

ORIGINAL RESEARCH

**ENHANCEMENT OF SOLUBILITY, DISSOLUTION RELEASE PROFILE AND GASTRIC TOLERANCE OF ACECLOFENAC BY INCLUSION COMPLEX WITH SKIMMED MILK FORMULATIONS****R. Satyasree**

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Submitted on: 15.08.17; Revised on: 20.08.17; Accepted on: 29.08.17**ABSTRACT:**

Aceclofenac, a non-steroidal anti-inflammatory drug is poorly soluble in water and is ulcerogenic. Milk has been used against the gastric disturbances caused by non-steroidal anti-inflammatory drugs. In this study, skimmed milk (SKM) is used as a carrier for inclusion complex (IC) due to its surface-active property and amino acid content. Thus, through IC with SKM solubility and dissolution rate were enhanced whereas ulcerogenicity is reduced. IC of aceclofenac were prepared with SKM by solvent evaporation using Rota evaporator and evaluated for solubility, dissolution, solid state characterization, ulcerogenicity studies.

KEY WORDS: Dissolution, Gastric Tolerance, Skimmed Milk.

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INTRODUCTION

Solubility is defined in quantitative terms as the concentration of the solute in a saturated solution at a certain temperature and in qualitative terms, it may be defined as the spontaneous interaction of two or more substances to form a homogeneous molecular dispersion. A saturated solution is one in which the solute is in equilibrium with the solvent. The solubility of a drug may be expressed as parts, percentage, molarity, molality, volume fraction,

and mole fraction. Drug solubility is the maximum concentration of the drug solute dissolved in the solvent under specific condition of temperature, pH and pressure. The drug solubility in saturated solution is a static property where as the drug dissolution rate is a dynamic property that relates more closely to the bioavailability rate [1]. The solubility of a drug is described in various descriptive terms which is based on the amount of drug dissolved in solvent and discussed in Table-1.

Table 1: TYPES OF SOLUBILITY

Descriptive terms	Appropriate volume of solvent in milliliters per gram of solute
Very soluble	Less than 1
Freely soluble	From 1-10
Soluble	From 10-30
Sparingly soluble	From 30-100
Slightly soluble	From 100-1000
Very slightly soluble	From 1000-10000
Insoluble	More than 10000

Need of Solubility:

- Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules.
- Solubility is one of the important parameter to achieve desired concentration of drug in systemic circulation for pharmacological response to be shown.
- Currently only 8% of new drug candidates have both high solubility and permeability. Nearly 40% of the new chemical entities currently being discovered are poorly water soluble.

Solubility of drug is largely due to: Polarity of the solvents, that is, to its dipole moment. A polar

solvent dissolves ionic solutes and other polar substances.

1. The ability of solute to form hydrogen bond with solvent.
2. Also, depends on the ratio of the polar to non-polar groups of the molecule
3. As the length of a non-polar chain of an aliphatic alcohol increases, the solubility of the compound in water decreases.
4. Straight chain monohydric alcohols, aldehyde, ketones, and acids with more than four or five carbons cannot enter the hydrogen bonded structure of water and hence are only slightly soluble.

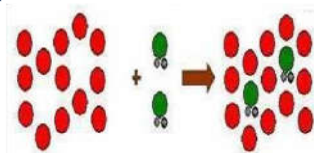
Figure 1 : Process of Solubilization

Step 1: Holes opens in the solvent



Step2: Molecules of the solid breaks away from the bulk

Step 3: The freed solid molecule is intergrated into the hole in the solvent



Drug absorption from the GI tract can be limited by a variety of factors most significant contributor being poor aqueous solubility and poor membrane permeability of the drug molecule. When administered an active agent orally it must first dissolve in gastric and/or intestinal fluids before it can permeate the membranes of the GIT to reach systemic circulation. [2] Hence, two areas of pharmaceutical research that focus on improving the oral bioavailability of active agents include; enhancing of solubility and dissolution rate of poorly water soluble drugs. The BCS is a scientific

framework for classifying a drug substance based on its aqueous solubility and intestinal permeability. As for BCS class II &

IV drugs rate limiting step is drug release from the dosage form and solubility in gastric fluid and not the absorption, so increasing the solubility in turn increase the bioavailability for BCS class II & IV drugs [3]. BCS

Classification System with examples of different drug is discussed in

Table 2 :Bio- Pharmaceutical Classification System [4]

BCS class I	High Solubility High permeability	β Blockers like Propranolol, Metoprolol
BCS class II	Low Solubility High Permeability	NSAID's like Ketoprofen Anti-epileptics like Carbazepine

BCS class III	High solubility Low Permeability	B blockers Atenolol, Antagonists Ranitidine	H2
BCS class IV	Low Solubility Low Permeability	Diuretics like Furosemide, Hydrochlorothiazide	

Techniques for Solubility Enhancement:

When the solubility of substances in aqueous media is limited, formulation strategies are required early in the drug discovery and they remain of critical importance for lead substance selection and commercial drug product development [5]. Various techniques have been used in attempt to improve solubility and dissolution rates of poorly water soluble drugs which include as following:

- a) Particle Size reduction
- b) Nanonization
- c) Co-solvency
- d) Hydrotropy
- e) pH Adjustment
- f) Sonocrystallization
- g) Supercritical Fluid (SCF) Process
- h) Solid Dispersion
- i) Inclusion Complexation
- j) Self-Emulsifying or Self-Micro Emulsifying Systems
- k) Liquisolid Methods

In these techniques carrier plays an important role in improving solubility and dissolution rate. Polymers, superdisintegrants, surfactants are extensively studied in recent years for dissolution enhancement in drugs. This part of this review discusses technological overview and effect of polymers, superdisintegrants and surfactants on dissolution enhancement of drugs while describes the role and applications of cyclodextrins, carbohydrates, hydrotropes, dendrimers, acids and miscellaneous carriers in enhancing dissolution of drugs

a) Particle Size Reduction:

The solubility of drug is often intrinsically related to drug particle size; as a particle becomes smaller, the surface area to volume ratio increases. [6] The larger surface area allows greater interaction with the solvent which causes an increase in solubility. Conventional methods of particle size reduction, such as comminution and spray drying, rely upon mechanical stress to disaggregate the active compound. Particle size reduction is thus permitting an efficient, reproducible, and economic means of solubility enhancement. However, the mechanical forces inherent to comminution, such as milling and grinding, often impart significant amounts of physical stress upon the drug product which may induce degradation. The thermal stress which may occur during comminution and spray drying is also a concern when processing thermo sensitive or unstable active compounds. Using traditional approaches for nearly insoluble drugs may not be able to enhance the solubility up to desired level. Micronization is another conventional technique for the particle size reduction. Micronization increases the dissolution rate of drugs through increased surface area, it does not increase equilibrium solubility. Decreasing the particle size of these drugs, which cause increase in surface area, improve their rate of dissolution. [7] Micronization of drugs is done by milling techniques using jet mill, rotor stator colloid mills and so forth micronization is not suitable for drugs having a high dose number because it does not change the saturation solubility of the drug. These processes were applied to griseofulvin, progesterone, spironolactone diosmin, and

fenofibrate. For each drug, micronization improved their digestive absorption, and consequently their bioavailability and clinical efficacy. Micronized

fenofibrate exhibited more than 10-fold (1.3% to 20%) increase in dissolution in at 30 minutes biorelevant media^[8,9]

Figure 2 : COLLOIDAL MILL



b) Nanonization:

Recently, various nanonization strategies have emerged to increase the dissolution rates and bioavailability of numerous drugs that are poorly soluble in water. Nanonization broadly refers to the study and use of materials and structures at the nanoscale level of approximately 100 nm or less.^[10] Nanonization can result in improved drug solubility and pharmacokinetics, and it might also decrease systemic side-effects^[11]. For many new chemical entities with very low solubility, oral bioavailability enhancement by micronization is not sufficient because micronized product has the tendency to agglomerate, which leads to decrease effective surface area for dissolution, the next step is nanonization.

