DESIGN AND EVALUATION OF MULTIPARTICULATE CONTROLLED RELEASE DRUG DELIVERY SYSTEMS FOR PAIN MANAGEMENT IN CHRONIC RHEUMATOID ARTHRITIS AND ANKYLOSING SPONDYLOSIS WITH LEFLUNOMIDE AND INDOMETHACIN.

SYNOPSIS OF THE THESIS

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S Y N O P S I S

Topic

‘‘Design and Evaluation of Multi-particulate Controlled Released Drug delivery Systems for pain management in chronic rheumatoid arthritis and ankylosing spondylosis with Leflunomide and Indomethacin”

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TOPIC:

‘‘Design and Evaluation of Multi-particulate Controlled Released Drug delivery Systems for pain management in chronic rheumatoid arthritis and ankylosing spondylosis with Leflunomide and Indomethacin”

BACKGROUND OF THE STUDY:

Effective and appropriate pain management is of great concern to both physicians and surgeons to give comfort to patients in distress of acute pain. It occurs with trauma. Sometimes such pain is reversible and needs intervention till correction of the underlying problems.

The concern of this research work is chronic pain associated with osteoarthritis, rheumatoid arthritis and ankylosing spondylosis which arise from path physiological conditions which evade diagnosis and hence focused treatment.

Effective and successful pain management therapy would depend not only on the choice of an appropriate drug molecule but also on the delivery system. In this research investigation the choice of appropriate drugs are leflunomide and indomethacin.Through factorial design and a few multi-particulate delivery systems these drugs will be evaluated using various biodegradable polymers such as ethylcellulose and acrylic co-polymers (various grades of eudragit RS100 and RL 100) ,and plasticizers such as polyisobutylene in the preparation of microspheres.

The challenge of this research investigation will be centered around the development of drug delivery systems which is optimum, from both technical and biopharmaceutical angle. As many of the drugs reach to the general circulation at a rate which is modulated by the physiological processes in the body and not by the delivery system. Thus the objective would be to deliver the leflunomide and indomethacin from the designed delivery systems in the required amount at a desired control rate to the target organ over the predetermined time period.

In contrast with conventional dosage forms, the delivery of the therapeutic requirement of the drug to the target organ, tissue, cell or receptor for the desired time period to elicit a pharmacological effect is a task often approximated but rarely satisfactorily achieved.

Administration of a drug by per oral or parenteral route is rapidly absorbed through the gastrointestinal mucosa or the tissue surrounding the injection site. Therefore drugs are transferred to the systemic circulation from where the drug is carried to and distributed to all the body organs differentially giving a plasma concentration versus time profile following a unit dose of an oral or i.v bolus of

a drug. Generally a curve that reaches its peak gradually after administration and gradually begins to decline in systemic elimination.

To achieve effective therapeutic results in pain management it is essential to maintain a constant therapeutic plasma level of the candidate drug between the medication intervals. In such case a greater dose of the candidate drug has to be administered with concomitant higher plasma level, much above the therapeutic level with toxic manifestation. To circumvent this if we reduce the dose in that case the blood level of the candidate drug during the period of pain management would produce blood plasma levels which fall much of the period of treatment remain below the threshold of pain control efficacy.

In this case the fraction of the administered pain relieved drug is utilized by the patient in decreasingly small amount. In conventional dosage forms for pain management it is only possible by repeat administration leading to non-compliance and discomfort to the patient. Never- the- less continuous pain management drug administration from conventional dosage forms is impossible and impractical. Development of state–of –the-art Hi-tech Novel Drug Delivery made it possible so that the rate of absorption of pain management candidate drug can be positioned in the affected body at a rate by controlling the rate of release of the pain management candidate drugs from fabricated drug delivery systems. This research work is exhibited to lead to the development of more practical and highly promising method which would provide the desired blood levels of the chronic pain management drugs over the desired period of time. It is intended to design and evaluate some multi-particulate drug delivery systems for pain management in chronic arthritis and ankylosing spondylosis.

Products fabricated with leflunomide and indomethacin will produce good result because their way of action are different.Indomethacin acts on the symptoms of pain where as leflunomide acts on the cause of pain by suppressing the over acting on immunity mechanism.

PAIN MANAGEMENT:

It is that branch of medicine employing an interdisciplinary approach to easing the suffering and improving the quality of life of those living with pain [1]. Pain usually resolves promptly once the underlying trauma or pathology has healed, and is treated by one practitioner, with drugs such as analgesics and (occasionally) anxiolytics. Treatment approaches to long term pain include pharmacologic measures, such as analgesics, tricyclic antidepressants and anticonvulsants, interventional procedures, physical therapy, physical exercise, application of ice and/or heat, and psychological measures, such as biofeedback and cognitive behavioral therapy[2] . In the treatment of chronic rheumatoid Arthritis pain, whether due to malignant or benign processes, the exact

medications recommended will vary with the country and the individual treatment center.

Classification of pain and its management:

Pain is classified as (1) mild (2) mild to moderate (3) moderate to severe pain.

Mild pain

Neck and back pain, which improve without specific treatment, headache, and facial pain, are in this class of pain. In the pain keeps returning, there are preventive approaches which can help the people to relieve by doing some exercise. In many cases the pain deals to change in way of behavior. Paracetamol (acetaminophen), or a non steroidal anti-inflammatory drug such as ibuprofen [3] is use for this case.

Mild to moderate pain

Defines as pain that may persist or progress over a long period of time. Paracetamol, an

NSAID and/or paracetamol in a combination product with a weak opioid such as hydrocodone used to provide greater relief than their separate use.

Moderate to severe pain

Chronic muscular pain in the arms, lower back, legs and neck are under this class of pain.

