

**An overview of Chrono-pharmaceuticals drug delivery systems: Recent advances**Minakshi Verma¹, T. Maity², Ashish K. Gupta³, Renu Singh², Vani Sharma², A. Mittal², B C Nandy², Anil K. Gupta^{2*}¹ Department of Pharmacy, G.S.V.M., Medical College Kanpur, Uttar Pradesh, India² Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India³ Department of Pharmacy, V.B.S., Purvanchal University, Jaunpur, Uttar Pradesh, India.

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ABSTRACT

Chrono-pharmaceuticals includes pharmaceutical application of "Chronobiology" in drug delivery. Chronobiology is the study of biological rhythms and their responses to other metabolic functions of body. Diseases such as bronchial asthma, hyper-cholesterolemia, ulcer, diabetes, arthritis, myocardial infarction, angina and hypertension show symptomatic changes due to circadian rhythmicity. The chronobiology, chronopharmacology and chronotherapeutics of pain have been extensively reviewed. For instance, there is a circadian rhythm in the plasma concentration of C - reactive protein and interleukin-6 of patients with rheumatoid arthritis. Rheumatoid arthritis, level of C - reactive protein increases early morning leading to enhanced pain and inflammation. Chronotherapy for all forms of arthritis using NSAIDs should be timed to ensure that the highest blood levels of the drug coincide with peak pain. Circadian rhythms of behavior in mammals are known to be robust and precise. Also several drugs cause alterations to 24 hours rhythms leading to illness and altered homeostatic regulation. Alteration in biological rhythm is also a novel concept of adverse effects, which can be minimized by optimizing the dosing schedule.

Key words: Chronobiology, chronopharmacology, chronotherapeutics.

1. INTRODUCTION:**1.1 Modified release dosage form" (MRDF) [1-3]:**

In 1985 the USP adopted the term "Modified release dosage form" (MRDF) to avoid confusion between the different manufacturer's descriptive terms. It is defined as one for which the drug release characteristics of time course and/or location are chosen to accomplish convenience objectives not offered by conventional dosage forms such as solutions, ointments or promptly dissolving dosage forms.

The USP also recognizes two distinct types of modified release dosage forms:-

- i) Extended release and
- a) Prolonged/sustained release or
- b) Controlled release.
- ii) Delayed release

i) Extended release dosage form:

It is one that at least a two-fold decrease in dosage frequency compared to the conventional immediate release dosage form after single administration.

Extended release dosage form are further subdivided into two categories, those are-

a) Prolonged/sustained release:

Prolonged or sustained release dosage forms are designed to release the drug substance slowly over an extended period of time

b) Controlled release:

Controlled release systems imply some predictability and reproducibility of Drug release. Initially a loading dose is released to obtain a rapidly achieved therapeutic blood level, followed by a slower, more constant release of the drug where the amount eliminated from the body is being constantly replaced. Controlled release products provide prolonged delivery of a drug while maintaining its blood concentration within therapeutic limits.

ii) Delayed release dosage form:

It is defined as one that releases the drug substance at a time other than immediate release of drug (enteric coated tablet which will not release the medication in the acidic environment of the stomach but allow its release in the less acidic environment of the intestines). These types of systems show a burst or sustained release of drug immediately after a predetermined lag time and as per

the need mechanical rhythms in our body, which make known chronotherapy.

1.2 Chronotherapy and Chronopharmaceutics:

1.2.1 Concept of chronotherapy and chronopharmaceutics [4-5]:

Chrono-pharmaceutics includes pharmaceutical application of "Chronobiology" in drug delivery. Chronobiology is the study of biological rhythms and their responses to other metabolic functions of body. There are three types of mechanical rhythms in our body:

(a) *Circadian rhythms:*

The term "circadian" was obtained from Latin words "circa" meaning "about" and "dies" meaning "day". Oscillations in our body that are completed within 24 hours are termed as circadian rhythms.

(b) *Ultradian rhythms:*

Oscillations that are completed in a shorter duration of less than 24 hours are termed as ultradian rhythms (more than one cycle per day).

(c) *Infradian rhythms:*

Oscillations that are completed in more than 24 hours are termed as infradian rhythms (less than one cycle per day)

For development of a chronotropic or pulsatile drug delivery system, thorough knowledge of pathogenesis of disease and role of circadian rhythm in its pathophysiology is required. Hence these systems are generally designed for the diseases having enough scientific background to justify their need for chronotropic systems as compared to conventional drug delivery systems.

1.2.2 Diseases show symptomatic changes due to circadian rhythmicity [4-6]:

Diseases such as bronchial asthma, hyper-cholesteremia, ulcer, diabetes, arthritis, myocardial infraction, angina and hypertension show symptomatic changes due to circadian rhythmicity.

➤ Aggravations of asthmatic attacks occur in early morning or after midnight due to low lung function promoted by circadian changes at that time.

➤ Also cardiovascular diseases like angina, hypertension, myocardial infraction and stroke etc. are more prone in early morning.

➤ Circadian changes also contribute in lipid metabolism in patients as well as in normal subjects, leading to complication in cholesterol synthesis in patients.

➤ Role of circadian changes in glucose level and insulin synthesis has been extensively studied.

➤ Peptic ulcer also favors the nocturnal acid breakthrough due to circadian variation.

➤ Patients with osteoarthritis tend to have less pain in the morning and more at night; while those with rheumatoid arthritis, have pain that usually peaks in the morning and decreases throughout the day.

1.2.3 ARTHRITIS [6-9]:

The chronobiology, chronopharmacology and chronotherapeutics of pain have been extensively reviewed. For instance, there is a circadian rhythm in the plasma concentration of C - reactive protein and interleukin-6 of patients with rheumatoid arthritis. Rheumatoid arthritis, level of C - reactive protein increases early morning leading to enhanced pain and inflammation. Chronotherapy for all forms of arthritis using NSAIDs should be timed to ensure that the highest blood levels of the drug coincide with peak pain.

Arthritis and other rheumatic conditions are a leading cause of disability in the United States, second only to heart disease as a factor contributing to work inability. It is estimated that 40 million Americans are affected, and by the year 2020, more than 59 million Americans could have arthritis. Approximately \$15.2 billion is spent on direct medical costs, with the total costs of medical care and lost wages totaling nearly \$65 billion annually.

Rheumatoid arthritis is a chronic, inflammatory, autoimmune disorder characterized by symmetrical joint involvement, usually of the hands and feet. The etiology is unknown and the immunologic changes may be initiated by a number of factors. Rheumatoid arthritis is estimated to occur in 1% to 2% of the population and is three times more common in women than men. Onset may be at any age, but it most often occurs between the ages of 25 and 50 years.