There are different techniques used for nanonization of drug including Wet milling, Homogenization, Emulsification-solvent evaporation technique, Pear milling, Spray drying etc.^[12,13] There are many examples of nanonization of drugs.

c) Cosolvency:

The solubility of poorly soluble drugs in water can be increased by mixing it with some water miscible

solvent in which the drug is readily soluble. This process is known as cosolvency and the solvent used in combination are known as cosolvent. Cosolvent system works by reducing the interfacial tension between the aqueous solution and hydrophobic solute. It is also commonly known as solvent blending. There is a dramatic change in the solubility of drugs by addition of organic cosolvent into the water. The cosolvents are having hydrogen acceptor or donor groups with a small hydrocarbon region. The hydrophobic hydrocarbon region usually interferes with the hydrogen bonding network of water which consequently reduces the intermolecular attraction of water while the hydrophilic hydrogen bonds ensures water solubility.^[14,15]

d) Hydrotropy:

Hydrotropy is a solubilization phenomenon whereby addition of large amount of a second solute results in an increase in the aqueous solubility of existing solute. Concentrated aqueous hydrotropic solutions of sodium benzoate, sodium salicylate, urea, nicotinamide, sodium citrate, and sodium acetate have been observed to enhance the aqueous solubilities of many poorly water-soluble drugs.^[16,17]

e) pH Adjustment:

Poor water soluble drug may potentially dissolve in water by applying a pH change. To access the solubility of this ~ 25 ~ The Pharma Innovation Journal approach, the buffer capacity and tolerability of the selected pH are important to consider. Solubilized excipients that increase environmental pH within the dosage form to a range higher than pKa of weakly acidic drugs increase the solubility of that drug, those excipients that act as alkalinizing agents may increase the solubility of weakly basic drugs.^[18,19]

f) Sonocrystallisation:

Recrystallization of poorly soluble materials using liquid solvents and antisolvents has also been employed successfully to reduce particle size. The novel approach for particle size reduction based on crystallization by using ultrasound is Sonocrystallisation. Sonocrystallisation utilizes ultrasound power characterized by a frequency range of 20-100 kHz for inducing crystallization. It's not only enhances the nucleation rate but also an effective means of size reduction and controlling size distribution of the active pharmaceutical ingredients. Most applications use ultrasound in the range 20 kHz-5 MHz.^[20]

g) Supercritical Fluid (Scf) Process:

The number of applications and technologies involving supercritical fluids has also grown explosively. It has been known for more than a century that supercritical fluids (SCFs) can dissolve nonvolatile solvents, with the critical point of carbon dioxide, the most widely used supercritical fluid. It is safe, environmentally friendly, and economical. The low operating conditions (temperature and pressure) make SCFs attractive for pharmaceutical research. A SCF exists as a single phase above its critical temperature (Tc) and pressure (Pc). SCFs have properties useful to product processing because

they are intermediate between those of pure liquid and gas (i.e., liquid-like density, gas-like compressibility and viscosity and higher diffusivity than liquids). Moreover, the density, transport properties (such as viscosity and diffusivity), and other physical properties (such as dielectric constant and polarity) vary considerably with small changes in operating temperature, pressure, or both around the critical points. Hence, it is possible to fine-tune a unique combination of properties necessary for a desired application. These unique processing capabilities of SCFs, long recognized and applied in the food industry, have recently been adapted to pharmaceutical applications. Commonly used supercritical solvents include carbon dioxide, nitrous oxide, ethylene, propylene, propane, n-pentane, ethanol, ammonia, and water. Once the drug particles are solubilized within SCF, they may be recrystallized at greatly reduced particle sizes. The flexibility and precision offered by SCF processes allows micronization of drug particles within narrow ranges of particle size, often to sub-micron levels. Current SCF processes have demonstrated the ability to create nano suspensions of particles 5-2,000nm in diameter.

Several pharmaceutical companies, such as Nektar Therapeutics and Lavipharm, are specializing in particle engineering via SCF technologies for particle size reduction and solubility enhancement. Several methods of SCF processing have been developed to address individual aspects of these shortcomings, such as precipitation with compressed antisolvents process (PCA), Rapid Expansion of Supercritical Solutions, Gas Antisolvent Recrystallization, Precipitation with Compressed Fluid Antisolvent, Impregnation or infusion of polymers with bioactive materials, Solution enhanced Dispersion by Supercritical

Fluid, solution enhanced dispersion by SCF (SEDS), supercritical antisolvents processes (SAS) and aerosol supercritical extraction system (ASES).^[21]

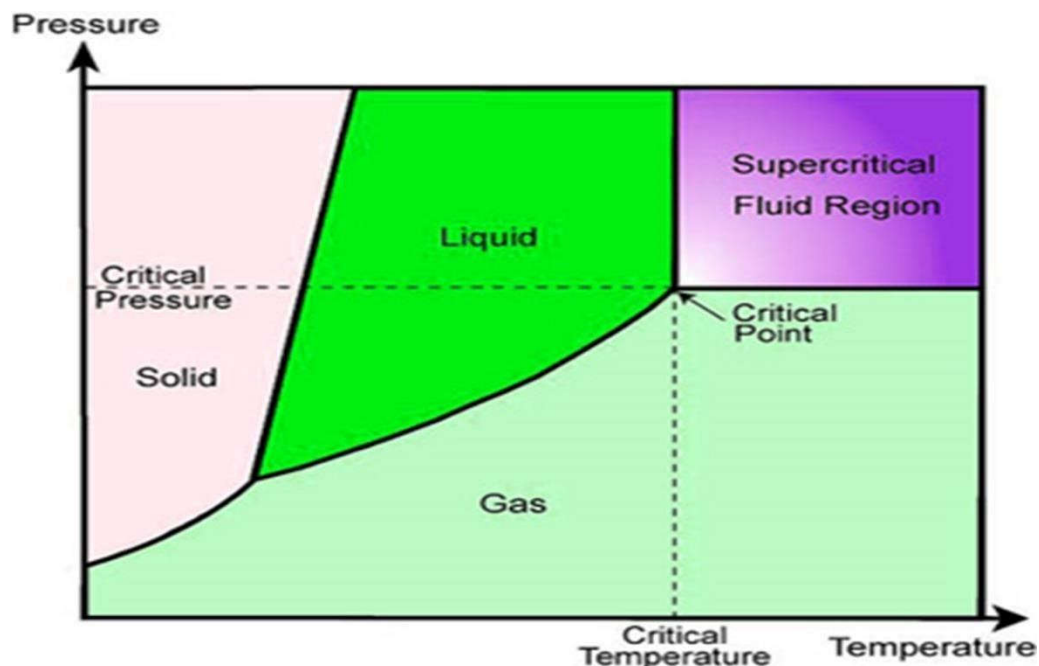


Figure 3: Super critical fluid process

h) Solid Dispersion:

The concept of solid dispersions was originally proposed by Sekiguchi and Obi, who investigated the generation and dissolution performance of eutectic melts of a sulfonamide drug and a water-soluble carrier in the early 1960s [22]. Solid dispersions represent a useful pharmaceutical technique for increasing the dissolution, absorption, and therapeutic efficacy of drugs in dosage forms. The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The most commonly used hydrophilic carriers for solid dispersions include polyvinylpyrrolidone (Povidone, PVP), polyethylene glycols (PEGs), Plasdone- S630. Surfactants like Tween-80, docusate sodium, Myrj-52, Pluronic-F68, and sodium lauryl sulphate (SLS) also find a place in the formulation of solid dispersion. The solubility of celecoxib, halofantrine, and ritonavir can be improved by solid dispersion using suitable

hydrophilic carriers like celecoxib with povidone (PVP) and ritonavir with gelucire. Various techniques to prepare the solid dispersion of hydrophobic drugs with an aim to improve their aqueous solubility are listed here:

1. Fusion Process:

In the fusion method of preparation, the carrier is heated to a temperature just above its melting point and the drug is incorporated into the matrix. The mixture is cooled with constant stirring to homogeneously disperse the drug throughout the matrix. Several mechanisms could operate during the process of dispersion. If the drug has a high degree of solubility in the carrier, the drug could remain “dissolved” in the solid state, yielding what is known as a solid solution. Particle size reduction under these conditions proceeds to the ultimate level leading to molecular dispersion of the drug in the carrier matrix. These systems show very high drug dissolution rates compared to control samples. If, on the other hand, the solubility of the drug in solid state is not so high, crystallites of the drug

become dispersed in the matrix. Such systems show only moderate increases in dissolution rates. A third mechanism is the conversion of a drug to an amorphous form in the presence of the matrix, again exhibiting different dissolution rates and solubility. Other factors that may play a role include solubilizing effect conferred by the carrier itself, improved wetting or decreased surface hydrophobicity, complexation, and crystallization of the drug in a metastable polymorphic form of altered thermodynamic properties. An important limitation of the fusion method of preparation is the exposure of drugs to elevated temperatures, particularly if the carrier is a high-melting solid and the drug is heat sensitive. [23]

2. Solvent method:

In the solvent method of preparation, the carrier and the active ingredient are dissolved in a suitable organic solvent. This solvent is evaporated at an elevated temperature or under vacuum. As the solvent is being removed, super saturation occurs followed by simultaneous precipitation of the constituents resulting in a solid residue. The coprecipitate is then dried under vacuum to drive out any solvent freely adhering to the particle surface. However, there is a possibility ~ 26 ~ The Pharma Innovation Journal of the formation of a solvate within the crystal lattice. This presents a problem in terms of pharmaceutical acceptance since most of the solvents used are non-aqueous (organic) and toxic. Hence, removal of even trace amounts of the solvent is implied.