Morphine is the gold standard of choice, followed by Oxycodone, Hydromorphone, Oxymorphone and Fentanyl in the form of a transdermal patch designed for chronic pain

management. Diamorphine, Methadone and Buprenorphine are used less frequently. Pethidine is not recommended for chronic pain management due to its low potency, short duration of action, and toxicity associated with repeated use. Amitriptyline is prescribed for chronic muscular pain in the arms, lower back, legs and neck. While opioids are often used in the management of chronic pain, high doses are associated with an increased risk of opioid overdose.[4]

Opioids

Opioid medications can provide a short, intermediate or long acting analgesia depending upon the specific properties of the medication and whether it is formulated as an extended release drug. Opioid medications may be administered orally, by injection, via nasal mucosa or oral mucosa, rectal,

transdermal, intravenously, epidurally and intrathecally. In chronic pain conditions the drug can be applied in combination of a long acting or extended release medication with a shorter acting medication for breakthrough the pain (exacerbations). Most opioid treatment is oral (tablet, capsule or liquid), but suppositories and skin patches can be prescribed.

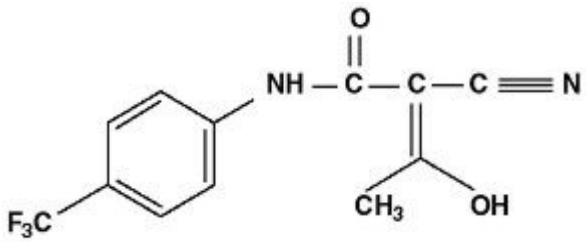
Non-steroidal anti-inflammatory drugs

The other major group of analgesics is Non-steroidal anti-inflammatory drugs (NSAID). This class of medications does not include acetaminophen, which has minimum anti-inflammatory properties. However, acetaminophen may be administered as a single medication or in combination with other analgesics (both NSAIDs and opioids). The alternatively prescribed NSAIDs such as ketoprofen and piroxicam, have limited benefit in chronic pain disorders and with long term use is associated with significant adverse effects. The use of selective NSAIDs designated as selective COX-2 inhibitors have significant cardiovascular and cerebrovascular risks which have limited their utilization.[5][6]

Leflunomide

Now a day leflunomide have been chosen for treatment of rheumatoid arthritis. It also has been used occasionally to treat other diseases such as systemic lupus erythematoms or sporiatic arthritis. It is an immunosuppressive medicine which means that it works by reducing the activity of immune system. It is a pyrimidine synthesis inhibitor indicated for the treatment of adults with rheumatoid arthritis. [7] Leflunomide is a pyrimidine synthesis inhibitor. The chemical name for leflunomide is N-(4′-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide. It has an empirical formula $C_{12}H_9F_3N_2O_2$, a molecular weight of 270.2.

Following oral administration, leflunomide is metabolized to an active metabolite A77 1726 which is responsible for essentially all of its activity *in vivo*. Plasma levels of leflunomide are occasionally seen, at very low levels. Studies of the pharmacokinetics of leflunomide have primarily examined the plasma concentrations of this active metabolite.



A77 1726 (M1)

Absorption

Following oral administration, peak levels of the active metabolite occurs between 6 – 12 hours after dosing. Due to the very long half-life a loading dose of 100 mg for 3 days was used in clinical studies to facilitate the rapid attainment of steady-state level.

The drug inhibits dihydro orotate dehydrogenase in the de- novo- path way of pyrimidine. Leflunomide is a prodrug that is converted almost completely (98%) to an active metabolite A77-1726 (2- cyano-3-hydroxy-N-(4- trifluro-methyl phenyl) croton amide. It works by suppressing immune system since rheumatoid arthritis is caused by damage from an overactive immune system. This drug is taken by mouth, generally once daily. It is generally recommended to start with special higher dose.This action helps to reduce inflammation and to reduce pain and swelling. It also limits damage to the joints and helps to prevent disability in the long term. Because leflunomide acts to reduce the damage to the joints, rather than just to relieve the pain, it belongs to the so called disease modifying anti-rheumatic drug. [8]

Indications:

(1) To reduce signs and symptoms of rheumatoid arthritis.

(2) To inhibit structural damage as evidence by X-ray erosions and joint pain narrowing.

(3) To improve physical function of the affected joints.

(4) To reduce inflammation and pain.

(5) To help to prevent disabilities in long term.

Pharmacokinetics:

Leflunomide is metabolized to teriflunomide which is responsible for pharmacological action. Pharmacokinetics studies of leflunomide have primarily examined through study of the plasma concentration of teriflunomide. Plasma levels of leflunomide are occasionally detected, but at very low level. After oral administration, peak plasma levels of triflunomide occur between 6 - 12 hours after dosing.

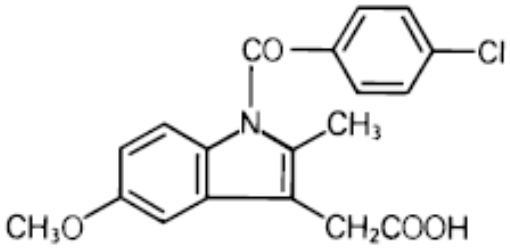
Due to its very long half life (approximately 2 week) a loading dose of 100 mg for 3 days is used in clinical studies to reach steady state levels quickly. Teriflunomide can be found as late as 2 years after termination of therapy. Aspirin or Indomethacin a non-steroidal anti inflammatory drug and/ or low dose corticosteroids may be continued during treatment with leflunomide. [9]

Storage Condition:

It is well stable at 25ºC temperature but stable in between 15-30ºC temperature. It is not moisture sensitive and can be stored in a light protected closed container. It is available in powder form and in tablet form.

