PT, Mazenko RS, Woolf EJ, Matuszewski BK. Monolithic silica liquid chromatography columns for the determination of cyclooxygenase II inhibitors in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2002; 779:249-57.

Werner U, Werner D, Mundkowski R, Gillich M, Brune K. Selective and rapid liquid chromatography-mass spectrometry method for the quantification of rofecoxib in pharmacokinetic studies with humans. J Chromatogr B Biomed Sci Appl. 2001; 760:83-90.

Zhang M, Moore GA, Gardiner SJ, Begg EJ. Determination of rofecoxib in human plasma and breast milk by high-performance liquid chromatographic assay. J Chromatogr B Analyt Technol Biomed Life Sci. 2004; 807:217-21.

2.7 Profile of Valdecoxib

2.7.1 Introduction

Valdecoxib is a selective cyclo-oxygenase-2 inhibitor which is used in the treatment of osteoarthritis and rheumatoid arthritis. At therapeutic concentrations, it does not inhibit Cyclo-oxygenase-1, the inhibition of which is responsible for gastrointestinal bleeding and ulcers.

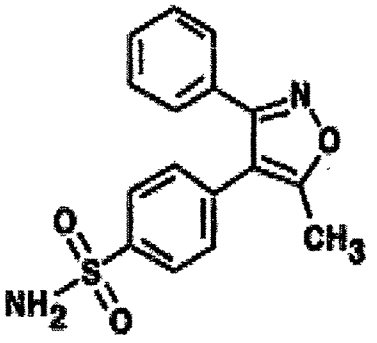
2.7.2. Description

Systematic name: 4-(5-methyl-3-phenyl-4-isoxazolyl) benzenesulfonamide

Molecular formula: $C_{16}H_{14}N_2O_3S$

Molecular weight: 314.36

Chemical structure of valdecoxib:



2.7.3 Physical properties

Description: Valdecoxib is a white crystalline powder, odourless or almost odourless.

Solubility: Relatively insoluble in water (10 μg/mL) at 25°C and pH 7.0, soluble in methanol and ethanol, and freely soluble in organic solvents and alkaline (pH=12) aqueous solutions.

2.7.4 Pharmacokinetics

Absorption

Valdecoxib achieves maximal plasma concentrations in approximately 3 hours. The absolute bioavailability of valdecoxib is 83% following oral administration of BEXTRA compared to intravenous infusion of valdecoxib.

Dose proportionality was demonstrated after single doses (1–400 mg) of valdecoxib. With multiple doses (up to 100 mg/day for 14 days), valdecoxib exposure as measured by the AUC, increases in a more than proportional manner at doses above 10 mg BID. Steady state plasma concentrations of valdecoxib are achieved by day 4.

No clinically significant age or gender differences were seen in pharmacokinetic parameters that would require dosage adjustments.

Distribution

Plasma protein binding for valdecoxib is about 98% over the concentration range (21–2384 ng/mL). Steady state apparent volume of distribution (Vss/F) of valdecoxib is approximately 86L after oral administration. Valdecoxib and its active metabolite preferentially partition into erythrocytes with a blood to plasma concentration ratio of about 2.5:1. This ratio remains approximately constant with time and therapeutic blood concentrations.

Metabolism

In humans, valdecoxib undergoes extensive hepatic metabolism involving both P450 isoenzymes (3A4 and 2C9) and non-P450 dependent pathways (i.e., glucuronidation). Concomitant administration of BEXTRA with known CYP 3A4 and 2C9 inhibitors (e.g., fluconazole and ketoconazole) can result in increased plasma exposure of valdecoxib.

One active metabolite of valdecoxib has been identified in human plasma at approximately 10% the concentration of valdecoxib. This metabolite, which is a less potent COX-2 specific inhibitor than the parent, also undergoes extensive metabolism and constitutes less than 2% of the valdecoxib dose excreted in the urine and feces. Due to its low concentration in the systemic circulation, it is not likely to contribute significantly to the efficacy profile of BEXTRA.

Excretion

Valdecoxib is eliminated predominantly via hepatic metabolism with less than 5% of the dose excreted unchanged in the urine and feces. About 70% of the dose is excreted in the urine as metabolites, and about 20% as valdecoxib N-glucuronide. The apparent oral clearance (CL/F) of valdecoxib is about 6 L/hr. The elimination half-life (T1/2) is approximately 8–11 hours.

2.7.7 Indications

- For relief of the signs and symptoms of osteoarthritis and adult rheumatoid arthritis.
- For the treatment of primary dysmenorrhea.

2.7.6 Dosage and Administration

Osteoarthritis and Adult Rheumatoid Arthritis

The recommended dose of valdecoxib Tablets for the relief of the signs and symptoms of arthritis is 10 mg once daily.

Primary Dysmenorrhea

The recommended dose of BEXTRA Tablets for treatment of primary dysmenorrhea is 20 mg twice daily, as needed.

2.7.7 Methods of analysis

Zhang et al, 2003a developed a sensitive and specific liquid chromatography-tandem mass spectrometry assay method to quantitate valdecoxib (I) and its hydroxylated

metabolite (II) in human plasma. The analytes (I and II) and a structurally analogue internal standard (IS) were extracted on a C(18) solid phase extraction (SPE) cartridge using a Zymark Rapid Trace automation system. The chromatographic separation was performed on a narrow-bore reverse phase Zorbax XDB-C(8) HPLC column with a mobile phase of acetonitrile:water (50:50, v/v) containing 10 mM ammonium acetate. The analytes were ionized using negative electrospray mass spectrometry, then detected by multiple reaction monitoring (MRM) with a tandem mass spectrometer. The precursor to product ion transitions of m/z 313-->118 and m/z 329-->196 were used to measure I and II, respectively. The assay exhibited a linear dynamic range of 0.5-200 ng/ml of I and II in human plasma with absolute recoveries from plasma at 91 and 86%, respectively. The lower limit of quantitation was 0.5 ng/ml for I and II. Acceptable precision and accuracy were obtained for concentrations over the calibration curve ranges (0.5-200 ng/ml). Sample analysis time for each injection was 5 min, a throughput of 70 human plasma standards and samples per run was achieved. The assay has been successfully used to analyze human plasma samples to support clinical phase I and II studies.

