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Development and Evaluation of Non-Steroidal Anti-Inflammatory Drug Loaded Albumin Microspheres

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ABSTRACT:
KEYWORDS:
DSC, CDR,Keteprofen , Microspher,UV analysis

I. INTRODUCTION

Sustained release systems include any drug delivery system that achieves slow release of drug over an extended period of time. More precisely, sustained drug delivery can be defined as "Sustained drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects.

In sustained release dosage forms, a sufficient amount of drug is initially made available to the body to cause a desired pharmacological response. The remaining fraction is released periodically and is required to maintain the maximum initial pharmacological activity for some desirable period of time in excess of time expected from usual single dose. A sustained release is facilitated through the consistent rejuvenation of drug molecules (Fig 1.1).

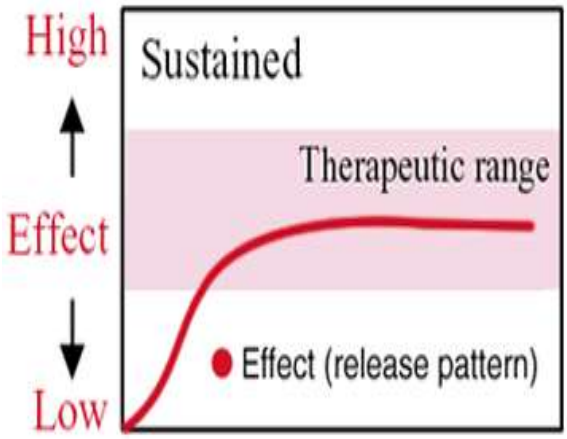

Fig 1.1 Sustained drug release effect

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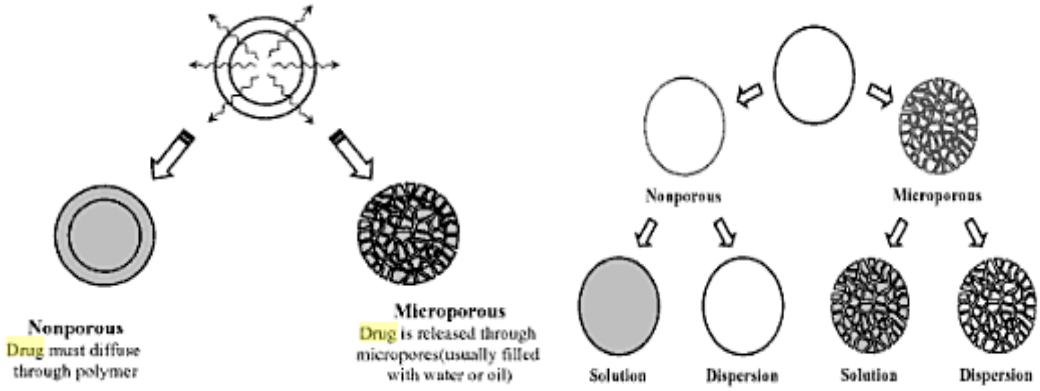

Fig 1.2 Sustained release mechanisms (a) Diffusion sustained reservoir systems.

1.2 MICROENCAPSULATION

Microencapsulation is a process by which solids, liquids or even gases may be enclosed in microscopic particles formation of thin coatings of wall material around the substances. There are various approaches in delivering a therapeutic substance to the target site in a sustained release fashion. One such approach is using microspheres as carriers for drugs.

1.2.1Microspheres

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 μm. They are made of polymeric, waxy or other protective materials that are biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. The natural polymers include albumin and gelatin, the synthetic polymer include polylactic acid and polyglycolic acid. The solvents used to dissolve the polymeric materials are chosen according to the polymer and drug solubility and stability, process safety and economic considerations. Microspheres are small and have large surface-to-volume ratio. At the lower end of their size range they have colloidal properties. The interfacial properties of microspheres are extremely important, often indicating their activity.

1.3 NATURAL POLYMERS

The use of natural biodegradable polymers to delivery drugs continues to be an area of active research despite the advent of synthetic biodegradable. Natural polymers remain attractive primarily because they are natural products of living organisms, readily available, relatively inexpensive, and capable of a multitude of chemical modifications. A majority of investigations of natural polymers as matrices in drug delivery systems have centered on proteins (e.g. collagen, gelatin, and albumin) and polysaccharides (e.g. starch, dextran). Most protein-based delivery systems have been formulated as solid cross linked microspheres in which the drug is dispersed throughout the polymer matrix. [8, 9]

In spite of the advent of many synthetic bio-degradable polymers, the use of natural polymers to delivery drugs looks to be an active area of research of compatibility due to natural origin, easy availability, cost effectiveness, eco friendliness, capable of multitude of chemical modifications and potentially degradable. Past research therefore studied and acknowledged various natural gum like agar, guar gum, chitosan, gelatin, carboxy methyl cellulose, xanthan, sodium alginate and lotus bean gum etc. for potential pharmaceutical and biomedical application.[10]

Albumin has been widely used in preparation of microspheres for drug delivery and other biomedical applications. It is a major plasma protein constituent, accounting for 355% of the total protein in human plasma. Since they were first described by Kramer, albumin microspheres have been extensively investigated in sustained release systems as vehicles for the delivery of therapeutic agents to local sites. The exploitable features of albumin include its reported biodegradation into natural products, its lack of toxicity, and its non antigenicity. The accumulation of albumin in solid tumours forms the rationale for developing albumin-based drug delivery systems for tumour targeting.Thus it has been used as a carrier for targeting drugs to tumours, and since the synovium of the rheumatoid arthritis patients shares various

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features observed in tumours, albumin-based delivery systems can be used to target drugs to the inflamed joint. Intravenous administration of the drugs coupled with albumin has been reported to improve the targeting efficiency of the drug to arthritic regions. The circulation half-lives of the drugs have been reported to dramatically increase when the drug is conjugated with albumin. Increasing the circulation half-life of the formulation by reducing its uptake by the reticulo endothelial system has been shown to improve the targeting efficiency of the formulation to the arthritic paws of rats. Achieving higher concentrations of the drug at the arthritic joint and minimizing its distribution to the other tissues would minimize the side effects associated with the drug. Targeting drugs to the inflamed joints, in the treatment of rheumatoid arthritis, would reduce the amount of drug required to control the disease, with possible additional reduction or even elimination of adverse side effects.[11, 12, 13]

1.3.1 Technologies used to prepare albumin microspheres

Novel drug delivery systems are the most solicitous branch of science, which involves multidisciplinary scientific approach, contributing to advanced drug delivery and human health care. The techniques utilized for the preparation should meet certain criteria. It should have the ability to incorporate high concentration of the drug. The particle size should be sustained by altering certain parameters like (1) type of albumin; (2) albumin concentration; (3) speed of agitation; (4) chemical cross-linking or heat denaturation; (5) Cross linking agent concentration or temperature; (6) addition or absence of surfactant ; (7) type of oil; and (8) mixing-cell with or without baffles. Further Particle size may be determined by laser diffraction technique. The selection of procedure depends upon the particle size required, route of administration, duration of drug action etc.., such that it releases the active ingredient over a prolonged period of time. For parenteral products the size of the particle should be minimized utmost such that they do not cause irritant action at the site if injection. The Albumin microspheres thus prepared should be stable and have a considerable shelf life.[14]

❖ **Different methods of microspheres manufacturing are**

Wax coating and hot melt:wax may be used to coat the core particles, encapsulating the drug by dissolution or dispersion in molten wax. The waxy solution or suspension is dispersed by high speed mixing into cold solution, such as cold liquid paraffin. The mixture is agitated for at least one hour. The external phase (liquid paraffin) is then decanted and the microspheres are suspended in a non- miscible solvent and allowed to air dry. Wax coated microspheres, while inexpensive and often used, release drug more rapidly than polymeric microspheres. Carnauba wax and beeswax can be used as the coating materials and these can be mixed in order to achieve desired characteristics.

Spray coating and pan coating: spray coating and pan coating employ heat-jacketed coating pans in which the solid drug core particles are rotated and into which the coating material is sprayed. The core particles are in size range of micrometersupto few millimetres. The coating material is usually sprayed at angle from the side into the pan. The process is continued until an even coating is completed. Coating a large number of particles may provide a safer and more consistent release pattern than coated tablets. In addition, several batches of microspheres cab be prepared with different coating thickness and mixed to achieve specific sustained release pattern.

Coacervation: This process is a simple separation of macromolecular solution into two immiscible liquid phases, a dense coacervate phase, which is relatively concentrated in macromolecules and a dilute equilibrium phase. In presence of only one macromolecule this process is referred to as simple coacervation. When two or more macromolecules of opposite charge are present, it is referred to as complex coacervation. Former one is induced by various parameters like change in temperature, addition of non-solvent or micro ions, which results in dehydration of macromolecules because they promote polymer-polymer interactions over polymer- solvent interaction. And the latter is induced by large number of variables like pH, ionic strength, macromolecule concentration, macromolecule ratio and macromolecular weight which results in a larger number of controllable parameters. These can be manipulated to produce microspheres with specific properties.

Spray drying: It is single step, closed-system process applicable to wide variety of materials, including heat-sensitive materials. The drug and polymer coating materials are dissolved in suitable solvent (aqueous or non-aqueous) or the drug may be present as a suspension in the polymer solution. Alternatively, it may be dissolved or suspended within an emulsion or coacervate system. For example, biodegradable polylactide microspheres can be prepared by dissolving the drug and the

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polymer in methylene chloride. The microsphere size is sustained by the rate of spraying, the feed rate of the polymer drug solution, the nozzle size, the temperature in drying and collecting chambers, and the size of the two chambers. The quality of the spray dried products are improved by the addition of plasticizers that promote the polymer coalescence and film formation and enhance the formation of smooth surfaced and spherical microspheres.

Solvent evaporation: This process is carried out in a liquid manufacturing vehicle. The albumin microspheres are dispersed in a volatile solvent, which is immiscible with the liquid manufacturing vehicle phase. A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microsphere. The mixture is then heated if necessary to evaporate the solvent. The solvent evaporation technique to produce microspheres is applicable to wide variety of core materials. The core materials may be either water soluble or water insoluble materials. Solvent evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous.

Precipitation: It is a variation on the evaporation method. The emulsion consists of polar droplets dispersed in a non-polar medium. Solvent may be removed from the droplets by the use of a co solvent. The resulting increase in the polymer concentration causes precipitation forming a suspension of microspheres.