Highly sensitive techniques such as differential scanning calorimetry (DSC), differential thermal analysis (DTA), thermogravimetric analysis (TGA), and less sensitive procedures like gravimetry and spectroscopy can be used to demonstrate complete solvent removal. [24]

3. Fusion-Solvent Method:

In the fusion methods, a carrier(s) is/are melted and the drug(s) is / are incorporated in the form of a solution. If the carrier can hold a certain proportion of liquid yet maintaining its solid properties, and if the liquid is innocuous, the need for solvent removal is eliminated. Otherwise, this method faces the same criticism of solvent retention

described before. This method is particularly useful for drugs that have high melting points or that are thermolabile. The feasibility of the method has been demonstrated for spironolactone and Griseofulvin dispersions in polyethylene glycol 6000. [25]

4. Spray Drying

In this type of preparation, the carrier and the active ingredient are dissolved or suspended in a suitable solvent. This solvent is evaporated by drying it to apply a stream of heated air to remove the solvent.

Due to the large surface area of the droplets, the solvent rapidly evaporates and solid

dispersion is formed quickly. [26,27]

5. Lyophilization (Spray Freeze Drying Method):

This method is used to avoid the heating during the preparation of thermosensitive drugs; spray freeze drying (SFD) has been successfully developed to prepare solid dispersions at ambient temperature, which was made significant development by the research work of William III. SFD technology involves the atomization of a feed liquid containing poorly water-soluble or insoluble APIs and excipients directly into a cryogenic liquid at ambient temperature to produce a frozen micronized powder that is subsequently dried. This process offers a variety of advantages compared to traditional technologies for solid dispersions, including amorphous structure and high surface area. [28]

6. Hot-melt Extrusion:

It is a very common method used in the polymer industry. But Speiser and Huttenrath were the first persons who use this technology for pharmaceutical purpose. A melt extrusion consists of the following sections: An opening to feed raw materials, a heated barrel that consists of extruder screws to convey and mix the fed materials, and an exit port, which consists of an optional die to shape the extruding mass. The Active ingredients and the carrier are fed into the heated barrel of extruder at a constant rate. When the mixture of active

ingredient and the carrier is conveyed through heated screws, it is transformed into its "fluid like state". This state allows intimate and homogeneous mixing by the high shear of extruder screws. An exit port, which consists of an optional die, shapes the melt in the required form such as granules, pellets, films, or powder. An important advantage of the hot melt extrusion method is that the drug/carrier mix is only subjected to an elevated temperature for about one minute, which enables drug that are somewhat thermolabile to be processed. [29,30]

Inclusion Complexation:

Among all the solubility enhancement techniques, inclusion complex formation technique has been employed more precisely to improve the aqueous solubility, dissolution rate, and bioavailability of poorly water soluble drugs. Inclusion complexes are formed by the insertion of the nonpolar molecule or the nonpolar region of one molecule (known as guest) into the cavity of another molecule or group of molecules (known as host). The major structural requirement for inclusion complexation is a snug fit of the guest into the cavity of host molecule. The cavity of host must be large enough to accommodate the guest and small enough to eliminate water, so that the total contact between the water and the nonpolar regions of the host and the guest is reduced. Various techniques are used to prepare for making inclusion complexes of poor soluble drugs with an aim to improve their aqueous Solubility are listed here:

a) **Kneading:**

The method involves the formation of paste of cyclodextrin with guest molecules by using small quantity of either water or ethanol to form kneaded mass. Kneaded mass can be dried at 45 °C and pulverized.

b) **Melting:**

Excess quantity of guest melted, mixed with powdered cyclodextrin, after cooling excess quantity of guest is removed by washing with weak complex forming solvent. The method restricted to sublimable guest like menthol.

c) **Solution –enhanced dispersion by the Supercritical fluids(SEDs):**

SEDs is novel, single step method, which can produce solid drug-cyclodextrin complexes. The optimization of processing conditions is essential to achieve the optimum complexation efficiency and to compare with drug cyclodextrin complexation methods described earlier in the literature (e.g. kneading, freeze drying, spray drying etc). Advantages over other methods are:

- Preparation of solid-cyclodextrin complexes in single step process,
- Achievement of high complexation efficiency (avoidance of excess cyclodextrin)
- Possibility to minimize the contact of drug with cyclodextrin during the process.
- Achievement of enhanced dissolution rate of the drug (which is comparable to the dissolution behavior of micronized drug-cyclodextrin) ^[31,32]

d) **Co-evaporation/Solvent evaporation method:**

To the alcoholic solution of guest, aqueous solution of host is added and stirred for sometimes and evaporated at room temp until dried mass obtained, pulverized and sieved and fraction is collected.

e) **Microwave Irradiation:**

This method is developed for rapid organic synthesis and reactions, which require shorter reaction time and higher yield product.

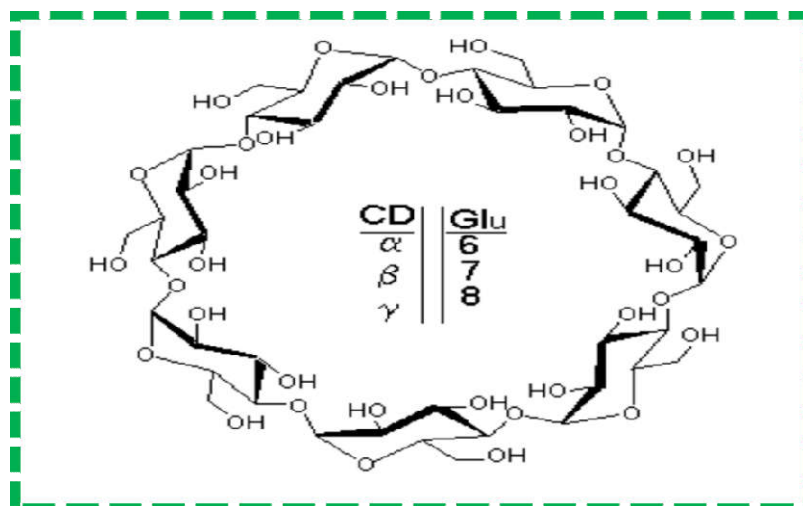


Figure 4 : Dissolution behavior of cyclodextrin

- f) **Freeze Drying/Lyophilisation technique:** The required stoichiometric quantity of host and guest were added to aqueous solution of cyclodextrin and this suspension stirred magnetically for 24 hours, and resulting mixture is freeze dried at 60 °C for 24 hours.
- g) **Spray drying/Atomization:** In this method, host solution prepared generally in ethanol: water 50% v/v. To this guest is added and resulting mixture is stirred for 24 hr. at room temperature and solution is spray dried by observing following conditions-air flow rate, atomizing air pressure, inlet temperature, outlet temperature, flow rate of solution etc. Product obtained by passing through 63-160 micrometer granulometric sieve.^[33]
- j) **Self-Emulsifying or Self-Micro Emulsifying Systems:**

Self-emulsifying or self-micro emulsifying systems use the concept of in situ formation of emulsion in the gastrointestinal tract. The mixture of oil, surfactant, co-surfactant, one or more hydrophilic solvents and co-solvent forms a transparent isotropic solution that is known as the self-emulsifying drug delivery system (SEDDS), in the absence of external phase (water) and forms fine o/w emulsions or microemulsions spontaneously upon dilution by

the aqueous phase in the GIT and is used for improving lipophilic drug dissolution and absorption. The ease of emulsification could be associated with the ease of water penetrating the various liquids crystalline or gel phases formed on the surface of the droplet. One of the advantages of SEDDS in relation to scale up and manufacture is that they form spontaneously upon mixing their components under mild agitation and they are thermodynamically stable. The drawbacks of this system include chemical instabilities of drugs and high surfactant concentrations. The large quantity of surfactant in selfemulsifying formulations (30-60%) irritates GIT. Most selfemulsifying systems are limited to administration in lipid filled soft or hard shelled gelatin capsules due to the liquid nature of the product. Interaction between the capsule shell and the emulsion should be considered to prevent the hygroscopic contents from dehydrating or migrating into the capsule shell. A Neoral-R is an example of self microemulsifying drug delivery system (SMEDDS). Depending on the dose level, the relative bioavailability of cyclosporine- α administered. A Neoral-R could be 174-239% of the bioavailability of cyclosporine- α from Sandimmune-R, the originally marketed formulation. Emulsion droplet size is a major

factor influencing bioavailability of drugs from emulsion formulations, with small droplet radii enhancing the plasma levels of drugs, in part due to direct lymphatic uptake. Since SMEDDS contain high concentration of surfactants, they should be limited to oral applications and may not be advisable for long term use due to the potential of causing diarrhea. [34]

k) Liquisolid Methods:

When the drug dissolved in the liquid vehicle is incorporated into a carrier material which has a porous surface and closely matted fibers in its interior as cellulose, both absorption and adsorption take place; i.e. the liquid initially absorbed in the interior of the particles is captured by its internal structure, and after the saturation of this process, adsorption of the liquid onto the internal and external surfaces of the porous carrier particles occur. Then, the coating material having high adsorptive properties and large specific surface area gives the liquisolid system the desirable flow characteristics. Liquisolid solid system is acceptably flowing and compressible powdered forms of liquid medications. In the concept of liquisolid system, liquid drugs having low aqueous solubility dissolved in suitable non-volatile solvents, converted in to free flowing and radially compressible powder by simple admixture with selected powdered excipients referred as carrier and coating materials. Microcrystalline and amorphous cellulose and silica powders may be used as coating materials. [35]

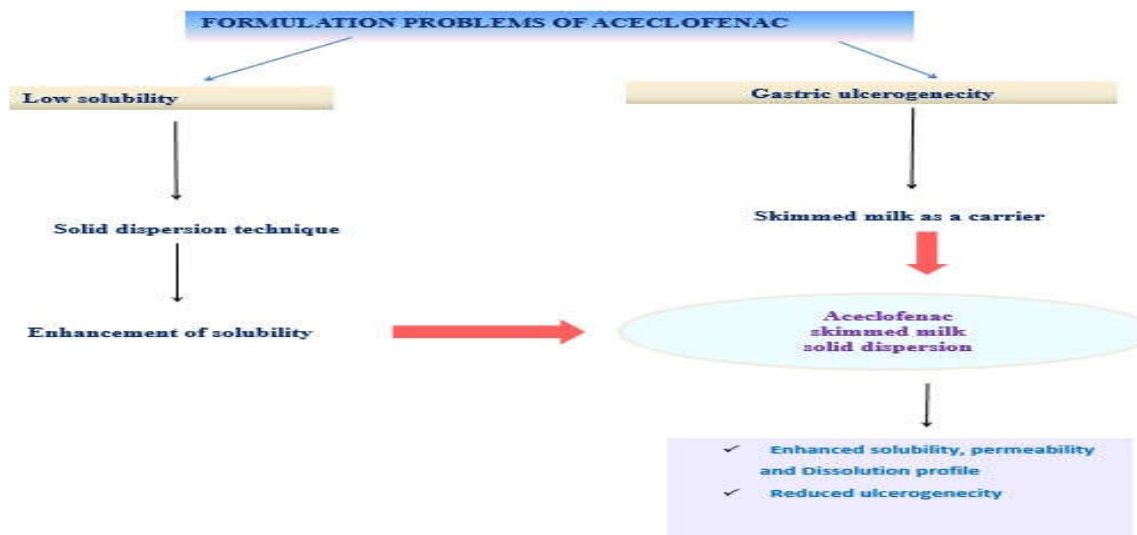
AIM AND OBJECTIVE:

The main aim of the present investigation is to formulate aceclofenac in the form of an inclusion complex to enhance the solubility and dissolution release profile of the drug. Moreover, we have used skimmed milk as a carrier to reduce the ulcerogenicity of aceclofenac. This is because the drug is an NSAID. These drugs generally show their mechanism of action by inhibiting COX enzyme and thus inhibiting the biosynthesis of Prostaglandins. However, the major side effect of NSAIDs is ulcers.

Justification for using 'skimmed milk' as a drug carrier

- ✦ Easily available
- ✦ Biodegradable
- ✦ Inexpensive
- ✦ Does not exhibit toxicity problems as experienced with PEG and PVP.
- ✦ Aminoacids have been suggested either as additives in peroral application or in the form of aminoacid salts to reduce gastrointestinal disorders arising due to aceclofenac like NSAIDs.
- ✦ The surface-active agents - casein micelles, globular proteins, lipoprotein particles and amino acid content are expected to be the reason for increased permeation of the drug from the solid dispersion.

OBJECTIVE:



MATERIALS AND METHOD:

Table 3: List Of Chemicals

Sl. No	CHEMICALS USED	SUPPLIED BY
1	ACECLOFENAC	Dr. Reddy's Laboratories Pvt. Ltd, Hyderabad
2	SKIMMED MILK	Local store
3	HCL	S D fine chemical Ltd.
4	METHANOL	S D fine chemical Ltd.

Table 4: LIST OF EQUIPEMENTS

Sl.No	INSTRUMENTS	MAKE AND PLACE	MODEL
1	Rota Evaporator	Cyber lab (HEIDOLPH), Germany	CR-2001
2	Orbital shaker	DBK Instruments, Mumbai	OSR30

3	Micro Centrifuge	Vignan Labs, Mumbai	RM12C
4	Tablet Dissolution Test Apparatus	LAB INDIA, Mumbai	DS8000
5	U.V Visible Spectrometer	Shimadzu Japan	UV1800S

DRUG PROFILE

Nonproprietary name - Aceclofenac

Chemical name - 2-[2-[2-[(2,6-dichlorophenyl) amino] phenyl] acetyl oxyacetic acid

Molecular formula- C₁₆H₁₃Cl₂NO₄

Molecular weight- 354.18472 g/mol

Description -It is non-steroidal anti-inflammatory drug for the treatment of pain and inflammation

Physicochemical profile

Description - White or almost white, crystalline powder.

Melting point - 149-153° C

Solubility - Practically insoluble in water, freely soluble in acetone, soluble in alcohol

Dissociation

Constant - pKa 4.65

Log P - 1.87 at pH 1.2 (n-octanol vs. acidic buffer)

Pharmacokinetic profile

Absorption - It is well-absorbed orally, with peak plasma levels occurring within 1.5 to 3 hours.

Half-life - 4h

Volume of distribution - 25 L Protein binding - >99%

t_{max} - 1.5-3 hrs

METHODS

Preparation of SKM powder

Accurately measured volume (100ml) of SKM was dried at 40C, 100rpm under vacuum for

40–60min using rotary vacuum evaporator (Hei-VAP advantage/561-01300, Heidolph, Germany). The obtained sticky semisolid mass was placed in desiccator for 48–72h to get free flowing powder

(100ml milk yielded about 10.11g powder) and passed through a sieve

65 and stored in well tight container till further use

Preparation of Aceclofenac loaded IC

Accurately weighed (1g) was Aceclofenac mixed with SKM (10, 20, 30, 40, 50, 60ml) in a /suspension was subjected for same procedure described above to obtain well-dried free flowing Aceclofenac loaded IC powder

Preparation of PM'S

Weighed amount of Aceclofenac (1g) was uniformly mixed with 1, 2, 3, 4, 5 and 6g of SKM powder separately using a mortar and pestle. The prepared mixtures were kept in a desiccator over calcium chloride at room temperature for 72h and passed through sieve #65 and stored in well tight container until further use.

Solubility studies of IC and PM

Excess amounts (100mg) of Aceclofenac, its IC and PMs with SKM were added in 2ml clicklokmicrocentrifuges (Tarsons Products Pvt. Ltd., Kolkata, India) filled with 1ml of pH 1.2 buffer (SGF) and they were kept in a orbital shaking incubator at 25C for 48h. The solutions were filtered through nylon membrane filter (pore diameter: 0.2mm and membrane diameter: 13mm) using a syringe filter unit. The samples were diluted appropriately and drug dissolved was measured using UV-visible spectrophotometer at 331nm (UV 3200, Labindia, Mumbai, India).

In-vitro dissolution release study

The dissolution rates of Aceclofenac pure drug (10mg), dose (10mg) equivalent amount of

Aceclofenac-loaded IC and PM was determined using the USP dissolution apparatus II (DS 8000, Labindia, India). The dissolution medium was simulated gastric fluid (SGF) at 37C and stirred at 100rpm. Samples of the dissolution medium were withdrawn at predetermined time intervals (5, 10, 15, 30, 45, 60, 90, 120min), filtered through Millipore membrane of 0.2mm pore diameter and assayed for the drug content using UV-visible spectrophotometer at 331nm (UV-3200, Labindia, India). Dissolution efficiency (DE) was calculated from the area under the dissolution curve (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time^[32].

The relative dissolution rate (RDR) was determined by dividing the DE of PM and IC with Aceclofenac (control) separately.

Differential scanning calorimetry

To determine the crystallinity and characterize the solid-state interaction between ACECLOFENAC and SKM 3mg each of the Aceclofenac, SKM powder, PM and IC were weighed separately, placed in aluminium pans and analyzed using a differential scanning calorimeter (Shimadzu DSC-60, Tokyo, Japan). The scanning speed was 5C/min in the range 0–300C.

X-ray diffraction

X-ray diffraction patterns of Aceclofenac, SKM powder, PM and IC was recorded with an X-ray diffractometer (Shimadzu, Maxima XRD-7000, Tokyo, Japan) under conditions of

“Cu” radiation target, nickel filter, 40kV voltage, 30mA current with a scan range of 10–

80with a scan speed of 3/min and an intensity range of 0–1500 counts.

Scanning electron microscopy

To determine the surface morphology of Aceclofenac, SKM powder, PM and IC were analyzed using a SEM (Joel 1200 EX-11, Tokyo, Japan) under various magnifications.