Indomethacin

Indomethacin is a non steroidal anti-inflammatory drug and it inhibits prostaglandin synthesis. However it has gastrointestinal irritation and CNS side effect as a result of rapid drug release properties. Chemically it is acetic acid derivatives (Methylated indole). It reduces prostaglandin biosynthesis in human system. Indomethacin is a non-steroidal anti-inflammatory indole derivative designated chemically as 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1-indole-3-acetic acid. Indomethacin is practically insoluble in water and sparingly soluble in alcohol. It has a pKa of 4.5 and is stable in neutral or slightly acidic media and decomposes in strong alkali. The structural formula is:



Chemically it is a methylated indole derivative. Its chemical formula is $C_{19}H_{16}ClNO_4$.

and molecular weight is 357.8. Indomethacin is a typical a non steroidal anti-inflammatory drug (NSAID) that also exhibits analgesic and antipyretic activity. Indomethacin may cause serious adverse effects and should not be used as a simple analgesic or antipyretic. Indomethacin is a poorly soluble, highly permeable (class II) drug, its oral absorption is often controlled by the dissolution rate in the gastrointestinal tract. [10]

Indications:

Indomethacin is used to treat osteoarthritis and control acute pain. Anti inflammatory effects of Indomethacin are believed to be due to inhibition of cyclo oxygenase in platelets which leads to the blockage of prostaglandin synthesis. Antipyretic action may be due to action on the hypothalamus resulting in an increased peripheral blood flow, vasodilation and subsequent heat dissipation. It is effective for relieving joint pain, swelling and tenderness,

increasing grip strength and decreasing duration of morning stiffness. It is estimated to be approximately 20 times more potent than aspirin.

Pharmacokinetics:

Oral indomethacin has excellent bioavailability. Its bioavailability is 100% following oral administration. After oral administration, the peak plasma level occurs between 4-5 hours. Indomethacin for poorly soluble, highly permeable (class II) kind of drug needs larger amount for absorption. The in-vitro drug release study follows first order diffusion controlled dissolution. More than 85% of drug was released over 6 hours at pH 6.2 for all types of normal dosage forms.[11]

Objective rationality of combination of Leflunomide and Indomethacin in the formulation.

The micro spheres approach has been widely used to overcome drug problems related to bad taste, poor aqueous or lipid solubility, inadequate chemical and an enzymatic stability, incomplete absorption across a variety of biological membranes, distribution and premature/ rapid/ slow metabolism to inactive species.[12] The NSAIDs share certain therapeutic actions and side effects. Gastric pain, mucosal erosion/ ulceration, blood loss and gastrointestinal (GI) toxicity are the major drawback of NSAIDs.[13]

Management of rheumatoid arthritis is done by first line of medication followed by second line of medications. Treatment with first line of medication is done with acetyl salicylate (aspirin), naproxen, ibuprofen, etodolac and indomethacin. These drugs can reduce tissue inflammation, pain and swelling. Newer NSAIDs include selective cox-2 inhibitors have often anti inflammatory effect with less risk of stomach irritation and bleeding risk. Second line of medication is done with disease modifying anti-rheumatoid drugs. Rheumatoid arthritis requires medications other than NSAIDs and corticosteroids to stop progressive damage of cartilage, bone and adjacent soft tissues by inhibiting the enzyme cyclo oxygenase. Leflunomide is this kind of drug. They are used for long period of time, even years at varying doses.Hydroxy chloroquine, sulfasalozine, D-penicillamide are used as disease modifying anti- rheumatic drugs.

AIM OF THE STUDY:

Recently pharmaceutical researches are clearly divided into two paths. One deal with new chemical entities corresponding pharmacological research and related works, and the second one comprises the efforts to formulation existing as well as newer drugs in suitable dosage form to increase their intrinsic efficacy and decrease their inherent toxicity. For last few decades the formulation scientists

have directed their efforts in developing controlled drug delivery systems. Due to rapid release of indomethacin from its conventional dosage forms it is necessitated the formulation of a controlled release dosage form. In the present investigation a novel procedure is developed for preparation of controlled release micro spheres. For these two synthetic polymers Eudragit RS 100 and Eudragit RL 100 are chosen for formulation of micro spheres. Depending on the solubility profiles of the drug and polymers we have chosen the solvent evaporation technique for micro spheres formulation.

The kinetic function governing the release profile of a drug from a single micro spheres is determined mainly by a restricted number of parameters such as drug content, particle shape, size, surface area and porosity. The release kinetics of leflunomide and indomethacin from the prepared micro spheres are determined by in- vitro analysis.

To design experimental preformulation studies by design of experiments and also by functional analysis as to check the feasibility of some multi-particulate drug delivery systems for pain management in chronic arthritis both osteoarthritis and rheumatoid arthritis and also ankylosing spondylosis to enhance and improve the quality of life. Once the feasibility studies are through a few multi-particulate drugs will be fabricated and evaluated using various biodegradable polymers such as ethylcellulose and acrylic co-polymers (various grade of eudragit RS100 and RL100), plasticizer such as polyisobutylene.

PLAN OF WORK:

First phase of study includes:

(1) General introduction and study of the path physiology of rheumatoid arthritis and osteo arthritis.
(2) Literature survey of leflunomide and indomethacin and some polymers.

Second phase of study includes:

(1) General survey on drug delivery system.
(2) Study on pharmacology, toxicity, side-effects, adverse-effects, precautionary measures during use of the drugs under investigation.
(3) Analytical process development and determination of standard calibration curve.

Third phase of study includes:

(1) Data analysis of micro particulate drug delivery systems.
(2) Mechanism of drug release from various delivery systems.
(3) Preformulation study of prepared drugs.