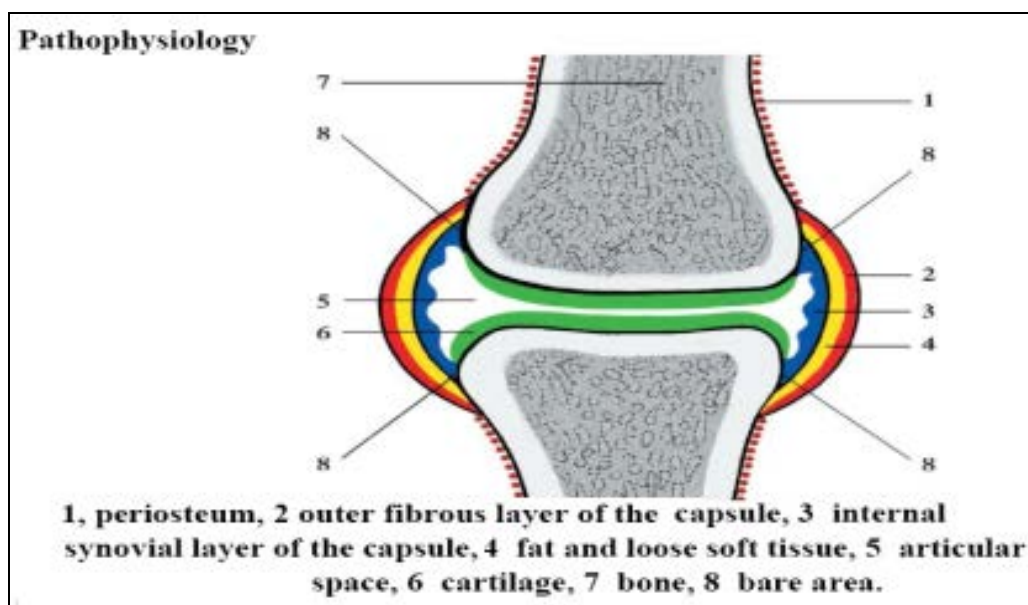


Figure 1.1: Schematic drawing of synovial joint

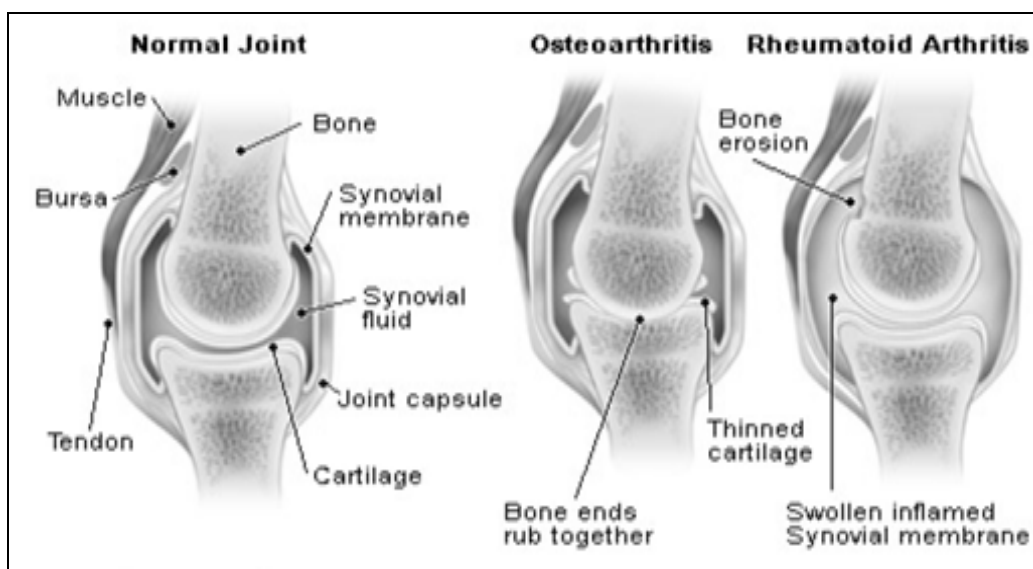


Figure 1.2: Schematic drawing of Normal and Arthritic joints

1.2.3.1 Main Symptoms of Arthritis:

Painful and swollen joints (**figure 1 & 2**), especially on hands, wrists, elbows, knees and feet, fatigue muscular pain, loss of appetite and Joint stiffness has been seen and the peak point of pain feelings has been seen mainly in the morning or after long resting periods.

1.2.3.2 Epidemiology of RA:

Rheumatoid arthritis is one of the most disabling types of arthritis, afflicting more than two million Americans. The prevalence of RA in most industrialized countries varies between 0.3% and 1%, whereas in developing countries it is at the lower end of this range. It affects approximately 1 per cent of world population including India.

1.2.3.3 Etiology:

Arthritis is affecting approximately 0.5%–1% of the global adult population. RA occurs two to three times more often in women than in men. The incidence is largely consistent racially and geographically, and the peak age of onset lies between the ages of 45 and 65 years. RA is regarded as an autoimmune disease in that in genetically susceptible patients, certain putative antigens that are presented by macrophages produce T-cell-mediated auto reactivity against a joint component.

1.2.3.4 Causes of Rheumatoid Arthritis:

The exact cause of RA is unknown.

The following are factors related to disease development:

- **Genetic Factor:** inheritance, passed from parent to child.
- **Environmental Factor:** These factors include: geographic location/climate, level of development, smoking, hormonal level and some infections, mainly viral ones.
- **Gender and Smoking Factor:** This disease, like any other, may appear in different stages of life; most cases, however, start between age 30 and 50 in both sexes, although it is more common in women. A potential risk factor is cigarette smoking.
- **Non genetic host factors (hormonal and pregnancy factors):** The increased risk of RA in females has led to considerable effort in examining the role of hormonal and pregnancy factors in disease occurrence. In general, male sex hormones, particularly testosterone, are lower in men who have RA by contrast levels of female sex hormones are not different between RA cases and controls.

1.2.3.5 Treatment for Arthritis:

In the fifties and early sixties, the treatment of RA revolved around the use of high-dose aspirin, other non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and subsequently, disease modifying anti rheumatic drugs (DMARDs).

- **Non-steroidal anti-inflammatory drugs (NSAIDs)**

The last few years or so have seen the advent of several new therapeutic agents for RA. Firstly, safer NSAIDs or 'Coxibs' have become available. The list which began with celecoxib seems to be expanding fast with the addition of rofecoxib, valdecoxib and etoricoxib etc; they relieve pain while they can also reduce stiffness and inflammation. They reduce the incidence of serious gastrointestinal events by about 80% of that observed with conventional NSAIDs. Patients likely to benefit most with Cox-2 inhibitors include elderly, those taking steroids or anticoagulants and those with past history of peptic ulcers. At present there is uncertainty about the efficacy of even conventional NSAIDs as substitutes for low dose aspirin in coronary artery disease (CAD) prophylaxis. Patients taking low dose aspirin for CAD or stroke must not discontinue it.

- **Corticosteroids**

Prednisone and Methyl-prednisolone, such drugs act directly on the immune system and lower its response to the antigen. Other side effects of corticosteroids include weight gain, osteoporosis, easy bruising, muscle weakness, and thinning of the skin. They can also worsen

diabetes and glaucoma and increase risk for cardiovascular diseases.

- **Disease modifying anti-rheumatic drugs (DMARDs)**

These included gold, d-penicillamine, chloroquine and hydroxychloroquine, Sulphasalazine, Methotrexate, sulfasalazine, hydroxychloroquine and cyclosporine, Leflunomide.

- **Biologics**

These include most notably, anti-TNF antibody or 'infliximab', soluble TNF- α receptor or 'etanercept' and interleukin-1 receptor antagonist or 'anakinra'. The other very important new agent is leflunomide.

- **Botanical Medicines**

Curcumin or turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), tropical almond (*Terminalia chebula*), tribulus (*Tribulus terrestris*), ashwagandha (*Withania somnifera*), and coriander also called Chinese parsley (*Coriandrum sativum*). Oleoresin gum extracts of boswellia (*Boswellia serrata*), with 37.5–65 percent boswellic acid, exert potent anti-inflammatory actions via inhibition of proinflammatories such as leukotrienes.

1.2.4 Designing of chronotropic systems [10-11]:

Circadian rhythms of behavior in mammals are known to be robust and precise. The efficacy and toxicity of many drugs depends upon the relationship between the dosing schedule and the 24 hour rhythms of biochemical, physiological and behavioral processes. Also several drugs cause alterations to 24 hours rhythms leading to illness and altered homeostatic regulation. Alteration in biological rhythm is also a novel concept of adverse effects, which can be minimized by optimizing the dosing schedule.

Traditionally, drug delivery was only concerned with drug absorption which should be predictable from gut or site of injection. Besides this, second generation drug delivery was meant to achieve perfection in continuous and constant rate delivery of bioactive agents. Since living organisms do not show "zero order" requirement or response to drugs and they are predictable resonating dynamic systems, so they require different amounts of drug at predictably different time within circadian cycle which will maximize desired and minimize undesired drug effects.

1.2.4.1 Rationalities of developing chronotropic systems:-

(a) Treatment of diseases in which circadian rhythms play important role in their pathophysiology (chronopharmacotherapy)

(b) Minimize the degradation of drugs in upper gastrointestinal tract (proteins and peptides)

(c) For programmed delivery of hormones (since continuous release dosage forms may lead to disturbance in normal feedback mechanism of body as well as development of resistance may also take place) and

(d) For delivery of those drugs which develop biological tolerance (e.g. nitroglycerines) or undergo extensive first pass metabolism and also that are targeted to specific site of gastrointestinal tract e.g. colon.

Chronotropic systems are based on the concept of chronopharmaceutics in which there is a transient release of certain amount of drug within a short period of time immediately after a predetermined off-release period.

1.2.4.2 Numerous methodologies have been developed to design chronotropic systems to achieve desired drug-release profile in a pulsatile fashion:

- i) Timed-release/time-dependent chronotropic systems.
- ii) Stimuli dependent systems (pulsatile drug delivery systems).

Timed-release/time-dependent chronotropic systems:

These types of systems show a burst release of drug immediately after a predetermined lag time.

Depending on methodologies applied to design them, these systems can be further classified into following subtypes:

• Reservoir systems with rupturable polymer coating:

These systems may be either single unit or multiparticulate reservoir systems with outer rupturable barrier. Upon entry of water within the systems, a hydrostatic pressure develops which leads to rupturing of surrounding polymeric layer resulting drug release from the core of system. Pressure buildup required to rupture the coating can be achieved by using swelling agents, gas producing effervescent agents or osmogens. Rate of water permeation and mechanical resistance of the outer membrane are major factors affecting the lag time. Drug release mechanism is based on either diffusion or dissolution according to the nature of drug.