Zhang et al, 2003b developed a simple, sensitive and specific automated SPE-LC-MS-MS method for the determination of valdecoxib (I), its hydroxylated metabolite (II) and carboxylic acid metabolite (III) in human urine. The analytes (I, II and III) and a structural analogue internal standard (I.S.) were extracted on a C(18) solid-phase extraction cartridge using a Zymark Rapid Trace automation system. The chromatographic separation was performed on a narrow-bore reverse phase HPLC column with a mobile phase of acetonitrile-water (50:50, v/v) containing 10 mM 4-methylmorpholine (pH 6.0). The analytes were ionized using negative electrospray mass spectrometry, then detected by multiple reaction monitoring with a tandem mass

spectrometer. The precursor to product ion transitions of m/z 313-->118, m/z 329-->196 and m/z 343-->196 were used to measure I, II and III, respectively. The assay exhibited a linear dynamic range of 1-200 ng/ml for I and II and 2-200 ng/ml for III in human urine. The lower limit of quantitation was 1 ng/ml for I and II and 2 ng/ml for III. Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges. Run time of 5.5 min for each sample made it possible to analyze a throughput of 70 human urine samples per run. The assay has been successfully used to analyze human urine samples to support clinical phase I and II studies.

Ramkrishna et al, 2004 developed and validated a simple, sensitive and specific HPLC method with UV detection (210 nm) for quantitation of Valdecoxib in human plasma. The analyte and an internal standard (Rofecoxib) were extracted with diethyl ether/dichloromethane (70/30 (v/v)). The chromatographic separation was performed on reverse phase ODS-AQ column with an isocratic mobile phase of water/methanol (47/53 (v/v)). The lower limit of quantitation was 10 ng/ml, with a relative standard deviation of <20%. A linear range of 10-500 ng/ml was established. This HPLC method was validated with between-batch and within-batch precision of 1.27-7.45 and 0.79-6.12%, respectively. The between-batch and within-batch bias was 0.74-7.40 and -0.93 to 7.70%, respectively. Frequently coadministered drugs did not interfere with the described methodology. Stability of Valdecoxib in plasma was excellent, with no evidence of degradation during sample processing (autosampler) and 30 days storage in a freezer. The method was reported to be suitable for bioequivalence studies following single dose in healthy volunteers.

2.7.8 References

Ramakrishna NV, Vishwottam KN, Wishu S, Koteshwara M. Quantitation of Valdecoxib in human plasma by high-performance liquid chromatography with ultraviolet absorbance detection using liquid-liquid extraction. J Chromatogr B Analyt Technol Biomed Life Sci. 2004; 802:271-5.

Zhang JY, Fast DM, Breau AP. Development and validation of an automated SPE-LC-MS/MS assay for valdecoxib and its hydroxylated metabolite in human plasma. J Pharm Biomed Anal. 2003a; 33:61-72.

Zhang JY, Fast DM, Breau AP. Determination of valdecoxib and its metabolites in human urine by automated solid-phase extraction-liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2003b; 785:123-34.

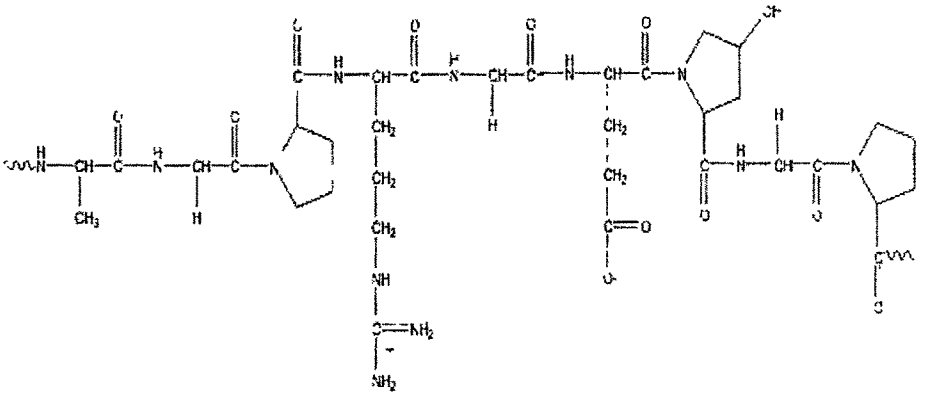
2.8 Profile of Gelatin

2.8.1 Preparation (Oppenheim, 1987)

Gelatin is obtained by the partial hydrolysis of animal collagenous tissues such as skins, tendons, ligaments and bones. The hydrolysis of collagen can be conducted under acidic or alkaline conditions. Type A gelatin, prepared under acidic conditions, has an iso-electric point between pH 7 and 9. It usually exists as a large, well hydrated polycation in water. Type B gelatin, prepared from an alkali treated precursor, has a lower isoelectric point of around pH 5. It exists a large polyanion.

2.8.2 Structural unit

Gelatin contains a large number of glycine (almost 1 in 3 residues, arranged every third residue), proline and 4-hydroxyproline residues. A typical structure is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-.



2.8.3 Description

Gelatin is available as off-white to tan flakes or powder.

2.8.4 Properties

It is a bio-degradable and biocompatible. In addition it is non-toxic having low antigenicity and relatively inexpensive.

2.8.5 Solubility (Oppenheim RC, 1987)

Gelatin is practically insoluble in ethanol, chloroform, fixed and volatile oils and ether, as would be expected for a polyelectrolyte, whereas it is soluble in hot water as well as in a mixture of glycerol and water. In cold water, gelatin slowly swells and softens when immersed, gradually absorbing 5-10 times its weight of water.

2.8.6 Incompatibilities

It is incompatible with tannins and formaldehyde.

2.8.7 Preparation of gelatin microspheres

Simple coacervation

Simple coacervation is a process involving the addition of a strongly hydrophilic substance (nonsolvent or salt) to a solution of colloid. This added substance causes two phases to be formed, one phase rich in colloid droplets and the other poor in such droplets. This process depends primarily on the degree of dehydration produced. Addition of alcohol or sodium sulphate, as typical hydrophilic substances, to an aqueous solution of gelatin can lead to phase formation. When suitable conditions, including the presence of suitable nuclei are prevalent, microcapsules are formed. For preparation of gelatin microcapsules, to a 10 percent dispersion of gelatin in water, the core material is added with continuous stirring at a temperature of 40°C. Then a 20 percent sodium sulfate solution or ethanol is added at 50 to 60 percent by final total volume, in order to induce the coacervation. Then, in order to insolubilize the coacervate capsules suspended in the equilibrium liquid, a hardening agent such as glutaraldehyde or formaldehyde is added. The resulting microcapsules are washed, dried and collected.

