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Innovative formulations for the controlled and site-specific delivery of anti-inflammatory drugs

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Abstract

Pharmaceutical technology has introduced a promising pathway in the future of medicinein particular nanotechnological innovations have provided the opportunity to design and develop efficient drug delivery systems able to target and treat several diseases, including those mediated by inflammation. The engineering of drug delivery systems can be used to target tissues involved in the pathology under treatment, to avoid early drug biological environmental degradation and to modulate drug pharmacokinetics. Glucocorticoids and non-steroidal anti-inflammatory drugs are the most commonly prescribed drug categories worldwide for the treatment of disorders associated with inflammation.

Although glucocorticoids can be highly effective in treating inflammation, their systemic application is limited due to the high incidence of serious adverse effects, mainly in long-term treatment. Non-steroidal anti-inflammatory drugs are a heterogeneous group of compounds and most of them have unfavorable pharmacokinetics and pharmacodynamics, leading to adverse effects, such as gastrointestinal disorders. Therefore, the need for drug delivery systems for long term administration of anti-inflammatory drugs witha well-controlled release profile is evident. The aim of this review is to assess innovative colloidal drugs carriers, in particular liposomes and nanoparticles, with special focus on site-specific delivery for particularly problematic tissues such as the gastrointestinal tract, joints and eyes.

Introduction

Inflammation is an immediate response of the body to tissue and/or cell damage by pathogens and noxious stimuli such as chemical or physical injury. This damage initiates the activation of transcription factors controlling the expression of many inflammatory mediators, such as eicosanoids, biological oxidants, cytokines, adhesion factors, and digestive enzymes (proteases, hyaluronidase, collagenase, and elastase). Acute inflammation is a short-term response which usually results in healing, as leukocytes infiltrate the damaged region removing the stimulus and repairing the tissue. The acute phase is characterized by the induction of inflammatory genes, like NF-κB and other transcription factors, where moderate amounts of inflammatory mediators are produced [1].

Conversely, chronic inflammation is a prolonged, deregulated and maladaptive response involving active inflammation, tissue destruction and repeated attempts at tissue repair. The chronic phase may last from months to years and is marked by a dramatic increase in the production of inflammatory mediators. Such persistent inflammation is associated with many chronic human conditions and diseases, including allergy, atherosclerosis, cancer, arthritis and autoimmune disorders [2].

Numerous anti-inflammatory drugs target the production of inflammatory eicosanoids and their side effects are often a result of their inhibition of eicosanoid production (Figure 1). Eicosanoids, so called as they derive from a unsaturated fatty acid with 20-carbon atoms, i.e., arachidonic acid (eicosatetraenoic acid), are obtained from membrane phospholipids and synthesized *de novo* at the time of cellular stimulation. Arachidonic acid is cleaved from membrane-bound phosphatidylcholine by phospholipase A2. Arachidonic acid can then follow one of two enzymatic pathways, leading to the production of inflammatory mediators e.g., the pathway initiated by cyclooxygenase (COX) produces prostaglandins. Glucocorticoids (GC) have assumed a major role in the treatment of a wide spectrum of diseases with an inflammatory-mediated component as they

have a wide range of effects on virtually every phase and component of the inflammatory responses. However, their systemic application is limited due to the high incidence of serious adverse effects related to their unfavorable pharmacokinetics and pharmacodynamics (Table I) [3-5]. When GC are administered either intravenously or orally, their pharmacokinetics follow first-order release kinetics [6] with an initial high plasma concentration, then a rapid exponential clearance. This means that high and frequent doses must be given if an effective concentration has to arrive at the inflamed target sites. A drug delivery system able to provide a slow, zero-order release kineticsmaintaining, at the same time, the plasma drug concentration below the toxicity level, can thus achieve therapeutic drug level overcoming the unfavorable pharmacokinetics [7].

Moreover, the poor bioavailability of drugs in organs and tissues such as the eyes and joints makes the treatment of inflammatory conditions one of the most challenging endeavours the pharmacologists have to face. Therefore, there is a clear need for the development of formulations capable of promoting drug penetration and maintaining therapeutic level with a reasonable administration frequency [8-10].

Non-steroidal anti-inflammatory drugs (NSAID) are a heterogeneous group of compounds with different structural classes, which do not include a steroid nucleus derived biosynthetically from cholesterol in their chemical structure withvarious pharmacokinetic and pharmacodynamic properties. NSAID share the same mode of action so they are also known as cyclooxygenase (COX1 and COX2) inhibitors [11, 12]. NSAID may be grouped as salicylates (with aspirin itself being a prominent member), arylalkanoic acids (diclofenac, indomethacin, nabumetone, sulindac), 2-arylpropionic acids or profens (ibuprofen, flurbiprofen, ketoprofen, naproxen), N-arylanthranilic acids or fenamic acids (mefenamic acid, meclofenamic acid), pyrazolidine derivates (phenylbutazone), oxicams (piroxicam, meloxicam), sulfonanilides (nimesulide) and others (Table II) [5, 13]. The way in which aspirin and non-aspirin NSAID inhibit the COX enzyme differ fundamentally, as aspirin permanently inhibits the COX by non-competitive and irreversible acetylation. Conversely, non-aspirin NSAID inhibit the COX enzyme competitively and reversibly

during only part of their dosage interval [14].

Although NSAID are effective in relieving pain and reducing inflammation, their efficacy is limited in many cases, as high systemic drug concentrations may lead to potentially serious adverse events, such as gastrointestinal ulceration or bleeding, hypertension and/or cardiovascular events, acute renal impairment and hepatotoxicity [15]. In addition to the inhibition of COX-1 and -2, certain NSAID, such as diclofenac, can also affect inflammation pathways by inhibiting leukotriene formation [16].

Several approaches have been proposed to overcome the drawbacks in the use of GC and NSAID. For this aim, several drug delivery systems controlling drug release and sustaining therapeutic levels over extended period of time have been investigated [17-19].

The most promising approach seems to be the delivery of a drug via micro- and nanoparticles as they meet three major objectives i.e., enhancement of drug penetration, controlled release and targeting [18, 20]. Moreover, injectable nanoparticulate drug delivery systems are particularly interesting as they take advantage of the unique micro-anatomy of the inflamed tissue blood capillaries, which have gaps between the lining endothelial cells, making vessels leaky. Inflamed tissues can discriminate between micro- and nanoparticles, as only the nanoparticles can extravasate into these tissues [21].

The engineering of functional systems on a sub-micron scale has been applied to improve drug efficacy through controlled release and targeting of the therapeutic compound. These systems can bring better management of drug pharmacokinetics, pharmacodynamics non-specific toxicity, immunogenicity and biorecognition. Depending on their particle charge, surface properties, and relative hydrophobicity, nanotechnology-based delivery systems can be designed to overcome physiological barriers and provide time protection for the encapsulated drug, prolonging exposure by controlled release [22]. This has also led to the solution of various solubility-related problems connected to poorly soluble drugs and can be used to target mononuclear phagocyte systems to allow region-specific delivery and minimize side effects in other organs [23]. Clinical trials are

being carried out on several nanomaterials for medical applications. Food and Drug Administration (FDA) have approved some for use in humans. Some nanomaterials are in the proof-of-concept stage. They are investigated bothin cell-culture and small-animal models by researcher laboratories [24]. The physicochemical characteristics of a drug also influence the release rate and its therapeutic effects, consequently. However, in the case of nanoparticles, both the surface charge and the binding of the drug to the particles have been found to be more important than the drug loading itself [25]. This review will focus on innovative colloidal systems for the sustained delivery of GC and NSAID such as liposomes, microparticles, polymeric and lipid nanoparticles.

Site-specific drug delivery

Gastrointestinal drug delivery

The colon is a location of inflammatory bowel disease (IBD), including ulcerative colitis and Crohn disease, making it an important target with challenging aspects for intact and quantitative amount of drugs, as it is the last step of the digestive tract. Indeed, drugs have to face a deleterious environment after oral administration as they pass through the mouth(saliva enzymes), stomach (pH 1-3) and the small intestine (enzymatic release and pH 3-6). Therefore, high drug doses and/or frequent administration are common to circumvent degradation by stomach acidic pH, or small intestine digestive enzymes, where side-effects may be problematic. Although enemas are often used to target drugs to the colon, this procedure has its drawbacks i.e., it is cumbersome and not well-accepted, limited to the distal part of the colon and is associated with the risk of local complications, including bleeding and/or perforation [26].

In addition to the absorption of nutrients, intestinal epithelial cells have a barrier function governed by the formation of an epithelial monolayer with tight junctions and a signal-transduction function. Abnormal permeability has been observed in IBD patients in both affected and non-affected intestinal areas [27]. However, the increased permeability also offers therapeutic potential as nanoformulations may accumulate in the gaps among the cells. The enhanced permeability and retention effect are observed in the tumour vasculature. Furthermore, the damaged intestinal epithelial cells seem to lose some of their polarity leading to an apical expression of basolateral membrane proteins. For an instance, the expression of the transferrin receptor was reported to be enhanced on both the apical and basolateral side of enterocytes in the inflamed colonic mucosa of IBD patients [28].

Apart from size and charge, little is known about the ideal properties of a nanocarrier system which is to passively to target the inflamed intestinal mucosa and reach maximum retention time in the tissue. Inorganic particles (e.g. silver or silica particles), biodegradable or non-biodegradable

polymeric carriers and lipid based systems have all been shown to target the inflamed intestine. Therefore, although the nature of the carrier material itself may have little influence on the efficacy of targeting it does affect drug release kinetics. PEGylation of the surface, i.e., the process of covalent attachment of poly (ethylene glycol) (PEG) polymer chains, and enhanced surface hydrophilicity reduce particle opsonisation, increasing systemic circulation time and extravasation rate across inflamed leaky vasculature [29]. Several drug delivery systems have been developed to target the gastrointestinal tract, including micelles [30], liposomes [31] and nanoparticles [26].

As particulate drug delivery systems selectively accumulate in areas of intestinal inflammation when delivered orally. They are particularly well-suited for the treatment of IBD. They allow the local delivery of therapeutics to areas of inflammation without the need to target ligands on the particle surface [32]. Most of microparticular systems designed for IBD therapy are in the size range of 10-300 μm. Therefore, they are too large for targeting to the inflamed intestinal tissue or for internalization by immune competent cells. Their improved therapeutic efficacy can be attributed to a reduction of drug absorption during small intestinal passage and to slower colonic passage due to the streaming effect. Even if further reducing the size of drug delivery systems to a micro- or nanometre scale might increase colonic residence time. Size reduction can also provide additional benefits for IBD therapy. A size dependent accumulation of micro- and nanoparticles can be observed specifically in the inflamed intestinal regions [28]. This effect was first described for negatively charged polystyrene particles administered orally in a trinitrobenzene sulfonic acid induced (TNBS) rat model of colitis for three days [32].

Dendritic cells and macrophages are parts of the innate intestinal immune system. Both cell types have the ability of uptake bacteria or particles smaller than 4 μm by phagocytosis. In IBD they are highly activated and increased in number, with enhanced phagocytotic activity [33]. Thus preferential uptake of nanoparticles and smaller microparticles by antigen presenting cells may also account for the specific accumulation observed in inflamed intestinal areas. Invading immune cells do not only present a target to improve localization of a drug to the inflamed tissue, but they may

also transport the nanoformulations to the target site for time delayed or triggered drug release [28]. Recently, this cell mediated drug delivery approach has been intensively researched for the cancer therapy, as well as for various autoimmune or inflammatory conditions, such as lung inflammation or microbial infections [34]. However, to the best of our knowledge, the concept has not yet been evaluated in the context of IBD.