Fourier transform infra-red spectroscopy

Fourier transform infra-red spectra of Aceclofenac, SKM powder, PM and IC were subjected to FTIR analysis (Bruker AlphaT-1020, Ettlingen, Germany). Potassium bromide pellets were prepared on KBr press. The samples were prepared using KBr discs by means of KBr pellet press at a pressure of 8 tons. The spectra were recorded over the range of

4000cm⁻¹ to 400cm⁻¹

Assessment of ulcerogenicity

Male and female Wistar rats (100–200g) were divided into three groups each consists of six animals. Group I served as the standard (80mg/kg body weight); group II served as PM test group (80mg equivalent amount of PM); group III served as the IC test group (80mg equivalent amount of Aceclofenac -loaded IC). The animals were fasted for 72h before the test substance administration but had free access to water. The test substance was administered by oral gavage to each rat as a single dose, using an intubation needle fitted onto a syringe of appropriate size. The dose administered to each individual rat was calculated according to its body weight recorded on the day of test substance administration. The study was performed with the approval of Institutional Animal Ethical Committee, School of Pharmacy, Anurag Group of Institutions, Hyderabad, India. Following completion of the experiment, rats were killed using ether anesthesia and stomach was taken for macroscopic observation

RESULTS AND DISCUSSION:

Milk consists of water (88%), lactose (5%), fat (3%), protein(3.3%), and minerals (0.7%). Milk can be described as a multiple component system in which proteins and fat globules form colloidal particles dispersed in the aqueous phase (Fox & Mc Sweeny, 1998). The fat content of the milk can be simply isolated by gravity or centrifugation. The protein portion of SKM is composed of 80% caseins and 20% whey proteins. The principle components of caseins and whey proteins are α_1 -, α_2 -, β -, and κ -casein and α -, β -lactoglobulin and bovine serum albumin, respectively (Atherton & Newlander, 1977; Fox & Mc Sweeny, 1998; Walstra et al., 1999; Gaucheron, 2005; Walstra et al., 2006; Livney, 2010). The major protein fractions of SKM are casein. The majority of the casein proteins of SKM available in the form of colloidal particle identified as the casein micelle. It is implicit that these micelles encapsulate hydrophobic ACECLOFENAC inside with its surface-active property improves its solubility and dissolution release in gastric or dissolution media. These drug entrapped micelles have a porous nature, which may responsible for the release of encapsulated ACECLOFENAC molecule from the IC into media. The other model also recommended is casein submicelle model. According to this model, there are small aggregates of whole casein, consisting of 10–100 casein molecules, called submicelles (Atherton & Newlander, 1977; Walstra, 1990; Walstra et al., 1999). It is assumed that there are two different types of submicelle: with and without κ -casein. Submicelle rich in κ -casein absorb surface location and show surface activity. The second principle protein fractions of SKM are whey proteins, which are surface active molecules and more aqueous soluble than casein (Atherton & Newlander, 1977; Fox & Mc Sweeny, 1998; Walstra et al., 1999, 2006; Sahin & Arslan,

2007). However, in this present research work we have not evaluated which component in SKM responsible more to formation of an IC, we expect that casein micelles and whey proteins are concerned as they have significant surface activity to solubilize ACECLOFENAC entrapped in micelles and associating with hydrophilic whey protein portions. In this study, SKM was selected as carrier for formulation of PM and IC of

ACECLOFENAC to improve solubility, release profile and reduce ulcerogenicity of ACECLOFENAC.

Drug content, solubility and dissolution release studies

Drug content data for the PM and IC formulations are depicted in Table 1. From the results it has shown that the drug content in the formulated IC and PM was within the range of $\pm 3\%$ theoretical amount, indicating the method adopted for formulation was suitable and reproducible. Free ACECLOFENAC was found to have a solubility of 0.064 mg/ml. The solubility was enhanced (0.16 mg/ml) 2.5-fold using the IC technique (IC4, Table 1).

Likewise, the solubility of ACECLOFENAC was increased (0.087 mg/ml) 1.4-fold in PM4. The enhanced aqueous solubility of ACECLOFENAC observed with IC was possibly due to a reduction in particle size and formation of an amorphous state, while in the case of PM, the surface active agents and amino acid content of the milk may be responsible for better solubility. The hydrophilic casein micelles entrap hydrophobic ACECLOFENAC, thus improving the aqueous solubility (Vijaya Kumar & Mishra, 2006; Ankush et al., 2012). The results suggest that the SKM was able to enhance the solubility of ACECLOFENAC in enzyme free SGF. The dissolution profiles of the PM and IC formulations as compared to free ACECLOFENAC are depicted in Figure 1A and B, respectively. Free drug showed a percentage cumulative drug release (% CDR) of only 9.59 \pm 0.8 within 15 min. The % CDR of

PM formulations ranged from 20.91 \pm 1.38 to 43.50 \pm 1.59, whereas in case of IC it varied from 24.34 \pm 3.19 to 99.21 \pm 2.82. The results showed that the % CDR from IC and PM was 2–9 times higher than free ACECLOFENAC within 15 min. The best % CDR was shown by formulations IC4 and PM4, 96.74 \pm 2.30 and 41.0 \pm 5.12, respectively, with in 15 min.

It is evident that the % CDR continues to enhance with the increasing amounts of SKM in the formulations. However, beyond drug SKM ratio of 1:4, the % CDR was not considerably improved. This may be because of higher quantities of carrier involved which may take more time to dissolve (Gaucheron, 2005; Ahire et al., 2010). The IC showed higher % CDR in comparison to PM because the presence of carrier also prevents aggregation of fine drug particles, increasing dissolution of the drug. Furthermore, the presence of carrier inhibits the crystal growth of the drug assisting in faster dissolution (Patel et al., 2008). Accordingly, DE was

also significantly higher for IC formulations compared to free ACECLOFENAC in enzyme free SGF medium (p50.01) (Table 1). The RDR greater than one indicates dissolution enhancement and in this work noticed more than one for IC formulations (Table 1). Interestingly, the IC formulation (IC4) increased dissolution of ACECLOFENAC compared to other formulations. The in-vitro studies revealed that IC4 and PM4 were best of all the formulations. The solubility and dissolution rates exhibited by these formulations were maximum and better than other formulations. Therefore, IC4 and PM4 were selected for further studies.

Table 5: Formulation chart of ACECLOFENAC-loaded IC and PM.

Formulation code	ACECLOFENAC: skimmed milk/powder	Drug content (%)	Solubility (mg/ml)	DE	RDR
Free ACECLOFENAC-		-	0.06410.0012	42.35	-
PM ₁	10:10	98.0301.94	0.06430.0024	47.24	1.12
PM ₂	10:20	97.0402.43	0.04650.0070	54.79	1.29
PM ₃	10:30	99.0202.44	0.08300.0065	60.58	1.43
PM ₄	10:40	99.8703.24	0.09870.0044	63.98	1.51
PM ₅	10:50	98.9102.32	0.06500.0053	66.06	1.56
PM ₆	10:60	99.0101.37	0.04580.0047	67.4	1.59
IC ₁	10:10	100.002.22	0.08490.0044	53.12	1.25
IC ₂	10:20	101.0001.98	0.05190.0046	64.71	1.53
IC ₃	10:30	99.0002.66	0.06680.0034	83.02	1.96
IC ₄	10:40	98.0001.98	0.16030.0032	91.51	2.16
IC ₅	10:50	102.0001.38	0.06050.0054	93.6	2.21
IC ₆	10:60	100.0001.86	0.03680.0055	95.01	2.24

Solid state characterization

The mechanism of improved dissolution rate of ACECLOFENAC from IC, the solid-state properties of this system was investigated by means of DSC, XRD and SEM.

Differential scanning calorimetry

In order to determine the solid state of the ACECLOFENAC in PM and IC with SKM, DSC studies were performed on the ACECLOFENAC, PM and IC separately. As shown in Figure 2, the DSC

curve of ACECLOFENAC showed one endothermic peak at 204.7C, corresponding to its melting point, whereas SKM's peaks has been shown to be at 148.6 and

208.3C. The DSC plots of the PM and IC showed almost all similar two broad peaks at about 144.0 and 200.0C. As the DSC peak of the IC is broad, it could be concluded that the complete conversion of crystalline state of ACECLOFENAC to amorphous state in IC.