Complex coacervation

Complex coacervation can be induced in systems having two dispersed hydrophilic colloids of opposite electric charges. Neutralization of the overall positive charges on one of the colloids by the negative charge on the other is used to bring about separation of the polymer-rich complex coacervate phase. The gelatin-gum arabic (gum acacia) system is the most studied complex coacervation system. Complex coacervation is possible only at pH values below the isoelectric point of gelatin. It is at these pH values that gelatin becomes positively charged, but gum arabic continues to be negatively charged. A typical complex coacervation process using gelatin and gum arabic colloids is as follows: The core material is emulsified or suspended either in the gelatin or gum arabic solution. The aqueous solution of both the gelatin and gum arabic should each be below 3 percent by weight. Then, the gelatin or the gum arabic solution (whichever was not previously used to suspend the core material) is added into the system. The temperature of the system must be higher than the gel point of an aqueous gelatin solution (greater than 35°C). The pH is adjusted to 3.8-4.3 and continuous mixing is maintained throughout the whole process. The system is cooled to 50'C and the gelled coacervate capsule walls are insolubilized by either adding glutaraldehyde or another hardening agent or adjusting the pH. The microcapsules are washed, dried and collected.

Emulsification solvent extraction method

In this method, the drug is dispersed or dissolved in the gelatin solution. Then, the drug containing dispersion or solution is emulsified in vegetable or paraffin oil containing an emulsifier. After water in oil emulsion is formed, the water from the internal phase is extracted by the use of a water extracting agent such as isopropyl alcohol or acetone.

The formed microspheres are then separated by centrifugation or filtration and washed with a solvent which can remove the residual oil.

Emulsification chemical cross-linking

In this method, the drug is dispersed or dissolved in the gelatin solution. Then the drug containing dispersion or solution is emulsified in vegetable or paraffin oil containing an emulsifier. After water in oil emulsion is formed, gelatin in the internal phase is cross-linked using formaldehyde or glutaraldehyde. Stirring is continued for a definite interval of time to ensure complete cross-linking and then the formed microspheres are separated by filtration or centrifugation and washed with a solvent which can remove the residual oil. Finally the microspheres are washed with water to remove the residual cross-linking agent and dried.

2.8.8 Gelatin microspheres in drug delivery

Tabata et al, 1989 prepared glutaraldehyde cross-linked gelatin microspheres for targeting interferon to macrophages. Interferon was incorporated into the gelatin microspheres by cross-linking gelatin in presence of the drug. The rate of interferon release from the microspheres was found to be dependent on the extent of cross-linking. Incubation in phosphate buffered saline solution (PBS) without collagenase did not result in degradation of gelatin microspheres or interferon release within two days of observation. This indicated that IFN was released only by degradation of the gelatin backbone. When the microspheres containing ^{125}I-labeled IFN were added to the suspension of mouse peritoneal macrophages, the microspheres were phagocytosed and degraded gradually in the interior of the macrophages.

Narayani and Rao, 1990 prepared sustained release gelatin microspheres loaded with propranolol hydrochloride (P-HCl) in order to reduce its toxicity. The microspheres were prepared by polymer dispersion technique. The microspheres were characterized

by FTIR and optical microscopy. The FTIR studies indicated that the microspheres were completely free from the dispersion medium. The morphological characteristics of the microspheres from the optical microscopic studies indicated the smooth, spherical nature of the microspheres. The in vitro release of P-HCI from gelatin microspheres at physiological pH 7.4 and at 37°C in phosphate buffer indicated that the drug was released in a controlled fashion following zero-order. Further the release of P-HCI extended from 6 to 10 hours as compared to P-HCI which was released within 4 minutes.

Cortesi et al, 1999 investigated the use of oxidized dextran as a cross-linker for the preparation of gelatin microspheres. Microspheres were prepared by thermal gelation method and their dissolution kinetic was examined. The microspheres were characterized for swelling behavior and dissolution tests. The results indicated that oxidized dextran can form a cross-linked gelatin network which can reduce the dissolution of gelatin. Gelatin microspheres treated with oxidized as well as native dextran, slowed down the release of the model anti-tumor drug TAPP-Br. They concluded that oxidized dextran is an alternative means of cross-linking gelatin microspheres for achieving controlled release of the drugs.

Morimoto et al, 2000 investigated the use of gelatin microspheres for pulmonary delivery of salmon calcitonin. They prepared positively charged as well as negatively charged gelatin microspheres from acidic and basic gelatin respectively. The in-vitro release studies indicated that the release from positively charged microspheres was rapid compared to the negatively charged microspheres. The pulmonary absorption of salmon calcitonin from gelatin microspheres was established by measuring its hypocalcaemic effect in rats. It was observed that the pharmacological availability of

salmon calcitonin from positively charged microspheres was significantly higher than that from the negatively charged microspheres.

Morita et al, 2001 prepared gelatin microspheres by co-lyophilization with polyethylene glycol as a protein-micronization adjuvant. Aqueous solutions containing gelatin and PEG at various mixing ratios were freeze dried. The lyophilizates were dispersed in methylene chloride and subjected to particle size analysis. The particle size decreased as the PEG: Gelatin ratio increased. By using this technology, gelatin microparticles with an average size of less than 10μm, with high purity of more than 90% and with good dispersibility could be obtained with high yield. The gelatin microparticles were then encapsulated into biodegradable PLGA/PLA microspheres by solid-in-oil-in-water emulsion process. The entrapment efficiency was highly dependent on the particle size and the size distribution. They concluded that the gelatin microspheres prepared by this method can be useful for studying and developing various drug delivery systems.

Saravanan et al, 2003 prepared diclofenac sodium loaded gelatin magnetic microspheres for intra-arterial administration. The formulated microspheres were characterized by drug loading, entrapment efficiency, magnetite content, FT-IR spectroscopy, particle size analysis, optical microscopy, scanning electron microscopy, and in vitro release studies. The data obtained from the in vitro release studies were applied to various kinetic models. The FTIR revealed no drug/polymer interaction. The average particle size was between 36 to 61 microm depending on quantity of magnetite and gelatin used. Optical microscopy and SEM showed spherical and compact nature of microspheres. The formulated microspheres released the drug for a period of 42 to 78 hours depending on drug loading. The release was

diffusion controlled at lower drug loading and dissolution/diffusion controlled at higher drug loading.