Intra-articular drug delivery

Despite of extended pharmaceutical and clinical researches, there are still unmet requirements in the treatment of rheumatic diseases such as osteoarthritis and rheumatoid arthritis. Treatment of irreversible joint damage is achieved through oral, parenteral or intra-articular (i.a.) drugs. Although injections of medications directly into the joint cavity are frequently prescribed to reduce the systemic exposure of anti-arthritic drugs, often clinical improvement obtained is only transient as the drugs readily leave the joint and distribute throughout the body [35]. Despite some practical drawbacks, the direct delivery of a drug to an affected joint offers the possibility of reaching high drug concentrations at the site of action with limited systemic toxicity. Intra-articular administration is advantageous for drugs with low bioavailability. Nevertheless, depending on their chemical structure, some active compounds are rapidly cleared from the joint, requiring numerous injections which may lead to infection and/or joint disability [36]. An innovative technique able to maintain a therapeutic concentration, over longer periods, is the administration of depot formulations which generally contain corticosteroids. Despite their clinically proven efficacy, these depot formulations do have a significant drawback which limits their use i.e., their crystalline nature [37]. In fact, these drugs may generate inflammatory conditions with an i.a. injection, leading to transient crystal-induced arthritis; wich has been observed in 10% of patients and disappears within a few days [38]. This reinforces the need to develop drug delivery systems for i.a. use that would function as depot able to gradually release the active substance and provide a local sustained drug action.

Nowadays, liposomes can be considered as beneficial i.a. drug delivery system [39]. Moreover, microspheres injected i.a. represent one of the most studied delivery systems to decrease i.a. drug clearance [40] and nanoparticles generally contain a specific cartilage-binding moiety [41].

The incorporation of the drug within microspheres of sufficient size can extend drug joint residence time since the synovium is leaky and particle size conditions molecular flux. There is an upper limit (40-250 nm radius) to the size of particles that can escape freely from the joint cavity. Possibly the simplest means of prolonging drug residence time within joints is to formulate microspheres bigger

than 250 nm, exploiting size inclusion [42]. Drug is released as the carrier is progressively degraded. The fate of biocompatible microspheres is size dependent i.e., those with a diameter < 10 μm (optimally between 1 μm and 4 μm). They are phagocytized in the synovial fluid by synovial resident and recruited macrophages, without evoking a neutrophil response [43]. Larger microspheres are not phagocytized, but are sequestered as subsynovial granulation plaques, surrounded by giant cells [44]. Macrophages comprise approximately 30% of the cells in the intima and subintima in normal joints and this proportion increases with age and progression of arthritis [45]. The synovium is rich in cells that have the potential to take up and sequester a large microsphere load and, upon breakdown of the carrier; the encapsulated drug may be freed. If the drug is then able to diffuse out of the macrophage, it will become available to the surrounding milieu. Therefore, the time required for microsphere degradation to occur has the potential to prolong drug retention time within the synovial joint. Both the release rate and the reservoir capacity can be tailored by specific technological parameters such as the polymer type [35].

Ocular drug delivery

Due to ocular anatomical and physiological drug absorption barriers, which include the relative impermeability of the corneal epithelial membrane, tear dynamics, nasolacrimal drainage and the high efficiency of the blood-ocular barrier, designing a drug delivery system to target a particular tissue of the eye has become a major challenge for scientists in the field [46]. It is common for only 1% or less of a topically applied dose to be absorbed across the cornea and reach the anterior segment of the eye. Pulse entry is favoured even if highly undesirable pharmacokinetic characteristics are associated with the use of eye-drops. The initial high drug concentration found in tears, followed by a rapid decline, poses potential toxicity risk and prompts the need for frequent dosing [47]. Attempts to overcome the toxicity are associated with the high initial concentration without the necessity for frequent dosing, form a challenging task, particularly when potent drugs are involved. Nasolacrimal drainage is the principle element involved in precorneal drug loss leading to poor ocular bioavailability. It is also the major route of entry into the circulatory system for drugs that are applied topically. Indeed, the systemic exposure to potent drugs through nasolacrimal drainage after topical administration can suffice to cause systemic toxicity. The delivery of drugs to the posterior segment of ocular tissue is also difficult. The aforementioned factors responsible for the poor ocular bioavailability are coupled to the blood-retinal barrier that limits the efficacy of the intravenous route in posterior drug delivery. Thus, the most acceptable method for posterior drug delivery is intravitreal injection. Intravitreal injection is associated with a high risk of complications like retinal detachment and endophthalmitis [48]. It may also lead to complications such as vitreous hemorrhage, infection, lens and/or retinal injury [49].

, Large and often toxic doses are required to attain therapeutic efficacy because only a small fraction of the drug administered orally or by other extravascular routes, reaches the targeted retina [50]. A considerable amount of effort has been made in ophthalmic drug delivery since the 1970s and the various approaches attempted in the early stages can be divided into two main categories:an improvement in bioavailability and controlled release drug delivery. Indeed, the improvement of the

poor bioavailability of topically applied ophthalmic drugs is a major challenge in ocular drug therapy sincedrug absorption must overcome the severe constraints imposed by the eye [51]. Vesicular systems not only help in providing controlled ocular delivery by preventing drug metabolism due to the enzymes present on the tear/corneal epithelial surface, but they also provide controlled action at the corneal surface. Indeed, a suitable carrier system for ophthalmic drug delivery should not only provide suitable biopharmaceutical properties, but also ocular tolerability. Without adequate regulatory guidance for ocular drugs novel formulations can pose unique challenges to those involved in designing non- clinical programmes and choosing species, strains and ocular toxicity parameters. During the last few decades, several drug delivery systems, mainly liposomes and nanoparticles, have emerged as novel strategies for the delivery of anti-inflammatory drugs [52-58]. The advantages of liposomes are complete biodegradability, biocompatibility, relative non-toxicity and their ability to install an intimate contact with the corneal surface. However, there are some disadvantages that are slightly difficult to overcome. Their structure makes liposomes less stable than polymeric and lipid nanoparticles, which are characterized by the presence of a solid matrix, raising concerns on liposome long-term shelf life [59]. Although a change in lipid composition may lead to more stable liposomes (e.g., by increasing the amount of cholesterol), it must be noted that this may run a risk for controlled/prolonged drug release profile. Moreover, the lower stability generally leads to a faster release of the drug compared to nanoparticle formulations, making liposomes less suitable for intraocular use, where drug release is required over long time periods. The binding affinity of liposomes to the cornea suggests that the interaction is probably electrostatic in nature. As the corneal epithelium is coated thinly with negatively charged mucin, liposome that have a positive surface charge expose to more stable adsorption [60].

Innovative glucocorticoids drug delivery systems

Liposomal carriers

Small size, amphiphilicity and biocompatibility of liposomes make them promising systems for drug delivery. In fact, liposomes may have a positive, negative, or neutral surface charge, depending on their chemical composition (type of phospholipids, drug substances and other lipid molecules). They can accommodate both hydrophilic and lipophilic drugs in their aqueous compartment and lipid bilayer, respectively. A major obstacle to cutaneous drug delivery is the permeation characteristics of the stratum corneum, which limits drug transport, making this route of administration frequently insufficient for medical use. Several studies have reported that deformable liposomes are able to improve *in vitro* skin delivery of various drugs [61] and to penetrate intact skin, *in vivo*, transferring therapeutic amounts of drugs [62]. Betamethasone was encapsulated in the aqueous compartment of liposomes by the use of cyclodextrins combining the advantages of cyclodextrin inclusion complexes and deformable liposomes. This leads to a new concept described as drug-in-cyclodextrin-in-deformable liposomes. Deformable liposomes made of soybean phosphatidylcholine or dimyristoylphosphatidylcholine and sodium deoxycholate as edge activator were compared to classical non-deformable liposomes. In comparison with non-deformable liposomes, these new vesicles showed improved encapsulation efficiency, good stability and higher *in vitro* diffusion percentages of encapsulated drug [63]. The release kinetics is directly correlated to the encapsulation efficiency, which is closely related to the betamethasone concentration in cyclodextrin complex solution [64]. In an *ex vivo* study, the authors showed that classical and deformable liposomes do not remain intact when penetrating the deepest layers of the skin and that phosphatidylcholine acts as a penetration enhancer. Betamethasone is released from the vesicles, after free drug molecules diffuse through the stratum corneum and partition into the viable skin tissue (Figure 2). It was observed that the encapsulation into the lipid bilayer significantly enhanced

the accumulation of betamethasone in the epidermis of pig ear skin [65]. Negatively charged liposomes significantly enhanced betamethasone penetration in the epidermidis compared to positively charged or neutral liposomes [66].

Banciu et al. investigated the effects of methyl prednisolone disodium phosphate on the production of angiogenic/inflammatory factors in the B16.F10 murine melanoma model. Furthermore, the viability and proliferation of tumor and endothelial cells was evaluated *in vitro*. Long-circulating liposomes were prepared with appropriate amounts of dipalmitoylphosphatidylcholine, cholesterol and poly (ethylene glycol) (PEG) - conjugated distearoylphosphatidylethanolamine. Tumors from mice treated with glucocorticoid long-circulating liposomes showed statistically significant growth inhibition compared with control tumors. Different corticosteroids were incorporated and the ranking order of the reducing effects of liposome-gluocorticoids on *pro*-angiogenic protein production was: liposome-budesonide disodium phosphate > liposome-dexamethasone disodium phosphate > liposome-prednisolone phosphate > liposome-methylprednisolone disodium phosphate [67].

A recent study was aimed at the development of a targeted liposomal delivery system capable of delivering drugs in glomerular endothelium. Dexamethasone was formulated in immunoliposomes and conjugated with anti-E-selectin antibodies (AbEsel liposomes) as a model drug. Its pharmacological activity was then studied in antiglomerular basement membrane glomerulonephritis. Accumulation of AbEsel liposomes in the kidney was 3.6 times higher than non-targeted IgG liposomes, whereas there was a comparable accumulation of both liposomes in the clearance organs (liver and spleen) and in heart and lungs. In glomeruli, the AbEsel liposomes colocalized with the endothelial cell marker CD31. Quantitative RT-PCR analysis of laser-microdissected arterioles, glomeruli and postcapillary venules demonstrated that targeted delivery of dexamethasone by AbEsel liposomes led to a 60–70 % reduction in glomerular endothelial expression of P-selectin, E-selectin, and vascular cell adhesion molecule (VCAM)-1. A single administration of dexamethasone-AbEsel liposomes sufficed to reduce albuminuria 1 week later

[68-69]. When dexamethasone was delivered directly to the lung as a liposomal formulation, there was a significant increase in retention and a significant reduction in the systemic side-effects [70]. Therefore, liposome-entrapped dexamethasone was evaluated in an animal acute lung injury model. The authors demonstrated that dexamethasone administered as a free drug or in a liposomal formulation was able to reduce lipopolisaccaride-induced acute lung injury. This may be attributed to a suppression of the release of the eicosanoid acid metabolites via an inhibitory effect on phospholipase A2. The suppressive action of the liposomal dexamethasone preparation was far greater than that of the free drug. This elevated suppressive effect may be related to a liposome-mediated dexamethasone availability [71]. A study using a rat animal model was aimed for determining whether dexamethasone in liposomes is capable of down-regulating ventilator-induced lung inflammation or not. Liposomal formulation of the glucocorticoid dexamethasone was reported to inhibit important ventilator-induced lung inflammation parameters [72].

Ulmansky reported that sterically stabilized betamethasone hemisuccinate liposomes in tne nanometer size range (averagely 80 nm), had excellent therapeutic efficacy when injected intravenously (i.v.) in an adjuvant arthritis model of rheumatoid arthritis in Lewis rats. The liposome lipid composition was hydrogenated soybean phosphatidylcholine/cholesterol/polyethyleneglycol–distearoyl phosphoethanolamine with a mole ratio of 55:40:5 [73]. The authors evaluated therapeutic efficacy in the adjuvant arthritis rat model. Liposomes were administered either i.v. or subcutaneous, comparing nano-drugs to those of the free drugs, and to arthritis biological modulators, such as infliximab and etanercept. They reported that treatment with both liposomal nano-drugs significantly decreased the severity of adjuvant arthritis when administered at disease peak. The therapeutic effect was evident within 24-48 h after administration of the drug and lasted for 4-13 days [74].