(4) Validation of equipments.
(5) Formulation design through factorial analysis.
(6) Design of delivery system.
(7) Determination of percentage of yield, flow properties.
(8) In-vitro dissolution study.
(9) Characterization of solid dosage forms by Differential Scanning Calorimetry. Scanning Electron Microscopy.
(10) Results and discussion.
(11) Summary and conclusion.

METHODS:

Analytical methods for the estimation of leflunomide and indomethacin.

Determination of λ max for leflunomide:

A 100 ml stock solution containing 1mg /ml of leflunomide was prepared by dissolving the drug with few drops of acetone and rest of the volume was adjusted with phosphate buffer (pH 7.2) with mild shaking. This stock solution was further diluted to10µg/ml and 5 µg/ml with the same phosphate buffer. Aliquots of these solutions were then taken in a quartz cell and scanned for determining λ max in the range of 200- 400 nm using phosphate buffer as blank in a double beam UV spectro photometer (Model UV-1700, Shimadzu).

Determination of λ max for indomethacin:

A 100 ml stock solution containing 1mg /ml of indomethacin was prepared by dissolving the drug with few drops of methanol and rest of the volume was adjusted with phosphate buffer (pH 7.2) with mild shaking. This stock solution was further diluted to10µg/ml and 5 µg/ml with the same phosphate buffer having the pH 7.2. Aliquots of these solutions were taken in a quartz cell and scanned for determining λ max in the range of 200- 400 nm using phosphate buffer as blank in a double beam UV spectro photometer (Model UV-1700, Shimadzu). [14]

Preparation of Standard curve for Leflunomide and indomethacin:

Accurately weighed, 10 mg of leflunomide and 10 mg of indomethacin separately into two volumetric flasks. These were dissolved in few drops of acetone and methanol respectively and the final volume was made to 100ml with phosphate buffer (pH 7.2).From these stock solutions a numbers of standard solutions containing 1,2,4,6,8,10 µg/ml were prepared. The absorptions of those solutions were noted against 260nm for leflunomide and against 319nm for indomethacin by UV/visible spectrophotometer (Model UV-1700,

Shimadzu).The room temperature was maintained an ambient temperature (25°C). [15]

Particle size analysis:

Particle size distributions in samples of microspheres were measured by sieve shaker machine. The different sieves having the numbers 10, 20, 25, 30, 40 and 50 were set in the sieve shaker machine, keeping the higher size of sieve in the upper most portions. The sieve shaker machine was kept on for 30 minutes. Due to three dimensional movements the particles of different ranges of micro spheres were well separated and retained in different sieves. The instrument was fitted with a special clumping device that ensured that sieves were held firmly and allowed them to be quickly removed and replaced. The vibratory action moved the micro spheres all over the sieve in a unique way providing faster and more efficient sieving, while the rapid vertical movements also help to keep the apertures free from blocking. The weight of micro spheres retained in different sieves was taken to evaluate the pattern of particle size distribution. Size distribution was determined by sieving the micro particles using a nest of standard sieves ad well as by optical microscopy. [16]

Angle of repose:

Angle of repose could be determined by the fixed funnel and free standing cone method. The method employed a funnel that was secures with its tip at a given height H above the graph paper that was placed on a flat horizontal surface. Granules /powder were carefully poured through the funnel, the apex at the conical pile just touch the tip of the funnel. [17] Thus with R being the radius of the base of the conical pile, then $\tan \alpha = H / R$ Where H and R were the height and radius of the granules cone.

Preparation of polymethyl- acrylate microspheres of leflunomide:

20 mg of leflunomide was weighed separately in five cleaned 50ml glass beakers.20mg, 40mg, 60mg, 80mg, 100mg of eudragit RL100 and eudragit RS100 mixture in the ratio of 1:4 were also weighed separately in five glass beakers. Leflunomide and Eudragit (RL 100 and RS 100 in the ratio of 4:1) were used in the ratio of 1: 1, 1: 2, 1: 3, 1: 4, 1: 5 giving the batch no.B_1, B_2, B_3, B_4, B_5 respectively.

Polymers were dissolved in acetone by stirring with a magnetic bar. The temperature was maintained at 15ºC to minimize the rapid evaporation of acetone. Stirring was continued until a smooth polymer solution was formed. The required amount of drug (which were weighed separately) and one drop of polyisobutylene was dispersed uniformly in the polymer solution. The resulting slurry was poured at a constant and steady stream in liquid paraffin. Its

temperature is maintained at 15º C .While adding it was being stirred at 800 rpm with an electrical stirrer (Remi, Bombay).The liquid paraffin had an absolute viscosity of 87.1 cp at 30ºC (determined by falling sphere method) obtained by blending heavy liquid paraffin with light liquid paraffin in the ratio of 1: 1. The salary was converted into spherical microcapsules .Stirring was continued for sufficient period of time to evaporate the acetone at ambient temperature. Petroleum ether was added to the extract the residual amount of acetone and to rigidize [18] the resultant microcapsules. The microcapsules were separated by filtration through a 100 mesh nylon cloth and washed with three to four portions of 100 ml cold petroleum ether to remove adhering liquid paraffin and dried at 40-45°C for 4 hours.

Preparation of Indomethacin micro spheres:

Microspheres were prepared by solvent evaporation technique. [19] The weighed quantity of eudragit RS 100 and eudragit RL 100 in the ratio of 1:10 were dissolved in acetone and weighed quantity of the Indomethacin was dissolved in small quantity of chloroform separately in small glass beakers. These two solutions were well mixed with the help of a magnetic stirrer for five minutes. This solution was added in a form of a continuous stream in the light liquid paraffin with continuous stirring. The stirring speed was kept around 400 r p m. and was continued for 4 hours to allow evaporating the smell of acetone. The micro spheres were filtered and washed with a portion of petroleum ether thrice. The washed micro spheres were dried in an oven at room temperature not exceeding 25°c.In this way five batches of micro spheres namely B1, B2, B3, B4, B5 were prepared using different drug: polymers ratio (1: 1, 1:2, 1:3, 1:4, 1:5).