Ueda et al. discovered time controlled explosion systems for water insoluble drugs in both single as well as multiple unit dosage forms. Both types of dosage forms contain a core of drug plus osmotic agent and super disintegrants. Finally the cores are coated with a protective polymeric rupturable layer and a top water insoluble semipermeable layer, which is the rate controlling membrane for influx of water into osmotic core.

In order to attain a better control over release pattern, water soluble polymer (mainly pH dependent) can be incorporated in insoluble polymeric membrane so that at elevated pH of small intestine, polymer starts dissolving

leading to weakening of membrane after a predetermined lag time. By variation in coat thickness as well as proportion of soluble and insoluble material in the coating, the lag time before drug release can be prolonged with better control and reliability and eventual disintegration of coating ensuring release of drug.

• Chronotropic systems dependent on changed membrane permeability:

Drug release in such type of systems is achieved by change in permeability of polymeric coating layer in presence of certain counter ions of surrounding media. Narisawa et al developed a device capable of pulsed-release depending on the change in diffusion properties of Eudragit RS. They studied and justified that cores of theophylline coated with Eudragit RS show very slow release in pure water but release rate increases significantly when the microcapsules are immersed in an organic acid solution containing succinic acid, glutaric acid, tartaric acid, malic acid or citric acid. The above phenomena occurs due to higher hydration of film containing quaternary ammonium groups in the polymer chain and that is not affected by succinic acid, suggesting that the quaternary ammonium groups of Eudragit RS are essential to produce unique drug release profile.

• Reservoir systems with soluble/eroding polymer coating:

This class of reservoir type pulsatile systems possess a barrier layer, which dissolves or erodes after a specific lag time followed by burst release of drug from the reservoir core. In these types of systems, the lag time prior to drug release is controlled by thickness of coating layer. A chronotropic system which consists of a drug containing core layered with HPMC and a top layer of enteric coating, the lag time before drug release will be dependent upon the thickness and viscosity grade of HPMC layer. Since drug release mechanism in these types of systems is dissolution, that's why, a high degree of drug solubility relative to dose of drug is essential for rapid release of drug after the lag period. Various grades of hydroxyl propyl methyl cellulose and Eudragit (acrylate) polymers have been studied to in an attempt to deliver drugs to various sites in gastrointestinal tract due to their solubility and eroding properties.

• Low density/floating systems:

Nowadays floating dosage forms are gaining importance as technological drug delivery systems with gastro-retentive behavior, offering several advantages in drug delivery. Like treatment of gastrointestinal disorders such as gastro-esophageal reflux, improved drug absorption (because of increased GRT), ease of administration and

better patient compliance. These systems are comprised of low density/floating pulsatile dosage forms which are retained in stomach for long time (4-12 hours) and not affected by variation in gastric pH, local environment or gastric emptying rate. These dosage forms may be either single unit (floating tablets) or multiparticulates (beads, pellets, granules, microspheres) with capability of gastro-retention.

These systems are specifically beneficial for drugs, either absorbed from the stomach or requiring local delivery in stomach. Generally polysaccharides are widely accepted in gastroretentive delivery systems because of their simplicity to formulate the drug delivery system and achieve the desired drug release profile.

i) Stimuli dependent systems (pulsatile drug delivery systems):

Such systems are novel drug delivery approaches meant for targeted drug delivery at specific site due to induction of certain physiochemical stimuli at target site. Release of certain enzymes, hormones, antibodies, pH of the site, temperature of the site, presence of certain cells, and concentration of biomolecules (glucose, neurotransmitters, inflammatory mediators), all are acted as stimuli to trigger the release of drug from these types of drug delivery systems.

• **Temperature sensitive pulsed- release delivery systems:**

Physiological temperature of various types of cells inside the body is not same due to their different metabolic functions. Certain cells possess some what different temperature (either higher or lower) with respect to other cells like tumor cells, in which cellular temperature is raised due to their higher metabolic rate. For targeting tumors, a pulsatile drug delivery system can be designed by utilizing thermo-responsive hydrogel system. As the name suggests, these polymers undergo swelling/deswelling phenomena in response to temperature change (at different metabolic rates of tumor cells) which modulates drug release from these systems.

• **Inflammation induced systems:**

Any physical or chemical stress (injury, fracture e.t.c), which may lead to inflammation, acts as a stimulus (due to hydroxyl radicals produced from inflammation responsive cells). In favor of this Yui and coworkers *et.al* designed and developed inflammation responsive pulsatile drug delivery system which responded to hydroxyl radicals and degraded in a limited manner. They utilized hyaluronic acid which is specifically hydrolyzed by hyaluronidase or free radicals present at

inflammatory site abundantly rather than normal tissue. Hence it became possible to treat patient with inflammatory diseases like rheumatoid arthritis, using NSAIDs incorporated into hyaluronic acid gels as a new implantable drug delivery system.

• **Enzyme dependent pulsatile-release systems:**

Such systems are generally developed for colonic delivery of drug since release rate of drug is dependent upon the catalysis of polymeric membrane by enzymes secreted by colonic microflora. Therefore these systems are more specific for targeting, independent of pH variations along the gastrointestinal tract. Numerous natural polysaccharides such as chondroitin sulphate, pectin, dextran, guar gum e.t.c have been investigated for their potential in designing colon specific drug delivery. Chronotherapy of rheumatoid arthritis has been tried by utilizing these polymers to deliver NSAIDs in colon after a lag time of 4-6 hours to relieve pain in early morning. Also pulsatile delivery of 5-aminosalicylic acid has been attempted in case of irritable bowel syndrome.

• **Glucose concentration dependent insulin release systems:**

It was depicted earlier that there is an increase in blood glucose concentration rhythmically in Diabetes-mellitus Type1. Several systems were developed which responded to changes in glucose concentration. One such stimuli induced system includes pH sensitive hydrogel containing glucose-oxidase enzyme immobilized in hydrogel. As the blood concentration of glucose rises, glucose-oxidase converts glucose into gluconic acid, which changes the pH of system. Due to change in pH, swelling of polymer takes place and this results into insulin release. Insulin decreases the blood glucose level and consequently the gluconic acid level also declines and system turns to deswelling and hence decreasing the insulin release. Examples of pH sensitive polymers include n, n-dimethyl amino ethyl methacrylate, chitosan, polyol e.t.c.

• **Intelligent gels responding to antibody concentration**

Resistance as well as tolerance towards antibiotic concentration is a common phenomenon shown by microbes in many of the infectious diseases. Hence in order to kill all microbes, both multiplying as well as in dormant phase, a pulsatile release of antibiotic is desired. Novel kind of gels have been developed that respond to change in antibiotic concentration to alter their swelling/deswelling characteristics.

- **pH sensitive pulsatile drug delivery systems :**
pH dependent polymers are widely accepted and most versatile approach to achieve a desired lag time before drug release in a chronotropic system. Either single unit or multiparticulate dosage forms, they show reliable and predictable drug release profile. These systems take the advantage of fact that there exists different pH environment at different parts of gastrointestinal tract. Hence utilizing pH dependent polymers, targeting at specific site of gastrointestinal tract is possible as well as a desired lag time can be achieved due to dependency of polymer solubility only at a particular pH of gastrointestinal tract. Generally pH dependent polymers include copolymers of methacrylic acid (various grades of Eudragit), phthalates, and carboxy methyl cellulose e.t.c. these polymers are utilized for enteric coating to protect the drug from degradation in upper G.I.T and attain drug release at specific part of intestine (according to solubility of polymer at particular pH and specific site of intestine) after a predetermined lag time. A number of chronotropic systems have been developed and marketed for chronotherapy utilizing pH dependent polymers for asthma, angina, rheumatoid arthritis, cancer, diabetes and ulcer e.t.c.

1.2.5 Dosage Form Development [10-16]:

1.2.5.1 Multi-Layered tablets or capsules:

Such systems are generally time controlled rupturable pulsatile drug delivery systems either in form of hard gelatin capsules or tablets. In case of capsules, drug filled in capsule-body is either for single pulse or multi-pulse release (in form of multiparticulates) which is coated over with a swelling layer followed by an external water insoluble semipermeable polymeric coating. Upon water ingress the swelling layer swells to attain a threshold hydrodynamic pressure required to rupture the outer coating and allowing the release of contents in surrounding medium. The time required by swelling layer to rupture outer coating serves the purpose of desired lag time required in chronotherapy of disease. The tablets are manufactured and coated on the same principle as that of double coated gelatin capsules.