Mladenovska et al, 2003 studied the effect of particle size on the biodistribution of 131I-BSA loaded gelatin microspheres after peroral application to BALB/c mice. To two groups, radiolabeled ^{131}I-BSA gelatin microspheres of different particle size, 1.2±1.1 μm and 7.0±1.2 μm, were administered orally. To the control group, a solution of ^{131}I-BSA was administered orally as well. Biodistribution was followed periodically within 15 days as the percent of total radioactivity present in the stomach and small intestine with Peyer's patches and mesentery, in colon with Peyer's patches, appendix and mesentery, in liver, spleen, blood, kidney, lungs and heart. Preliminary *in vitro* biodegradation and drug release studies confirmed the potential of gelatin microspheres to protect the antigen of interest from enzymatic degradation in the gut, and to release it in a controlled manner. The biodistribution data confirmed that particle uptake into Peyer's patches and passage to the liver and spleen *via* the mesentery lymph supply and nodes increased with decreasing particle size.

Sankar et al, 2003 prepared gelatin A microspheres (MS) of ketorolac tromethamine (KT) for intranasal systemic delivery in order to avoid gastro-intestinal complications, to improve patient compliance, to use as an alternative therapy to conventional dosage forms, to achieve controlled blood level profiles, and to obtain improved therapeutic efficacy in the treatment of postoperative pain and migraine. Gelatin A microspheres were prepared using the emulsification-crosslinking technique using glutaraldehyde as a cross-linking agent. Chitosan was used as a copolymer. All the prepared microspheres were evaluated for physical characteristics, such as particle size, incorporation efficiency, swelling ability, *in vitro* bioadhesion on rabbit small intestine and *in vitro* drug release characteristics in pH 6.6 phosphate

buffer. All the microspheres showed good bioadhesive properties. Gelatin A and chitosan concentrations, percentage of the crosslinking agent and also the drug loading affected significantly the rate and extent of drug release. The data indicated that the drug release followed Higuchi's matrix model.

Muvaffak et al, 2004 prepared 5-fluorouracil loaded gelatin microspheres using water-in oil polymerization technique using glutaraldehyde as a cross-linking agent. The influence of preparation compositions on microsphere recovery, particle size and morphology, swelling and degradation, 5-fluorouracil loading and release, and cytotoxicity were investigated. The concentrations of gelatin and glutaraldehyde influenced the size and surface properties of microspheres. The decrease in gelatin concentration and the increase in glutaraldehyde concentration resulted in the formation of smaller microspheres with smoother surface properties. Swelling values and the drug release were decreased as the amount of glutaraldehyde was increased. Cytotoxicity tests of free and entrapped 5-fluorouracil were carried out with MCF-7 breast cancer cell line. Free 5-fluorouracil produced an immediate effect, whereas entrapped 5-fluorouracil showed a prolonged cytotoxic effect.

2.8.9 References

Cortesi R, Esposito E, Osti M, Squarzoni G, Menegatti E, Davis SS, Nastruzzi C. Dextran cross-linked gelatin microspheres as a drug delivery system. Eur J Pharm Biopharm. 1999 ;47 (2):153-60.

Mladenovska K, Janevik, Glavas MD, Kumbaradzi EF, Goracinova K. Biodistribution of 131I-BSA loaded gelatin microspheres after peroral application to BALB/c mice-Particle size study. Acta Pharm 2003; 53: 187-197.

Morimoto K, Katsumata H, Yabuta T, Iwanaga K, Kakemi M, Tabata Y, Ikada Y.Gelatin microspheres as a pulmonary delivery system: evaluation of salmon calcitonin absorption. J Pharm Pharmacol. 2000;52:611-7.

Morita T, Horikiri Y, Suzuki T, Yoshino H. Preparation of gelatin microparticles by co-lyophilization with poly (ethylene glycol): characterization and application to entrapment into biodegradable microspheres. Int. J. Pharm. 2001; 219: 127-137.

Muvaffak A, Gurhan I, Hasirci N.Cytotoxicity of 5-fluorouracil entrapped in gelatin microspheres. J Microencapsul. 2004 May;21(3):293-306.

Narayani R, Rao KP. Sustained release of propranolol hydrochloride from biodegradable gelatin microspheres. Trends in Biomaterials & Artificial organs. 1990; 5: 22-8.

Oppenheim RC, Gelatin microspheres as drug carrier systems in "Polymers in controlled drug delivery" Illum L and Davis SS, eds., 1987, IOP Publishing limited, Bristol.pp-74.

Sankar C, Mishra B. Development and *in vitro* evaluations of gelatin A microspheres of ketorolac tromethamine for intranasal administration. Acta Pharm. 2003; 53: 101–110.

Saravanan M, Bhaskar K, Narayanan NV, Maharajan G, Pillai KS. Diclofenac sodium loaded gelatin magnetic microspheres for intra-arterial administration: formulation, characterization and in vitro release studies. Boll Chim Farm. 2003; 142:347-51.

Tabata Y, Ikada Y. Synthesis of gelatin microspheres containing interferon Pharm Res. 1989; 6: 422-427.

2.9 Profile of Chitosan

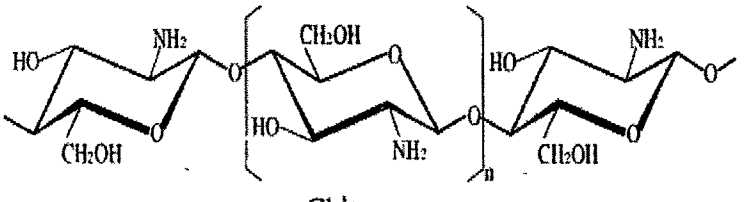
2.9.1 Preparation

Chitosan is obtained by deacetylation of aminoacetyl groups of chitin, which is the main component of shells of crabs, shrimp and krill by an alkaline treatment. The process of deacetylation is achieved by treating chitin with a strong solution of sodium hydroxide at elevated temperature. After deacetylation of chitin, dry chitosan flakes are obtained. They are milled to get fine mesh chitosan.

2.9.2 Chemical composition

Chitosan[α(1→4) 2-amino 2-deoxy β-D glucan], the deacetylated form of chitin, is a muco-polysaccharide having structural characteristics similar to glycosamines with a chemical formula of $(C_6H_{11}O_4N)n$. Chitosan is a biodegradable and biocompatible homo-polysaccharide (i.e. contain single type of monomeric unit) comprising of GLUCOSAMINE and N-ACETYLGLUCOSAMINE. It is marketed under a variety of forms with different molecular weights and degrees of deacetylation, or as chitosan base or salt.

2.9.3 Structural Formula



Chitosan

2.9.4 Description

Chitosan is a tasteless, free flowing, light brown coloured, low density powder.

2.9.5 Properties

Important characteristics of chitosan are its molecular weight, viscosity, degree of deacetylation, crystallinity index, number of monomeric units, water retention value,

pKa and energy of hydration Chitosan has a high charge density and adheres to negatively charged sulphates and chelates metal ions. It is bio-degradable, bio-compatible and non-toxic.