Freeze Drying: This technique involves the freezing of the emulsion and the relative freezing points of the continuous and dispersed phases are important. The continuous phase solvent is usually organic and is removed by sublimation at low temperature and pressure. Finally the dispersed phase solvent of the droplets is removed by sublimation, leaving polymer-drug particles.

Chemical and thermal cross- linking: Microspheres made from natural polymers are prepared by a cross-linking process; polymers include gelatin, albumin, starch and dextran. A water-oil emulsion is prepared, where the water phase is a solution of polymer that contains drug to be incorporated. The oil phase is a suitable vegetable oil or oil - organic solvent mixture containing an oil soluble emulsifier. Once the desired water-oil emulsion is formed, the water soluble polymer is solidified by thermal treatment or addition of a chemical cross-linking agent such as glutaraldehyde to form a stable chemical cross link as in albumin. If chemical or heat cross linking is used, the amount of chemical and the period and intensity of heating are critical in determining the release rates and swelling properties of the microspheres.

❖ **Advantages and disadvantages of NSAID loaded albumin microspheres**

The following advantages make them a promising means for the delivery of NSAIDs.

- Albumin Microspheres provide constant and prolonged therapeutic effect.
- Reduces the dosing frequency and thereby improve the patient compliance.
- They could be injected into the body due to the spherical shape and smaller size.
- Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
- Albumin microsphere morphology allows a controllable variability in degradation and drug release.
- Reduces GI toxic effects.
- Albumin has non-antigenic property and ability to control the physicochemical characteristics of the microspheres produced, depending on the cross-linking methods and characteristics of cross-linking agent.

Some of the disadvantages were found to be as follows

- The modified release from the formulations.
- The release rate of the sustained release dosage form may vary from a variety of factors like food and the rate of transit though gut.
- Differences in the release rate from one dose to another.
- Sustained release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity.
- Dosage forms of this kind should not be crushed or chewed.
- Larger size of extended release products may cause difficulties in ingestion or transit through the gut.
- Possibility of distal intestinal toxicological manifestations because of sustained release and enteric coated NSAID formulations.

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❖ **Mechanism of action**

Most NSAIDs act as non selective inhibitors of the enzyme cyclooxygenase (COX), inhibiting both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes. COX catalyzes the formation of prostaglandins and thromboxane from arachidonic acid (itself derived from the cellular phospholipid bilayer by phospholipase A_2. Prostaglandins act (among other things) as messenger molecules in the process of inflammation.

❖ **Classification of NSAIDs**

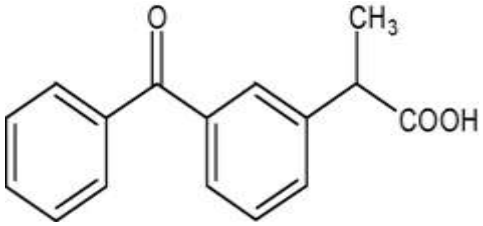
NSAIDs can be classified based on their chemical structure or mechanism of action.

1. Salicylates : Aspirin, Diflunisal, Salsalate
2. Propionic acid derivatives : Ibuprofen, Naproxen, Fenprofen, Ketoprofen, Flurbiprofen, Oxaprozin
3. Acetic acid derivatives : Indomethacin, Sulindac, Etodolac, Ketorolac, Diclofenac, Nabumetone
4. Enolic acid (Oxicam) derivatives :Piroxican, Meloxicam, Tenoxicam, Droxicam, Lornaxicam, Isoxicam
5. Fenamic acid derivatives : Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid
6. Selective COX-2 inhibitors (Coxibs) : Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib
7. Sulphonanilides :Nimesulide
8. Others : Licofelone.[21]

II. DRUG PROFILE[26, 27]

3.1.1 KETOPROFEN

Structural formula :



Molecular formula: $C_{16}H_{14}O_3$
Molecular weight : 254.29
IUPAC name :RS-2-(3-benzoyl phenyl) propionic acid.
Melting point: 93^0 -96^0C
Description: White or almost white, crystalline powder, odourless.
Solubility: Freely soluble in ethanol (95%), chloroform, ether, methanol and practically insoluble in water.
pKa : 4.45

Storage recommendations : Stored in tight containers.

In the present work Ketoprofen was chosen as the drug to be incorporated into the albumin microspheres. Ketoprofen (KP) is one of the most powerful inhibitors of cyclooxygenase at concentrations well within the range of therapeutic plasma concentrations (EC50 2µg/l). It produces reversible COX inhibition by competing with the substrate, arachidonic acid, for the active site of the enzyme. This inhibition results in a reduction in the tissue production of prostaglandins such as PGE2 and PGF2α. In addition to its effect on cyclooxygenase, Ketoprofen inhibits the lipoxygenase pathway of the arachidonic acid cascade. Ketoprofen is also a powerful inhibitor ofbradykinin, an important chemical mediator of pain and inflammation. It also stabilizes lysosomal membranes against osmotic change and prevents the release of lysosomal enzymes that mediate tissue destruction in inflammatory reactions. The short half-life and the low single dose administration make Ketoprofen as an ideal candidate for the formulation of sustained release dosage forms.

❖ **Mechanism of action**

Ketoprofen is a non steroidal anti-inflammatory drug with analgesic and antipyretic properties. Its anti-inflammatory effects are believed to be due to inhibition of both cylooxygenase-1 (COX-1) and cylooxygenase-2 (COX-2) which leads to the inhibition of prostaglandin synthesis, and leukotriene synthesis, to have anti brady-kinin activity, as well as to have lysosomal membrane-stabilizing action. Antipyretic effects may be due to action on the hypothalamus, resulting in an increased peripheral blood flow, vasodilation, and subsequent heat dissipation.

❖ **Pharmacological properties**

Ketoprofen is a non steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. KP has pharmacologic actions similar to those of other prototypical NSAIDs that are thought to be associated with the inhibition of prostaglandin synthesis. KP is used to treat rheumatoid arthritis, osteoarthritis, dysmenorrhoea, and to alleviate moderate pain.[28]

❖ **Pharmacokinetics**

The pharmacokinetics of Ketoprofen after IM, IV, or oral administration are best described by

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a linear, two-compartment model with first-order absorption and elimination, and pharmacokinetics of the drug after IV administration are best described by a two- or three- compartment model.

❖ **Pharmacokinetic data**

- Bio availability**:** The bioavailability of orally administered KP is approximately 100%.
- Protein binding**:** 99%
- Metabolism**:**KP is metabolized mainly by conjugation with glucoronic acid
- Half life**:** 2 ± 0.5 h
- Route of administration**:** Oral, IV, IM, Topical route.

❖ **Contraindications**

Ketoprofen is contraindicated in patients who have shown hypersensivity to it. KP should not be given to patients in whom aspirin or other NSAIDs induce asthma, urticaria, or other allergic-type reactions, because severe, rarely fatal, anaphylactic reactions to KP have been reported in such patients.

- Allergy to aspirin or NSAIDs
- Bleeding or blood cell disorder
- History of ulcer disease
- Severe impairment of kidney function

❖ **Adverse events:**

- Abdominal pain
- Changes in kidney function
- Constipation
- Diarrhea
- Headache
- Insomnia
- Indigestion
- Nausea and nervousness.

❖ **Therapeutic uses**

It is used as analgesic, anti-inflammatory and antipyretic actions and used in the treatment of rheumatoid arthritis and osteoarthritis.

❖ **Dosage:** 50 – 100 mg twice daily with food.[29]

3.2. POLYMER PROFILE [30,31,32,33,34]

3.2.1 BOVINE SERUM ALBUMIN

Synonyms: Bovine Serum Albumin; Bovine Plasma Albumin; BSA

Structure

The molecular weight of BSA has frequently been cited as 66,120 or 66,267, but it was revised in 1990 to 66,430. All three values are based on amino acid sequence information. BSA is a single polypeptide chain consisting of about 583 amino acid residues and no carbohydrates. At pH 5-7 it contains 17 intra chain disulfide bridges and 1 sulfhydryl group.

Physical Properties

Appearance **:** Powder - White to light tan
Solutions **:** Clear to slightly hazy and amber
PI in Water at 25°C **:** Endogenous Material - 4.7; 4.9
Fatty Acid Depleted **:** 5.3
pH of 1% Solution **:** 5.2-7
Intrinsic viscosity,η**:** 0.0413
Stability / Storage **:** If stored at 2-8 °C, BSA powders and BSA solutions were stable for a minimum of 2.5 years.

❖ **Solubility / Solution Stability**

Albumins are readily soluble in water and can only be precipitated by high concentrations of neutral salts such as ammonium sulfate. The solubility of powdered BSA in deionised water at 40 mg/ml and obtains clear to very slightly hazy, faint yellow solutions. The solution stability of BSA is very good (especially if the solutions are stored as frozen aliquots). In fact, albumins are frequently used as stabilizers for other solubilised proteins (e.g., labile enzymes). However, albumin is readily coagulated by heat. When heated to 50°C or above, albumin quite rapidly forms hydrophobic aggregates, which do not revert to monomers upon cooling. At somewhat lower temperatures aggregation is also expected to occur, but at relatively slower rates.

❖ **Product Description / Usage**

Albumins are a group of acidic proteins, which occur plentifully in the body fluids, and tissues of mammals and in some plant seeds. Unlike globulins, albumins have comparatively low molecular weights, are soluble in water, are easily crystallized, and contain an excess of acidic amino acids. Serum and plasma albumin is carbohydrate-free and comprises 55-62% of the protein present.

Albumin binds water, Ca^{2+}, Na^{+}, and K^{+}. Due to a hydrophobic cleft, albumin binds fatty acids, bilirubin, hormones and drugs. The main biological function of albumin is to regulate the colloidal osmotic pressure of blood. Human and bovine albumins contain 16% nitrogen and are often used as standards in protein calibration studies. Albumin is used to solubilise lipids, and is also used as a blocking agent in Western blots or ELISA applications. Globulin free albumins are suitable for use in applications where no other proteins should be present (e.g., electrophoresis).