Methylprednisolone hemisuccinate sodium salt was incorporated in nanoliposomes using the strategy of transmembrane ion gradient-driven remote loading of amphipathic weak acid or base drugs into PEGylated nanoliposomes. Overall, the features of the optimized nano-sterically

stabilized liposomes methylprednisolone hemisuccinate gave a high drug payload, high encapsulation efficacy, good storage stability at 4°C storage and superior pharmacokinetic and biodistribution properties, as well as a slow zero-order drug release [75]. This formulation showed superior therapeutic efficacy in mouse and rat models of diseases with inflammatory components like rheumatoid arthritis [73-74], in murine models of experimental autoimmune encephalomyelitis and cerebral malaria and in two types of mouse tumor models (the methylprednisolone hemisuccinate sodium salt-sensitive BCL-1 B-cell leukemia and the methylprednisolone hemisuccinate sodium salt-insensitive J6456 T-cell lymphoma) [75].

Tissue targeting of nano sterically stabilized liposomes (averagely 100 nm) was evaluated in an *in vivo* animal model of foreign body granuloma. Nano-sterically stabilized liposomes were made up of fully hydrogenated soybean phosphatidylcholine, methoxy 2-kDa polyethylene glycol–distearoyl phosphoethanolamin, cholesterol and were remote loaded with methylprednisolone hemisuccinate. Twenty-four h after i.v. administration of nano-sterically stabilized liposomes, their level increased two-fold in the vicinity of the implanted foreign body compared to the level observed in the controlateral (normal) leg or in the legs of control animals. Whilst the level of nano-sterically stabilized liposomes in the target site is dictated by the total administered dose, the ratio between the level of nano-sterically stabilized liposomes in tissue with an implanted foreign body and the contralateral healthy tissue seems to be constant, whatever the nature of the implant [76].

Microparticulate carriers

Budesonide, a potent non-halogenated corticosteroid, is currently in clinical use for the treatment of asthma, allergic rhinitis and IBD, due to its ability to inhibit the expression of several proinflammatory genes, such as interleukin (IL)-6, IL-8, and tumor necrosis factor. Kompella et al. assessed inhibition of budenoside expression of vascular endothelial growth factor (VEGF) in a retinal pigment epithelial cell line (ARPE-19) and determined whether subconjunctivally administered budesonide micro- and nanoparticles sustain retinal drug levels or not. Polymeric-

budesonide micro- and nanoparticles were formulated by a solvent-evaporation method. These particulate systems sustained retinal budesonide levels when compared with a solution form in a rat model and it was reported that budenoside is capable of reducing VEGF expression in retinal pigment epithelial cells through its glucocorticoid receptor activity [77].

Fialho et al. developed a microemulsion system intended for topical ocular administration of dexamethasone eye-drops. The microemulsion was prepared following the method called titration with the cosurfactant. Dexamethasone was dispersed in the oily phase (isopropyl myristate) and then the surfactant (Cremophor EL) and the water phase were added and rotated to form an oil-in-water macroemulsion. Next, the cosurfactant (propyleneglycol) was added to the macroemulsion previously prepared, and rotated until a transparent system was found. The test was carried out on male New Zealand white rabbits for both the microemulsion with drug and the vehicle alone. No alterations were found in any of the ocular structures of the animals treated with the new formulation. No significant corneal lesions, opacity, conjunctival chemosis, redness, discharge or iris alterations were observed in any of the rabbits. The microemulsion developed presented a greater bioavailability of the drug in the aqueous humour after its administration [78-79].

In clinical practice Cardillo et al. investigated the therapeutic response and ocular tolerance of a single intravitreal injection of 1 mg triamcinolone acetate in a controlled-release microsphere system in comparison with a single intravitreal injection of 4 mg triamcinolone acetate for the treatment of diffuse diabetic macular edema. In this preliminary report the author found that both the microsphere formulation and triamcinolone acetate injections may be well tolerated with long-term performance clearly favouring the former over triamcinolone solution [80].

Micelle systems, composed of the polyoxyethylated nonionic surfactant Pluronic1 F127 (F127) and cationic polyelectrolyte chitosan, were prepared with dexamethasone. All micelle systems were characterised by prolonged release profiles. The addition of chitosan significantly enhanced the *in vitro* dexamethasone release rate and transport across Caco-2 cell monolayers, as compared to the chitosan-free F127 micelle system. This colloidal carrier was well tolerated in rabbit eyes and no

clinically abnormal signs in various ocular structures were observed. Any increase in intraocular pressure in rabbits was used to evaluate dexamethasone ocular bioavailability. The AUC values showed a 1.7- and 2.4-fold increase in bioavailability of chitosan micelle systems as compared to a standard dexamethasone suspension [81].

Copolymers of polyhydroxyethylaspartamide, bearing in the side chains polyethylene glycol and/or hexadecylamine (PHEA-PEG, PHEA-PEG-C16 and PHEA-C16 respectively), have been studied as potential colloidal drug carriers for ocular drug delivery. *In vitro* permeability studies, performed on primary cultured rabbit conjunctival and corneal epithelia cells, using PHEA-C16 and PHEA-PEG-C16 as micelle carriers for dexamethasone alcohol and dexamethasone phosphate, reported that, in all cases, drug loaded PHEA-C16 and PHEAPEG- C16 micelles provided a better drug permeation across ocular epithelia greater than simple drug solutions or suspensions (Figure 3) [82-83].

Nanocarriers

Depending on the solubility and hydrophilic characteristic of the drug, several protocols of nanoparticles synthesis can be proposed. Ishihara et al. developed poly (d,l-lactic/glycolic acid) (PLGA) or poly (d,l-lactic acid) (PLA) nanoparticles with a diameter of less than 200 nm that encapsulated water-soluble corticosteroid derivatives for sustained release and targeting to inflammatory sites. The nanoparticles were prepared with PLGA (or PLA), zinc, betamethasone phosphate and surfactant by an oil in-water solvent diffusion method. In this method, the efficiency of encapsulating betamethasone phosphate in the nanoparticles and the particle size were significantly affected by various factors, such as PLGA (or PLA) concentration and the amount of zinc added [84]. The zinc content increased along with the betamethasone phosphate content of the nanoparticles, indicating the incorporation of the drug as a complex with zinc. Nanoparticles encapsulating a fluorescent dye (rhodamine) instead of betamethasone phosphate, were incubated with murine macrophages, resulting in the internalization of rhodamine encapsulated in the nanoparticles, indicating that the nanoparticles were internalized in cells by phagocytosis, as has

been previously reported [85].

PEG with uncharged, hydrophilic and non-immunogenic properties is an attractive material for surface modification of the nanoparticles to reduce opsonization. PLGA/PLA nanoparticles with PEG grafting escape renal exclusion and the mononuclear phagocytic system; therefore, they have enhanced half-lives in plasma [86-90]. Furthermore, these long-circulating nanoparticles preferentially accumulate in tumors and inflammation sites that have a leaky vasculature, due to enhanced permeability and retention effects [91].

Various types of nanoparticles encapsulating betamethasone phosphate were prepared from different mixtures of PLA/PLGA homopolymers and PEG-PLA/PLGA block copolymers by the solvent diffusion method [51]. In nanoparticles of the same PEG content, the drug loading of bethametasone phosphate decreased along with an increase in the molecular weight (6 to 16 kD) of the PLA/PLGA homopolymers. The release rate of betamethasone phosphate from nanoparticles is controllable by changing the compositions/molecular weight of the PLA/PLGA homopolymers and the blend ratio of homopolymers and block copolymers. The cellular uptake *in vitro* can be controlled by PEG density depending on the blend ratios of homopolymers and block polymers and by PEG loss, depending on the block polymer composition. Markedly increased blood circulation time and reduced liver uptake of PEGylated PLA/PLGA nanoparticles compared to non-PEGylated nanoparticles after intravenous administration to mice has previously been demonstrated [92]. All blended nanoparticles exhibited prolonged residence in the blood circulation compared to conventional non-stealth nanoparticles [93].

The anti-inflammatory activity of these stealth nanosteroids was evaluated in experimental arthritis models i.e., rats with adjuvant arthritis rats and mice with anti-type II collagen antibody induced arthritis (AbIA). Approximately 30 to 40 % remission of joint inflammation was accomplished within 1 day of treatment with stealth nanosteroids in adjuvant arthritis rats and the therapeutic benefit of the injection lasted for up to 9 days. Whereas the equivalent dose of conventional type nanosteroids and free betamethasone phosphate led to only a 15 % remission at the same dose.

Stealth nanosteroids were used in AbIA mice and, within 2 days, an extended sustained anti-inflammatory effect, which lasted for 8 days, was obtained. The authors concluded that if superior anti-inflammatory activity is to be obtained, as in the adjuvant arthritis rats, nanoparticles should exhibit, not only prolonged blood circulation but also sustained drug release at the lesion. The nanoparticle properties appear to be influenced by the surface PEG content, the size of the nanoparticles and the rate at which the nanoparticles loose PEG (Figure 4) [94]. Furthermore, it may well be that stealth nanosteroids are not only able to enhance the concentration of the drug at the target site, but also to lower the drug concentration at non-target tissues.

Dexamethasone nano-aggregate was prepared for the treatment of intimal hyperplasia caused by abnormal proliferation of smooth muscle cells. Triblock copolymers, made up of PEG and PLA, were synthesized with different chain lengths of PEG. The nano-aggregates had an average diametre of 200 to 300 nm. Due to their low solubility in water, *in vitro* dexamethasone nano-aggregates released out to the aqueous phase for relatively long periods. Different release profiles were obtained, depending on the length of PEG chains in triblock copolymer [95].

In an adjuvant-induced arthritis rat model N-(2-hydroxypropyl)-methacrylamide copolymer dexamethasone confirmed the arthrotropism of the formulation in this type of acute inflammation. It has been reported that both the increase in molecular weight and dexamethasone content will facilitate joint-targeting through increasing the circulation half-life of the conjugates and will presumably enhance inflammatory cell uptake at the inflammation sites [96].

The use of nanoparticle enabled sustained, site-specific methylprednisolone delivery onto the injured spinal cord was investigated in an animal study. This approach was designed to overcome the undesirable side effects of high-dose systemic drug administration whilst, at the same time, significantly enhancing delivery efficiency. Biodegradable and injectable poly (lactic-co-glycolic acid) (PLGA)-based methylprednisolone loaded nanoparticles carrying $1/20^{th}$ (by weight) of the current clinical dose of methylprednisolone were locally delivered at the injury site in a dorsal over-hemisection model of spinal cord injury. The data obtained demonstrated that sustained local

delivery of methylprednisolone loaded nanoparticles soon after spinal cord injury significantly decreased the reactivity of the early markers of injury/secondary injury, reduced lesion volume and improved functional outcomes after spinal cord injury. These results were obtained using a remarkably low dose of methylprednisolone i.e., 400 μg/animal. The sustained and local delivery of methylprednisolone onto the lesion site, facilitated by PLGA-based methylprednisolone loaded nanoparticles treatment, generated benefits which were apparent within the first 24 h after injury, with significantly increased expression of anti-apoptotic protein along with a decreased expression of pro-apoptotic related proteins [97]. An *in vivo* animal study investigated the anti-inflammatory effect of intravenous administered PLA nanoparticles encapsulating betamethasone phosphate on experimental autoimmune uveoretinitis (EAU) in Lewis rats. Nanoparticles were prepared using a modified oil-in-water solvent diffusion method. Experimental autoimmune uveoretinitis was induced with a subcutaneous injection of 50 mg of S-antigen peptide emulsified in the same volume of complete Freund's adjuvant containing Mycobacterium tuberculosis H37Ra. The rats were examined every other day for clinical signs of the disease and were graded from 0 to 4. The clinical scores of rats with autoimmune uveoretinitis reached its maximal severity on day 17, PLA betamethasone phosphate loaded nanoparticle-treated rats (100 mg) showed reduced clinical scores on day 15–31 compared to those of free betamethasone phosphate-treated rats (100 mg). Histopathological examination showed that PLA betamethasone phosphate loaded nanoparticle-treated rats displayed remarkable preservation of structural integrity, whereas betamethasone phosphate-treated rats (500 mg) showed mild disruption in inner and outer segments of all surviving photoreceptors. The authors concluded that systemically administered PLA betamethasone phosphate loaded nanoparticles effectively inhibit the development of autoimmune uveoretinitis by targeting and through sustained delivery of the corticosteroid drug [98-99].