2.9.6 Solubility

Chitosan is insoluble at alkaline and neutral pH, but forms salts with inorganic acids such as glutamic acid, hydrochloric acid, lactic acid and acetic acid. Upon dissolution, the amine groups of the polymer becomes protonated with a resultant positively charged soluble polysaccharides (RNH_3^+). However, chitosan salts are soluble in water, the solubility being dependent on the degree of de-acetylation. Chitosan with a low degree of de-acetylation (40%) has been found to be soluble upto pH 9.0, whereas chitosan with a degree of de-acetylation of about 85% is soluble only upto pH 6.5.

2.9.7 Incompatibilities

The pH of chitosan solution must be kept below 6.0 to prevent precipitation. Chitosan may best be formulated to the pH 2-3 region. The acid solution of chitosan is compatible with non-ionic polymers but is incompatible with sulphates and most anionic water soluble polymers.

2.9.8 Preparation of chitosan microparticles (Kas, 1997)

Ionotropic gelation

Chitosan solution in acetic acid are prepared and extruded dropwise through a needle into different concentration of aqueous solutions of magnetically stirred tripolyphosphate. The beads are removed from the counted ion by filteration, washed with distilled water and dried by an air jet and further air dried at ambient temperature.

Extrusion-spheronization

Goskonda and Upadrashta, 1993 prepared chitosan beads by extrusion-spheronization technology where the ingredients are mixed and the wet mass is passed through an extruder. The cylindrical extrudate obtained is immediately processed in a spheronizer.

Solvent evaporation technique

Chitosan is dissolved in an aqueous acetic acid solution. The solution is added to toluene and sonicated to form a w/o emulsion. Glutaraldehyde solution in toluene is added to the emulsion and stirred at room temperature to give cross-linked microspheres. The suspension is centrifuged. Following evaporation of solvent, microspheres are separated, washed with distilled water and dried.

Li et al, 1991 modified the solvent evaporation method and named it Dry-in-oil method. Here chitosan solution in acetic acid is dropped into oil under stirring. The system is warmed to 50°C and the pressure is reduced. When the solvent is evaporated completely the microsphere are separated, washed with sodium hydroxide solution, distilled water and diethyl ether and dried.

Multiple emulsion method

Multiphase emulsions are also prepared by the solvent evaporation technique by a three step emulsification process. Aqueous drug solution and oil phase containing emulsion stabilizers are combined to give a water-in-oil emulsion. Later the w/o emulsion is dispersed in the polymer solution. The solvent is evaporated under reduced pressure.

Spray drying method

Chitosan microspheres are prepared by using a spray drier apparatus. Microspheres have been prepared from solutions of different concentrations of chitosan in glacial acetic acid/water/acetone.

Precipitation/coacervation method

Berthold et al, 1996 prepared chitosan microsphere by a novel precipitation method using sodium sulfate as a precipitant. In this method chitosan is dissolved in acetic acid containing polysorbate 80. A solution of sodium sulfate is added dropwise during stirring and ultrasonication. The formation of microsphere is indicated by turbidity. The formed microspheres are purified by centrifugation and resuspended in demineralized water. This method avoided the use of organic solvents and glutaraldehyde for the preparation of chitosan microparticles with high loading efficiency and sustained release effect.

Chitosan microparticles can also be prepared by complex coacervation method. Sodium alginate, sodium carboxymethylcellulose, kappa-carregeenan and sodium polyacrylic acid are used in the complex coacervation procedure with chitosan. Here the microparticles are formed by ionic interaction between the oppositely charged polymers. Formulation of coacervate capsules of chitosan alginate and chitosan-kappa-carrageenan in a solution of sodium alginate through a hand operated syringe into potassium chloride or calcium chloride solution. The counterion solution consisted of chitin. The obtained capsules were agitated to harden in the counterion solution before washing and drying. Chitosan-alginate microparticles are also prepared to control release characteristics and physicochemical properties. Chitosan-CMC complex microparticles are used to immobilize cell culture.

Coating by chitosan

In this method, previously formed microparticles are coated with chitosan. HAS microsphere are prepared and added to various concentrations of chitosan-acetic acid solutions and mixed. The chitosan treated microparticles are filtered and dried.

2.9.9 Chitosan microspheres in drug delivery

Hassan and Gallo, 1993 developed magnetic chitosan microspheres containing oxantrazole for brain targeting. Magnetic chitosan microspheres containing Oxantrazole (MCM-OX) and Oxantrazole solution (OX-S) were administered intraarterially to male Fischer 344 rats with a magnetic field of 6000 G applied to the brain for 30 min. Animals were sacrificed at 30 min and 120 min after MCM-OX and OX-S treatments, and multiple tissues were collected and analyzed for OX by HPLC. The concentration of Oxantrazole when administered in microspheres was found to be 100 folds higher than that obtained after administration of oxantrazole solution. Within the MCM-OX treatment groups, ipsilateral OX concentrations were much greater, indicating target organ selectivity. A most interesting finding was that OX brain concentrations were similar at 120 min and 30 min after MCM-OX treatment. Thus, even in the absence of the magnetic field, MCM-OX was retained in the brain, possibly through cationic-anionic interactions with the blood-brain barrier.

Jameela and Jayakrishnan, 1995 prepared chitosan microspheres from 74% deacetylated chitin by the emulsion cross-linking method using glutaraldehyde as a cross-linking agent. A relatively novel antineoplastic agent, mitoxantrone, was incorporated into the microspheres and the drug release was studied in vitro into phosphate buffer for over 4 weeks at 27°C. Drug release was found to be effectively controlled by the extent of cross-linking. Only about 25% of the incorporated drug was released over 36 days from microspheres of high cross-linking density. Implantation of placebo chitosan microspheres in the skeletal muscle of rats was carried out in order to assess the biocompatibility and biodegradability of the microspheres. Histological analysis showed that the microspheres were well tolerated by the living tissue. However, no significant biodegradation of the material was

noticed over a period of 3 months in the skeletal muscle of rats. Data obtained indicate the possibility of using cross-linked chitosan microspheres as a drug carrier for sustained drug release for very long periods.

Wang et al, 1996 studied an orthogonal experimental design to optimize cisplatin loaded chitosan microspheres which were produced by emulsion chemical cross-linking technique. Seven factors and three levels for each factor that might affect the formulation of microspheres were selected and arranged in an L27 (3^{13}) orthogonal experimental table. The dependent variables selected were the trapping efficiency and the size distribution. A desirability function was calculated based on these two variables. Microspheres with high trapping efficiency could be produced by this method. The in-vitro release of cisplatin from chitosan microspheres in saline was retarded compared with that from the saline solution; the release of cisplatin from chitosan microsphere was suggested to be controlled by the dissolution and diffusion of the drug from the chitosan matrix.