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❖ **Applications**
- Antibody purification
- Binding and transport studies
- Blood banking reagents
- Culture media (microbial)
- Cell culture (general)
- Electrophoresis (M.W. standard)
- ELISA (blocking reagent)
- ELISA (non-specific binding)
- Enzyme systems
- Hapten carrier
- Immunocytochemistry
- Immunohematology
- Mitogenic assays
- Molecular biology
- Protein base or filler
- Protein supplement (controls)
- Protein standard (M.W., amino acids, nitrogen)
- RIA systems
- Serology

III. MATERIALS AND METHODS

The following materials of Pharma grade or the best possible LR were used as supplied by the manufacturer. The double distilled water was used in all experiments.

Table 4.1: List of chemicals used with grade and supplier

Sl. no.	Materials used	Grade			Manufacturer
1.	Ketoprofen	Pharma Grade			Acharya chemicals, Maharashtra, India
2.	Bovine serum albumin	Pharma Grade			S D fine-chem limited, Mumbai
3.	Petroleum ether	LR			S D fine-chem limited, Mumbai
4.	Sunflower oil	LR			S D fine-chem limited, Mumbai
5.	Paraffin liquid light	LR			S D fine-chem limited, Mumbai
6.	Paraffin liquid heavy	LR			S D fine-chem limited, Mumbai
7.	Ethanol	LR			S D fine-chem limited, Mumbai
8.	Acetone	LR			S D fine-chem limited, Mumbai
9.	Tween 80	LR			Central drug house (p) Ltd, Bombay
10.	Sodium hydroxide pellets	LR			Qualigens fine chemicals, Bombay
12.	Hydrochloric acid	LR			S D fine-chem limited, Mumbai
12.	Potassium dihydrogen phosphate	LR			Qualigens fine chemicals, Bombay
13.	Potassium chloride	LR			Qualigens fine chemicals, Bombay

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Sl.no.	Instrument	Manufacturer
1.	U.V Visible spectrophotometer	Shimadzu Corporation, Japan
2.	FTIR spectrophotometer	IR Affinity-1 Shimadzu Corporation, Japan
3.	Magnetic stirrer	Remi motors, Ahmedabad
4.	Mechanical stirrer	Remi motors, Ahmedabad
5.	Centrifuge	Remi motors, Ahmedabad
6.	SEM, JSM – 840A	JEOL, Japan
7.	Electronic balance	Citizen scales Pvt. Ltd
8.	Digital pH meter	Digisun Electronics, Hyderabad
9.	DSC	Mettler-Toledo star 822^{e} system, Switzerland
10.	Digital melting point apparatus	CL 725/726, Microcontroller based melting point apparatus
11.	XRD	Joel JDX-8030, Japan
12.	USP XXIII dissolution apparatus	Electrolab TDL-08L
13.	Hot air oven	Techno scientific, Bangalore
14.	Microscope	Mvtex SM-3JR

Table 4.2: List of instruments used

4.2 METHODS

4.2.1 Preformulation studies

❖ **Solubility study**

The solubility of KP in 10 mg/10 ml of solvent was carried out and it reveals that it is freely soluble in ethanol, chloroform, acetone, ether and soluble in

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benzene and strong alkali, but practically insoluble in water at 20° C.

❖ **Melting point determination**

Melting point of KP was determined by open capillary method.

❖ **Identification of Ketoprofen**

Identification of KP was carried out by FTIR spectrophotometry.

❖ **Determination of λ_{max}**

A solution of KP containing concentration 10µg/ml was prepared in ethanol and UV spectrum was taken using Shimadzu (UV-1800) double beam spectrophotometer and scanned between 200 to 400 nm. The maxima obtained in the graph were considered as $\lambda_{max}$for the drug KP.

4.2.2 Formulation design

Table 4.3: Formulation composition for Ketoprofen microspheres using Bovine serum albumin

Sl.no	Batch code	Drug: Polymer Ratio
1.	KP-1	1:1
2.	KP-2	1:2
3.	KP-3	1:3
4.	KP-4	1:4
5.	KP-5	1:5
6.	KP-6	1:6

❖ **Preparation of microspheres of Ketoprofen using Bovine serum albumin polymer**

Method used**:** Solvent evaporation method

The bovine serum albumin microspheres were prepared by solvent evaporation method reported by Tabassi et al[35] with some modifications. A 1% w/v solution of BSA was prepared in distilled water. KP is dispersed in above solution. This solution was dispersed in 100 ml of Sunflower oil containing 0.5ml Tween 80 in a 200 ml beaker. The dispersion was stirred at 600 rpm for 30 min. After the stirring time, microspheres were centrifuged, washed several times with petroleum ether and finally with acetone. The microspheres were dried at 50˚C and stored in desiccator.

4.2.3 Evaluation of Ketoprofen loaded albumin microspheres

❖ **Drug polymer interaction (FTIR) study** [36]

FTIR spectroscopy was performed on Fourier transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm^{-1}. FTIR study was carried on KP, physical mixture of KP and polymer, KP microspheres and blank microspheres.

❖ **Surface morphology(SEM)** [37]

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan). Dry KP microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of KP microspheres were taken by random scanning of the stub.

❖ **Frequency distribution analysis**

Determination of average particle size of KP microspheres was carried out by optical microscopy in which stage micrometer was employed. A minute quantity of KP microspheres was spread on a clean glass slide and average size of 300 KP microspheres was determined in each batch. In order to be able to define a size distribution or compare the characteristics of particles with many different diameters, the size distribution can be broken down into different size ranges, which can be presented in the form of a histogram. Histogram presents an interpretation of the particles size distribution and enables the percentage of particles having a given equivalent diameter to be determined.

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❖ **Percentage yield**

Percentage practical yield is calculated to know about percentage yield or efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of KP microspheresrecovered from each batch in relation to the sum of starting material. The percentage yield of prepared KP microsphereswas determined by using the formula:

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

❖ **Determination of percentage drug entrapment efficiency (PDE)** [38, 39]

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula:

$$\text{PDE} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

❖ **Calibration curve of Ketoprofen in Phosphate buffer pH 7.0**

- Scanning of Ketoprofen by UV-spectrophotometer in Phosphate buffer pH 7.4

Standard stock solution of Ketoprofen was prepared by dissolving accurately
weighed 10 mg of Ketoprofen in Phosphate buffer pH 7.0 in 100 ml volumetric flask. The volume was then made up mark by using Phosphate buffer pH 7.0, so as to get the solution of 100 µg/ml.

- **Procedure for Calibration curve of Ketoprofen in phosphate buffer (pH 7.0) at λmax 258 nm**

From the Ketoprofen standard stock solution (100µg/ml). From this solution, aliquots of 0.2, 0.4, 0.6, 0.8 and 1.0 ml were transferred to the series of 10 ml volumetric flasks and final volume is made with phosphate buffer pH 7.0, so as to get drug concentrations of 2.0 to 10.0 µg/ml respectively. The absorbences of these drug solutions were estimated at λmax 258 nm. This procedure was performed in triplicate to validate the calibration curve.

❖ **Theoretical drug content** was determined by calculation assuming that the entire KP present in the polymer solution used gets entrapped inKP microspheres, and no loss occurs at any stage of preparation of KP microspheres.

❖ **Practical drug content**was analyzed by using the following procedure, Weighed amount of KP microspheres equivalent to 100 mg of KP was dissolved in 100 ml of phosphate buffer pH 7. This solution was kept overnight for the complete dissolution of the KP in phosphate buffer pH 7. This solution was filtered and further diluted to make a conc. of 10 µg/ml solution. The absorbance of the solutions was measured at 258 nm using double beam UV-Visible spectrophotometer against distilled water as blank and calculated for the percentage of drug present in the sample.

❖ **In vitro dissolution studies**

➢ Calibration curve of Ketoprofen in Phosphate buffer pH 7.0 The procedure for the calibration curve of Ketoprofen is same as mentioned under Percentage drug entrapment efficiency.

Dissolution studies were carried out by using USPXXIII dissolution test apparatus by rotating basket method in phosphate buffer pH 7 for 12 h. The dissolution media were maintained at a temperature of 37 ± 5^0 C. The speed of rotation of basket maintained was 50 rpm. The samples were withdrawn at 1 h min intervals.

➢ **Procedure for in vitro dissolution studies :** Ketoprofen albumin microspheres equivalent to 100mg were placed in basket in each dissolution vessel to prevent floating. 5 ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were passed through Whatmann filter paper and the amount ofKP released was determined by UV absorption spectroscopy at 258 nm. Dissolution profiles of the formulations were analyzed by plotting drug release versus time plot. Data obtained was also subjected to kinetic treatment to understand the release mechanism.

❖ **Kinetics of drug release**

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s t), first order [Log(Q_0-Q) v/s t], Higuchi's square root of time (Q v/s $t^{1/2}$) and Korsemeyer Peppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q_0-Q) is the cumulative percentage of drug remaining after time t.

In short, the results obtained from in vitro release studies were plotted in four kinetics models of data treatment as follows

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- Cumulative percentage drug release Vs. Time (zero order rate kinetics)
- Log cumulative percentage drug retained Vs. Time (first order rate kinetics)
- Cumulative percentage drug release Vs. √T (Higuchi's classical diffusion equation)
- Log of cumulative percentage drug release Vs. log Time (Peppas exponential equation)

❖ **Differential Scanning Calorimetry (DSC)** [40]

The physical state of KP in the microspheres was analyzed by Differential Scanning Calorimeter (Mettler-Toledo star 822[e] system, Switzerland). The thermograms of the KP, physical mixture of KP and polymer, KP microspheres and blank microspheres were obtained at a scanning rate of 10°C/min conducted over a temperature range of 25–300°C, respectively.

❖ **X-Ray power Diffractometry (XRD) study** [41]

X-ray diffractometry of the KP, physical mixture of KP and polymer, KP microspheres were performed by a diffractometer using model (Joel JDX-8030, Japan) equipped with a graphite crystal monochromator (Cu-Kα) radiations to observe the physical state of KP in the microspheres.

The IR Spectrum of KP was found to be similar to the standard spectrum of KP. The spectrum of KP shows the following functional groups at their frequencies shown in Fig 5.1.

IV. RESULT

PREFORMULATION STUDIES

❖ **Solubility study**

The solubility of KP in 10 mg/10 ml of solvent was carried out and it reveals that it is freely soluble in ethanol, chloroform, acetone, ether and soluble in benzene and strong alkali, but practically insoluble in water at 20° C.

❖ **Melting point determination**

The melting point of KP was found to be 94°C.