The effect of budesonide (100 pM to 10 μM) on VEGF secretion, expression of VEGF mRNA and cytotoxicity were determined in ARPE-19 cells. Secretion and mRNA expression studies were also performed in the presence of a glucocorticoid receptor antagonist (RU486) to determine the

involvement of glucocorticoid receptor in the observed effects of budesonide. PLA nano- and microparticles containing budesonide were prepared by a solvent evaporation technique and the particles were characterized for size, morphology, encapsulation efficiency and *in vitro* release. Budesonide PLA nano- and microparticles were administered subconjunctivally to one eye of Sprague-Dawley rats and drug levels in the retina, vitreous, lens and cornea of both eyes were determined at the end of 1st, 7th and 14th days. Budesonide inhibited VEGF secretion and mRNA expression in ARPE-19 cells, in a dose-dependent manner, at concentrations devoid of cytotoxicity. RU486 treatment prevented budesonide-mediated inhibition of VEGF secretion and VEGF mRNA expression. Budesonide-PLA nano- (345 nm) and microparticles (3.6 μm), with an encapsulation efficiency of 65 % and 99 %, respectively, sustained budesonide release *in vitro*. Both budesonide PLA nano- and microparticles produced sustained budesonide levels in the retina and other ocular tissues, after subconjunctival administration (Figure 5) [77].

Polymeric micelles were prepared using N-isopropylacrylamide, vinyl pyrrolidone and methacrylate as monomers in the presence of N,N-methylene bis-acrylamide and triethyleneglycol dimethacrylate as cross-linking agents. Dexamethasone-containing nanosuspensions, made up of these temperature- and pH-sensitive micellar nanoparticles, were evaluated for their efficacy to deliver ocular drugs in an animal model of uveitis induced by intravitreal injection of the endotoxin into the rabbits' eyes. Topical administration of prepared nanosuspensions reduced uveitis symptoms, which were quantified by the Hogan score. Furthermore, the nano-formulations significantly reduced inflammation after lipopolissacaride injection [100].

Lipid nanoparticles combine several advantages and avoid some of the aforementioned disadvantages for other colloidal carrier systems, when optimized, lipid nanoparticles show high physical stability, protection of incorporated labile drugs against degradation and excellent *in vivo* tolerability. However, these systems generally show a low drug payload capacity and drug expulsion during storage, due to the transition of highly ordered lipid particles. These disadvantages can be remedied by using structured lipid matrices in solid lipid nanoparticle formulations and

surface modification of the particles.

Nanostructured lipid carriers loaded with dexamethasone acetate, were prepared by an emulsification-ultrasound method and were relatively uniform in size (178 ± 4 nm) with a negative zeta potential (-38 ± 4 mV). The average drug entrapment efficiency was 91 ± 3 %. *In vitro* release tests indicated dexamethasone acetate loaded nanostructured lipid carriers possessed sustained release characteristics and that there was an almost 80% accumulative release percentage at 23 h. The carrier exhibited an average peak concentration of dexamethasone acetate (7.6 μg/ml) in the pleural exudate after intravenous administration to an experimental model of γ-carrageenan-induced pleuritis rats, which was 8.3 times higher than that of dexamethasone acetate (0.9 μg/ml). The γ-carrageenan-induced oedema test showed that the anti-acute inflammatory activity of dexamethasone acetate loaded nanostructured lipid carriers was stronger than that of the free drug at the same drug concentration. Moreover, biodistribution results clearly indicated that this carrier preferentially accumulated in livers and lungs of the mice after intravenous injection. These results indicated that injectable nanostructurated lipid carriers may serve as an excellent carrier for dexamethasone acetate, greatly enhancing the selective effect on inflammatory sites (Figure 6) [101].

Dexamethasone acetate was incorporated into the nanostructure of lipid carriers, made up of a binary mixture of solid lipid and a spatially different liquid lipid as a carrier by a film dispersion-ultrasonication method. The authors indicated that this drug delivery system significantly improved the *in vitro* release, biodistribution, pharmacokinetic and therapeutic properties of dexamethasone acetate with remarkably prolonged drug circulation time in plasma and increased drug accumulation in the liver. The formulation had a hepatoprotective effect on CCl4-induced mice and exhibited a significant hepatoprotective effect compared with dexamethasone acetate solution [102].

Solid lipid nanoparticles (SLN) for the lung-targeting delivery of dexamethasone acetate by intravenous administration were developed. Dexamethasone acetate loaded SLN colloidal suspensions were prepared by the high pressure homogenization method. The authors reported that

the *in vitro* drug release profile showed that the initial burst release of the drug from SLN was about 68% during the first 2 h, followed by a gradual release of the drug over the next 48 h. The concentration of dexamethasone acetate in the lung reached a peak at 0.5 h post dexamethasone acetate loaded SLN injection. There was a 17.8-fold increase in the area under the curve (AUC) of the drug delivered by SLN, compared to the AUC reached by the dexamethasone acetate solution. These results indicate that SLN may be used for lung targeting drug carriers for lipophilic drugs, such as dexamethasone acetate [103].

Triamcinolone acetonide is a corticosteroid drug, currently administered by intravitreal injection, for a broad spectrum of inflammatory, edematous and angiogenic ocular diseases [104-105]. Triamcinolone acetonide was encapsulated in nanostructured lipid carriers (NLC), so as to increase its bioavailability by ocular instillation. A recent paper reported on nanometric (200 nm), unimodal and negatively charged NLC loaded with the fluorescent lipid marker Nile red (NR-NLC) and triamcinolone acetonide (TA-NLC), which were produced by high pressure homogenization. Sections of sliced eye tissue were obtained for histopathological study to determine the behaviour of NLC after being used as eye-drops. The retinal fluorescence of NR gradually increased with time, peaking 40 min after administration, decreasing thereafter and almost disappearing at 160 min after administration. The data obtained from this study on mice, evidence the possibility of drug delivery to the posterior segment by NLC carriers, making these nanoparticles a promising approach to provide selective and prolonged drug concentration in the eye and avoid the problematic intravitreal injections. Therefore, colloidal nanoparticle carriers appear to be efficacious for ocular absorption enhancement of drugs. This may be due to multidimensional mechanisms i.e., prolonged drug residence time in the ocular surface and conjunctival sac, by sustained drug release from the delivery system and/or by reduced precorneal drug loss [106].

By definition, drug nanocrystals are nanoparticles, being as they are, composed of 100% drug without any matrix material, with a size range typically between 200 and 500 nm [107]. Several methods may be used to reduce the particle size of a drug e.g., bottom-up and top-down

technologies. The product obtained is a suspension of drug nanocrystals in a liquid stabilized by a surfactant or polymer, showing an increased dissolution velocity and higher saturation solubility in comparison to micronized drugs. The physicochemical properties are dependent on the type of stabilizer. The nanosuspension has to be transformed into a solid, dry multiparticulate product, by means of lyophilization or spray-drying procedures. Usually high temperatures have been applied in this process to obtain a controlled-release dosage form with better physical stability, thus the method is not suitable for thermolabile drugs [59]. Pargaonkar et al. proposed that a combination of sonication and layer-by-layer coating can be used to produce monodispersed suspensions of dexamethasone microcrystals. This technique produced diffusion-controlled drug release via the polyelectrolyte multilayer nano-thick shell [108].

In summary, liposomes as glucocorticoid delivery systems show superior therapeutic efficacy compared to free drug in animal models of acute and chronic inflammation. The loading and release of glucocorticoids from polymeric nanoparticles improve drug pharmacokinetic profiles and anti-inflammatory effects at lower dosages compared to free drugs. Lipid nanoparticles have an excellent in vivo tolerability but a low payload capability. In particular, nanoliposomal formulations for the delivery of drugs to colonic inflamed lesion seem a promising therapeutic approach to treat IBD but evidences from clinical trials are needed.

Innovative non steroidal anti-inflammatory drug delivery systems

Liposomal carriers

Jubeh et al. studied the adhesion properties of charged liposomes to the healthy and inflamed (colitis-induced) rat intestinal epithelium. An *ex vivo* comparison of cationic, anionic and neutral multilamellar liposomes (800±50 nm) in the colon sacs of dinitrobenzene sulfonic acid treated rats showed a strong adherence of cationic liposomes to the healthy epithelium, while no binding of neutral or negatively charged liposomes was observed. Conversely, the negatively charged liposomes preferentially adhered to the inflamed tissue, while cationic liposomes only showed a weak binding [109].

Diclofenac delivered in liposomes carrying hyaluronan or collagen as surface ligands, showed reduced side effects and toxicity, when given as intra-articular injection in an osteoarthritis model in comparison to the control. The therapeutic availability of diclofenac was enhanced with antioxidant (co-enzyme Q10 and ascorbyl palmitate) loaded diclofenac liposomes in comparison to the conventional liposomes. *In vivo* drug targeting studies showed an increase in AUC, therapeutic availability of diclofenac in air pouch fluid and air pouch fluid/serum diclofenac concentration ratios from antioxidant loaded liposomes compared to conventional liposomes and drug solution. Thus these surface loaded antioxidant liposomes produced a targeting effect in inflammation induced rats [110].

Niosomes, non-ionic surfactant-based liposomes, were also investigated along with traditional liposomes, for the local delivery of diclofenac sodium to treat arthritis [33]. Moreover, both liposome- and niosome-containing diclofenac were embedded in carbopol or sodium carboxymethyl cellulose gels to further prolong the release of the active substance together with better joint retention, leading to novel drug delivery systems, lipogelosomes and niogelosomes, respectively. Radiolabelled diclofenac-containing lipogelosomes, injected into arthritic rabbit joints, presented the longest retention times, when compared to the other formulations. Moreover, there was a slow

release of the radioisotope, 67% of the initially injected radioactivity was still present at 24 h post-injection [111]. This type of carrier has mainly been studied in an attempt to target the articular cartilage in diseases such as osteoarthritis.

Diclofenac was incorporated into phospholipid vesicles (liposomes) in an attempt to transport a sufficiently therapeutic concentration of the drug across the skin, to no avail. This failure is most likely due to the fact that conventional liposomes are not able to cross the skin barrier [112, 113]. Cevc et al. developed ultradeformable drug carriers that trespass the intact skin spontaneously, probably under the influence of the naturally occurring, but previously overlooked, transcutaneous hydration gradient. Ultradeformable vesicle-encapsulated diclofenac was able to penetrate deep into the soft tissue under the drug application site, in animal experiments. Therefore, the reach of the carrier-associated agent may exceed, by more than one order of magnitude, the maximum agent penetration from the reference diclofenac-loaded gel. Ultradeformable vesicles can even carry a therapeutic quantity of diclofenac into the knee being treated with reasonable reproducibility. Cevc et al. then showed that the improved NSAID delivery by deformable liposomes is due to the driving force provided by the osmotic gradient between the outer and inner layers of the stratum corneum [114].

A liposomal formulation was developed so as to avoid the systemic side-effects and low bioavailability of oral formulations of celecoxib. To this aim, the Celecoxib was encapsulated into multilamellar vesicles made up of 1,2-distearoyl-sn-glycero-3-phosphocholine and variable amounts of cholesterol. Encapsulation efficiency, loading and release of celecoxib decreased with increasing cholesterol content due to a competition between celecoxib and cholesterol for the co-operativity region of the phospholipids. Multilamellar vesicles prepared without cholesterol exhibited the lowest ability for celecoxib retention after 72 h [115].

An approach that can further improve liposomal drug delivery is the use of bioadhesive polymers, e.g., poly (alkyl cyanoacrylate), chitosan, hyaluronic acid, to prolong the residence time of the ocular preparation in the precorneal region [116]. Modified-release liposome formulations have

been investigated to improve the treatment of posterior segment by maintaining the drug concentration, enhancing direct delivery and minimizing intraocular toxicity [117].