Genta et al, 1997 prepared bioadhesive microspheres of chitosan containing acyclovir for ophthalmic delivery. They cross-linked microsphere by either chemical treatment or heat treatment. They concluded that incorporation of acyclovir in the microspheres improved the bioavailability compared to the raw drug and the drug residence time in the eye when incorporated in microspheres is higher compared to the plain drug.

Lim and Wan, 1998 investigated the effect of magnesium stearate on the chitosan microspheres. The microspheres were prepared by an emulsification-coacervation technique using pentasodium tripolyphosphate as a counter ion. The chitosan microspheres obtained showed a high degree of aggregation. Incorporation of magnesium stearate in the disperse phase reduced the aggregation resulting in

discrete, spherical microspheres with smooth surfaces. An increase in the magnesium stearate content led to an increase in the particle size in case of blank microspheres but in case of propranolol hydrochloride loaded microspheres; there was a decrease in the particle size with an increase in the magnesium stearate content. The DSC analysis data suggested that the magnesium stearate was converted to stearic acid during the preparation process. The release of propranolol hydrochloride from the microspheres was fast, irrespective of the content of magnesium stearate. Drug encapsulation efficiency was enhanced when a greater amount of magnesium stearate was used.

Kumbhar et al, 2002 prepared diclofenac sodium loaded chitosan microsphere using three different cross-linking agents viz. glutaraldehyde, sulphuric acid and heat treatment. The cross-linking takes place at free amino groups in all the three cases, as evidenced by FTIR. The results indicated that with an increase in the extent of cross-linking the particle size decreases. Glutaraldehyde cross-linked microspheres have smaller particle size than the other microspheres. Moreover, the glutaraldehyde cross-linked microspheres shows slowest drug release while the formaldehyde cross-linked microspheres shows fastest drug release. The release mechanism slightly deviates from the Fickian process.

Portero et al, 2002 investigated the use of reacetylated chitosan microspheres for controlled release of antimicrobial agents amoxicillin and metronidazole in the gastric cavity. Two different microencapsulation approaches were developed and optimized in order to encapsulate hydrophilic (amoxycillin) and hydrophobic (metronidazole) compounds efficiently. The reacetylated chitosan microspheres exhibited a controlled water swelling capacity and gelified at acidic pH, resulting in prolonged release of the encapsulated antibiotics. The reacetylation time was found to be a key factor that affects not only drug release, but also encapsulation efficiency and anti-microbial

activity of the encapsulated compound. The last two parameters were also dependent on drug solubility in the reacetylating agent. Using short reacetylation time periods, it was possible to efficiently control the release of both hydrophilic and lipophilic antibiotics while maintaining their activity against different bacteria. Consequently, reacetylated chitosan microspheres are promising vehicles for the controlled delivery of anti-microbial agents to the gastric cavity and, hence, for the eradication of Helicobacter pylori, a pathogen strongly associated with gastric ulcers and possibly gastric carcinoma.

Turan et al, 2002 investigated the use of Chitosan microspheres for sustained-release of recombinant human interleukin-2 (rIL-2). The effects of the different variables on the microspheres prepared using precipitation technique, on the characteristics of the microsphere were investigated. The average diameter of microspheres was between 1.11-1.59 μm and encapsulation efficiency of Recombinant IL-2 was between 75-98%. Formulation factors had no effect on the microsphere size. The microspheres released Recombinant IL-2 release in a sustained manner for a period of over 3 months. The encapsulated rIL-2 remained biologically active and could be completely recovered from the release medium. The efficacy of rIL-2 loaded chitosan microspheres was studied using two model cells, HeLa and L-strain cell lines. Chitosan microspheres were added to the cells at different concentrations, and the amount of rIL-2 was assayed using the ELISA kit. Cell culture studies indicated that microspheres were uptaken by cells, and rIL-2 was released from the microspheres. Cellular uptake of rIL-2-loaded microspheres was dose dependent. They concluded that chitosan microsphere is a suitable carrier for rIL-2 delivery.

Dhawan and Shingla, 2003 prepared nifedipine loaded chitosan microspheres by emulsification phase-separation method. A high level of entrapment of nifedipine in

the microspheres was achieved. The microspheres exhibited excellent swelling properties. Differential scanning calorimetry, X-ray diffractometry, and scanning electron microscopy confirmed that at 1.84% loading, nifedipine was dispersed molecularly. The microspheres exhibited faster release at low loadings compared to high loadings. Fitting the data to the coupled Fickian/case II equation, showed that at low loadings polymer relaxation coefficients (k2) were high. As the polymer content increased in the microspheres, the value of n (diffusional exponent characteristic of the release mechanism) approached one, which is indicative of zero order.

2.9.10 References

Berthold A, Cremer K, Kreuter J. Preparation and characterization of chitosan microspheres as drug carrier for prednisolone sodium phosphate as model anti-inflammatory drug. J. controlled release, 1996; 39:17-25.

Dhawan S, Singla AK. Nifedipine loaded chitosan microspheres prepared by emulsification phase-separation. Biotech Histochem. 2003; 78:243-54.

Genta I, Conti B, Perugini P, Pavanetto F, Spadaro A, Puglisi G. Bioadhesive microspheres for ophthalmic administration of acyclovir. J. Pharm.Pharmacol. 1997; 49: 737-742.

Gosconda SR, Upadrashta SM. Avicel RC-591/chitosan beads by extrusion spheronization technology. Drug dev. Ind. Pharm. 1993; 19:915-927.

Hassan EE, Gallo JM. Targeting anticancer drugs to the brain. I: Enhanced brain delivery of oxantrazole following administration in magnetic cationic microspheres. J Drug Target. 1993; 1:7-14.

Jameela SR, Jayakrishnan A. Glutaraldehyde cross-linked chitosan microspheres as a long acting biodegradable drug delivery vehicle: studies on the in vitro release of mitoxantrone and in vivo degradation of microspheres in rat muscle. Biomaterials. 1995; 16:769-75.

Kas SH, Chitosan: properties, preparations and applications to microparticulate systems. J. Microencapsulation. 1997; 14:689-711.

Kumbhar SG, Kulkarni AR, Aminabhavi TM. Cross-linked chitosan microspheres for encapsulation of diclofenac sodium; Effect of cross-linking agent. J. Microencapsul.2002; 19: 173-180.