1.2.5.2 Press coated tablets:

These are timed release formulations, simple to manufacture, comprised of an inner core that contains an active pharmaceutical ingredient and excipients surrounded by an outer layer that dissolves or disintegrates slowly to produce the lag time. The core is placed between two layers of polymer and directly compressed by flat punches of tableting machine.

Surrounding polymeric layers protect the drug from release before the desired lag time, hence effective delivery in chronotherapy as it allows the drug release at the point in circadian cycle when clinical signs develop and increase. Drugs that treat cardiovascular disease (nifedipine, nitrendipine, amlodipine, diltiazem e.t.c) and asthma (theophylline, budesonide) had been attempted to formulate such dosage forms. Sawada *et al.* prepared timed release compression coated tablets of nifedipine for chronotherapy of angina and compared its invitro-in vivo release profile with sustained release formulation.

1.2.5.3 Core-cup-tablets:

The system consists of three different components, a core tablet containing the active ingredient, an impermeable outer shell and a top cover layer-barrier that should be removed at predetermined time. Ideally, the drug should be released after a complete removal of the top cover layer, with the lag time being controlled by the characteristic properties of the material in the top cover. The impermeable coating cup consisted of cellulose acetate propionate and the top cover layer of hydrophilic swellable materials such as polyethylene oxide, sodium alginate or sodium carboxymethylcellulose. The system releases the drug after a certain lag time generally due to the erosion of top cover layer. The quantity of material, its characteristics (viscosity, swelling, gel layer thickness) and the drug solubility was found to modify lag time and drug release. The lag time increases when quantity of top layer increases, whereas drug release decreases.

1.2.5.4 Multiparticulate systems:

Such systems have been designed on the basis of various methodologies of designing pulsatile drug delivery system discussed earlier (like time controlled, stimuli induced or externally regulated pulsatile drug delivery systems). Various types of multiparticulate dosage forms are: Pellets, microsponges, microspheres, granules, nanoparticles and Beads e.t.c. Multiparticulate dosage forms are gaining much more importance over single unit dosage forms due to their potential advantages over single unit dosage forms. A no. of multiparticulate pulsatile drug delivery systems has been developed for chronotherapy.

For instance, colonic delivery of theophylline in form of microspheres and coated pellets for nocturnal asthma, formulation of pellets and microspheres of NSAIDS (indomethacin, ibuprofen, flurbiprofen, meloxicam, aceclofenac, didophenac) for chronotherapy of rheumatoid arthritis and floating beads of alginates encapsulating the active drug component in core, have

been attempted to deliver many of the drugs which are absorbed in upper gastrointestinal tract. Numerous advanced technologies have been developed in designing of pulsatile release multiparticulate dosage forms and many of them are FDA approved and being marketed.

1.2.5.5 Pulsincap systems:

These are the well designed pulsatile release drug delivery systems capable of releasing drug at a pre determined time. Drug formulation is contained within the insoluble capsule body which is sealed by means of a hydrogel plug. On oral administration the water soluble capsule cap dissolves in the gastric juices and hydrogel plug swells. At a controlled and predetermined time point after the ingestion, the swollen plug is ejected from the pulsincap dosage form after which the encapsulated dosage formulation is then released.

1.2.5.6 Infusion pumps:

These are externally and internally controlled, pre-programmed systems and sensitive to modulated enzymatic or hydrolytic degradation, pH, magnetic fields, ultrasound, electric fields, temperature, light and mechanical stimulation. Infusion pumps recently in the

market that have been referred as “chronomodulating infusion pumps” for drug delivery application include the “Melodie”, “Programmable-Synchromed”, “Panomat V5 infusion”, and the “Rhythmic Pumps”. The insulin reservoir is refilled once a month or every 3 months at a physician’s office by inserting a needle through the skin into the pump (a local anesthetic is first used).

1.2.5.7 Chronomodulating-microchips:

Micro-fabrication technology is an alternative method to achieve pulsatile or chronopharmaceutical drug release. Santini *et al.* reported a solid-state silicon microchip that can provide controlled release of single or multiple chemical substances on demand.

1.2.5.8 Advances in pulsatile drug delivery:

Chronotropic systems are one of the interesting novel drug delivery systems emerging for chronotherapy due to advanced technologies and desired therapeutic application. Among these, multiparticulate systems (beads, pellets, microspheres e.t.c) are gaining more importance than single unit systems due to their potential benefits over them. Various pulsatile technologies (**table 1**) have been developed on the basis of approaches discussed previously.

Table 1: List of some Marketed chronotropic pulsatile drug delivery system [2-7, 10-16]

Sl. No.	Technology	Mechanism	Proprietary name and dosage form	API	Disease
1.	OROS®	Osmotic Mechanism	Covera-HS® XL tablet	Verapamil HCl	Hypertension
2.	CODOS®	Multiparticulate pH dependent system	Verelan® PM;XL tablets	Verapamil HCl	Hypertension
3.	DIFFUCAPS®	Multiparticulate system	Innopran®; XL tablets	Verapamil HCl, Propranolol HCl	Hypertension
4.	Three dimensional printing®	Externally regulated system	Their Form®	Diclofenac Sodium	Hypertension
5.	Pulsincap™	Rupturable system	Pulsincap™	Dofetilide	Hypertension

2. RECENT ADVANCES:

Some researchers those who worked on chronotropic drug delivery systems are summarised and given as follows. Singhai SK *et al.* [17], surveyed on recent advances in chronopharmacology and requirement of an appropriate technology to deliver the drug at specific time and site led to the development of novel type of drug delivery systems as “chronotropic drug delivery systems”. Rationale behind designing these drug delivery systems is to release the drug at desired time (pathophysiological need of disease), which results into improved therapeutic efficacy and patient-compliance. These systems are meant for treatment of those diseases that are caused due to circadian changes in body and

when zero order drug release is not desired. These drug delivery systems are designed to release the drug within a short period of time, immediately after a predetermined lag time. Chronotropic systems are promising drug delivery systems in asthma, peptic-ulcer, cardiovascular diseases, arthritis, attention-deficit syndrome in children and hypercholesteremia e.t.c. This article mainly focused on diseases requiring chronotropic systems, approaches to design them, recent technologies for chronotherapy and currently available marketed formulations. Tiwari G *et al.* [18], reviewed on the colon as a site where both local and systemic delivery of drugs can take place. Local delivery could, for example, allow topical treatment of inflammatory bowel disease.

Treatment could be made more effective if it were possible for drugs to be targeted directly on the colon. Systemic side effects could also be reduced. Colon specific systems might also allow oral administration of peptide and protein drugs, which are normally inactivated in the upper parts of the gastrointestinal tract. This review also focused on evaluations of CDDS in general. Yang L *et al.* [19], studied the necessity and advantages of colon-specific drug delivery systems have been well recognized and documented. In the past, the primary approaches to obtain colon-specific delivery achieved limited success and included prodrugs, pH- and time-dependent systems, and micro flora-activated systems. Precise colon drug delivery requires that the triggering mechanism in the delivery system only respond to the physiological conditions particular to the colon. The focus of this review was to provide detailed descriptions of the four systems, in particular, and in vitro/in vivo evaluation of colon-specific drug delivery systems, in general. Kinget SM *et al.* [20], studied on specific targeting of drugs to the colon and it is recognized to have several therapeutic advantages. Drugs which are destroyed by the stomach acid and/or metabolized by pancreatic enzymes are slightly affected in the colon, and sustained colonic release of drugs can be useful in the treatment of nocturnal asthma, angina and arthritis. Treatment of colonic diseases such as ulcerative colitis, colorectal cancer and Crohn's disease is more effective with direct delivery of drugs to the affected area. Likewise, colonic delivery of vermicides and colonic diagnostic agents require smaller doses. This article was aimed at providing insight into the design considerations and evaluation of colonic drug delivery systems. For this purpose, the anatomy and physiology of the lower gastrointestinal tract are surveyed. Furthermore, the biopharmaceutical aspects are considered in relation to drug absorption in the colon and hence various approaches to colon-specific drug delivery are discussed. Jain A *et al.* [21], surveyed on the ability of delivering of drugs to the human colon in a specific manner has become feasible over the years. Targeting pharmaceutical drugs to the colon makes it possible to achieve local or systemic drug delivery to this site. To deliver the compounds in a non-degraded form to the lower part of the gastrointestinal tract, they must first of all pass through the stomach, the upper part of the intestine and must use the characteristics of the colon to specifically release the drugs in this part of the digestive tract. A final overview of the various approaches to target drugs to the colon utilizing natural polysaccharides, the limits and the future developments in this field with these