Microparticulate carriers

Microencapsulation is one of the novel drug-delivery technologies employed to sustain drug release for the safe and effective delivery of NSAID [17]. Maghsoodi et al. prepared microparticles of naproxen with EUDRAGIT L100 and AEROSIL using the emulsion solvent diffusion method to prevent local gastrointestinal irritation, which is one of the major side effects of NSAID drugs after oral ingestion [118]. Poly n-butylcyanoacrylate (PNBCA) nanocapsules of indomethacin were prepared by interfacial polymerization, in a study by Miyazaki et al. [119]. Yapel et al. prepared ketoprofen-loaded microspheres with a polymeric blend using the spray-drying technique [120]. Kumbar et al. encapsulated diclofenac sodium into cross-linked chitosan microspheres and investigated the effect of the cross-linking agent [121]. Palmieri et al. prepared ketoprefen controlled-release microspheres using the emulsion/solvent evaporation method at + 15 °C in order to avoid the formation of semisolid particles [122]. Gonçalves et al. developed chitosan microspheres using the simple coacervation method and cross-linking using epichlorohydrin or glutataldehyde for the controlled release of diclofenac sodium [123]. Lu et al. reported that the mean residence time of flubiprofen is two-fold that of injection of the drug suspension [124]. Similar observations were registered for a celecoxib solution compared to celecoxib-embedding chitosan microspheres, where a 10-fold increase in the celecoxib concentration in the joint was achieved after i.a. injection [125].

Most microsphere systems explored in the context of IBD therapy are in the size range of 10-300 μm, making them too large to specifically target the inflamed intestinal tissue, or to be internalized by immune competent cells. The drug delivery systems are based on non-coated or coated cores of chitosan (Figure 7) [126] and chitosan-Ca-alginate [127]. Even multidrug systems are feasible as demonstrated on the example of a combined sulfasalazine and betamethasone formulation [128]. By

combining different controlled release technologies, such as polymeric or swellable particle matrix, enzymatic cleavage of the drug carrier and pH sensitive coating not only improve the colon targeting efficacy but also increase the risk of insufficient drug release due to pathophysiological idiosyncrasies. Some passive targeting potential, in particular to the innate immune system, may be attributed to small microparticulate formulations (< 10 μm). Mladenovska et al. studied chitosan-Ca-alginate picroparticles prepared by spray drying and observed that they were 6.2- 8.6 μm in size and not enteric coated [127]. The 5-ASA release from this system is controlled by the swelling of the particles with relatively low dissolution at pH 1.2. At pH 6.8, the release rate was comparable to the one in pH 1.2, as the lower solubility of 5-ASA at this pH compensates for the higher deprotonation of the chitosan. Only in the presence of colonic enzymes and bile salts was there a faster release due to matrix degradation and increased solubility of 5-ASA. The modelling of release kinetics revealed a major influence on the drug dissolution and its diffusion in the aqueous paths created in the matrix. Biodistribution studies confirmed the colon targeting of the formulation, as lower systemic bioavailability from the microparticles was observed than from the free 5-ASA solution. Most of the 5-ASA was delivered to the colon, reaching a peak after 10 h. There was a notable microparticle distribution to the spleen, associated to the uptake of smaller microparticle fractions of < 4 μm by gut-associated lymphatic tissues. Furthermore, there was a significant percentage of particle retention in the stomach, as highly charged particles adhered to the stomach mucosa and the acetylated metabolite of 5-ASA accumulated in the exposed tissue.

The colon targeting of the formulation was improved compared to the non-coated formulation with 56% of 5-ASA in the colon after 10 h and 40% after 24 h. Thus the optimal time-frame for drug release was considered to be 10 to 15 h.. Freeze-drying prepared 5-ASA loaded N-succinyl-chitosan matrixes had a comparable *in vivo* anti-inflammatory effect to negatively charged spray-dried microparticles (5.1 ± 2.2 μm) [129].

There are several classes of NSAID that have a different chemical structure, serum half life and pharmacokinetics, which have proven to be an effective alternative to steroidal agents in the topical

management of ocular inflammations. Currently, these drugs have been used topically in the management of postoperative inflammation, inhibition of intraoperative miosis, treatment of seasonal allergic conjunctivitis, prevention and treatment of cystoid macular oedema and in pain control [130]. Substantial efforts have been directed towards the development of ocular drug delivery systems able to prolong drug retention i.e., allowing it to remain in contact with the cornea for longer periods thus increasing bioavailability [59]. Celecoxib, is a small molecule COX-2 inhibitor capable of inhibiting prostaglandin secretion, VEGF expression and oxidative stress in retinal cells. The periocular delivery of celecoxib to the retina is several-fold higher than is systemic administration. The retinal delivery of celecoxib following periocular administration can be sustained for a few months using sustained-release microparticulate systems. Therefore, a periocular approach of administering the drug by injection closer to the retina for celecoxib delivery was studied, to avoid systemic effects [131]. Although intravitreal injections and surgical implants avoid these systemic effects, they are associated with complications such as retinal detachment and cataracts. As the sclera is shown to be more permeable than the cornea and has a large surface area for sustained drug delivery, transcleral routes are emerging as alternatives to topical and intravitreal modes of administration for the treatment of retinal disorders. Through this route, drugs are administered adjacent to the sclera and reach the retina after passing the sclera and underlying tissues, including the choroid-Bruch layer and the retinal pigment epithelial layer [132]. The principle elimination pathway for celecoxib (loss to the systemic circulation) after periocular administration is through the periocular circulation and lymphatics, which is about an order of magnitude higher than loss to the systemic circulation by choroidal circulation. The pharmacokinetic modeling further indicate that suspensions are better than solutions in increasing delivery duration to the retina following periocular administration, which is applicable for celecoxib as well as other drugs. Moreover, Amrite et al. also demonstrated, by the use of simulations, that celecoxib delivery to the retina can be sustained for prolonged periods by designing systems able to release celecoxib slowly after administration into the periocular tissue [133]. Simulations using

celecoxib as a model drug have demonstrated that slow release particulate systems with low clearance by the periocular blood and lymphatic circulation allow for better sustainment of celecoxib delivery to the retina following periocular administration [134].

Sustained release delivery systems, such as particulate systems, can be designed to provide prolonged delivery of the drug to the retina. Amrite and Kompella investigated the distibution of particulate systems after periocular administration and reported that therapeutically effective levels of celecoxib in the retina can be achieved at 2 months post-periocular administration of celecoxib-PLGA microparticles > 200 nm in diameter. Small nanoparticles (20 nm) are rapidly cleared from the site of administration after periocular administration and are not suitable for sustained delivery to the retina. Therefore, they argued that polymeric microparticles sustain retinal delivery of celecoxib better than nanoparticles due to their lower surface, volume ratio and much lower clearance from the periocular site of administration [134].

It has been shown that the transscleral route provides significantly higher levels of the drugs to the retina than the systemic mode of administration. Ayalasomayajula and Kompella demonstrated that celecoxib delivery to the retina is 54-fold higher following subconjunctival administration that can be obtained by systemic administration. The authors also demonstrated that the delivery of the drug to the contralateral eye is similar to the intraperitoneal and subconjunctival routes of administration. Based on their assessments, > 95% of the celecoxib is available to the ipsolateral retina through local routes and there is most likely a direct penetration of celecoxib through the sclera and choroid-RPE as well as into the retina through the transscleral pathway [135].

Microemulsion systems might well offer a solution to the problem of poor delivery to the cornea, by sustaining the release of the drug as well as by providing a higher drug penetration into the deeper layers of the eye. Moreover, they have the potential of increasing the solubility of the drug in the ophthalmic delivery vehicle. Klang et al. carried out an *in vivo* evaluation of the corneal penetration of indomethacin from a commercial hydro-poly (ethylene glycol) ocular solution and compared it to a negatively and to a positively charged microemulsion. The microemulsion formulations provided

significantly higher indomethacin levels to the aqueous humor and sclera-retina than did the commercially available eye-drop solution [136]. Furthermore, the spreading coefficient of the positively charged microemulsion on cornea was 4-fold higher than that of the negatively charged microemulsion, revealing that positively charged microemulsions have better wetability properties on the cornea than either commercial solutions or negatively charged microemulsions. These data were attributed to the enhanced residence time of the drops on the epithelial layer provided by the positive charges, thus permitting a better drug penetration through the cornea to the internal eye tissues. To date, a range of other NSAID has been formulated in a microemulsion in an attempt to sustain release and, in general, these studies have shown that it is possible to delay the effect of a drug incorporated into a microemulsion, thereby improving bioavailability [78, 137].

Nanocarriers

Nanoparticles, composed of various biodegradable polymers e.g., PLA, poly(alkyl cyanoacrylate) (PACA), PLGA, poly(epsilon-caprolactone) (PCL), as well as natural polymers such as chitosan, gelatin, sodium alginate and albumin, have shown promising results over the last decade for efficient NSAID drug delivery to the tissues [10, 138].

Lamprecht et al. were the first to explicitly focus on nanoparticles and their passive accumulation in inflamed intestinal areas for the development of improved drug carrier systems [32].

The treatment of IBD and local delivery of NSAID can be done using nanoparticle matrices that have been engineered to favourably collapse within the targeted area, taking advantage of specific characteristics of the inflamed colon, such as pH [139], release of oxidative species [140] and osmotic systems [141]. Moreover, biomaterials such as hydrogels have biological, physical, and chemical characteristics that make them major candidates for nanoparticles vectorization. Hydrogels are a network of hydrophilic polymer chains thay may contain up to 99% of water. Hydrogels can protect the drug and/or the drug vector until such times as the targeted organ is reached. They then dissolved under particular conditions including pH, time, temperature or

enzyme activity. As aforementioned, the hydrogel has to be selected and/or engineered to be degraded at a specific gastrointestinal location, such as the colon [142].

One of the most important issues associated to polymeric nanoparticles is their burst release in the first 60 to 120 min due to amorphous drug adsorbed close to the particle surface and the huge surface area provided by the nanoscaled carriers. If applied orally, this initial drug release from PLGA/PLA/PCL nanosystems will take place during the gastric or small intestinal passage and the drug will either be degraded in the gastric environment or taken up into systemic circulation, thus reducing the available drug amount at the target site in the distal ileum and colon and increasing adverse effects. Pertuit et al. demonstrated the therapeutic potential of polymeric nanoparticles for the treatment of IBD with 5-ASA, also known as mesalazine or mesalamine, as a target drug delivery systems to inflammation tissues [143]. They covalently coupled 5-ASA to PCL and prepared particles from this modified polymer by using the emulsion solvent evaporation and nanoprecipitation method, giving particles of 200 to 330 nm in size, an almost neutral charge and 3.4±0.5 μg/ml loading rates. In a TNBS mouse model of colitis, oral administration of 5-ASA PLGA nanoparticles, produced by double emulsion solvent diffusion (0.5 mg/kg body weight 5-ASA) proved slightly less efficient than 5-ASA solution at 100 mg/kg and comparable to 30 mg/kg 5-ASA solution as quantified via clinical activity score [142]. Mahajan et al. developed nanoparticles of comparable size with high negative zeta potential (−50mV). Drug loading rates were surprisingly high at 1.5% to 2.5%, even if a substantial burst release (> 50%) of the very hydrophilic drug from the carrier can be expected. Both orally and rectally applied nanoparticles improved the clinical activity compared to the free 5-ASA solution and maintained activity after *in vivo* dosing of acetic acid in a colitis model [144].