Li YP, Machida Y, Sannan T, Nagai T, Preparation of chitosan microspheres containing fluorouracil using the dry-in-oil method and its release characteristics. S.T.P Pharm.Sci. 1991; 1:363:368.

Lim LY and Wan LSC. Effect of magnesium stearate on chitosan microspheres prepared by an emulsification-coacervation technique. J. microencapsul. 1998; 15: 319-333.

Portero A, Lopez RC, Criado MT, Alonso MJ. Reacetylated chitosan microspheres for controlled delivery of anti-microbial agents to the gastric mucosa. J Microencapsul. 2002; 19:797-809.

Turan OS, Akbuga J, Aral C. Controlled release of interleukin-2 from chitosan microspheres. J. Pharm. Sci. 2002; 91:1245-51.

Wang, Y. M., Sato, H., Adachi, I., Horikoshi, I. Optimization of the formulation design of chitosan microspheres containing cisplatin. J. Pharm. Sci. 1996; 85: 1204-1210.

2.10 Profile of Bovine serum albumin

2.10.1 Chemical composition

It belongs to the class of serum proteins called albumins, which make up about half of the protein in plasma and are the most stable and soluble proteins in plasma. The MW of BSA is 67 X 10^3. It contains 59 lysines, and 30-35 of these are primary amines capable of reacting with a conjugation site of linkers.

2.10.2 Description

It is available as white to off-white flakes or powder.

2.10.3 Properties

Albumin is a biodegradable, non-toxic and non-antigenic material.

2.10.4 Solubility

Albumin is practically insoluble in ethanol, chloroform, fixed and volatile oils. It is soluble in water.

2.10.5 Incompatibilities

Strong acids and oxidizing agents

2.10.6 Preparation of BSA microspheres

Thermal denaturation **(Filipovic and Jalsenjak, 1993)**

In this method, albumin is dissolved in water and the drug is either dispersed or dissolved in albumin solution. This aqueous phase is then emulsified in external oily phase to get a w/o emulsion. This emulsion is then added to another portion of oil which is heated at temperatures of around 130°C. The high temperature used denatures albumin to form the microspheres. The mixture is then cooled to room temperature and the microspheres are separated by filtration or centrifugation and washed with a solvent to remove the oil phase.

Emulsification chemical cross-linking

In this method, albumin solution containing the drug dispersed or dissolved is emulsified in the oily phase to give an o/w emulsion. Glutaraldehyde or formaldehyde is added to cross-link the albumin present in the internal phase of the emulsion. The cross-linking reaction is continued for a definite interval of time and then the microspheres formed are separated by centrifugation or filtration and washed with a solvent to remove the oil and finally with distilled water to remove the residual crosslinking agent.

Spray drying **(Giunchedi et al, 2000)**

In this method, the drug is suspended or dissolved in aqueous solutions of albumin and the solution or the suspension is spray dried using a spray drier to obtain microspheres. Definite process conditions of inlet temperature, outlet temperature and flow rate are used to get a definite particle size of the microspheres.

Coacervation method **(Bayomi, 2004)**

In this method, the microspheres are formed by interaction between negatively charged egg albumin molecules in phosphate buffer, pH 7.2, or sodium hydroxide solution and positively charged chitosan molecules dissolved in diluted acetic acid to form an insoluble precipitate. This method avoids the use of organic solvents that are needed in conventional methods of microencapsulation.

2.10.7 BSA microspheres in drug delivery

Willmott et al, 1985 prepared adriamycin-loaded bovine serum albumin microspheres by a technique that allows preparation and administration to animals on the same day. Criteria adopted for injection were that microspheres should be stable and of a size such as to become trapped in capillary beds. Appropriate combinations of glutaraldehyde concentration, stirring speed and continuous phase viscosity were

used to fulfill these criteria. After systemic administration, the rats were sacrificed at intervals and major visceral organs examined for entrapped microspheres and serum for released drug. Microspheres sieved out in the first capillary bed encountered, the lung, then following biodegradation they disappeared at a rate dependent on the amount of cross-linking agent used in their preparation. In contrast to bolus injection, serum drug levels after microsphere administration indicated an initial rapid release followed by a more protracted phase lasting at least 24 h. This latter observation is consistent with drug release during biodegradation of carrier.

Fujimoto et al, 1985 prepared biodegradable albumin microspheres containing mitomycin C by heat denaturation in oil or by cross-linking with glutaraldehyde. These MMC microspheres released, in vitro, about 20% of the contained MMC for over 3 days, and they were intra-arterially infused into albino rabbits and Wistar rats, as a preclinical model of intra-arterial infusion treatment for patients with inoperable hepatic tumor. The microspheres were infused into the femoral artery of rabbits with a VX-2 tumor implanted into the flank of the hindleg. High levels of MMC were maintained for several hours in the tumor and the entrapped MMC microspheres were detected within arterioles in the VX-2 tumors. The growth of VX-2 tumor was inhibited considerably, compared to findings in the control rabbits given conventional MMC. In the next studies, MMC microspheres were infused into the rat hepatic artery, and the levels of MMC in the hepatic vein blood were maintained at much the same concentration for over 2 hours after the infusion, in marked contrast to rapid decreases in the conventional MMC. Histologic findings revealed that MMC microspheres were entrapped within the hepatic arterioles for over 2 weeks and released biologically active MMC into the neighboring tissues for prolonged periods of time.

Filipovic et al, 1993 studied the effect of drug partition coefficient of the preparation and drug release of human serum albumin microspheres containing barbiturates. The microspheres were prepared by thermal denaturation method. The barbiturates used for the study were of similar general physicochemical properties but different partition coefficients. The drug content of the microspheres decreased with an increase in the partition coefficient due to the drug migration in the outer organic phase. The drugs with higher partition coefficient were released faster and to a greater extent than those with lower partition coefficients.

Tao et al, 1999 prepared adriamycin loaded magnetic albumin microspheres (ADM-MAMs) were prepared by the heat-stabilized protein methods. The physico-chemical properties of the prepared microspheres were examined and their cytotoxicity against tumor cells in vitro was assayed by a modified MTT method. The effect on the implanted gastric tumor in wistar rats was observed after administration of ADM-MAMs via alimentary canal in the presence of the external magnetic fields. The results showed that the ADM-MAMs were successfully prepared and had cytotoxic effect on tumor cells in vitro similar to the free ADM ($P > 0.05$). The inhibitory effects of ADM-MAMs on the implanted gastric tumor in vivo were significantly increased as compared with the controls ($P < 0.01$). Our results suggested that ADM-MAMs were a new type of adriamycin (ADM) preparation and its form alteration did not affect its anticancer effects.