❖ **Identification of Ketoprofen**

5.2 DRUG POLYMER INTERACTION (FTIR) STUDY

From the spectra of Ketoprofen, physical mixture of Ketoprofen and polymer, Ketoprofen microspheres and blank microspheres, it was observed that all characteristic peaks of KP were present in the combination spectrum, thus indicating compatibility of the KP and polymer. IR Spectr shown in Fig 5.1 to 5.4, data shown in table 5.1

Fig 5.1 IR Spectrum of Ketoprofen

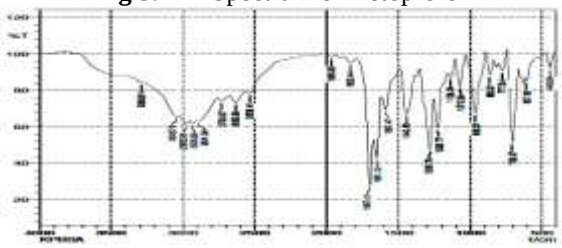

Fig 5.2 IR Spectrum of physical mixture of Ketoprofen and Bovine serum albumin

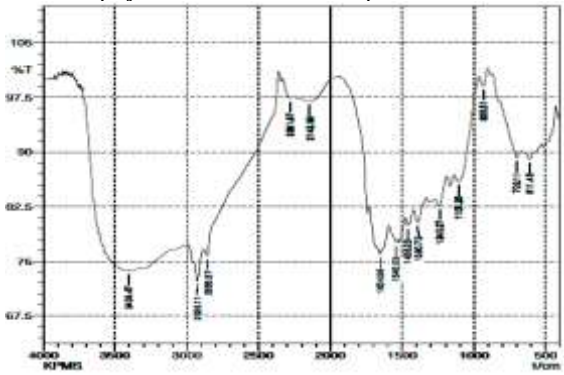

Fig 5.3 IR Spectrum of Ketoprofen microspheres using Bovine serum albumin

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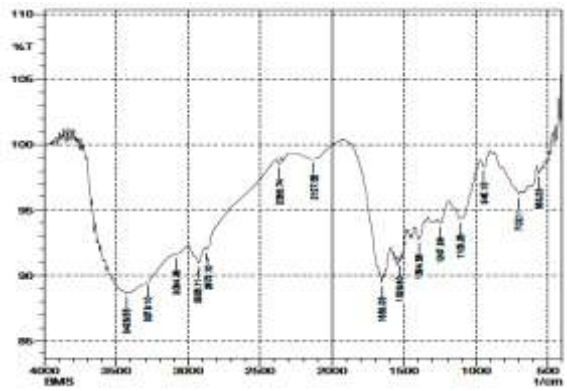

Fig 5.4 IR Spectrum of blank microspheres using Bovine serum albumin

Table 5.1 IR Spectrum data

Sl.no	IR Spectrum	Peaks(cm^{-1})	Groups	Stretching /Deformation
1	Ketoprofen	2885.6	C-H(alkyl)	Stretching
		1699.34	C=O(aromatic ketone)	Stretching
		1651.12	COOH(unsaturated carboxylic acid)	Stretching
		3043.77	O-H(Carboxylic acid)	Stretching
2	Physical mixture of Ketoprofen and Bovine serum albumin	2891.39	C-H(alkyl)	Stretching
		1697.41	C=O(aromatic ketone)	Stretching
		1651.12	COOH(unsaturated carboxylic acid)	Stretching
		3045.7	O-H(Carboxylic acid)	Stretching
3	Ketoprofen microspheres of Bovine serum albumin	2856.67	C-H(alkyl)	Stretching
		1654.98	C=O(aromatic ketone)	Stretching
		1645.03	COOH(unsaturated carboxylic acid)	Stretching
		2926.11	O-H(Carboxylic acid)	Stretching
4	Blank microspheres of Bovine serum albumin	2872.1	C-H(alkyl)	Stretching
		1653.05	C=O(aromatic ketone)	Stretching
		1629.6	COOH(unsaturated carboxylic acid)	Stretching
		3084.28	O-H(Carboxylic acid)	Stretching

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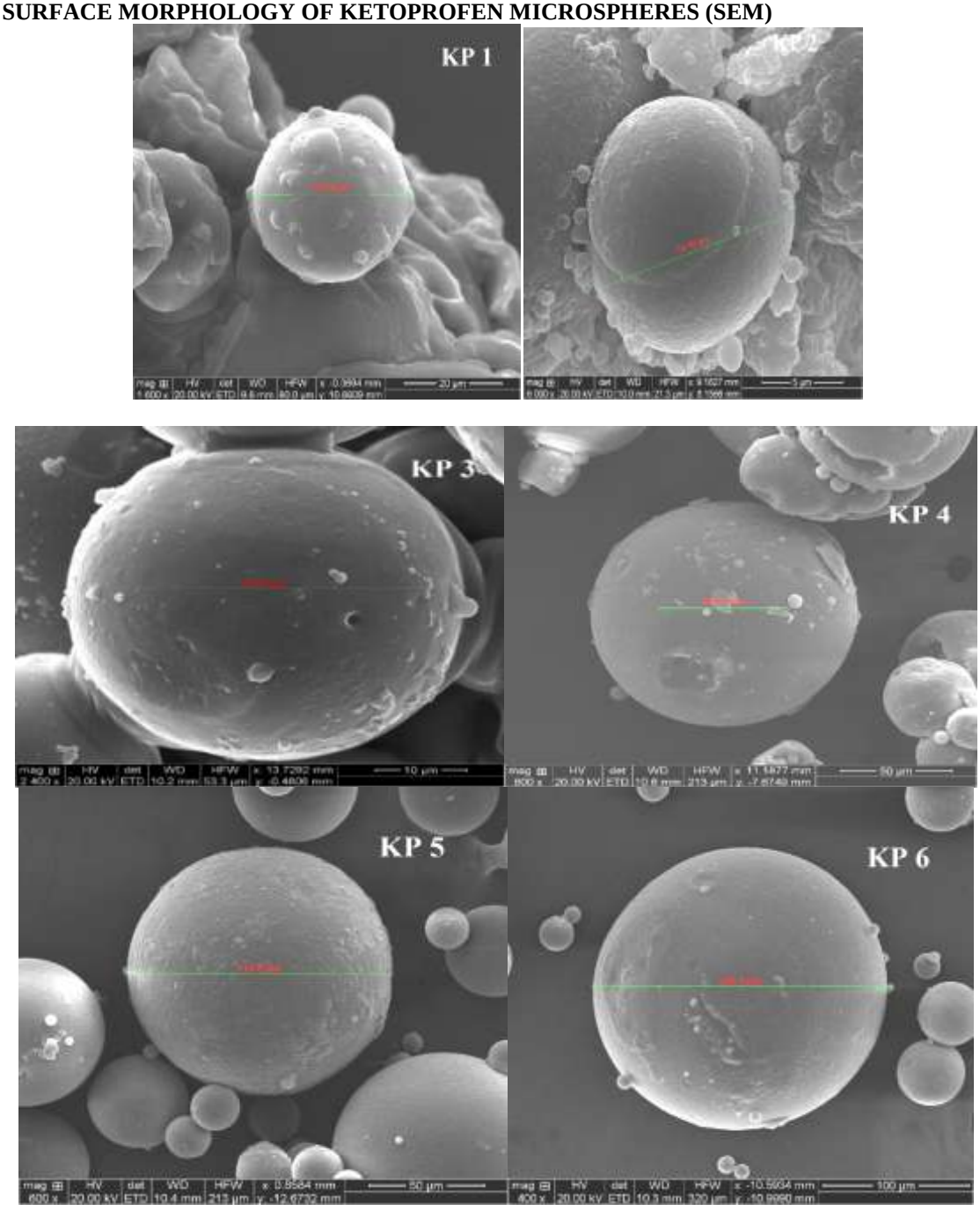
5.3 SURFACE MORPHOLOGY OF KETOPROFEN MICROSPHERES (SEM)



KP1, KP2, KP3, KP4, KP5 and KP6 refers to KP microspheres prepared by using BSA with drug: polymer ratio 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6

Fig. 5.5 SEM photographs of Ketoprofen microspheres

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5.4 FREQUENCY DISTRIBUTION ANALYSIS

❖ **Determination of Average particle size**

Table 5.2 Average diameter of Ketoprofen microspheres

Sl. No	Formulation code	Average size (µm)±SEM
1	KP1	13±2.73
2	KP2	30±6.53
3	KP3	53±8.62
4	KP4	69±9.97
5	KP5	115±7.17
6	KP6	203±5.32

SD=Standard deviation(n=3).

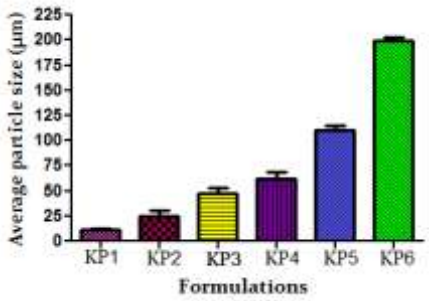

Fig 5.6 Average diameter of Ketoprofen microspheres

❖ **Frequency distribution analysis**

Table 5.3 Frequency distribution data of Ketoprofen microspheres

Size range (µm)	Number of particles					
	KP1	KP2	KP3	KP4	KP5	KP6
0-30	50	45	35	40		
30-60	65	90	45	70	25	
60-90	80	60	85	90	45	20
90-120	60	55	60	65	50	30
120-150	45	50	55	35	85	65
150-180			20		60	75
180-210					35	70
210-240						20
240-270						20