X-ray diffraction

Figure 3 shows the X-RPD of the free ACECLOFENAC, SKM, PM and IC. Considerably different X-RDPs are to be predicted if an IC is formed, because crystal structure will be altered (Green et al., 1991; Khan et al., 2001). In our study, it was noticed that most of the diffraction patterns of the PM were simply superimposition or summation of the ACECLOFENAC and SKM, with similar sharp peaks, similar d values and other characteristics as that of pure components. However, some alterations like peak

positions, diminutions in peak intensities, and little disparity in d values were noticed diffractograms of the PM (Table 2), demonstrating the possibility of interactions between SKM and ACECLOFENAC. On the other hand, the study of changes in peak positions, appearances, disappearances and relative intensity of the peaks of the ICs established somewhat soft diffraction patterns with considerable differences than those of PM and individual constituents. Additionally the d values of the IC were completely different from that of ACECLOFENAC and SKM (Table 2), which further supports the evidence that complexation did takes place between ACECLOFENAC and SKM. The X-RDPs further showed that the crystalline peaks in the diffractograms of the IC were wider and decreased intensities, indicating the amorphous nature of the ACECLOFENAC in the final IC. These results are accordance with those, previously explained by the DSC study. This could be recognized to the molecular inclusion of the ACECLOFENAC molecules in the center hydrophobic core of the SKM casein micelles leading to the inhibition of crystallization of the ACECLOFENAC.

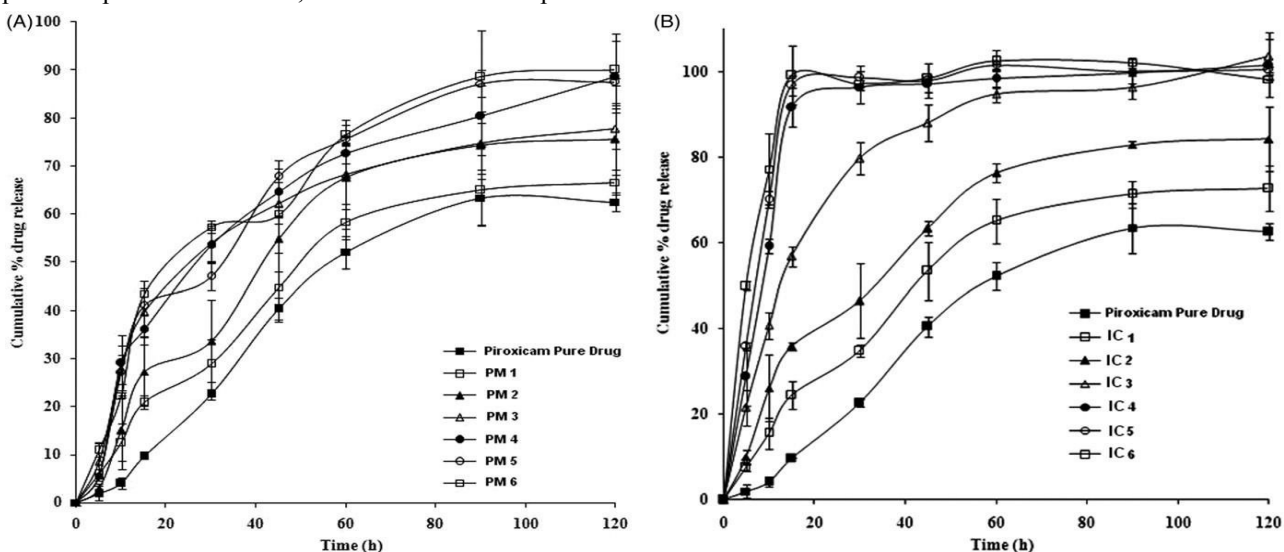


Figure 5: In-vitro dissolution release profiles of (A) free ACECLOFENAC versus PM with skimmed milk (B) free ACECLOFENAC versus IC with skimmed milk

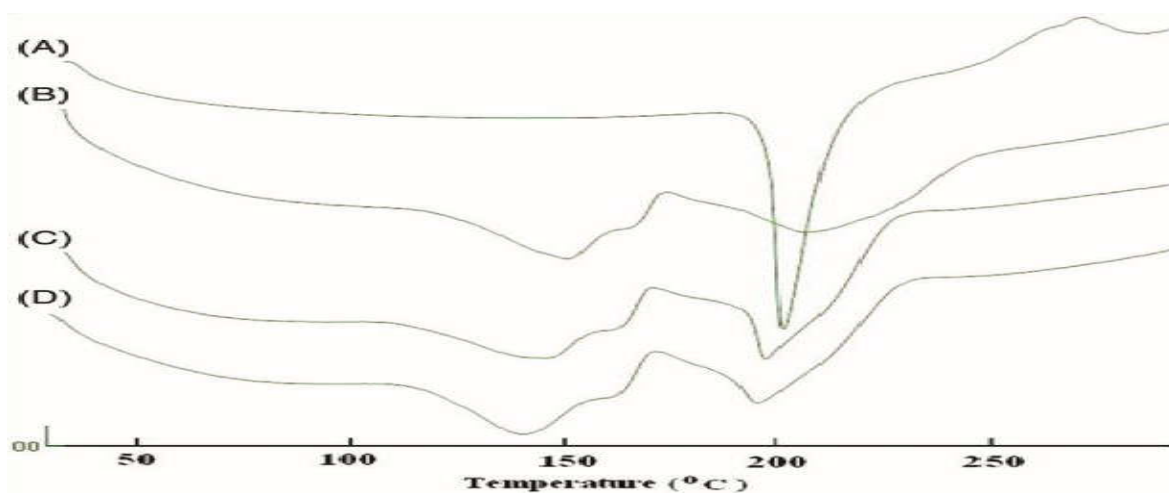


Figure 6: DSC thermograms of (A) free ACECLOFENAC, (B) skimmed milk powder, (C) physical mixture and (D) inclusion complex.

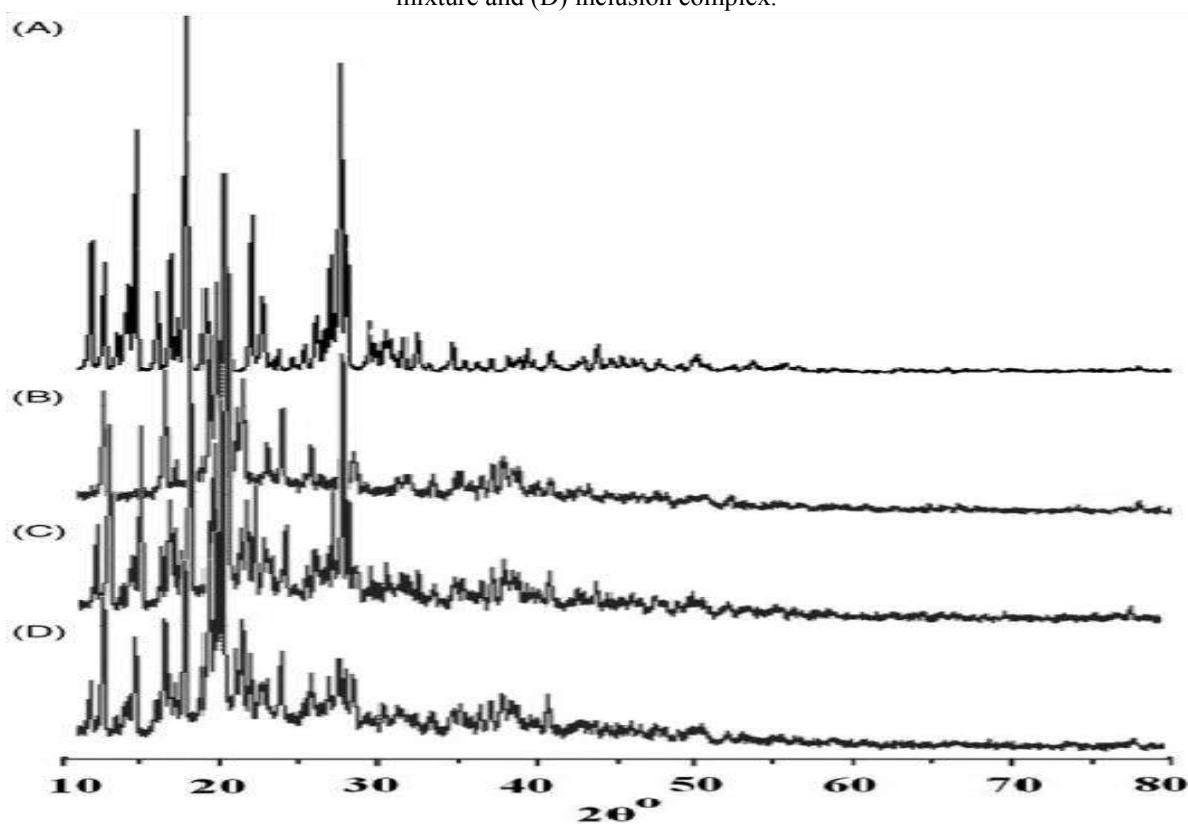


Figure 7: XRD patterns of (A) free ACECLOFENAC, (B) skimmed milk powder, (C) physical mixture and (D) inclusion complex

Table 6: X-ray powder diffraction of piroxicam, skimmed milk, PM, IC expressed as 2 θ , peak intensity, width, inter planar distance and relative diffraction intensity