Guinchedi et al, 2000 prepared albumin microspheres for ocular delivery of piroxicam. The microspheres were prepared by spray drying technique. The prepared microspheres were evaluated for the drug content, particle size, surface morphology and in-vitro drug release. The in-vitro drug release studies indicated that loading of piroxicam into the microparticles led to a remarkable improvement in the rate of

dissolution of the drug and with an increase in the albumin: piroxicam ratio there is an increase in the rate of drug dissolution. The pharmacokinetic profile of piroxicam was investigated in albino rabbits. The albumin-piroxicam microspheres resulted in greater bioavailability of piroxicam than commercial eye-drops.

Sahin et al, 2002 investigated the use of bovine serum albumin microspheres loaded with Terbutaline sulphate for passive lung targeting. The microspheres were prepared by emulsion polymerization method using glutaraldehyde as the cross-linking agent. All microspheres were spherical and smooth with the mean particle size in the range of 22-30 μm. Drug release from the BSA microspheres displayed a biphasic pattern characterized by an initial fast release, followed by a slower release. The released amount decreased with an increase in the glutaraldehyde concentration. In the absence of trypsin, the time required for complete degradation of microspheres was increased from 144 to 264 hours when the glutaraldehyde concentration increased from 0.1 to 0.7 ml. In the presence of trypsin, a linear relationship was obtained between the degradation rates and trypsin concentrations, indicating that saturation was not reached under the experimental conditions. Biodistribution studies indicated that the degree of uptake by the lungs was higher than that of the other organs. All these results demonstrated that terbutaline sulfate loaded microspheres can be used for passive lung targeting.

Almond et al, 2003 used albumin microspheres as a drug carrier for the chemotherapeutic agent mitoxantrone in order to reduce its systemic toxicity. The authors have shown that intratumoral injection of antineoplastic drugs can provide high localized drug concentrations with greatly reduced systemic toxicity. Using albumin microspheres as a drug carrier, localized and sustained release of chemotherapeutic drugs has been achieved by intratumoral injection, thus increasing

the intratumoral dose and antitumor efficacy. Microspheres provide the advantages of localized, prolonged drug release. The efficacy and toxicity of intratumoral free mitoxantrone or mitoxantrone-loaded albumin microspheres were evaluated in a murine breast cancer model. In the same model, a combination of these two therapies was also evaluated. Results indicated that intratumoral mitoxantrone, especially in microsphere preparations, significantly improved survival and decreased systemic toxicity.

Dubey et al, 2003 investigated the effect of heating temperature and time on the characteristics of 5-fluorouracil loaded albumin microspheres. The microspheres were prepared by emulsification followed by thermal denaturation of albumin present in the internal phase of the emulsion. Three temperatures and three different stirring times were used for the preparation of the microspheres. The effect of these variables on the mean particle size, particle size distribution and entrapment efficiency was studied. They concluded that both the heating temperature and time affect the mean particle size, particle size distribution and entrapment efficiency. Hence the choice of the temperature and heating time should be carefully selected which gives the particles of desired size range with high entrapment efficiency.

Gomez et al, 2004 prepared cytarabine loaded albumin microspheres and these microspheres were immersed in a poly (lactide-co-glycolide) (PLGA) film to constitute a comatrix system to develop a prolonged form of release. The microspheres were prepared by emulsification method. These cytarabine-loaded microspheres were used to prepare PLGA-comatrices. Kinetic release studies indicated that total cytarabine release only takes place in the presence of protease, probably due to the fact that glutaraldehyde establishes covalent links with the amine side group of the drug and cross-links it with the protein matrix. A slower kinetic

release of the drug was obtained from PLGA-comatrices, although only 80% of the included cytarabine was released on day 7. The comatrices were subcutaneously implanted in the back of rats. The drug was detected in plasma 10 days. The mean residence time (MRT) of the drug administered by these comatrices was 87-91 times larger when compared to the value obtained when the drug was administered in solution by intraperitoneal injection. The histological studies show that a degradative process of the comatrices takes place. The comatrices do not damage surrounding tissue; a normal regeneration of the implanted zone was observed.

2.10.9 References

Almond BA, Hadba AR, Freeman ST, Cuevas BJ, York AM, Detrisac CJ, Goldberg EP.Efficacy of mitoxantrone-loaded albumin microspheres for intratumoral chemotherapy of breast cancer. J Control Release. 2003; 91:147-55.

Bayomi MA. Aqueous preparation and evaluation of albumin-chitosan microspheres containing indomethacin. Drug Dev Ind Pharm. 2004; 30:329-39.

Dubey RR, Parikh JR, Parikh RR. Effect of Heating Temperature and Time on Pharmaceutical Characteristics of Albumin Microspheres Containing 5-Fluorouracil. AAPS PharmSciTech. 2003; 4(1): article 4.

Fujimoto S, Miyazaki M, Endoh F, Takahashi O, Okui K, Sugibayashi K, Morimoto Y.Mitomycin C carrying microspheres as a novel method of drug delivery. Cancer Drug Deliv. 1985; 2:173-81.

Filipovic J and Jalsenjak I. Microspheres of human serum albumin with barbiturates: Effect of drug partition coefficient on preparation and drug release. J.Pharm.Pharmacol. 1993; 45:394-399.

Gomez C, Blanco MD, Bernardo MV, Olmo R, Muniz E, Teijon JM. Cytarabine release from comatrices of albumin microspheres in a poly(lactide-co-glycolide) film: in vitro and in vivo studies. Eur J Pharm Biopharm. 2004; 57:225-33.

Guinchedi P, Chetoni P, Conte U, Saettone MF. Albumin microspheres for ocular delivery of piroxicam. Pharmacol Commun. 2000; 6: 149-153.

Sahin S, Selek H, Ponchel G, Ercan MT, Sargon M, Hincal AA, Kas HS. Preparation, characterization and in vivo distribution of terbutaline sulfate loaded albumin microspheres. J. Control. Release. 2002; 82:345-58.

Tao K, Chen D, Chen J, Tian Y, Wu Z, Wang X, Yang X. Preparation of adriamycin magnetic albumin microspheres and their experimental antitumor effects in vitro and in vivo. J Tongji Med Univ. 1999; 19:295-9.

Willmott N, Cummings J, Stuart JF, Florence AT. Adriamycin-loaded albumin microspheres: preparation, in vivo distribution and release in the rat. Biopharm Drug Dispos. 1985; 6:91-104.