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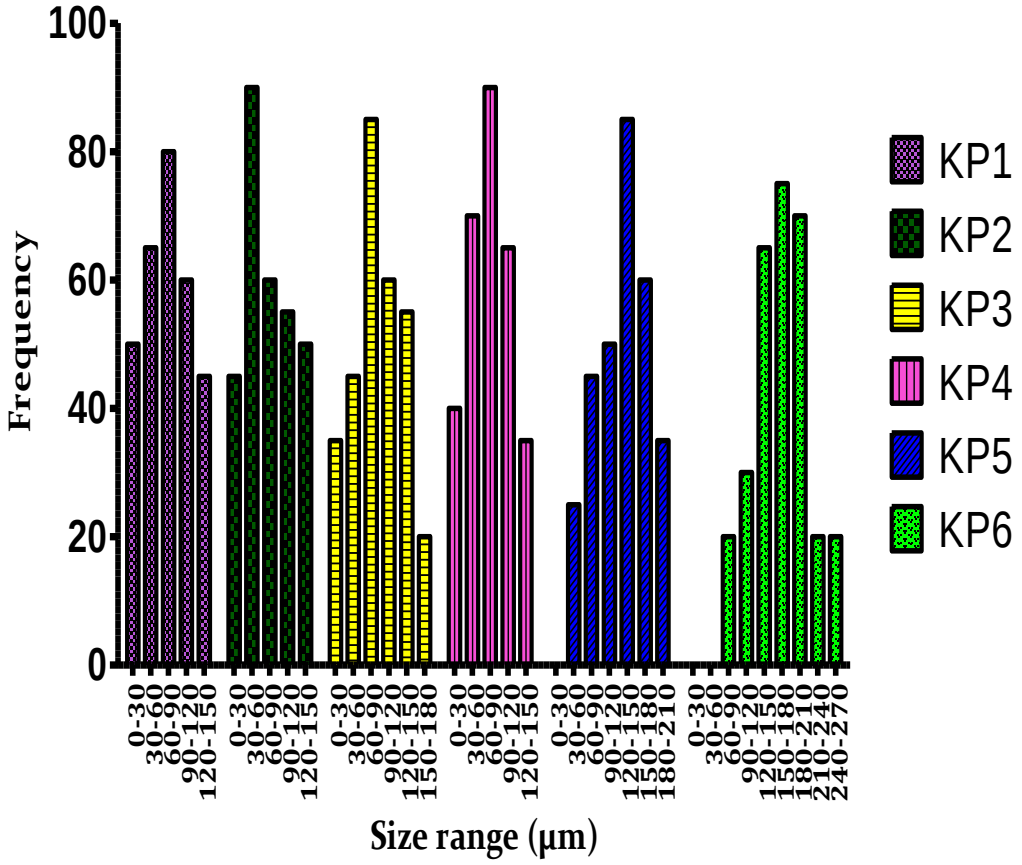
Fig. 5.7 Frequency distribution of Ketoprofen microspheres



5.5 STANDARD CALIBRATION CURVE

Table 5.4 Data for the standard calibration curve of Ketoprofen in pH 7 at 258 nm

Sl.no	Concentration (µg/ml)	Absorbance (λ_{max}258 nm)
		pH 7
1	0	0
2	2	0.090
3	4	0.177
4	6	0.268
5	8	0.349
6	10	0.432

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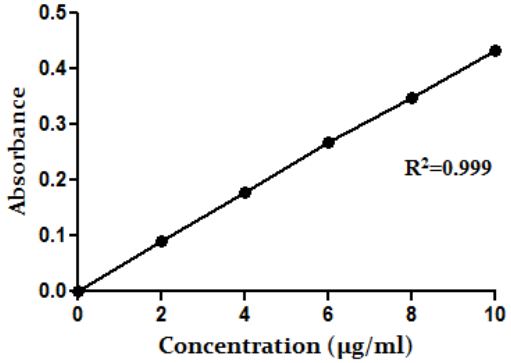

Fig. 5.8 Standard calibration curve for Ketoprofen in pH 7 at 258 nm

5.6 PERCENTAGE DRUG ENTRAPMENT EFFICIENCY

Table 5.5 Drug entrapment efficiency of Ketoprofen microspheres

Sl.no	Formulation Code	Percentage yield	Drug content (%)	Entrapment efficiency (%)
1	KP1	50.91	20.42	26.00±2.30
2	KP2	66.46	19.82	46.60±1.50
3	KP3	79.10	17.99	67.00±3.50
4	KP4	83.86	16.88	73.00±1.05
5	KP5	90.40	15.02	86.20±4.07
6	KP6	96.99	12.32	96.50±1.09

SD=Standard deviation(n=3).

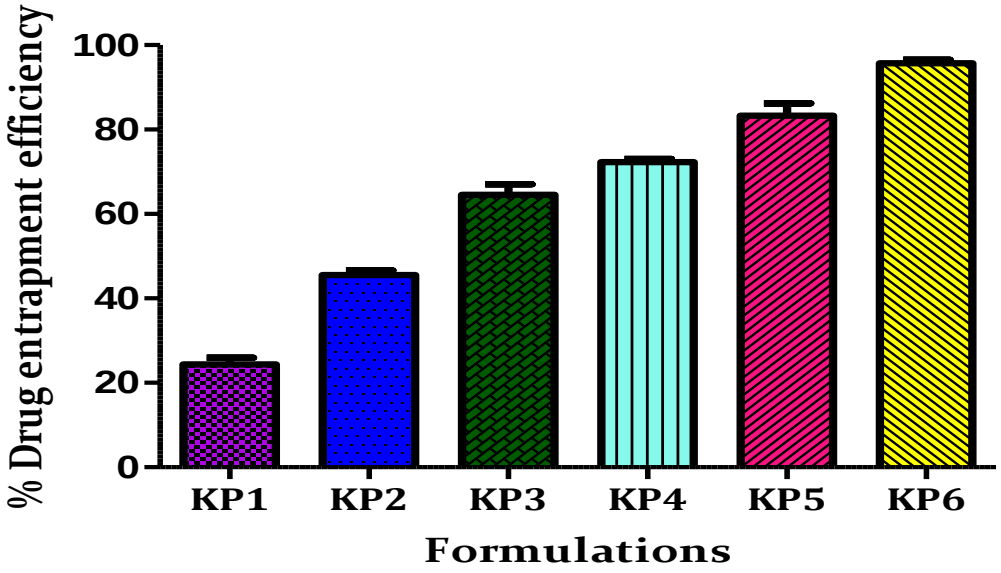

Fig. 5.9 Drug entrapment efficiency of Ketoprofen microspheres

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5.7 IN VITRO DISSOLUTION STUDIES

The standard calibration of Ketoprofen was carried out in pH 7 at 258nm shown in Table 5.4 and Fig 5.8

Sl.no.	**Table 5.6** In vitro release data of Ketoprofen microspheres**Time (h)**	**% Cum. drug release**		
		KP1 ± SD	**KP2 ± SD**	**KP3 ± SD**
1	0	0	0	0
2	1	16.51 ± 0.62	15.45 ± 0.67	12.35 ± 0.60
3	2	23.22 ± 0.49	21.47 ± 0.67	18.37 ± 0.62
4	3	29.58 ± 0.69	27.56 ± 0.61	24.31 ± 0.60
5	4	36.59 ± 0.68	33.66 ± 0.58	30.31 ± 0.61
6	5	43.48 ± 0.67	40.42 ± 0.61	36.34 ± 0.56
7	6	50.36 ± 0.60	46.53 ± 0.59	42.33 ± 0.59
8	7	56.72 ± 0.67	52.30 ± 0.47	48.37 ± 0.57
9	8	63.52 ± 0.52	59.38 ± 0.55	54.36 ± 0.61
10	9	70.48 ± 0.68	65.48 ± 0.52	60.37 ± 0.63
11	10	77.36 ± 0.56	72.25 ± 0.67	66.39 ± 0.61
12	11	83.39 ± 0.59	78.41 ± 0.72	72.39 ± 0.62
13	12	90.54 ± 0.53	84.49 ± 0.62	78.35 ± 0.59
% Cum. drug release				
KP4 ± SD		**KP5 ± SD**	**KP6 ± SD**	
0		0	0	
10.52 ± 0.64		7.64 ± 0.50	7.32 ± 0.59	
15.63 ± 0.57		13.38 ± 0.54	7.55 ± 0.55	
21.50 ± 0.60		17.78 ± 0.60	10.57 ± 0.66	
27.37 ± 0.62		23.57 ± 0.68	15.32 ± 0.57	
32.56 ± 0.59		28.45 ± 0.60	17.48 ± 0.66	
38.40 ± 0.56		34.01 ± 0.58	21.48 ± 0.58	
43.68 ± 0.60		38.65 ± 0.60	25.58 ± 0.57	
49.43 ± 0.56		44.44 ± 0.69	28.07 ± 0.66	
54.62 ± 0.64		49.52 ± 0.73	32.49 ± 0.58	
60.49 ± 0.52		54.54 ± 0.66	45.48 ± 0.66	
66.33 ± 0.60		59.56 ± 0.56	51.43 ± 0.69	
71.55 ± 0.56		64.35 ± 0.66	59.18 ± 0.63	

SD=Standard deviation(n=3).The differences in mean of % cumulative drug release between batch series 'KP1-KP6' were significant ($p < 0.0001$).

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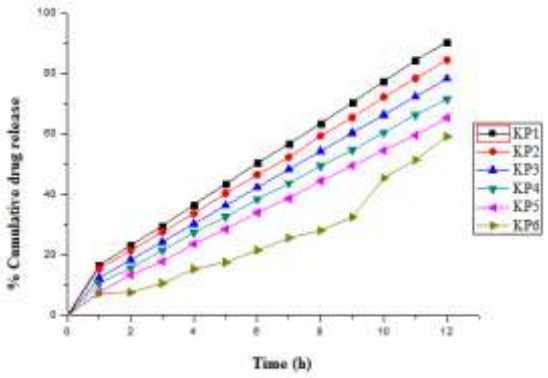

Fig. 5.10 Comparative in vitro release profile of Ketoprofen microspheres

5.8 RELEASE KINETICS OF KETOPROFEN MICROSPHERES

Table 5.7 Zero order release kinetics data of Ketoprfoen microspheres

Sl.no.	Time (h)	% Cum. drug release		
		KP1 ± SD	KP2 ± SD	KP3 ± SD
1	0	0	0	0
2	1	16.51 ± 0.62	15.45 ± 0.67	12.35 ± 0.60
3	2	23.22 ± 0.49	21.47 ± 0.67	18.37 ± 0.62
4	3	29.58 ± 0.69	27.56 ± 0.61	24.31 ± 0.60
5	4	36.59 ± 0.68	33.66 ± 0.58	30.31 ± 0.61
6	5	43.48 ± 0.67	40.42 ± 0.61	36.34 ± 0.56
7	6	50.36 ± 0.60	46.53 ± 0.59	42.33 ± 0.59
8	7	56.72 ± 0.67	52.30 ± 0.47	48.37 ± 0.57
9	8	63.52 ± 0.52	59.38 ± 0.55	54.36 ± 0.61
10	9	70.48 ± 0.68	65.48 ± 0.52	60.37 ± 0.63
11	10	77.36 ± 0.56	72.25 ± 0.67	66.39 ± 0.61
12	11	83.39 ± 0.59	78.41 ± 0.72	72.39 ± 0.62
13	12	90.54 ± 0.53	84.49 ± 0.62	78.35 ± 0.59

% Cum. drug release		
KP4 ± SD	KP5 ± SD	KP6 ± SD
0	0	0
10.52 ± 0.64	7.64 ± 0.50	7.32 ± 0.59
15.63 ± 0.57	13.38 ± 0.54	7.55 ± 0.55
21.50 ± 0.60	17.78 ± 0.60	10.57 ± 0.66
27.37 ± 0.62	23.57 ± 0.68	15.32 ± 0.57
32.56 ± 0.59	28.45 ± 0.60	17.48 ± 0.66
38.40 ± 0.56	34.01 ± 0.58	21.48 ± 0.58
43.68 ± 0.60	38.65 ± 0.60	25.58 ± 0.57
49.43 ± 0.56	44.44 ± 0.69	28.07 ± 0.66
54.62 ± 0.64	49.52 ± 0.73	32.49 ± 0.58
60.49 ± 0.52	54.54 ± 0.66	45.48 ± 0.66
66.33 ± 0.60	59.56 ± 0.56	51.43 ± 0.69
71.55 ± 0.56	64.35 ± 0.66	59.18 ± 0.63

SD=Standard deviation(n=3).The differences in mean of % cumulative drug release between batch series 'KP1-KP6' were significant ($p < 0.0001$).