	2 θ	Interplanar Dis (d Value)	Peak Int. (I)	Rel. Diff. Int. (I/I _o)	Peak width (λ)
					0.1876
Compound					
Pure piroxicam	11.646	7.592	679	35	
	12.483	7.085	574	30	0.1890
	14.508	6.100	1269	66	0.1799
	15.859	5.583	388	20	0.1812
	16.699	5.304	590	31	0.1801
	17.698	5.007	1930	100	0.1786
	27.398	3.252	1624	84	0.1891
	27.793	3.207	711	37	0.1814
Pure SKM powder	12.513	7.067	290	31	0.2084
	16.408	5.398	314	33	0.1962
	19.162	4.627	406	43	0.2201
	19.602	4.524	534	57	0.2292

	20.002	4.435	942	100	0.2127
	21.244	4.178	281	30	0.2072
Physical mixture	12.512	7.068	431	56	0.2014
	14.540	6.086	388	51	0.1895
	16.396	5.401	229	30	0.2137
	17.733	4.997	558	73	0.1877
	19.164	4.627	340	45	0.2195
	19.604	4.524	423	55	0.2264
	20.003	4.435	763	100	0.2090
	21.779	4.077	222	29	0.1771
	26.805	3.323	213	28	0.1729
	27.448	3.246	558	73	0.1870
	27.838	3.202	228	30	0.1821
Optimized ACECLOFENAC inclusion complex 12.5557.044313390.2156with SKM					
	16.460	5.381	254	31	0.2152
	17.770	4.987	357	44	0.2073

19.643	4.515	456	57	0.2379
20.050	4.425	807	100	0.2269

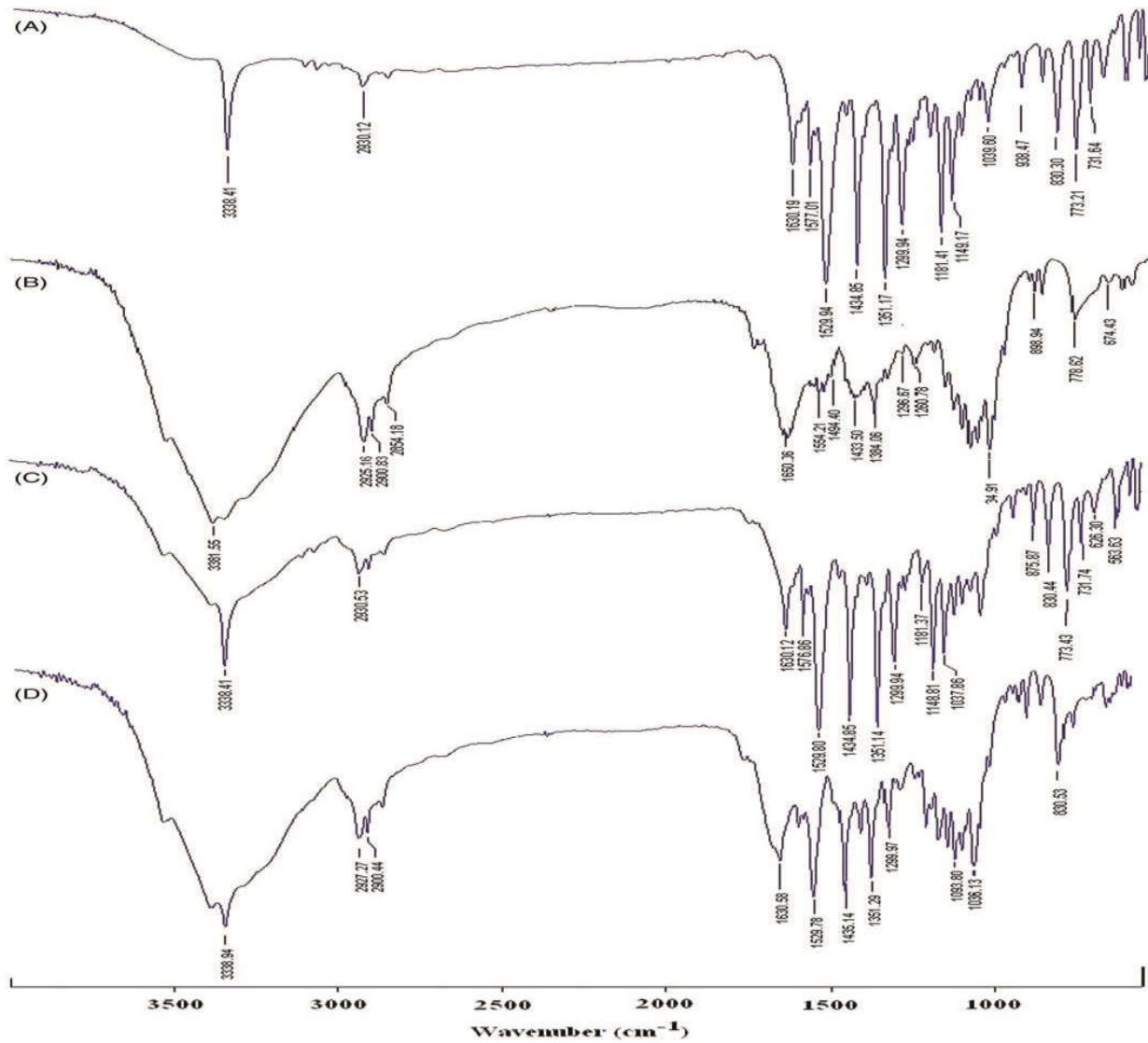


Figure 8: FTIR spectra of (A) free ACECLOFENAC, (B) skimmed milk powder, (C) physical mixture and (D) inclusion complex

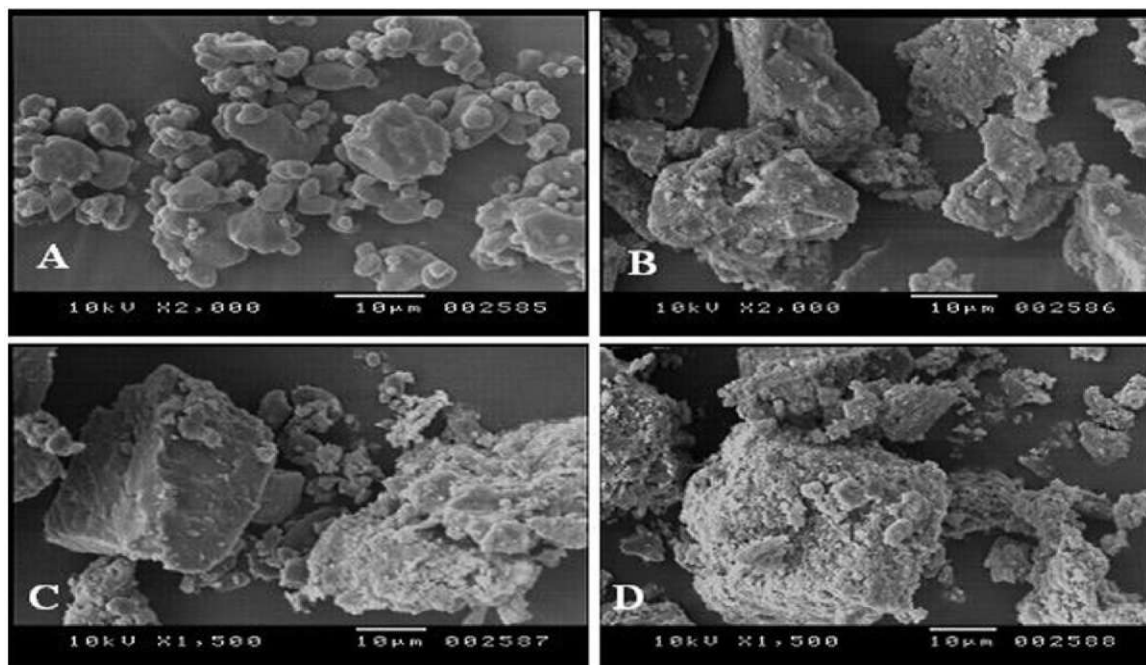


Figure 9: SEM images of (A) free ACECLOFENAC, (B) skimmed milk powder, (C) physical mixture and (D) inclusion complex.

Table 7: Characteristic peaks of free ACECLOFENAC, skimmed milk, PM and IC with skimmed milk in FTIR spectra.