natural polymers is discussed which leads to the conclusion. Sinha VR *et al.* [22], reviewed on natural polysaccharides, which are now extensively used for the development of solid dosage forms for delivery of drug to the colon. The rationale for the development of a polysaccharide based delivery system for colon is the presence of large amounts of polysaccharidases in the human colon as the colon is inhabited by a large number and variety of bacteria which secrete many enzymes e.g. D-galactosidase, amylase, pectinase, xylanase, D-xylosidase, dextranase, etc. A large number of polysaccharides have already been studied for their potential as colon specific drug carrier systems, such as chitosan, pectin, chondroitin sulphate, cyclodextrin, dextrans, guar gum, inulin, amylose and locust bean gum. Recent efforts and approaches exploiting these polysaccharides in colon-specific drug delivery were discussed. Hovgaard L *et al.* [23], studied on Polysaccharides, which has over the years been used widely in pharmaceutical, chemical, and biochemical drug delivery. This family of natural polymers has an appeal to the area of drug delivery as it is comprised of polymers with a large number of derivatizable groups, a wide range of molecular weights, varying chemical compositions, and for the most part, a low toxicity and biodegradability, yet a high stability. The main scope of this review is to relate the polysaccharides available now to the rapidly growing field of colonic drug delivery. Polysaccharides have been applied to the area as controlled release coatings, matrices, macromolecular carriers, and biodegradable carriers. A final overview of the various approaches to obtain colon-specific delivery by using polysaccharides and a summary of available in vitro and in vivo testing methods will lead to the conclusion that polysaccharides at this point appear to be very promising compounds for use in obtaining colon-specific drug delivery systems. Laine L *et al.* [24], surveyed on nonsteroidal anti-inflammatory drugs (NSAIDs), which are probably the most common cause of gastroduodenal injury in the United States today. Approximately half of patients who regularly take NSAIDs have gastric erosions, and 15%–30% have ulcers when they are examined endoscopically. However, the incidence of clinical gastrointestinal (GI) events caused by NSAIDs is much lower. Clinical upper GI events may occur in 3%–4.5% of patients taking NSAIDs, and serious complicated events develop in approximately 1.5%. However, the risk varies widely in relationship to clinical features such as history of ulcers or GI events, age, concomitant anticoagulant or steroid use, and NSAID dose. This review discussed the risks of clinical GI disease in NSAID users, the predictors of increased risk, and

strategies for prevention of NSAID-associated GI disease. Derle DV *et al.* [25], reviewed on the nonsteroidal antiinflammatory drugs, which are among the most widely prescribed and used drugs in the community for rheumatologic as well as nonrheumatologic conditions, which include acute and chronic pain; biliary, ureteric colic; dysmenorrhoea; fever; and other applications that derive from the suppression of prostaglandin synthesis. Almost all nonsteroidal antiinflammatory drugs irritate gastric mucosa and enhance ulceration by blocking protective action of the prostaglandins on gastric mucosa, causing ulcer formation not only in stomach but also in lower part of oesophagus and in duodenum too. This review focused on the adverse effects of nonsteroidal antiinflammatory drugs, severity of these adverse effects and attempts made to reduce the side effects through the concomitant use of other drugs. Bondeson J *et al.* [26], focused on rheumatoid arthritis (RA), which is probably the most common source of treatable disability. A major problem in modern rheumatology is that the mechanism(s) of action of the currently used disease-modifying antirheumatic drugs (DMARDs) remain unclear. Many of these drugs entered rheumatology mainly through clinical intuition and have been used for decades. This review summarized the recent advances in determining the mechanisms of action of the currently used DMARDs, with particular emphasis on their effects on the induction of TNF- α and interleukin 1 (IL-1) in mononuclear phagocytes. Alan J *et al.* [27], surveyed on therapeutic advances in rheumatoid arthritis (RA), which have largely focused on the development of non-steroidal antiinflammatory drugs (NSAIDs) with improved characteristics compared with aspirin. For example, greater potency, safety, improved tolerance in the elderly and reduced frequencies of dosing have been achieved. However, these agents are generally considered to be palliative treating of the symptoms of the disease. The development of disease modifying drugs (DMD), also known as second line drugs, for RA has not been very successful. Most of the agents that are currently used in this category were originally used to treat other diseases such as malignancy (cyclophosphamide, methotrexate), Wilson's disease (D-penicillamine) and tuberculosis (gold salts). Unfortunately, none of the agents is ideal and each has potentially serious side-effects. There have been several attempts to develop agents with new mechanisms of action that hopefully will greatly improve these current therapies. Takagi T *et al.* [28], reviewed on orally administered, immediate-release (IR) drug products in the top 200 drug product lists from the United States

(US), Great Britain (GB), Spain (ES), and Japan (JP), which were provisionally classified based on the Biopharmaceutics Classification System (BCS). The provisional classification was based on the aqueous solubility of the drugs reported in readily available reference literature and a correlation of human intestinal membrane permeability for a set of 29 reference drugs with their calculated partition coefficients. In summary, more than 55% of the drug products were classified as high-solubility (Class 1 and Class 3) drugs in the four lists, suggesting that in vivo bioequivalence (BE) may be assured with a less expensive and more easily implemented in vitro dissolution test. Akhgari A *et al.* [29], studied to evaluate the effect of two factors (ratio of Eudragit S100 and Eudragit L100 and the coating level) on indomethacin release from pellets in order to optimize coating formulations for colonic delivery. Coating formulations were designed based on the full factorial design. Sinha VR *et al.* [30], investigated to develop a single unit, site-specific drug formulation allowing targeted drug release in the colon. Tablets were prepared using polysaccharides or synthetic polymer as binders. These included xanthan gum, guar gum, chitosan and Eudragit E. Indomethacin was used as a model drug. The prepared tablets were enteric coated with Eudragit-L 100 to give protection in the stomach. The above study showed that chitosan could be successfully used as a binder, for colon targeting of water insoluble drugs in preference to guar gum when used in the same concentration. Additionally, formulations developed with chitosan and Eudragit E would be highly site specific since drug release would be at a retarded rate till microbial degradation or polymer solubilization takes place in the colon. Takeuchi H *et al.* [31], studied on the solid dispersion particles of indomethacin (IMC), which were prepared with different types of silica, non-porous (Aerosil 200) or Poroussilica (Sylysia 350) by using spray-drying method. Powder X-ray diffraction analysis showed that IMC in solid dispersion particles is in amorphous state irrespective of the type of silica formulated. The dissolution rate of IMC from the solid dispersion particles with Sylysia 350 was faster than that of Aerosil 200 irrespective of IMC content. In stability test, amorphous IMC in the solid dispersion particles with each silica particles did not crystallize under storing at severe storage conditions (40°C, 75% RH) for 2 months, while amorphous IMC without silica easily crystallized under same conditions. Lovrečich M *et al.* [32], studied on the effect of ageing on the release of indomethacin from coprecipitates with Eudragit RS, Eudragit E and blends of these polymers was studied. DSC thermograms were

carried out to control the glass transition temperature (T_g) of polymers and the physical state of indomethacin after 2 and 3 years of storage in closed containers at room temperature. The rotating disk method was used on compressed powders in order to test drug release under controlled conditions. Data treatments were carried out in order to verify modifications in the diffusion coefficient and mechanism of drug release. Results indicated that, for all the systems investigated, randomness was reduced within the polymeric network during storage, and this observation was confirmed by the appearance of a peak at the polymeric T_g. The drug diffusion coefficient from Eudragit RS was not influenced by storage, while, in the case of Eudragit E and a blend of polymers, a significant reduction of the diffusion coefficient was noticed after 3 years, probably due to an interaction between the drug and Eudragit E. Rusua D *et al.* [33], synthesized the indomethacin (1H-indole-3-acetic acid, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl), through a new method, which reduces some stages from the previous methods. Both the structure of the finished product and the structures of the intermediaries were investigated by chromatographic methods (TLC, chromatography on column, GC-MSD) and spectroscopic methods (UV, IR, ¹H-NMR, ¹³C-NMR). The chromatographic and spectroscopic studies proved that these had a special analytical value and they serve to control synthesis and to identify the compounds in all the stages of the process. Chena X *et al.* [34], was investigated acid-base reactions of solid materials (drug-exipients interaction of indomethacin). In a typical experiment, 500mg of pure form indomethacin were mixed with 500mg of sodium bicarbonate. The combination of these solid-state characterization techniques is demonstrated to be essential to detect and monitor acid-base reactions in solid materials, which are impossible to monitor using solution-chemistry methods. The reaction kinetics at 66% RH fits the Jander equation very well, which is consistent with a diffusion-controlled mechanism. Casellaa R *et al.* [35], studied on the solubility, dissolution behavior, complex-binding constant, crystallinity and enthalpy. The results showed indomethacin solubility was improved by complex formation with cyclodextrin. There was little difference among the various complex solubilities. Indomethacin dissolution was improved by complex formation with the exception of one complex. Indomethacin dissolution profiles were found to differ and were unrelated to either the complexed indomethacin content or binding constant. Indomethacin dissolution profiles were found to be related to the complex crystallinity and enthalpy.