Protection from burst release was observed when mesalamine was coupled to the surface of 3-(aminopropyl)-trimethoxysilane activated silica nanoparticles, via a succinimid anhydride linker. No drug release was observed in PBS pH 7.4, but complete release occurred within 48 h in simulated intestinal fluid, due to enzymatic cleavage by enzymes, such as pancreatin. An 8 h lag

time in drug release was observed and is thought to suffice to allow the nanoparticles dosage form to reach the colon and avoid absorption in the gut and small intestine. It was observed that fluorescently labelled particles accumulate in the inflamed tissue to a 6-fold higher degree than in to healthy regions, in the TNBS model. Clinical activity of mesalamine silica nanoparticles was comparable to a 5-ASA solution at 100 mg/kg and clearly superior to 5-ASA solution at 50 and 30 mg/kg. Making it evident that a significant dose reduction can be achieved employing this formulation strategy. A peptide-loaded polymeric nanoparticle formulation was also reported as a novel therapeutic approach in IBD treatment [145].

In topical administration onto problematic tissue, like the eye without bioadhesion, nanoparticles are eliminated from the precorneal site almost as quickly as are aqueous solutions. This prompted investigation into the use of mucoadhesive polymers to the negatively charged cornea. The cationic polymer chitosan, a deacetylated chitin, has attracted a great deal of attention because of its unique properties, such as acceptable biocompatibility, biodegradability and ability to enhance the paracellular transport of drugs.

Araujo et al. developed a corneal device to study any modifications in the corneal structures after application of nanomedicines or free drugs to the eye [29]. The usual conditions for the permeation studies had to be modified to reproduce the behaviour when eye-drops are administered; leading to the development of a new tetra-compartmental pharmacokinetic model. These corneas can be subjected to scanning electron microscopy, to determine possible modifications of the corneal structures after the permeation studies. Chitosan was the most studied polymer, with several reports claiming its low toxicity and good biocompatibility following topical administration to rabbits and cell cultures [146, 147]. There are few studies on *in vivo* toxicology and NSAID-loaded nanoparticles and no clinical studies have yet been performed. So as to improve the ocular availability of the drug, polymeric nanoparticle suspensions were prepared from poly (lactide-co-glycolide) and poly (lactide-co-glycolide-leucine) biodegradable polymers and loaded with diclofenac sodium. Nanoparticle suspensions were prepared by the emulsion and solvent

evaporation technique and characterized on the basis of physicochemical properties, stability and drug release features. *In vitro* release showed that the nanoparticles had an extended-release diclofenac sodium profile. To verify the absence of irritation towards the ocular structures, blank nanoparticle suspensions were applied to the rabbit eye and a modified Draize test performed. Polymer nanoparticles seemed to be devoid of any irritant effect on the cornea, iris, or conjunctiva for as long as 24 h after the application in rabbit eyes [148].

Corneal permeation of two anti-inflammatory drugs i.e., diclofenac and flurbiprofen loaded to cyclodextrins or polymeric nanoparticles was evaluated. An *ex vivo* study was performed in order to determine any differences in their corneal permeation compared to free drug and/or commercial eye drops. Some studies have proven that polymeric nanoparticles of poly (D-L lactic-coglycolyc) acid and poly-epsilon-caprolacton are efficacious in the intraocular targeting of NSAID, with poly-epsilon-caprolacton being more effective in increasing flurbiprofen corneal permeation [149, 150].

Flurbiprofen and ibuprofen were also incorporated into polymeric nanoparticle suspensions, prepared from Eudragit RS100R and RL100R polymer resins, with the aim of improving the drug availability at an intra-ocular level to prevent myosis being induced during extracapsular cataract surgery. Nanosuspensions were prepared by a quasi-emulsion solvent diffusion technique. The resulting nanoparticles showed a controlled drug release profile from the nanoparticles. *In vivo* anti-inflammatory efficacy was assessed in the rabbit eye after induction of an ocular trauma (paracentesis) and drug-loaded nanosuspensions showed no toxicity on ocular tissues. Moreover, the inhibition of the miotic response to the surgical trauma was comparable to a control eye-drop formulation [151-153].

Nanoparticle systems have the potential to improve the reach of aspirin in the posterior chamber, whilst, at the same time, to mask its undesiderable toxic effects. Reduction of the particle size causes minimal irritation, increase in precorneal residence time, overcomes poor drug solubility, reduces the therapeutic dose requirement and chances of toxicity, compared to the conventional formulations [154]. Biodegradable and biocompatible albumin nanoparticles have proven to be

particularly efficacious in the administration of various ocular drugs [25].

Dias et al. developed an aspirin loaded albumin nanoparticle prepared by the desolvation based coacervation method of microencapsulation to deliver aspirin to the posterior chamber of the eye. An *in vitro* release kinetics study shows that nanoparticles are capable of releasing the drug for as long as 72 h with 50% release occurring at 12 h, unlike the free drug, which peaks at 1/2 h and saturates at 1 h, allwoing this system to overcome the burst effect of the free drug [154].

One of the challenges in ophthalmic drug delivery is to increase the residence time of the formulation without causing irritation or blurring [155]. This can be achieved by the addition of biodegradable polymers, such as viscosity enhancers. The authors used xanthan gum (0.5%) to enhance the viscosity of the system. The release kinetics, in the presence of artificial tear and artificial tear containing lysozyme, was studied to simulate the ocular conditions and the release profile is quite similar in both cases. A 50 % drug release is observed around 30 h in the presence of tears and in 40 h in presence of lysozyme. An almost 100 % release is attained at about 80 h. The release profiles in the presence of artificial tears and lysozyme show that nanoparticles are capable of releasing aspirin in a slow and sustained manner, even in the presence of tear fluid. However, comparison with the release profiles of control (in the absence of tear fluid) shows that, although there is the same release pattern, they are not identical. That is to say, a slightly slower release pattern can be observed in the presence of tear solution. This finding may be due to the presence of charged moieties in the system, which affects the drug release. The pharmacokinetic pathway was simulated through the three compartment diffusion chamber. The first significant barrier a topically administered drug encounters is the cornea. It then has to pass through the aqueous humor to reach the anterior plane of the vitreous. After crossing the thick vitreal gel, it reaches the posterior plane of vitreous and retrovitreal tissue. Therefore, the drug, which reaches the third compartment with phosphate buffer in the middle, may be considered equivalent to the drug reaching the anterior plane of the posterior chamber. Similarly, the drug reaching the third compartment crossing the vitreous in the middle is equivalent to the drug that arrives at the posterior plane of the vitreous and

retrovitreal structures.

It has been observed that the amount of drug reaching the receiving compartment through vitreous is lower than that found in the presence of phosphate buffer in the middle compartment. Indeed, only about 11% the entrapped drug was detected at the end of 72 h in the presence of vitreous, whereas 75% of the drug reaches the third compartment in the presence of phosphate buffer at 72 h. The pharmacological efficacy of the formulation was determined by measuring the total inhibition of platelet aggregation produced when platelet rich plasma was incubated for 1 h with 0.18 mg/ml of aspirin loaded albumin nanoparticles. An almost total inhibition of platelet aggregation can be achieved with aspirin nanoparticles. Similar results were also obtained using one third of the therapeutic dose of the free drug. Aspirin released from nanoparticles inhibit platelet aggregation in a time dependent manner. Prevention of platelet aggregation was almost 50% at the end of 1 h compared to control platelet rich plasma. Indicating that drug-loaded nanoparticles are pharmacologically active and sustained release of aspirin from the carrier prevents platelet aggregation [154].

The limiting factor for the development of transdermal delivery systems for therapeutic agents is the low permeability of drug molecules through the stratum corneum and numerous studies have been carried out in an effort to enhance the permeability of drug molecules. There are various drug pathways that may be chosen in the stratum corneum: the transcellular pathway, intracellular pathway and trans-accessory pathway, but the transcellular pathway seems to be the most suitable. It has been reported that the space of this pathway is 50-70 nm. Tomoda et al. developed indomethacin (model drug) and coumarin 6 (fluorescent marker)-loaded PLGA nanoparticles with diameters of 100 nm and studied the permeability of the nanoparticles through rat abdominal skin. A higher amount of indomethacin was delivered through the skin when indomethacin was loaded in nanoparticles than when indomethacin was in free molecules. Moreover, iontophoresis was applied to enhance nanoparticles permeability. When iontophoresis with 3 V/cm was applied, the

indomethacin permeability was much higher than that obtained by the simple diffusion of nanoparticles through the skin [156].

Other researchers attempted to verify the penetration of nanoparticles across the skin, but found that only a few nanoparticles were capable of penetrating passively into the skin through hair follicles, whilst most of the nanoparticles were primarily restricted to the uppermost stratum corneum layer and were not able to penetrate the skin [157]. As a rule, the amount of the drug loaded into the nanoparticles increases as does the size of the nanoparticles. Conversely, the larger the nanoparticles, the less capable they are of permeating into the skin. Therefore, so as to achieve maximum drug penetration, both the drug loading efficiency and the nanoparticle size should be taken into consideration when they are applied for topical drug administration. Even if fewer large nanoparticles deposited into the whole skin than did the smaller nanoparticles, the ratio of the amount of nanoparticles in the dermis to the epidermis increased as did the nanoparticle size. This phenomenon may be explained by the encapsulation of more feeding drug within each of the larger nanoparticle, even if fewer of these nanoparticles permeated into the dermis. Since the particle size could influence the penetration of the nanoparticles, the polydispersity index would also affect the penetration. Therefore, reducing the polydispersity index would decrease the variance of the penetration. The permeation rate increased with an increase in the nanoparticle concentration, but that there was a gradual fall in the extent of the increase. This may be due to the fact that the skin was saturated and the permeation reached a limit value when the concentration gradually increased, therefore, an appropriate concentration with the maximal penetration and the minimum drug-cost should be considered when nanoparticles are used for topical drug administration. On basis of *in vitro* experiment results, the biodegradable PLGA nanoparticles could sustain drug release in the skin over a prolonged period [158].

Sashmal et al. designed and evaluated NSAID (flurbiprofen as model drug) loaded nanoparticles with suitable size range to concentrate at inflammation sites by solvent diffusion nano-precipitation

method. Optimized formulations were found to have a polymer-drug ratio of 19:1 and a nonsolvent-solvent ratio of 4:1 to maximize both releases after 8 h and particle size [159].

Two different chitosan nanocarriers i.e., nanoparticles and nanoemulsion, were developed to prolong indomethacin precorneal residence time and improve ocular bioavailability, the main limitations in indomethacin management of post-operative inflammation and intraocular irritation after cataract extraction. Chitosan-nanoparticles were developed by modified ionic gelation of chitosan with tripolyphosphate, while nanoemulsion was prepared by the spontaneous emulsification technique. *In vitro* release studies, performed under sink conditions, revealed small initial burst release during the first hour, followed by slow gradual drug release of 76 and 86% from nanoparticles and nanoemulsion respectively over a 24 h period. *In vivo* studies and histopathological examination revealed that rabbit eyes treated with nanoemulsion showed clearer healing of corneal chemical ulcer with moderate effective inhibition of polymorph nuclear leukocytic infiltration than did nanoparticle preparation. Moreover, it was possible to achieve a therapeutic concentration of indomethacin in the cornea after topical instillation of chitosan nanoemulsion to rabbits, throughout the study period, with a fairly high indomethacin level in the inner ocular structure, aqueous humor. These levels were significantly higher than those obtained after the instillation of indomethacin solution [160].

SLN, like liposomes, are preferentially employed in parenteral and/or topical drug administration. However, as their stability is increased due to their solid matrix, they are capable of withstanding also the conditions found in the upper gastrointestinal tract. Lipid nanoparticles with solid matrix demonstrated high drug loading (both hydrophilic and lipophilic drugs), long-term shelf stability and hassle-free large-scale production [161].

Attama et al. preparated sodium diclofenac–loaded lipid nanoparticles by combining the homolipid from goat (goat fat) to a phospholipid, with high encapsulation efficiency applying the hot high-pressure homogenization technique [162].