FT-IR Study:

Determination of interaction between drug and polymer was performed using FT-IR analysis. FT-IR of leflunomide blank, polymer blank, and drug loaded polymer were studied with potassium bromide pellets using Perkin-Elemer model 883 spectroscope in range 400 -4000 cm-^{1} and the resolution was 2 cm^{-1} at Indian Institute of Chemical Biology , Kolkata. Same study had been done with indomethacin also.

SEM Study:

For morphology and surface characteristics, prepared micro spheres were coated with gold in an argon atmosphere. The surface morphology of the microspheres was then studied by Scanning Electron Microscope (model quanta 200mk 2 make Fer Netherland) at Bose Institute, Kolkata.

In vitro drug release study:

In vitro drug release studies were carried out for all products in USP type II fitted with six rotating basket [Campbell Electronics, Mumbai, India].The release of leflunomide from microcapsules to the surrounding sink solution of pH 7.2 phosphate buffer media was studied. The concentration of leflunomide was determined spectrophotometrically at 260 nm and cumulative amount of the drug released was determined from calibration curve. The dissolution studies were conducted on microspheres in different particle size ranges. However for purpose of this reporting, the dissolution kinetic of the optimum size of 25 meshes was taken up for detailed kinetics studies.[20]

RESULTS AND DISCUSSION:

Leflunomide

Drug entrapment and content uniformity studies:

No significant differences in drug loading for micro spheres made of different polymer solution viscosity were noted. However, the drug loading increases as the concentration of polymer was increased relative to drug concentration. The analysis of drug content showed a maximum entrapment efficiency of 91.7 ± 0.62% at the drug polymer ratio of 1:5. Overall, drug entrapments were found to range between 84.4 ± 0.22% and 91.7 ± 0.62%.

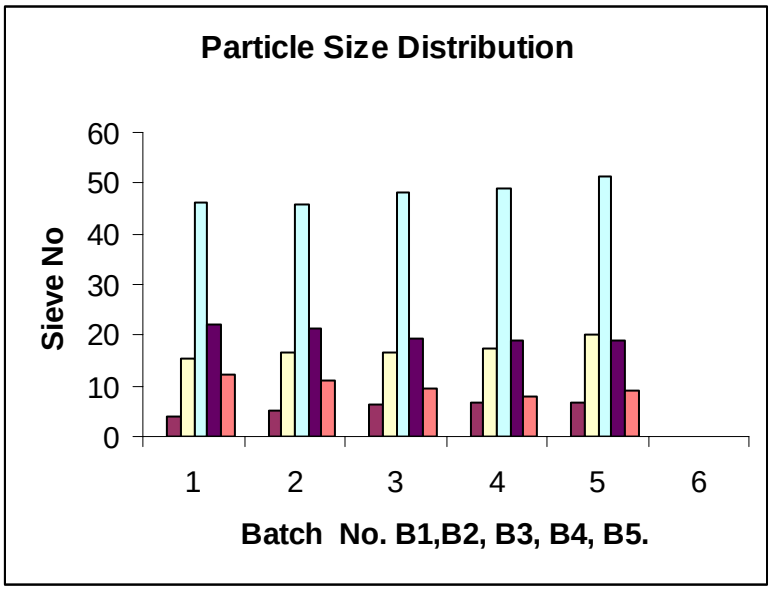
Initially, solvents for polymer, concentration of polymer solution, temperature of the system and viscosity of liquid paraffin were optimized. The polymer concentrations as well as the solvent concentration was assessed such that it would be easily pourable in the processing vessel and no pin holes would be present on the surface of the microcapsules. Liquid paraffin of high viscosity posed resistance to the microspheres to assume a spherical shape. Low viscosity of the liquid paraffin led to gradual adherence of the microspheres, resulting in the formation of an agglomerated mass. Application of heat to flash off acetone led to sudden agglomeration of the microspheres and formation of pin holes in the microcapsules due to rapid evaporation of acetone. The uniformity of drug contents in each batch indicated reproducibility of the manufacturing method.

Particle size analysis:

Results of sieve analysis revealed that microcapsules prepared were confined within 10–50-mesh sieve sizes and maximum amount of microspheres were retained by 25-mesh sieve. The particle size of the micro spheres using a different ratio of drug polymers differs significantly at the same stirring speed.

There was formation of microspheres with large and irregular sieve due to an increase in solution viscosity of the polymers. Hence, higher agitation speed was required to prepare micros spheres of the same size as that of single polymers alone.

Graphical presentation of particle size distribution of different batches of leflunomide microspheres.



Where 1, 2,3,4,5 represent B1, B2, B3, B4, and B5 respectively.