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Table 5.8 First orderrelease kinetics data of Ketoprofen microspheres

Sl.no.	Time (h)	Log % Cum. drug remain to be released		
		KP1 ± SD	**KP2 ± SD**	**KP3 ± SD**
1	0	2 ± 0.000	2 ± 0.000	2 ± 0.000
2	1	1.922 ± 0.003	1.927 ± 0.003	1.943 ± 0.002
3	2	1.885 ± 0.002	1.895 ± 0.003	1.912 ± 0.003
4	3	1.848 ± 0.004	1.860 ± 0.003	1.879 ± 0.003
5	4	1.802 ± 0.004	1.822 ± 0.003	1.843 ± 0.003
6	5	1.752 ± 0.005	1.775 ± 0.004	1.804 ± 0.003
7	6	1.696 ± 0.005	1.728 ± 0.004	1.761 ± 0.004
8	7	1.636 ± 0.006	1.678 ± 0.004	1.713 ± 0.004
9	8	1.562 ± 0.006	1.609 ± 0.005	1.659 ± 0.005
10	9	1.470 ± 0.010	1.538 ± 0.006	1.598 ± 0.006
11	10	1.355 ± 0.010	1.443 ± 0.010	1.526 ± 0.008
12	11	1.193 ± 0.016	1.334 ± 0.014	1.441 ± 0.009
13	12	0.975 ± 0.024	1.190 ± 0.017	1.335 ± 0.012
% Cum. drug release				
KP4 ± SD		**KP5 ± SD**		**KP6 ± SD**
2 ± 0.000		2 ± 0.000		2 ± 0.000
1.952 ± 0.003		1.965 ± 0.002		1.967 ± 0.002
1.926 ± 0.002		1.938 ± 0.002		1.966 ± 0.002
1.895 ± 0.003		1.915 ± 0.003		1.951 ± 0.003
1.861 ± 0.003		1.883 ± 0.003		1.928 ± 0.002
1.829 ± 0.003		1.855 ± 0.003		1.917 ± 0.003
1.790 ± 0.003		1.819 ± 0.003		1.895 ± 0.003
1.751 ± 0.004		1.788 ± 0.004		1.872 ± 0.003
1.704 ± 0.004		1.745 ± 0.005		1.857 ± 0.004
1.657 ± 0.006		1.703 ± 0.006		1.829 ± 0.003
1.597 ± 0.005		1.658 ± 0.006		1.736 ± 0.005
1.527 ± 0.007		1.607 ± 0.006		1.686 ± 0.006
1.454 ± 0.008		1.540 ± 0.008		1.611 ± 0.006

SD=Standard deviation(n=3).The differences in mean of % cumulative drug release between batch series 'KP1-KP6' were significant ($p < 0.0001$).

Table 5.9 Higuchi matrix release kinetics data of Ketoprofen microspheres

Sl.no.	√T (h)	% Cum. drug release		
		KP1 ± SD	**KP2 ± SD**	**KP3 ± SD**
1	0	0	0	0
2	1.000	16.51 ± 0.62	15.45 ± 0.67	12.35 ± 0.60
3	1.414	23.22 ± 0.49	21.47 ± 0.67	18.37 ± 0.62
4	1.732	29.58 ± 0.69	27.56 ± 0.61	24.31 ± 0.60
5	2.000	36.59 ± 0.68	33.66 ± 0.58	30.31 ± 0.61
6	2.236	43.48 ± 0.67	40.42 ± 0.61	36.34 ± 0.56
7	2.449	50.36 ± 0.60	46.53 ± 0.59	42.33 ± 0.59
8	2.645	56.72 ± 0.67	52.30 ± 0.47	48.37 ± 0.57
9	2.828	63.52 ± 0.52	59.38 ± 0.55	54.36 ± 0.61
10	3.000	70.48 ± 0.68	65.48 ± 0.52	60.37 ± 0.63
11	3.162	77.36 ± 0.56	72.25 ± 0.67	66.39 ± 0.61
12	3.316	83.39 ± 0.59	78.41 ± 0.72	72.39 ± 0.62

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13	3.464	90.54 ± 0.53	84.49 ± 0.62	78.35 ± 0.59
% Cum. drug release				
KP4 ± SD		**KP5 ± SD**	**KP6 ± SD**	
0		0	0	
10.52 ± 0.64		7.64 ± 0.50	7.32 ± 0.59	
15.63 ± 0.57		13.38 ± 0.54	7.55 ± 0.55	
21.50 ± 0.60		17.78 ± 0.60	10.57 ± 0.66	
27.37 ± 0.62		23.57 ± 0.68	15.32 ± 0.57	
32.56 ± 0.59		28.45 ± 0.60	17.48 ± 0.66	
38.40 ± 0.56		34.01 ± 0.58	21.48 ± 0.58	
43.68 ± 0.60		38.65 ± 0.60	25.58 ± 0.57	
49.43 ± 0.56		44.44 ± 0.69	28.07 ± 0.66	
54.62 ± 0.64		49.52 ± 0.73	32.49 ± 0.58	
60.49 ± 0.52		54.54 ± 0.66	45.48 ± 0.66	
66.33 ± 0.60		59.56 ± 0.56	51.43 ± 0.69	
71.55 ± 0.56		64.35 ± 0.66	59.18 ± 0.63	

SD=Standard deviation(n=3).The differences in mean of % cumulative drug release between batch series 'KP1-KP6' were significant ($p < 0.0001$).

Table 5.10 Peppas release kinetics data of Ketoprofen microspheres

Sl.no.	**Log T (h)**	**Log % Cum. drug release**		
		KP1 ± SD	**KP2 ± SD**	**KP3 ± SD**
1	0	0	0	0
2	0	1.218 ± 0.016	1.189 ± 0.018	1.091 ± 0.020
3	0.301	1.366 ± 0.009	1.332 ± 0.013	1.264 ± 0.014
4	0.477	1.471 ± 0.010	1.440 ± 0.009	1.386 ± 0.010
5	0.602	1.563 ± 0.008	1.527 ± 0.007	1.482 ± 0.008
6	0.698	1.638 ± 0.006	1.607 ± 0.006	1.560 ± 0.006
7	0.778	1.702 ± 0.005	1.668 ± 0.005	1.627 ± 0.006
8	0.845	1.754 ± 0.005	1.719 ± 0.003	1.685 ± 0.005
9	0.903	1.803 ± 0.003	1.774 ± 0.004	1.735 ± 0.004
10	0.954	1.848 ± 0.004	1.816 ± 0.003	1.781 ± 0.004
11	1.000	1.889 ± 0.003	1.859 ± 0.004	1.822 ± 0.004
12	1.041	1.926 ± 0.003	1.894 ± 0.004	1.860 ± 0.003
13	1.079	1.957 ± 0.002	1.927 ± 0.003	1.894 ± 0.003
% Cum. drug release				
KP4 ± SD		**KP5 ± SD**	**KP6 ± SD**	
0		0	0	
1.021 ± 0.026		0.883 ± 0.029	0.864 ± 0.034	
1.192 ± 0.016		1.126 ± 0.017	0.877 ± 0.031	
1.332 ± 0.012		1.250 ± 0.014	1.024 ± 0.027	
1.437 ± 0.009		1.372 ± 0.012	1.185 ± 0.016	
1.513 ± 0.007		1.454 ± 0.009	1.242 ± 0.016	
1.584 ± 0.006		1.532 ± 0.007	1.332 ± 0.011	
1.640 ± 0.005		1.587 ± 0.006	1.408 ± 0.009	
1.694 ± 0.004		1.648 ± 0.006	1.448 ± 0.010	
1.737 ± 0.005		1.695 ± 0.006	1.512 ± 0.007	
1.782 ± 0.003		1.737 ± 0.005	1.658 ± 0.006	
1.822 ± 0.003		1.777 ± 0.004	1.711 ± 0.005	

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1.855 ± 0.003	1.815 ± 0.004	1.772 ± 0.004

SD=Standard deviation(n=3).The differences in mean of % cumulative drug release between batch series 'KP1-KP6' were significant ($p < 0.0001$).