Lipid nanoparticles are able to improve drug solubilisation in the gastrointestinal tract due to their

ability to retain a poorly soluble substance in a solubilised state and to enhance solute-solvent interactions also after mixing with endogenous solubilizers such as bile acids andphospholipids.. Moreover, the protective effect of lipid nanoparticles, coupled with their sustained/controlled release properties, prevents premature degradation of the drugs/macromolecules and improves their stability in the gastrointestinal tract [163]. Their nanoparticulate state facilitates their uptake by M cells in Peyer's patches, which, in turn, enables the carrier system to bypass the effect of first-pass metabolism, through lymphatic absorption. Lipids are able to promote oral absorption of the encapsulated drugs via selective lymphatic uptake. Moreover, small particles, ranging between 120 and 200 nm rarely undergo blood clearance by the reticuloendothelial system (i.e., liver and spleen filtrations are avoided). The reduction of side effects (i.e., stomach toxicity) and masking of taste are also two relevant goals for oral administration of lipid nanoparticles [164].

Moreover, it has been postulated that the carrier size may well play an essential role in achieving efficient carrier penetration into cartilage. This aspect was investigated and reported by Rothenfluh et al for nanoparticles coated with collagen II-binding peptide. The authors used fluorescence measurement and demonstrated a 14.9-fold preferential accumulation of nanoparticles with an average diametre of 38 nm within the cartilage relative to 96 nm diameter nanoparticles. This significant difference was attributed to the 60 nm pore size of the dense collagen network [41].

Diclofenac was also incorporated in SLN made up of a combination of homolipid from goat (goat fat) and phospholipids and were evaluated for diclofenac sodium delivery to the eye using bio-engineered human cornea, produced from immortalized human corneal endothelial cells, stromal fibroblasts and epithelial cells CEPI 17 CL 4. Encapsulation efficiency was high, with a sustained diclofenac release and high permeation [162].

In another study with PLGA, Vega et al. applied an experimental design to optimize a formulation of flurbiprofen loaded nanoparticles where the particle surface was coated with Poloxamer 188. Drug entrapment efficiencies of over 90 % were obtained, and *in vitro* release studies showed an initial burst release, followed by a slower release, which completed after 90 min. *In vivo* studies

performed in rabbits demonstrated that the formulations did not induce toxicity or irritation. Nanoparticle formulations were compared to commercial eye-drops after the induction of inflammation by instillation of sodium arachidonate. The results indicated a very good anti-inflammatory efficacy for flurbiprofen-loaded PLGA nanoparticles, providing controlled and continuous drug delivery [165-167].

Lipid nanoemulsions were studied as carriers for NSAID, such as diclofenac and flurbiprofen. The use of lipid emulsions as drug carriers was impeded by two major limitations i.e., limited extravasation into extravascular spaces and rapid clearance by the reticuloendothelial system. Improved extravasation and minimization of rapid clearance can be achieved by decreasing the size of the colloidal particles or by ligand specific delivery or a combination of these. Targeted drug delivery with specific ligand at an optimal rate improves the efficacy, therapeutic index and reduces the possibility of undesirable side effects of the drug. The inflammatory tissue (like tumor tissue) exhibits physiologic features of extravasation, passive accumulation of albumin due to hypoalbuminemia and expression of albondin receptors for receptor-mediated endocytosis for albumin [168]. Wunder et al. reported the passive accumulation of albumin at the inflammatory arthritic paws. The properties of albumin, such as ready availability, biodegradability and lack of toxicity and immunogenicity make it an ideal ligand for drug delivery [169]. Kandadi et al. developed albumin coupled diclofenac lipid nanoemulsions for targeted delivery to inflammatory sites. The lipid nanoemulsions were prepared by the hot homogenization and ultrasonication process. Formulations were injected into rats with granuloma air pouch induced inflammation. The main purpose of using stearylamine in the formulation was to attach albumin ligand to the emulsion globules. The stearylamine contained a long (C-18) fatty chain and amino group, which was able to hold the albumin intact at the globule surface after attachment. Intravenous administration of diclofenac lipid nanoemulsions produced a sustained *in vivo* anti-inflammatory effect in a rat paw oedema model.

These results, conducted in inflammation (granuloma air pouch model) induced rats, show a better

pharmacokinetic profile and a higher therapeutic availability of albumin coupled diclofenac lipid nanoemulsions, than that of drug solution. This clearly indicated the preferential accumulation of albumin coupled diclofenac at the inflammatory sites [168].

Drug nanocrystals can be considered as a universal formulation approach for poorly soluble drugs. The striking advantage is that the drug nanocrystals can be applied through various administration routes, including ophthalmic administration, to create systems with prolonged retention times. Moreover, drug nanocrystals systems are simple to produce. Currently there are few studies investigating NSAID in the form of nanocrystals as the major prerequisite for a nanocrystal formulation is the hardness of the drug crystals themselves [29].

In summary, microencapsulation is a useful technique to incorporate NSAID to reduce toxicity and improve efficacy, several evidence have been accumulated to target colonic IBD in particular of 5-ASA. Nanoparticles can also deliver efficiently NSAID to the colon in chemical induced colitis animal model and to the internal posterior chamber of the eye without toxic local effects. In particular, solid lipid nanoparticles can improve gastrointestinal absorption of the incorporated drug with preferential accumulation at inflamed sites. Nevertheless especially for intra-articular and ocular drug delivery only proof of concept studies have been performed without robust evidences about efficacy and toxicity.

Conclusions

Anti-inflammatory therapy usually necessitates frequent dose administration and, as the adverse effects of glucocorticoids and non-steroidal anti-inflammatory drugs are well known, this often implies a high risk of an increase in patient noncompliance. The last few years have witnessed the study and development of various approaches aimed at overcoming these problematic issues. At time of writing, although numerous pharmaceutical companies are working towards the commercialization of some advanced systems, further development is needed to achieve more satisfactory results, especially those involving *in vivo* studies and human clinical trials. As there is a paucity of clinical studies on ocular and intra-articular drug delivery-system approache in literature and those that have been published are mainly on liposomes, these approaches are, to-date, still in the experimental stage. Therefore, we urge more research on the *in vivo* qualities of such systems so that these studies may reach the clinical stage and be of benefit.

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Figure legends

Figure 1 Flowchart showing the mechanism of action of glucocorticoids (GC) and non steroideal anti-inflammatory drugs (NSAID). Arachidonic acid (AA) is released from cell membrane in response to noxious stimuli. It is cleaved by phospholipase A2 (PLA2) and it becomes the substrate of cyclooxygenase (COX) in order to generate prostaglandins (PG).

Figure 2 Amount of betamethasone in the epidermis, dermis and receptor medium of Franz cells (μg/cm2). R1: solution of betamethasone in absolute ethanol, R2: aqueous solution of betamethasone–HPγCD inclusion complexes and R3: dispersion of phosphatidylcholine and betamethasone in Hepes buffer (n = 9). Figure from reference 65.

Figure 3 Dexamethasone concentrations in aqueous humour after administration to rabbits. Circles are PHEA-PEG-C16 micelles; squares are dexamethasone suspension. Each data point corresponds to the mean dexamethasone concentration in ng/ml + SEM determinated in the aqueous humour at each sample time. *Student's t-test, $P < 0.05$. Figure from reference 82.

Figure 4 Paw inflammation rate of AA rats. Arthritis was induced in Lewis rats as described under Materials and Methods. Inflammation rate (percentage) = (measured leg volume - leg volume without adjuvant)/(leg volume on day 0 - leg volume without adjuvant) × 100. The mean (point) ± S.D. (line) of seven rats from days 0 to 9 in each group is shown. A, ○; type A stealth nanosteroid (40 μg); •, type A vehicle; ▵, BP (40 μg); ▿, BP (120 μg). **, $p < 0.01$ (40 μg of type A versus 120 μg of BP). B, ○, type A stealth nanosteroid; ▵, type B stealth nanosteroid; ▿, type C stealth nanosteroid; □, type D stealth nanosteroid; □, type NS nanosteroid. **, $p < 0.01$ (type A versus type NS). ††, $p < 0.01$(types B-D versus type NS). Figure from reference 94.

Figure 5 Budesonide nano- and microparticles sustained ocular tissue levels of budesonide after subconjunctival administration in rats. Budesonide was administered in the eyes of rats, either in the form of a solution (50 or 75 μg to one eye; small and large open circles, respectively), nanoparticles (50 μg to one eye; small filled circle), or microparticles (75 μg to one eye; large filled circle), and drug levels were estimated in (A) retina, (B) vitreous, (C) cornea, and (D) lens. Data are expressed as the mean ± SD of results in four experiments. Data are shown for the ipsilateral eye. Drug levels were below detection limits in the contralateral eye. Also, budesonide levels were below detection limits on day 14 in the solution and nanoparticle groups. Figure from reference 77.

Figure 6 Concentration of dexamethasone acetate (DA) in the pleural exudate after the intravenous administration of free DA and DA-NLCs to rats with experimental pleuritis (dose, 3 mg/kg). Each point represents the mean ± S.D. of 5 rats for each group. Figure from reference 101.

Figure 7 Drug distribution study in various parts of gastrointestinal tract following oral administration of Eudragit®S-100 coated chitosan microspheres bearing 5-ASA. Figure from reference 126.

Figure 1

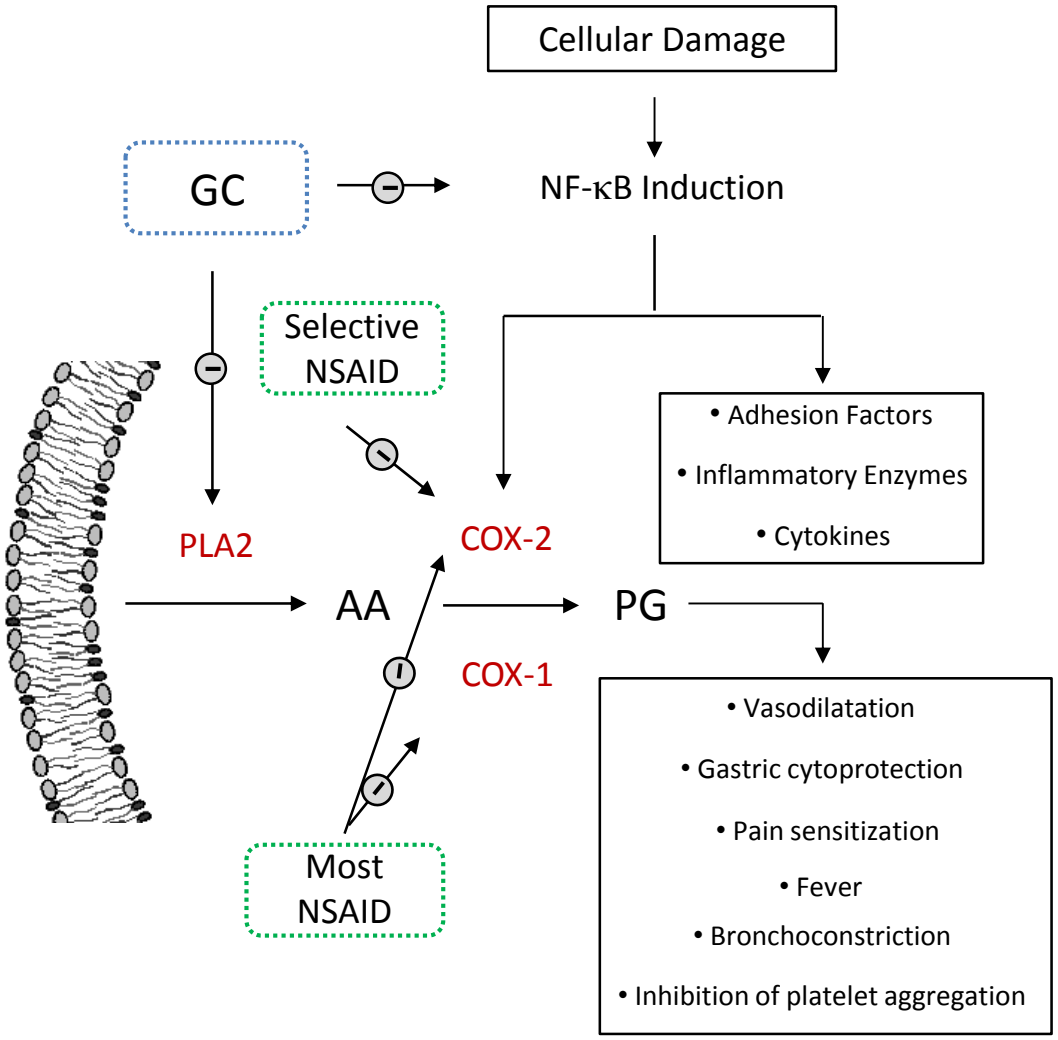
Cellular Damage
GC
NF-κB Induction
Selective NSAID
• Adhesion Factors
• Inflammatory Enzymes
• Cytokines
PLA2
COX-2
AA
PG
COX-1
• Vasodilatation
• Gastric cytoprotection
• Pain sensitization
• Fever
• Bronchoconstriction
• Inhibition of platelet aggregation
Most NSAID