Table: Distribution of different particle sizes of different batches of leflunomide in weight percentage:

Sieve No	B_1	B_2	B_3	B_4	B_5
10	4.1±0.12	5.3± 0.21	6.2± 0.13	6.6± 0.22	6.7± 0.12
20	15.4± 0.08	16.4± 0.19	16.7± 0.11	17.4± 0.31	20.1±0.23
25	46.1± 0.22	45.7± 0.31	48.3±0.15	49.0±0.16	51.2±0.11
30	22.2±0.09	21.5±0.11	19.5 ±0.22	19.0±0.21	18.8±0.10
40	12.2±0.12	11.1±0.16	9.3±0.08	8.0±0.13	9.2±0.14

*** All values are expressed as mean± SD, n=3**

FT-IR studies of leflunomide:

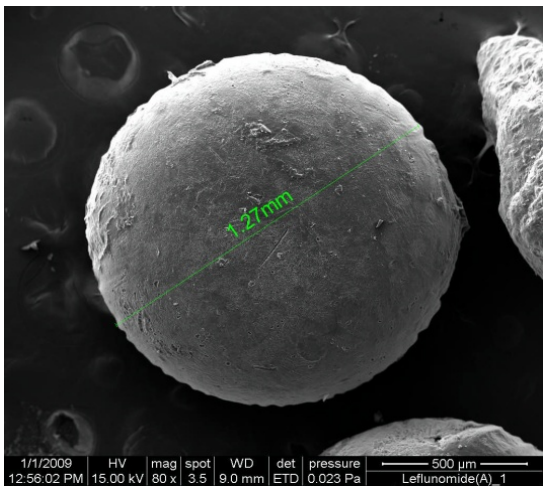
FT-IR spectra study showed no change in the fingerprint region of pure drug spectra. This confirmed the absence of drug to polymer interaction. FT-IR

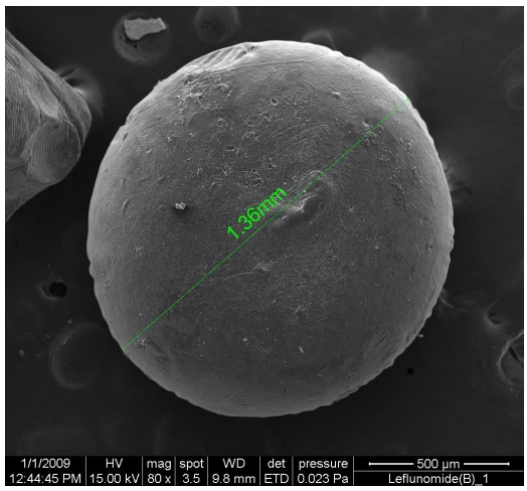
spectra revealed that there was no such interaction between the drug and the polymers used for micro sphere formulation.

Scanning Electron Microscopy(SEM):

For morphology and surface characteristics, prepared micro spheres were coated with gold in an argon atmosphere. The surface morphology was then studied by Scanning Electron Microscope.SEM study showed that the particles made of eudragit RL 100 and eudragit RS100 was spherical. The SEM image of leflunomide containing microspheres was shown in figure below. The microspheres were discrete, free-flowing and spherical. There was hardly any pore visible, indicating a total enveloping of the core by the coat. But, at a higher concentration, pores were observed, which might have been formed during the solvent evaporation process. Presence of pores was detected on the surface, which increased in size and number with respect to time after dissolution, indicating leaching of the drug through these channels.

Figure
Scanning Electron Microscopy (Leflunomide):





A) Leflunomide micro sphere after dissolution. (X 75)

B) Leflunomide micro sphere before dissolution (X 75)

In vitro dissolution studies:

In-vitro dissolution studies of all batches of microspheres were determined and analyzed. Microspheres made of eudragit RS 100 and eudragit RL 100 showed good flow properties and maximum release tendency. The release of drug from microspheres was gradual without producing a dose dumping effect and it was sufficiently prolonged as the coating thickness was increased.

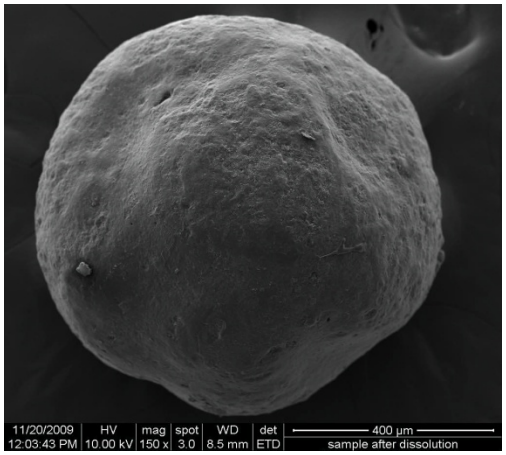
The microspheres with drug- polymer ratio 1: 1 and 1: 2 showed linearity by using pseudo first order kinetics. The difference between first order diffusion models was noted to be minimum. For microspheres with drug- polymer ratio1: 3, 1: 4 and 1: 5, best fit kinetic model was squire root equation, i.e. Higuchi matrix diffusion. The combination of polymers at polymer to polymer ratio 1:2 helped to leach out the drug from its matrices and exhibited an initial rapid drug release for the first 2-3 h and then a slower drug release, which could be best explained by Higuchi's spherical matrix release. Thus, on overall analysis of the kinetic data, it may be inferred that the release of leflunomide from the microspheres was predominantly diffusion rate controlled and followed the Higuchi equation.

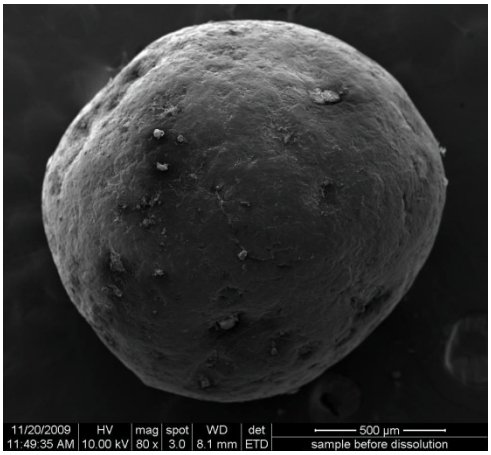
Indomethacin

A total of five batches of indomethacin microspheres namely B_1, B_2, B_3, B_4, B_5 were prepared by solvent evaporation technique and by optimizing the proportion of polymers, stirring speed and bed temperature. Batch no.B_4 containing eudragit RS 100 and eudragit RL 100 in the ratio 1: 9 and drug polymer ratio 1:4 was selected as best formulation because of its favourable size, good flow properties, angle of repose 10.54, tapped density 0.811, bulk density 0.724, Carr's index 8.747, entrapment efficiency and also due to its sustained drug release effect.