The complex-binding constants were found to support a theory reported previously that cyclodextrin ring cavity solvation was the predominant factor responsible for complex formation. Dixit M *et al.* [36], studied on indomethacin, an anti-inflammatory drug, exhibits poor water solubility and flow properties, poor dissolution and poor wetting. Consequently, the aim of this study was to improve the dissolution of Indomethacin. Microsphere containing Indomethacin was produced by spray drying using acetone and water in the ratio of 40:60 (v/v) as solvent system to enhance dissolution rate. The prepared formulations were evaluated for in vitro dissolution and solubility. It was believed that spray drying of Indomethacin is a useful tool to improve dissolution. Hence this spray drying technique can be used for formulation of tablets of Indomethacin by direct compression with directly compressible tablet excipients. Liu H *et al.* [37], studied on microbially triggered colon-targeted osmotic pump (MTCT-OP). The gelable property at acid condition and colon-specific biodegradation of chitosan were used to: (1) produce the osmotic pressure, (2) form the drug suspension and (3) form the in situ delivery pores for colon-specific drug release, respectively. These results showed that MTCT-OP based on osmotic technology and microbially triggered mechanism had a high potential for colon-specific drug delivery. Mohammed E *et al.* [38], developed a sensitive, selective and accurate high-performance liquid chromatography-mass spectrometry (LC-MS) assay for the determination of selected non-steroidal anti-inflammatory drugs (NSAIDs), namely diclofenac sodium (DIC), flufenamic acid (FLU), indomethacin (IND) and ketoprofen (KET), either individually or in mixtures. The examined drugs were injected onto Shim-pack GLC-CN column and were eluted with a mobile phase consisting of acetonitrile and 20 mM ammonium acetate solution (5:1 v/v)/pH 7.4 at a flow rate 1 ml min⁻¹. Within-day and between-day precision were in the range of 0.8–9.1% of the R.S.D. Mean recovery percentages of the individual compounds from laboratory-made mixtures and pharmaceutical. Javadzadeh Y *et al.* [39], investigated on the potential of liquisolid systems to improve the dissolution properties of a water-insoluble agent (indomethacin). In this study, different formulations of liquisolid tablets using different co-solvents (non-volatile solvents) were prepared and the effect of aging on the dissolution behaviour of indomethacin liquisolid compacts was investigated. Liquisolid compacts containing propylene glycol as vehicle produced higher dissolution rates in comparison with liquisolid compacts containing PEG 400 or Tween 80 of the same

concentration. The DSC and XPD results showed no changes in crystallinity of the drug and interaction between indomethacin and excipients (Avicel and silica) during the process. Bogdanovaa SV *et al.* [40], prepared a series of indomethacin/nicotinamide binary system-melts as models to study some aspects of the potential physico-chemical interactions between indomethacin and nicotinamide. The melts with a low drug content (e.g. 7.5 and 9.1% melts) were found to be in a metastable physical state probably due to the amorphous nature of the indomethacin or its complex. They can be in situ transformed into 1% indomethacin aqueous solution. Chowdary KPR *et al.* [41], prepared mucoadhesive microcapsules of indomethacin by an emulsification-ionic gelation process employing sodium carboxy methylcellulose, methylcellulose, Carbopol and hydroxy propyl methyl cellulose along with alginate and the microcapsules were evaluated for release kinetics and ulcerogenic activity. The resulting microcapsules were discrete, free flowing, multinudeate, monolithic and spherical. The microcapsules exhibited good mucoadhesive property in the in vitro wash-off test. Release from some microcapsules fulfilled the official (USP 23) drug release test-2 requirement of indomethacin extended release capsules. A 62-80 % reduction in ulcerogenic activity was observed with these microcapsules when compared to pure drug indomethacin. Hubert PH *et al.* [42], described a fully automated method, which enabled the determination of indomethacin in plasma by reversed-phase HPLC following on-line sample enrichment and clean-up on a short pre-column. The plasma sample is introduced directly into the column switching system. The precolumn, filled with a pellicular bonded phase, is first washed with phosphate buffer, pH 7.4. The effects of changes in the pH and flow rate of the washing eluent are studied. Under the conditions selected, memory effects can be avoided, the absolute recovery of the drug is 70% and the limit of detection 10 ng ml^{-1} for a $100 \sim 1$ injection of plasma. At a concentration of 100 ng ml^{-1} , the relative standard deviations (RSD) are 1.7% (within-day) and 3.5% (between-day), respectively. Richard N U *et al.* [43], used indomethacin to manage raised intracranial pressure (ICP) in humans during neuroanaesthesia and neurosurgery. Indomethacin causes cerebral vasoconstriction and reduces cerebral blood flow (CBF) and, therefore, ICP. The data and model support the concept of indomethacin having limited uptake into the brain, with its effect on CBF being the result of its action on the endothelium, where it indirectly modifies the turnover of a compound regulating vascular

tone. Akhgari A *et al.* [44], evaluated the combination of pH-dependent and time-dependent polymers as a single coating for design of colon delivery system of indomethacin pellets. Eudragit S100 and Eudragit L100 were used as pH-dependent polymers and Eudragit RS was used as a time-dependent polymer. A statistical full factorial design was used in order to optimize formulations. The results of in vitro experiments indicated that the proposed combined time-dependent and pH-dependent polymethacrylate polymer coating may provide a colonic delivery system for indomethacin. Krishnaiah YSR *et al.* [45], found the non-steroidal anti-inflammatory drugs (NSAIDs) as a potential chemo preventive agents of colo-rectal cancer. Celecoxib, an NSAID, with selective cox-2 inhibition was proved to be effective for the prevention of colon cancer in patients with familial adenomatous polyposis (FAP) and sporadic Payps, matrix tablets containing either 20 or 30 % of guar gum are most likely to target Celecoxib for local action in the colon, differential scanning calorimetric studies indicated no possibility of interaction between Celecoxib and guar gum. Havpt S *et al.* [46], studied on celecoxib, a chemo-preventive drug for familial adenomatous polyposis and under trail for reducing post surgical colorectal malignancies, films made up of chitosen and guar gum were prepared characterized for equilibrium swelling, muco adhesion in vitro and in vivo degradation and loaded with Celecoxib. Short term dosing studies in vitro were performed in the HT-29 colon carcinoma cell line that was incubated with Celecoxib using the MTT test to assess IC 50. Arico S *et al.* [47], studied on celecoxib induces apoptosis in the colon cancer cell line HT-29 by inhibiting 3-phosphoinositide-dependent kinase activity. This effect was correlated with inhibition of phosphorylation of the PDK1 downstream subtract AKT / protein kinase B on two regulatory sites. Sinha VR *et al.* [48], studied to investigate the effects of cyclodextrin on the aqueous solubility and dissolution rate of celecoxib. Inclusion complexes were prepared by the kneading method and characterized by SEM, NMR, IR, DSC, and X-ray powder diffraction. Dissolution rate of the complexes was significantly greater than that of the corresponding physical mixtures and pure drug, indicating that the formation of indusion complex increased the solubility of the poorly soluble drug celecoxib. Vernon ES *et al.* [49], was surveyed on biological and chemical irritants which can be the cause of irritation in a variety of organ sites. It is becoming well understood that chronic irritation in any form can initiate and accelerate the cancer process in these same organs. This understanding comes in part from the many epidemiologic studies which point out