Figure 2

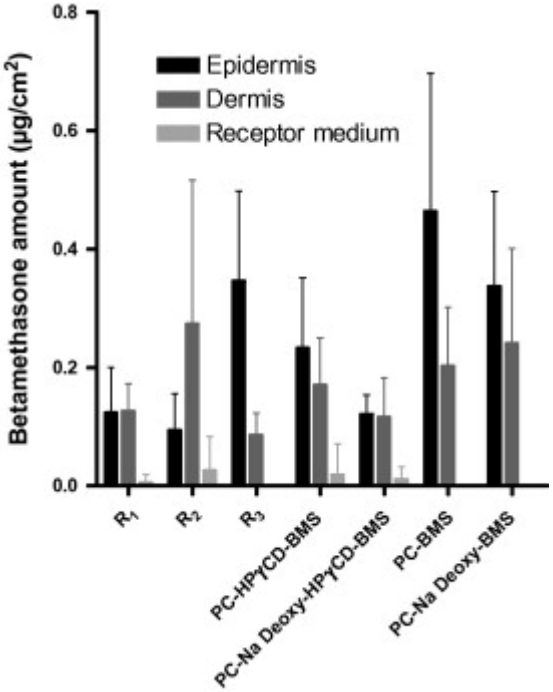
Epidermis
Dermis
Receptor medium
Betamethasone amount (µg/cm²)
0.8
0.6
0.4
0.2
0.0
R1
R2
R3
PC-HPγCD-BMS
PC-Na Deoxy-HPγCD-BMS
PC-BMS
PC-Na Deoxy-BMS

Figure 3

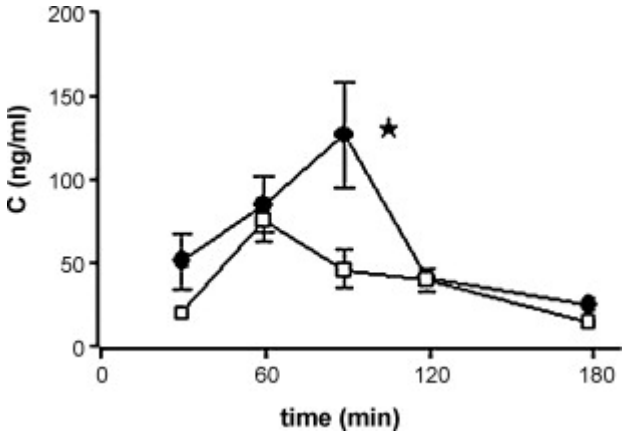

Figure 4

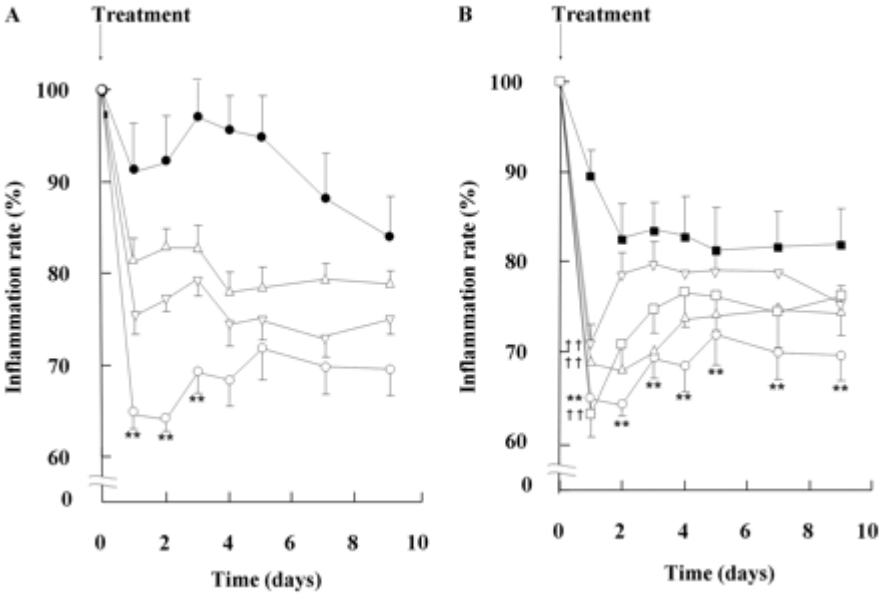
A
Treatment
Inflammation rate (%)
100
90
80
70
60
0
0
2
4
6
8
10
Time (days)
B
Treatment
Inflammation rate (%)
100
90
80
70
60
0
0
2
4
6
8
10
Time (days)

Figure 5

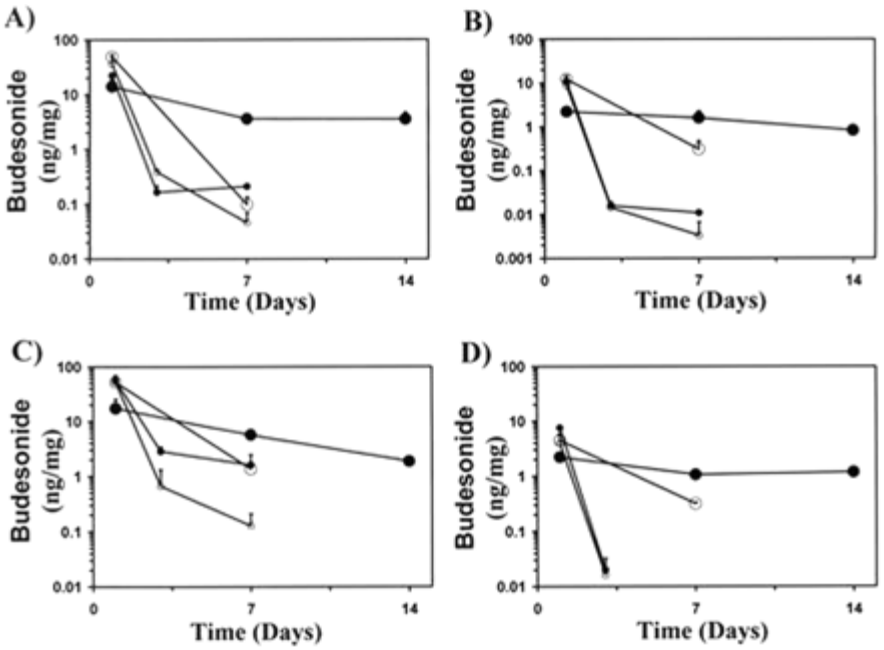

Figure 6

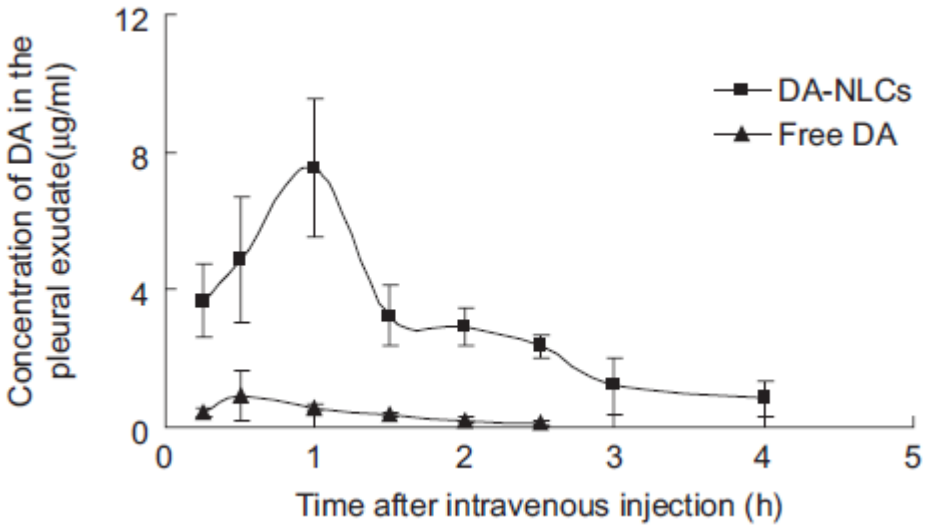
Concentration of DA in the pleural exudate(μg/ml)
12
8
4
0
0
1
2
3
4
5
Time after intravenous injection (h)
DA-NLCs
Free DA

Figure 7

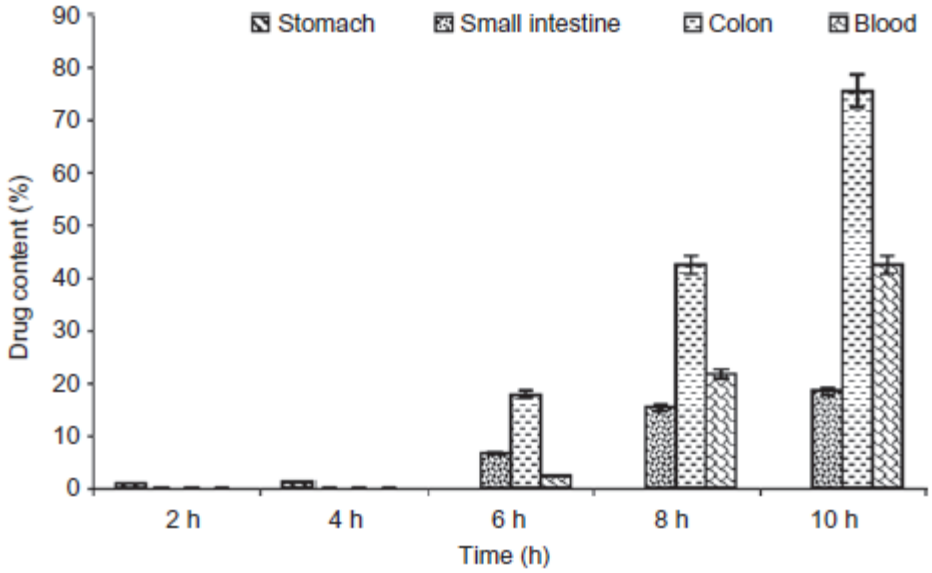
Stomach
Small intestine
Colon
Blood
Drug content (%)
90
80
70
60
50
40
30
20
10
0
2 h
4 h
6 h
8 h
10 h
Time (h)

Table I Pharmacokinetic properties of selected glucocorticoids (GC)

GLUCOCORTICOIDS	Anti-inflammatory potency	Half life (h)	Equivalent dose (mg)
Cortisol	1	8-12	20
Betamethasone	25	36-54	0.75
Dexamethasone	25	36-54	0.75
Fludrocortisone	10	12-36	-
6-α-Methylprednisolone	5	12-36	4
Prednisolone	4	12-36	5
Triamcinolone	5	12-36	4

Table II Pharmacokinetic properties of selected non steroideal anti-inflammatory drugs (NSAID)

NSAID	Time to peak plasma level (h)	Half life (h)	Binding to plasma protein (%)	Urinary excretion (%)
Acetylsalicylic acid	0.40	0.25	49	1.5
Diclofenac	2-3	1-2	99	65
Flurbiprofen	0.5-4	6	99	95
Ketoprofen	1-2	2	99	90
Ketolorac	0.5	4-6	99	90
Ibuprofen	0.5	2	99	90
Indomethacin	1-2	2.5	90	15
Naproxen	2-4	14	99	90
Sulindac	1-2	7-18	90	80
Celecoxib	2-4	11	97	57
Rofecoxib	2-3	17	87	72