The standard curve of Indomethacin was prepared in phosphate buffer solution (pH 7.2) at λ max 319 nm and the regression coefficient was found to be 0.997 with slope of 0.052 and Y- intercept of 0.002.

For morphology and surface characteristics, prepared micro spheres were coated with gold in an argon atmosphere. The surface morphology was then studied by Scanning Electron Microscope (Hitchi S-3600n Scanning Electron Microscope, Japan). The microspheres prior to in vitro dissolution studies showed uniform smooth surface. After the dissolution studies, the surfaces became rough and pore were formed on the surfaces of the microspheres. The existing small spores which were previously present became larger in size. Figures were given below:





Indomethacin microsphere before dissolution

Indomethacin microsphere after dissolution

Drug polymer interaction was studied using FTIR. There were no changes in the major peaks of indomethacin in the presence of eudragit RL100 and eudragit RS100.

It was noticed that mean particle size increased with increase in polymer concentration. Depending upon the drug - polymer ratios, the percentage of drug entrapment, the percentage of drug loading and percentage of yield in the microspheres were found to the ranges between 89.53% to 92.55%; 10.06% to 56.05% and 81.33% to 92.79% respectively in different batches.

The entrapment efficiency, drug loading were also found to be dependent on nature of polymer, polymer concentration and also on the stirring speed used in the formulation.

The flow property was expressed in terms of Carr's index. The Carr's index for all formulations was less than 20, which indicated excellent flow property.

The good flow property suggested that the microspheres could be easily handled during processing. Bulk and tapped densities showed good pack ability of the microsphers.

In the in-vitro release profile of indomethacin from all the preparations was examined for10 h. using the rotating paddle method specified in USP XXII at 100 rpm.

Microspheres equivalent to 75 mg of drug were placed in the basket and the medium was maintained at 37±0.5°c.The effect of variation in drug to polymer ratio on drug release was studied on indomethacin microspheres. Increase in polymer concentration resulted in a decrease in drug release rate. Sustain release up to 12h was achieved when drug polymer was taken up to 1: 4 ratio.

The release data obtained were fitted into various release kinetic models. The drug release kinetic profile was best fit in Higuchi matrix model. Kinetics followed by first order kinetics and zero order kinetics. It was found that the mechanism of drug release from microspheres was diffusion controlled. Among the different formulations of microspheres, the formulation B4 containing eudragit RS100 and eudragit RL100 was selected as best formulation because of its favorable size, good flow property (angle of repose 10.54, tapped density 0.811, bulk density0.724, Carr's index 8.747), entrapment efficiency 92.55% and also due to its sustained drug release effect. The microspheres prior to in-vitro dissolution studies showed uniform smooth surface.

After the dissolution studies, the surface became rough and pores were formed on the surface of the microspheres and spores which were previously present became larger in size. Accelerated stability study data of leflunomide and indomethacin microspheres were performed, the results proves that the formulation remains stable at 40°C /75% RH.Based on the in-vitro characterization and stability data it was concluded that indomethacin could be administered orally through the microspheres, which would be filled and dispensed in capsules.

SUMMARY:

Leflunomide, an immune modulatory pro-drug that is rapidly converted to its active metabolite possibly in the gut wall, plasma and in the liver, is microencapsulated by solvent evaporation technique using non-aqueous solution of polymethacrylate polymers to achieve its release from microspheres at a slower rate. At the optimal condition of process variables, such as stirring speed, temperature of the medium, drug polymer ratio and ratio of light liquid paraffin and heavy liquid paraffin maximum output is obtained. These microspheres are free-flowing in nature, discrete and uniform spherical in size as evidenced by scanning electron microscope.

The in-vitro release experiments are carried out in the stimulated intestinal fluid (pH 7.2, phosphate buffer) using United State Pharmacopoeia (USP) XXII apparatus II. The data obtained from the dissolution profiles are compared using different kinetics modules and the regression coefficients are compared.

The micro spheres made with both the polymers in different ratio exhibited a satisfactory drug release pattern. Therefore, it may be concluded that drug loaded microspheres are a suitable delivery system for leflunomide with a new choice of an economical, safe and more bioavailable formulation in the management of rheumatoid arthritis.

Indomethacin, a methylated indole derivative and a member of NSAIDs, having half life 2.6h to 6h is used for management of acute pain in ankylosing spondylosis, rheumatoid arthritis. It is extensively metabolized in the liver. The bioavailablity has been reported to be about 40%.Its control release dosage form is still not available. Therefore there are continued efforts to improve the pharmaceutical formulation of indomethacin in order to achieve an optimal therapy.

With the help of some polymeric substances such as eudragit RS 100 and eudragit RL 100 in different ratio, micro spheres can be produced by solvent evaporation method at a fixed rotational speed. The produced micro spheres are evaluated for determining the size distribution, drug entrapment efficiency and drug release behaviour in phosphate buffer.

Micro spheres prepared with different ratio of polymers, drug polymers, stirring speed, dropping height and bed temperature exhibit satisfactory drug release pattern as it release the drug in controlled fashion for extended period of time.

By optimizing dose in form of microspheres, release of drug at the site can be controlled which leads to overcoming the problem of dose related adverse effects.