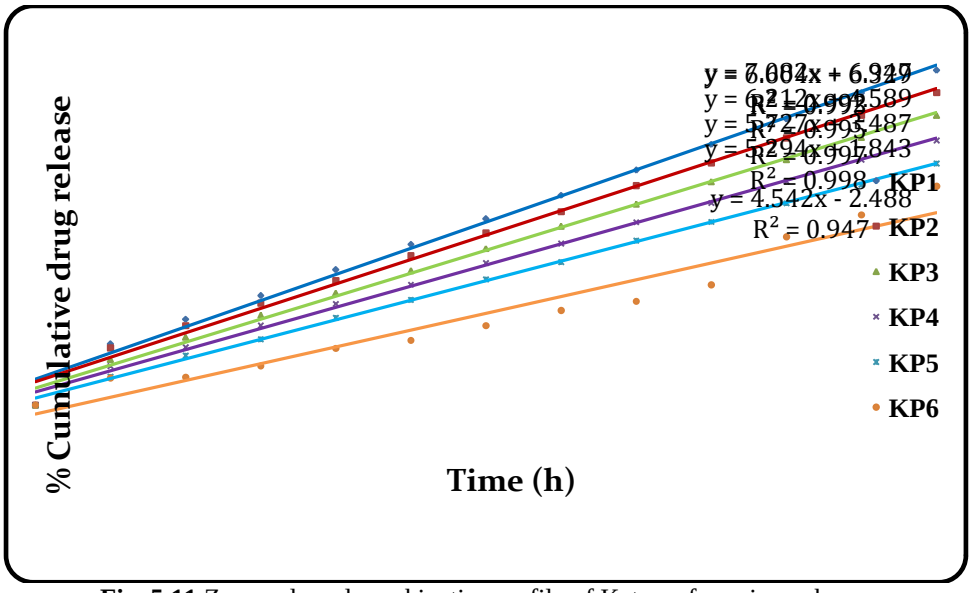

Fig. 5.11 Zero order release kinetics profile of Ketoprofen microspheres

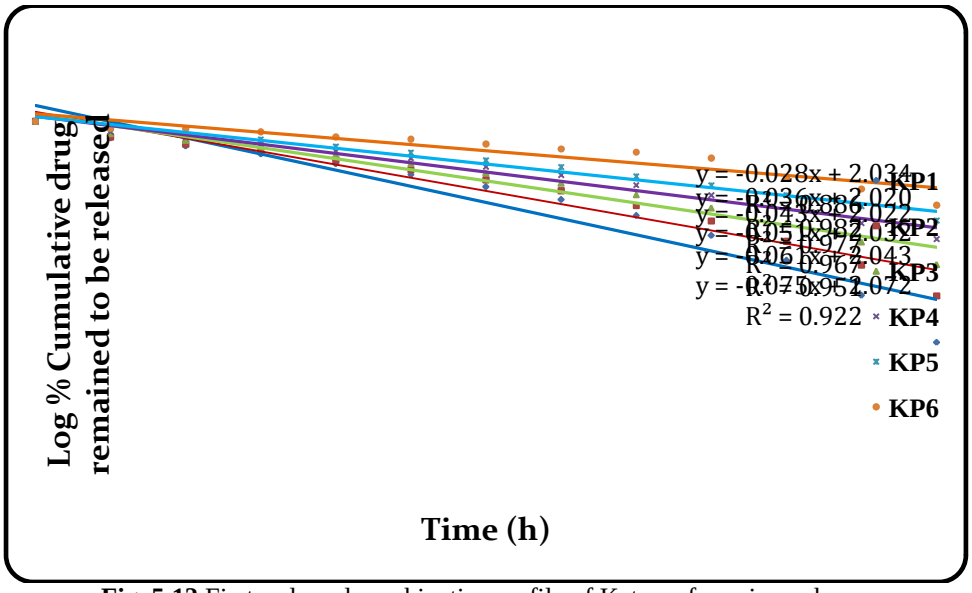

Fig. 5.12 First order release kinetics profile of Ketoprofen microspheres

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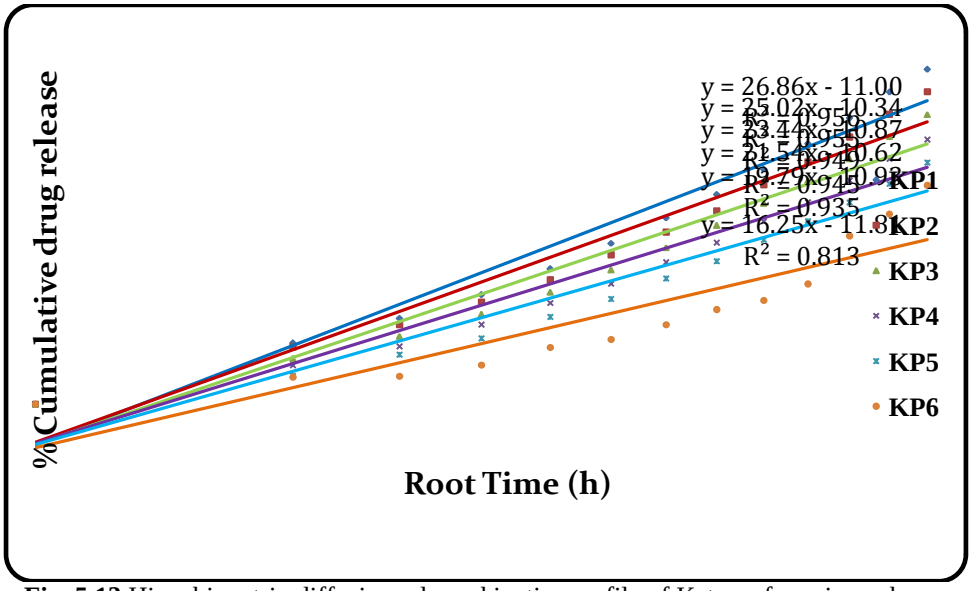

Fig. 5.13 Higuchi matrix diffusion release kinetics profile of Ketoprofen microspheres

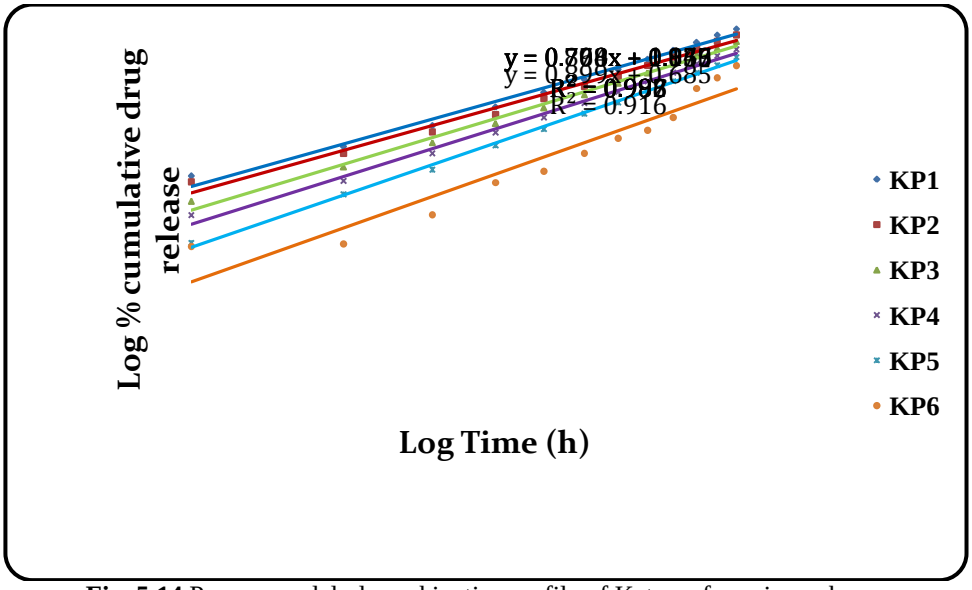

Fig. 5.14 Peppas modelrelease kinetics profile of Ketoprofen microspheres

Table 5.11 Regression co-efficient (r^2) values of different kinetic models and diffusion exponent (n) of Peppas model for Ketoprofen microspheres

Formulation	Zero order	First order	Higuchi Matrix	Peppas plot	
				r^2 value	'n' value
KP1	0.9927	0.9227	0.9758	0.9881	0.7082
KP2	0.9930	0.9512	0.9741	0.9868	0.7084
KP3	0.9959	0.9671	0.9764	0.9928	0.7634
KP4	0.9971	0.9773	0.9764	0.9937	0.7952

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KP5	0.9990	0.9820	0.9761	0.9978	0.8705
KP6	0.9472	0.8867	0.8587	0.9162	0.8992

5.9 DSC THERMOGRAMS

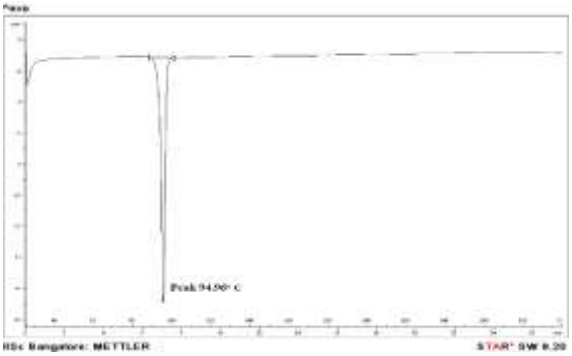

Fig 5.15 DSC thermogram of Ketoprofen

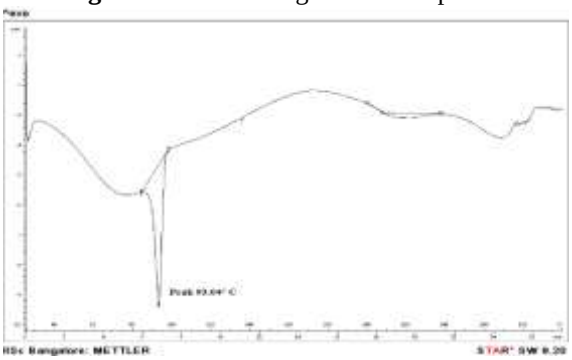

Fig 5.16 DSC thermogram of Physical mixture of Ketoprofen and Bovine serum albumin

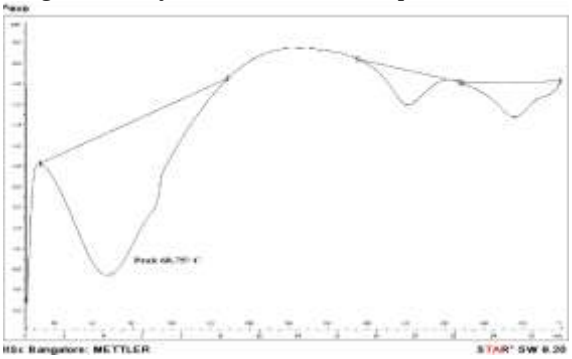

Fig 5.17 DSC thermogram of Ketoprofen microspheres using Bovine serum albumin

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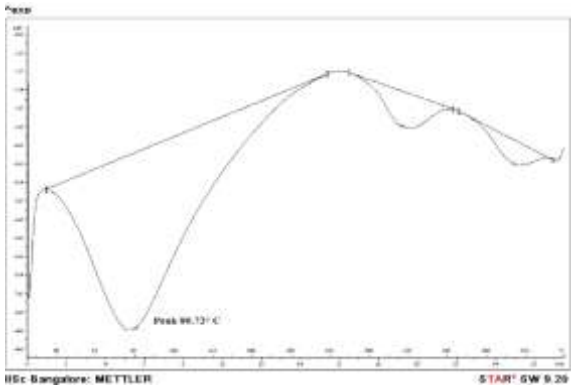