that chronic inflammation correlates with increased risk of developing cancer in that organ which is affected. These studies have primarily used non-steroidal anti-inflammatory drugs (NSAIDs) which block the COX pathways. Recent preclinical studies indicate that the LOX pathway also may be an important target for cancer prevention strategy. The expression of high levels of these enzymes in cancerous tissues can make them an obvious first target for cancer prevention strategies. Baboota S *et al.* [50], developed and validated a simple, economic, selective, precise, and stability-indicating HPLC method for analysis of celecoxib (CXB), a selective COX-2 inhibitor, both in bulk drug and in microemulsions. Reversed-phase chromatography was performed on a C18 column with methanol–water, 75:25 (% v/v), as mobile phase at a flow rate of 1.25 mL min⁻¹. Detection was performed at 250 nm and a sharp peak was obtained for CXB at a retention time of 4.8 ± 0.01 min. Linear regression analysis data for the calibration plot showed there was a good linear relationship between response and concentration in the range 0.27–80 µg mL⁻¹; the regression coefficient was 0.996 and the linear regression equation was $y = 48415x + 54359$. The detection (LOD) and quantification (LOQ) limits were 0.086 and 0.2625 µg mL⁻¹ respectively. The method was validated for accuracy, precision, reproducibility, specificity, robustness, and detection and quantification limits, in accordance with ICH guidelines. Statistical analysis proved the method was precise, reproducible, selective, specific, and accurate for analysis of CXB. The wide linearity range, sensitivity, accuracy, short retention time, and simple mobile phase imply the method is suitable for routine quantification of CXB with high precision and accuracy. Ali J *et al.* [51], studied to develop a hydrodynamically balanced system for celecoxib as single-unit floating capsules. Various grades of low-density polymers were used for formulation of these capsules. The capsules were prepared by physical blending of celecoxib and the polymer in varying ratios. The formulation was optimized on the basis of in vitro buoyancy and in vitro release in citrate phosphate buffer pH 3.0 (with 1% sodium lauryl sulfate). For gamma scintigraphy studies, celecoxib was radiolabeled with technetium-99m by the stannous reduction method. To achieve the maximum labeling efficiency the process was optimized by studying the reaction at various pH conditions and stannous concentration levels. The radiolabeled complex was added to the optimized capsule, and dissolution studies were performed to ensure that there was no leaching of radioactivity from the capsules. Gamma imaging was

performed in rabbits to assess the buoyancy of the optimized formulation. The optimized formulation remained buoyant during 5 hours of gamma scintigraphic studies in rabbits. Seedher N *et al.* [52], examined the solubility enhancement of 4 COX-2 inhibitors, celecoxib, rofecoxib, meloxicam, and nimesulide, using a series of pure solvents and solvent mixtures. Water, alcohols, glycols, glycerol, and polyethylene glycol 400 (PEG 400) were used as solvents and water-ethanol, glycerol-ethanol, and polyethylene glycol-ethanol were used as mixed-solvent systems. The greater the difference in the polarity of the 2 solvents in a given mixed solvent, the greater was the solubilization power. However, in a given mixed solvent system, the solubilization power could not be related to the polarity of the drugs. Significance of the solubility data in relation to the development of formulations has also been discussed in this study. Soliman SM *et al.* [53], aimed to develop suitable microemulsion gel systems for transdermal delivery that could assist dissolution enhancement of poorly water soluble celecoxib and thus improve its skin permeability. Long term oral administration of celecoxib causes serious gastrointestinal adverse effects, which makes it a good candidate for transdermal formulations, yet its low water solubility (4 mg/L) makes this challenging. The optimized formula (F12) was found to be superior to all other formulas. This formula increased the permeation rate of celecoxib up to 11 times compared to that of the cream. Its stability was retained after one year of storage under ambient conditions and its anti-inflammatory effect was significantly higher than that of celecoxib cream and the oral commercial formula. Skin irritancy and histopathological investigation of rat skin revealed its safety. The results revealed that the developed microemulsion gel has great potential for transdermal delivery of celecoxib. Kundlik M *et al.* [54], worked on celecoxib, which is a nonsteroidal anti-inflammatory drug that exhibits anti-inflammatory, analgesic, and antipyretic activities. Recently, considerable interest has been focused on the use of biodegradable polymers for specialized applications such as targeted release of drug formulations; meanwhile, microbeads drug delivery systems using various kinds of biodegradable polymers have been studied extensively during the past two decades.

Coated Celecoxib microbeads (1:1 ratio) showed cytotoxicity against HT-29 cells. DNA Fragmentation study confirms the better anti cancer activity of celecoxib microbeads against human colorectal adenocarcinoma cell line HT-29.

Hence the formulations can be effectively tested for its anticancer activity. Aumana JT *et al.* [55], treated cancer cells with the cyclooxygenase-2 inhibitor celecoxib show growth inhibition and induced apoptosis. This study was conducted to determine if the same processes are relevant to celecoxib's effects on human colorectal adenocarcinomas treated in vivo. The celecoxib pre-treated samples showed decreased expression levels in multiple genes involved in cellular lipid and glutathione metabolism; changes associated with diminished cellular proliferation. Celecoxib pre-treatment for 7 d in vivo is associated with alterations in colorectal adenocarcinoma gene expression which are suggestive of diminished cellular proliferation.

3. CONCLUSIONS:

Chronobiology is the study of biological rhythms and their responses to other metabolic functions of body. Diseases such as bronchial asthma, hyper-cholesterolemia, ulcer, diabetes, arthritis, myocardial infarction, angina and hypertension show symptomatic changes due to circadian rhythmicity. The chronobiology, chronopharmacology and chronotherapeutics of pain have been extensively reviewed. For instance, there is a circadian rhythm in the plasma concentration of C-reactive protein and interleukin-6 of patients with rheumatoid arthritis. Rheumatoid arthritis, level of C-reactive protein increases early morning leading to enhanced pain and inflammation. Chronotherapy for all forms of arthritis using NSAIDs should be timed to ensure that the highest blood levels of the drug coincide with peak pain. Circadian rhythms of behavior in mammals are known to be robust and precise. Also several drugs cause alterations to 24 hours rhythms leading to illness and altered homeostatic regulation.

4. REFERENCES:

1. Mathiowitz E. Encyclopedia of controlled drug delivery. John Wiley and Sons, Inc. New York, 2003, 698-726.
2. Fix JA. Oral controlled release technology for peptides: status and future prospects. *Pharm. Res.*, 1996, 13, 1760-1764.
3. Davis SS, Hardy JG, Fara JW. Transit of pharmaceutical dosage forms through the small intestine. *Gut.*, 1986, 27, 886-892.
4. Singhai SK, Singh VC, Nagar M, Gautam N, Trivedi P. Chronotherapy: A Novel Concept In Drug Delivery. *Der Pharmacia Lettre*, 2010, 2(3), 136-153.

5. Hanson GH. Analgesic, Antipyretic and Anti-inflammatory Drugs; Remington: The Science and Practice of Pharmacy, edited by A. R. Gennaro, Vol.2, 20th edition, Lippincott William & Wilkins, 1459-1460.
6. Loren L. Approaches to Nonsteroidal Anti-inflammatory Drug Use in the High-Risk Patient. *Gastroenterology*, 2001, 120, 594-606.
7. D'Acquisto F, May MJ, Ghosh S. An emerging theme in anti-inflammatory therapies. *Mol. Intervent.*, 2, 2002, 22-35.
8. Boobis A, Raelins M, Thomas S, Martin W. *Therapeutic Drugs*, Edited by Colin Dollery, Churchill Livingstone, 1999, M21- M24.
9. Goodman & Gilman's, *The Pharmaceutical Basis of Therapeutics*, edited by Hardmen, J. G. Tenth edition, Mc Graw Hill Medical Publishing Division, 708.
10. Basit A, Bloor J. Perspectives on colonic drug delivery. *Business briefing: Pharmtech*. 2003, 185-190.
11. Van den Mooter, G., Kinget, R., Oral colon-specific drug delivery: a review. *Drug Deliv*, 2: 81-93, 1995.
12. Ashford, M., Fell, J.T., Targeting drugs to the colon: delivery systems for oral administration. *J Drug Target*, 2: 241-258, 1994.
13. Rodriguez M, Vila-Jato JL, Torres D. Design of a new multiparticulate system for potential site-specific and controlled drug delivery to the colonic region. *J Control. Rel.*, 1998, 55, 67-77.
14. Azzaniga A, Iamartino P, Maffino G, Sangalli ME. Oral delayed release system for colonic specific drug delivery. *International Journal of Pharmaceutics*, 1994, 108, 77-83.
15. Sinha VR, Kumria R. Microbially triggered drug delivery to the colon. *Eur J Pharm Sci*, 2003, 18, 3-18.
16. Kosaraju SL. Colon-targeted delivery systems: Review of polysaccharides for encapsulation and delivery. *Crit Rev Food Sci Nut*, 2005, 45, 251-258.
17. Singhai SK, Chopra VS, Nagar M, Gautam N, Trivedi P. Chronotherapy: A Novel Concept In Drug Delivery. *Scholars Research Library, Der Pharmacia Lettre*, 2010, 2(3), 136-153.
18. Tiwari G, Tiwari R, Wal P, Wal A, Rai AK. Primary and novel approaches for colon targeted drug delivery – A review. *International Journal of Drug Delivery*, 2010, 2, 01-11.
19. Yang L, Chu JS, Fix JA. Colonspecific drug delivery: new approaches and in vitro/in vivo evaluation. *Int. J. Pharm.*, 2002, 235, 1-15.
20. Kinget R, Kalala W, Veroot L, Vanden MG. Colonic drug targeting. *Journal of Drug Targeting*, 1998, 6(2), 129-49.