CONCLUSION:

The present research work executed methodically and scientifically to achieve the objectives mentioned in the plan of work. The final conclusion may be drawn to highlight the following points: UV-Spectrophotometrically scan had been carried out for the determination of maximum wavelength (λ max) of leflunomide and indomethacin. It is revealed that λ max of leflunomide is 260nm and for indomethacin is 219nm.The particle sizes of the prepared microspheres were analyzed by sieve analysis and their interpretation had been done. Uniform particles had been achieved due to optimization of all the process variables.

The in vitro release data were evaluated applying the kinetic models of (a) zero order (b) first order(c) Higuchi (d) Korsmeyer equation. The regression coefficient values of different release kinetics equation postulated that in-vitro release profile of all formulations could be best expressed by zero order models. To confirm diffusional mechanism the data were fitted Korsmeyer's equation. The release component showed all were predominantly diffusion controlled. The result clearly indicated that microspheres prepared with leflunomide and indomethacin separately could be dispensed through empty capsules for patient compliance.

FUTURE APPLICATIONS:

The above study has got numerous future applications. With this modified fabricated drugs delivery system we can reduce the problem associated with conventional oral dosage forms like toxic manifestations and discomfort such as nausea, headache and dizziness to the patient.

High dose common pain killers raise the risk of heart disease in healthy people. It is the first evidence that so called NSAIDs pain relievers including some sold over the counter increase risk of heart disease. The risks are dose related and are mostly associated with dose of the drug. Most NSAIDs are associated with high increased cardio vascular mortality and morbidity.

In this investigation we shall try to minimize the pain with leflunomide and indomethacin with minimum side effects but with optimum efficacy. Development state –of-the-art Hi-tech Novel Drug Delivery System it is possible to manage the overdosing as well as missed dosing during treatment of the patient.

It is expected that with this work many patients suffering from acute pain due to various diseases like cancer, arthritis, and spondylosis and other diseases like post operative pain will be highly benefited.

This research work is promising the development of more practical and highly applicable method to provide the desired blood levels of the chronic pain management drugs over the desired period of time.

The methods applied during formulation of microspheres (solvent evaporation techniques) will minimize the formulation related problems during the time of bulk formulation.

REFERENCES:

1 Paul A. J. (1997). Chronic pain management: the essentials. U.K.: Greenwitch Medical Media.ISBN 1 900 151 855.

2. Main, Chris J.; Spanswick, Chtis C (2000). Pain management: an interdisciplinary approach. Churchill Livingstone. ISBN 0 443 05683 8.

3. Thienhaus, Ole; Cole, B. Eliot (2002). “The classification of pain” in wiener, Richard S, .Pain management: A practical guide for clinicians.CRC Press.p.29.

4. Jackson K C (2006). “Pharmacotherapy for neuropathic pain.” Pain practice: the official journal of World Institute of pain 6 (1): 27-33.

5. Munir MA, Enany N, Zhang JM (2007). "Non opioid analgesics". *Med. Clin. North Am.* 91 (1): 97–111.

6. Ballantyne JC (2006). “Opioids for chronic non terminal pain” South. Med. J. 99 (11): 1245-55.

7. Sanders S, Harisdangkul V, ‘Leflunomide for the treatment of rheumatoid arthritis and autoimmunity’, Am.J.Med.Sci.323 (4): 190-3.

8. Osiri M,Shea B, Welch V,Strand V, Tugwell P,Well G A ,‘Leflunomide for the treatment of rheumatoid arthritis’. Cochrane Database of Systematic Reviews.2002 (3) Art no CD 002047.

9. Breedveld F C, Dayer J M. ‘Leflunomide mode of action in the treatment of rheumatoid arthritis.’ Ann. Rheum Dis 2000; 59: 841-849.

10. Magithia, V; Geracis, A; rheumatoid arthritis: diagnosis and management. Am.J.Med, 2007, 120(11):936-9.

11. Beauvais A J, The first description of rheumatoid arthritis .Unabridge test of the doctoral dissertation presented in 1800. Joint Bone Spine .2001, 68(2): 130-143.

12. Silverman RB. The Organic Chemistry of Drug and Drug Action. 2nd Ed. New Delhi: Academic Press; 2004.

13. Robarts LJ, Morrow JD. Analgesic and anti- inflammatory agents and drugs employed in the treatment of gout. Ed. Goodman and Gilman’s The Pharmacological Basis of Therapeutics, 10th Ed. New York, Mc Graw-Hill; 2001. 687-732.

14. Karas A, Baykara T. Studies on Indomethacin insert prepared by water soluble polymers: The relation between dissolution rate and swelling behavior. II Farmaco. 2001; 56: 197-202.

15. Chowdary P K, Ramesh KV. Studies on micro capsulation of diltiazem. J. Pharm. Sci.1993; 55: 52-4.

16. Baidya S, Bedi S, Gupta B K.Desine and evaluation of microcapsules of diltiazem hydrochloride. Boll Chim Farmac Anno 2001; 14:1.

17. Singh B N, Kim K H. Floating drug delivery system: approach to oral controlled drug delivery via gastro-retention. J Control Rel. 2000; 63:235-259.

18. Fary A, Dubreuil F, Mohwal H. Mechanics of artificial microcapsules. 2004 New J. Phys. Doi: 10.1088/1367-2630/1/018.

19. Basu S K, Adhiyaman R. Preparation and characterization of nitrendipine-loaded Eudragit RL100 microspheres prepared by an emulsion –solvent evaporation, Trop. J Pharma Res 2008; 1033-41.

20. Costa P, Sousa LJM. Modeling and comparison of dissolution profiles. Eu. J. Pharm.Sci. 2001; 13: 123-133.

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