Fig 5.18 DSC thermogram of blank microspheres using Bovine serum albumin

5.9 XRD THERMOGRAMS

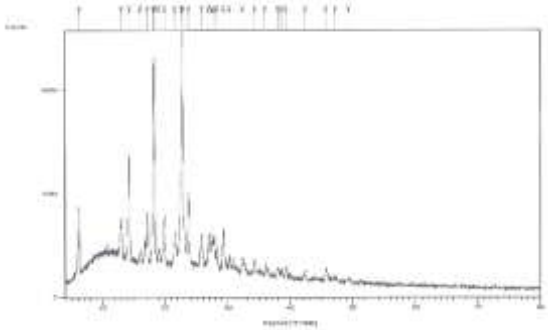

Fig 5.19 XRD thermogram of Ketoprofen

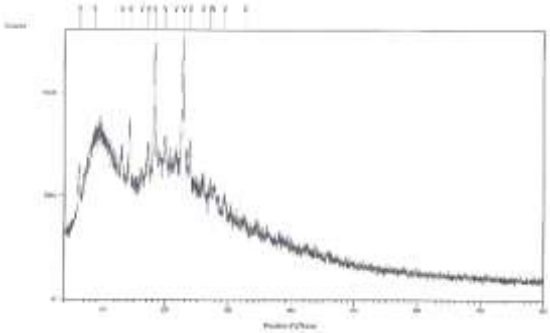

Fig 5.20 XRD thermogram of Physical mixture of Ketoprofen and Bovine serum albumin

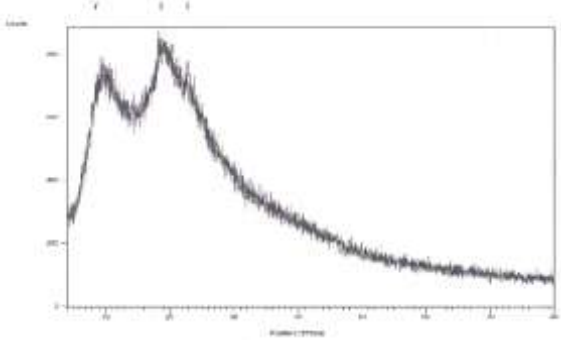

Fig 5.21 XRD thermogram of Ketoprofen microspheres using Bovine serum albumin

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V. DISCUSSION

The present study reports a novel attempt to prepare microspheres of the NSAID KP by using natural polymer like BSA as carrier for better treatment of rheumatoid arthritis, pain, inflammation etc., Microspheres of KP were prepared by solvent evaporation method using BSA. Various evaluation parameters were assessed, with a view to obtain sustained release of KP.

In the present work, total six formulations were prepared and the detailed composition is shown in Table.4.3. The prepared KP microspheres were subjected to FTIR, SEM, particle size, size distribution analysis, % yield, drug content, drug entrapment efficiency, invitro dissolution,release kinetics, DSC and XRD.

6.1 PREFORMULATION STUDIES

- The solubility of KP in 10 mg/10 ml of solvent was carried out and it reveals that it is freely soluble in ethanol, chloroform, acetone, ether and soluble in benzene and strong alkali, but practically insoluble in water at 20° C.
- The melting point of KP was found to be 94^{o}C, which complied with IP standards thus indicating purity of obtained drug sample.
- A solution of KP containing concentration 10µg/ml was prepared in ethanol and UV spectrum was taken using Shimadzu (UV-1800) double beam spectrophotometer and scanned between 200 to 400 nm. The maxima obtained in the graph were considered as $\lambda_{max}$for the drug KP.

6.2 EVALUATION OF KETOPROFENMICROSPHERES

6.2.1 Drug polymer interaction (FTIR) study

FTIR Spectra were obtained for KP, physical mixture of KP and polymer, KP microspheres, blank microspheres presented in Fig 5.1 to 5.4. The characteristic peaks of the KP were compared with the peaks obtained for physical mixture of KP and polymer, formulations were given in Table.5.1. The characteristics peaks found in KP, physical mixture and formulations, hence it appears there was no chemical interaction between KP and polymer and it can be concluded that the characteristics bands of KP were not affected after successful loading.

6.2.2 Surface morphology of Ketoprofen microspheres (SEM)

The surface morphology of the KP microspheres was studied by SEM. SEM photographs of the various formulations were shown in the Fig. 5.5.Surface smoothness of the KP microspheres was increased by increasing the polymer concentration, which was confirmed by SEM. At lower polymer conc. (1:1) rough and wrinkled surface of KP microspheres was obtained. [Fig. 5.5 (KP1)]and at higher polymer conc. (1:6) the KP microspheres with smooth surface was obtained[Fig. 5.5 (KP6)].

6.2.3 Frequency distribution analysis

As the KP to polymer ratio was increased, the mean particle size of KP microspheres was also increased Table 5.2 and Fig. 5.7.The significant increase may be because of the increase in the viscosity of the droplets (may be due to the increase in conc. of polymer solution). KP microspheres having a size range of10 to 240 µmTable 5.3 and Fig. 5.8with normal frequency distribution was obtained.

6.2.4 Percentage yield

The percentage yield for KP microspheres were 50.91%, 66.46%, 79.10%, 83.86% 90.40% and 96.99% for formulation KP1, KP2, KP3, KP4, KP5, KP6 respectively are given in Table 5.5.

6.2.5 Percentage drug entrapment efficiency

Entrapment efficiency increases with increase in the polymer conc. From the results it can be inferred that there is a proper distribution of KP in the microspheres and the deviation is within the acceptable limits.

The percent of drug content in the formulations was found to be in the range of 12.32% to 20.42%. The percentage entrapment efficiency was found to be 26.00% to96.50%. The results obtained are given in Table. 5.5 and their histograms shown in Fig.5.10.A maximum of 96.50% drug entrapment efficiency was obtained in the KP microspheres which were prepared by using BSA. It was further observed that the drug entrapment was proportional to the KP: polymer ratio and size of the KP microspheres. By increasing the polymer conc., the encapsulation efficiency was increased.

6.2.6 In vitro dissolution studies

The in vitro performance of KP microspheres showed prolonged and sustained release of KP. The results of the in vitro dissolution studies of formulations KP1to KP6are shown in Table 5.6 and Fig. 5.12. The study indicated that the amount of drug release decreases with an

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increase in the polymer concentration. The formulations KP1 showed a maximum of 90.54% and KP6 showed a minimum of 59.18% cumulative drug release.

6.2.7 Release kinetics of Ketoprofen microspheres

The plots of Cumulative percentage drug release V/s. Time, Cumulative percent drug retained V/s. root Time, Log Cumulative percent drug retained V/s. Time and Log Cumulative percent drug release V/s. Log Time were drawn and represented graphically as shown in Fig. 5.13 to 5.16 and Table 5.7 to 5.10 respectively. The slopes and the regression co-efficient of determinations (r^2) were listed in Table 5.11. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of Korsemeyer-Peppas model was found to be in the range of 0.5 to 1 for the KP microspheres prepared with BSA indicating Non-Fickian of drug through KP microspheres.

6.2.8 Differential scanning calorimetry (DSC)

In order to confirm the physical state of KP in the microspheres, DSC of the KP, physical mixture of KP and polymer, KP microspheres and blank microspheres were carried out and were shown in Fig. 5.17 to 5.20. The DSC trace of KP showed a sharp endothermic peak at 94.96°C, its melting point. The physical mixture of KP and polymer, blank microspheres showed the same thermal behavior 93.04°C as the individual component, indicating that there was no interaction between the KP and the polymer in the solid state. The melting point range of KP is between 93-96°C, thus indicating there is no change of KP in pure state, physical mixture of drug and polymer and the blank microspheres. The absence of endothermic peak of the KP at 94.96°C in the DSC of the KP microspheres suggests that the KP existed in an amorphous or disordered crystalline phase as a molecular dispersion in polymeric matrix.

6.2.9 X-Ray powder diffractometry (XRD)

In order to confirm the physical state of the KP in the microspheres, powder X-ray diffraction studies of the KP, physical mixture of KP and polymer, KP microspheres were carried out. X-ray diffractograms were shown in Fig. 5.19 to 5.21 and showed that the KP is still present in its lattice structure in the physical mixture where as it is completely amorphous inside the KP microspheres. This may be due to the conditions used to prepare the KP microspheres lead to cause completedrug amorphization.

vi. CONCLUSION

From the above experimental results it can be concluded that

- Preformulation studies like solubility, melting point, and UV analysis of KP were complies with IP standards.
- The FTIR Spectras revealed that, there was no interaction between polymers and ketoprofen. All the polymers used were compatible with the ketoprofen.
- Surface smoothness of the ketoprofen microspheres was increased by increasing the polymer concentration, which was confirmed by SEM.
- As the drug to polymer ratio was increased, the mean particle size of ketoprofen microspheres was also increased. ketoprofen microspheres with normal frequency distribution were obtained.
- Entrapment efficiency increase with increase in the polymer concentration. From the results it can be inferred that there was a proper distribution of ketoprofen in the microspheres and the deviation was within the acceptable limits.
- The study also indicated that the amount of drug release decreases with an increase in the polymer concentration. The in vitro performance of ketoprofen microspheres showed prolonged and sustained release of drug action.
- The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of Korsemeyer-Peppas model was found to be in the range of 0.5 to 1 for the ketoprofen microspheres prepared with BSA indicating Non-Fickian of drug through KP microspheres.
- The DSC and XRD data indicates that the ketoprofen is still present in its lattice structure in the physical mixture where as it was completely amorphous inside the ketoprofen microspheres. This may be due to the conditions used to prepare the ketoprofen microspheres lead to cause complete ketoprofen amorphization. The melting points of the ketoprofen was estimated by open

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capillaries and found agrees well with the DSC data.

- From the study it is evident that promising sustained release microspheres of ketoprofen maybe developed by solvent evaporation techniques by using polymer like BSA.

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