21. Jain A, Gupta Y, Jain SK. Perspectives of Biodegradable Natural Polysaccharides for Site-Specific Drug Delivery to the Colon. *J. Pharm. Pharmaceut. Sci.*, 2007, 10 (1), 86-128.
22. Sinha VR, Kumria R. Polysaccharides in colon-specific drug delivery *International Journal of Pharmaceutics*, 2001, 224, 19–38.
23. Hovaard L, Brondsted H. Current applications of polysaccharides in colon targeting. *Crit Rev Ther Drug Carrier Sys.*, 1996, 13(4), 185-223.
24. Laine L. Approaches to Nonsteroidal Anti-inflammatory Drug Use in the High-Risk Patient. *Gastroenterology*, 2001, 120, 594–606.
25. Derle DV, Gujar KN, Sagar BSH. Adverse Effects Associated with the Use of Nonsteroidal Antiinflammatory Drugs: An Overview. *Indian Journal of Pharmaceutical Sciences*, 2007, 1, 409-15.
26. Bondeson J. The Mechanisms of Action of Disease-Modifying Antirheumatic Drugs: A Review with Emphasis on Macrophage Signal Transduction and the Induction of Proinflammatory Cytokines. *Gen. Pharmac.*, 1997, 29(2), 127-150.
27. Lewis AJ, Glaser KB, Sturm RJ, Katherine I. Molnar K, Catherine CB. Strategies for the development of new antiarthritic agents *Int. J. Immunopharmac.*, 1992, 14 (3), 497-504.
28. Takagi T, Ramachandran C, Bermejo M, Yamashita S, Lawrence X Yu, Gordon L Amidon. A Provisional Biopharmaceutical Classification of the Top 200 Oral Drug Products in the United States, Great Britain, Spain, and Japan *American Chemical Society. Molecular Pharmaceutics*, 3 (6), 631-643.
29. Akhgari A, Garekani HA, Sadeghi F, Azimaie M. *International Journal of Pharmaceutics*, 2005, 305, 22-30.
30. Sinha VR, Kumria R. Binders for colon specific drug delivery: an in vitro evaluation. *International Journal of Pharmaceutics*, 2002, 249, 23-31.
31. Takeuchi H, Nagira S, Yamamoto H, Kawashima Y. Solid dispersion partides of amorphous indomethacin with fine porous silica particles by using spray-drying method *International Journal of Pharmaceutics*, 2005, 293, 155–164.
32. Lovrecich M, Nobile F, Rubessa F, Zingone G. Effect of ageing on the release of indomethacin from solid dispersions with Eudragits *International Journal of Pharmaceutics*, 1996, 131, 247- 255.
33. Rusua D, Cimpoiub C, Hodis T. The control over the new obtaining proœdeum of indomethacin1 *Journal of Pharmaceutical and Biomedical Analysis*, 1998, 17, 409–413.
34. Chena X, Griesserb UJ, Tea RL, Pfeiffera RR, Morrissa KR, Stowella JG, Byrna SR. Analysis of the acid–base reaction between solid indomethacin and sodium bicarbonate using infrared spectroscopy, X-ray powder diffraction, and solid-state nuclear magnetic resonance spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis*, 2005, 38, 670–677.
35. Casellaa R, Williamsb DA, Jambhekarb SS. Solid-state-cyclodextrin complexes containing indomethacin, ammonia and water. II. Solubility studies *International Journal of Pharmaceutics*, 1998, 165, 15–22.
36. Dixit M, Gowrav MP, Selvam P, Kulkarni PK. Preparation and characterization of spray Dried microsphere of indomethacin. *International Journal of Drug Formulation & Research*. Jan-Feb., 2011, 2 (1).
37. Liu H, Yang XG, Nie SF, Wei LL, Zhou LL, Liu H, Tang R, Pan WS. Chitosan-based controlled porosity osmotic pump for colon-specific delivery system: Screening of formulation variables and in vitro investigation. *International Journal of Pharmaceutics*, 2007, 332, 115–124.
38. Mohammed E, Novotny L, Hamza H. Determination of diclofenac sodium, flufenamic acid, indomethacin and ketoprofen by LC-APCI-MS *Journal of Pharmaceutical and Biomedical Analysis*, 2001, 24, 587–594.
39. JavadzadehY, Siah MR, Asnaashari S, Nokhodchi A. Liquisolid technique as a tool for enhancement of poorly water-soluble drugs and evaluation of their physicochemical properties, *Acta Pharm.*, 2007, 57, 99–109.
40. Bogdanovaa SV, Sidzhakovab D, Karaivanovaa V, Georgievaa SV. Aspects of the interactions between indomethacin and nicotinamide in solid dispersions *International Journal of Pharmaceutics*, 1998, 163, 1–10.
41. Chowdary KPR, Deepthi KS, Rao YS. Mucoadhesive Microcapsules of Indomethacin: Evaluation for Controlled Release and Ulcerogenic Activity *International Journal of Pharmaceutical Sciences and Drug Research*, 2009, 1(2), 74-79.
42. Hubert PH, Renson M, Crommen J. A fully automated high-performance liquid chromatographic method for the determination of indomethacin in plasma. *Journal of Pharmaceutical & Biomedical Analysis*, 1989, 7(12), 1819-1827.

43. Richard NU, Rasmussen M, Grant C, Martinez AM, Cold GE, Ludbrook GL. Pharmacokinetics and Pharmacodynamics Of Indomethacin: Effects On Cerebral Blood Flow In Anaesthetized Sheep Clinical and Experimental Pharmacology and Physiology, 2008, 35, 317–323.
44. Akhgari A, Sadeghi F, Garekani HA. Combination of time-dependent and pH-dependent polymethacrylates as a single coating formulation for colonic delivery of indomethacin pellets International Journal of Pharmaceutics, 2006, 320, 137–142.
45. Krishnaiah YS, Kumar BD, Satyanarayana S, Karthikeyan RS. Studies on the development of oral colon targeted drug delivery systems for celecoxib in the prevention of colorectal cancer. Journal of drug targeting, 2002, 10(3), 247-254.
46. Haupt S, Zioni T, Gati I, Kleinstern J, Rubinstein A. Luminal delivery and dosing consideration of local Celecoxib administration of colorectal cancer. European journal of pharmaceutical sciences, 2006, 28, 204-211.
47. Arico S, Pattingre S, Bauvy C, Gane P, Barbat A, Patrice, Dogno C, Denis EO. Celecoxib induces Apoptosis by inhibiting 3-hosphoinositide-dependent protein kinase-1 Activity in the Human colon cancer HT-29 cellline. The journal of Biological chemistry, 2002, 277, 27613-27621.
48. Sinha VR, Anitha R, Ghosh S, Amita, Kumria R, Bhinge JR, Kumar M. Physicochemical characterization and in vitro dissolution behaviour of celecoxib on cyclodextrin inclusion complexes Acta Pharm., 2007, 57, 47–60.
49. Vernon ES, Hawk ET, Viner JL, Lubet RA. Mechanisms and applications of non-steroidal anti-inflammatory drugs in the chemoprevention of cancer Mutation Research, 2003, 523–524, 137–144.
50. Baboota S, Faiyaz S, Ahuja A, Ali J, Shafiq S, Ahmad S. Development and validation of a stability-indicating hplc method For analysis of celecoxib (cxb) in bulk Drug and microemulsion formulations. Acta chromatographica, 2007, 18, 116-129.
51. Ali J, Arora S, Ahuja A, Babbar AK, Sharma RK, Khar RK. Formulation and Development of Floating Capsules of Celecoxib: In Vitro and In Vivo Evaluation. AAPS Pharm. Sci. Tech., 2007, 8 (4), Article 119.
52. Seedher N, Bhatia S. Solubility Enhancement of Cox-2 Inhibitors Using Various Solvent Systems. AAPS Pharm. Sci. Tech., 2003, 4 (3), Article 33.
53. Soliman SM, Malak NSA, Gazayerly ONE, Rehim AAA. Formulation of microemulsion gel systems for transdermal delivery of celecoxib: In vitro permeation, anti-inflammatory activity and skin irritation tests Drug Discoveries & Therapeutics, 2010, 4(6), 459-471.
54. Kundlik M, Girhepunje P, Krishnapiillai, Ranju S, Pal H, Gevariya B, Thirumoorthy N. Celecoxib loaded microbeads: a targeted drug delivery for colorectal Cancer. International Journal of Current Pharmaceutical Research, 2010, 2(1), 46-54.
55. Aumana JT, Churchb R, Lee SY, Watson MA, Fleshmanb JW, Mcleoda HL. Celecoxib pre-treatment in human colorectal adenocarcinoma patients is associated with gene expression alterations suggestive of diminished cellular proliferation. European journal of cancer, 2008, 44, 1754 –1768.