		Wave numbers (cm ⁻¹)		Characteristic wave Bond nature and bond S.No. Free		
ACECLOFENAC	IC	PM	number range (cm ⁻¹)	attributed (theoretical)		
1		2930	2927	2930	3100–2850	C–H stretching
2		830	830	830	900–675	H Bending
	773		774	773		
3		618	619	626	700–600	C–S stretching
4		1434	1435	1434	1600–1430	C ¹ / ₄ C, C ¹ / ₄ N ring stretching
	1529		1529	1529		
5		1351	1351	1351	1350–1430	Asymmetric stretching S(¹ / ₄ O) ₂
6		1149	1147	1148	1160–1120	Symmetric stretching S(¹ / ₄ O) ₂
7		3338	3338	3338	3350–3310	Secondary amine N–H stretching

Table 8: Permeation parameters of ACECLOFENAC,

Form of drug	ICDC (mg)	Area (cm ²)	PM and IC with ski-		J _{ss} (mg/cm ² /h)	K _p (cm/h)10 ⁷	ER
			Slope	mmmed milk.			
Free							
ACECLOFENAC10000		36.8	0.031	4566	0.00084	0.84	–
PM ₄	10000	36.8	0.040	4694	0.00108	1.08	1.29
IC ₄	10000	36.8	0.056	6360	0.00152	1.52	1.80

ICDC, initial drug concentration in donor compartment; Q2h, amount of drug permeated in 2h; ER, enhancement ratio; J_{ss}, steady state flux; K_p, permeability coefficient.

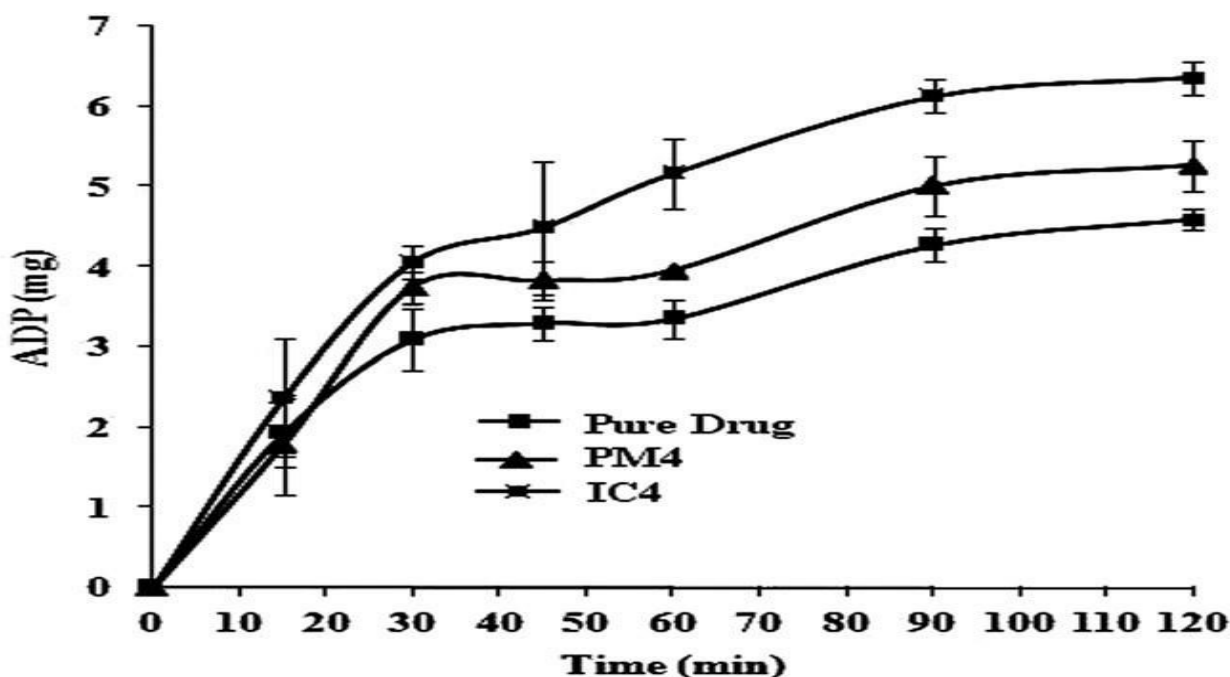


Figure 10: Ex-vivo permeation of ACECLOFENAC across rat intestine from physical mixture (PM₄) inclusion complex (IC₄) with skimmed milk (meanSD, n/3).

Scanning electron microscopy

The SEM results in Figure 4 show that in the case of PM, the particle size of ACECLOFENAC is almost the same, and on few SKM particles crystals of

ACECLOFENAC can be seen. In IC, ACECLOFENAC particles are in amorphous form, which concludes that decreasing in particle size was accomplished.

Table 9: Results of stability of piroxicam in inclusion complex (IC₄) and PM₄.

% cumulative drug release	For PM ₄ ^a		For IC ₄ ^b	
	Initial	RH for 90d	After storage at 40C/755%	
			Initial	RH for 90 days
0	0.000.00	0.000.00	0.000.00	0.000.00
5	5.620.06	7.021.22	28.766.75	25.883.44
10	29.15.71	27.342.39	59.101.70	60.012.22
15	35.962.96	32.412.23	91.674.45	94.264.53
30	53.533.71	50.761.29	96.393.71	98.532.56
45	64.565.01	61.882.67	97.111.97	99.342.43
60	72.562.07	73.181.54	98.272.07	99.792.45
90	80.376.42	79.453.46	99.642.66	98.661.36
120	88.677.53	86.295.72	101.537.53	99.874.98
Drug content	99.873.24	97.892.88	99.70.37	98.300.79

a_b f₂¹/483.47. f₂¹/485.34; p40.05.

Fourier transform infra-red spectroscopy

The FTIR spectra of samples are shown in Figure 5. FTIR spectra of ACECLOFENAC exhibits characteristic peaks of C–H stretching, aromatic CH bending, C–S stretching, C¼C, C¼N ring stretching, asymmetric S(¼O)₂ stretching, symmetric S(¼O)₂ stretching, secondary amine N–H stretching at 2930, 830, 773, 618, 1630, 1434, 1529, 1351, 1149 and 3338 wave numbers respectively (Table 3). In the spectra of IC optimized formulation (Figure 5D), the peaks characteristic to the SKM were present at almost same positions, whereas ACECLOFENAC peaks

were also present, but at a reduced rate of absorption, indicating the trapping of the drug inside the carrier matrix. None of the spectra showed any peaks other than those assigned to PRX drug and SKM, which indicates the absence of chemical interactions.

Ex-vivo intestinal permeation studies

The cumulative amount of drug permeated (Q) was plotted against time (Table 4 and Figure 6). The steady state flux (J_{ss}) calculated for the ACECLOFENAC, PM and IC were 0.00084, 0.00108 and 0.00152mg/cm² h¹, respectively. The permeability coefficient (K_p) of the drug through

intestine was found to be 0.84, 1.08, 1.52cmh¹ for ACECLOFENAC, PM and IC, respectively. The ER values for PM and IC were found to be 1.29

and 1.80, respectively, which indicates that the IC permeated more than PM than ACECLOFENAC.

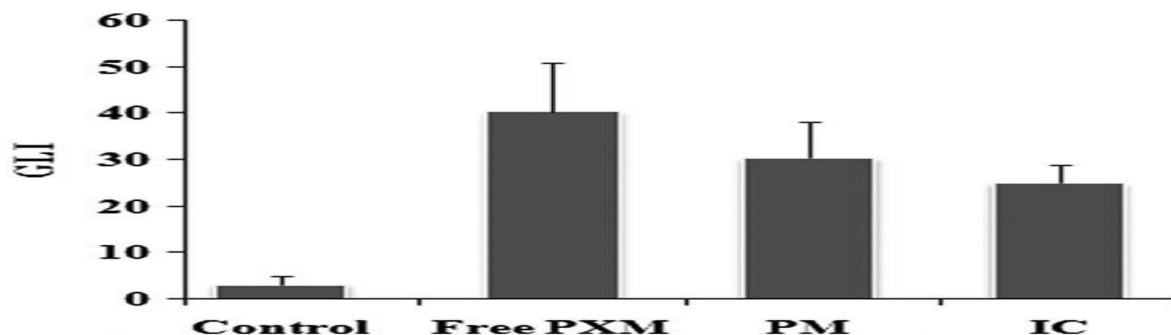


Figure 11: Gastric lesion index after administration to the rat of free ACECLOFENAC, PM and IC with SKM.

Assessment of ulcerogenecity

Macroscopic scoring

As shown in Figure 7 the average lesion index is higher for free ACECLOFENAC (40.3310.68) than for the PM (30.337.94) and for the IC with SKM (25.003.94). However, the difference between the GLI of normal control and free ACECLOFENAC is significant (p<0.001), free ACECLOFENAC and IC is significant (p<0.01), whereas the difference between PM and IC is not significant. The macroscopic images of individual stomach from each group were represented in Figure 8.

Histopathology

The histological pattern of the mucosal specimens was studied by examining the histology of the

treated and control samples. Stomach tissue with normal histological pattern observed in normal rats (Figure 9A), Stomach tissue with mucosal ulceration was observed in ACECLOFENAC-treated rats (Figure 9B) with a complete loss of the mucosa and neutrophil infiltration and fibrin deposition. At the periphery of the ulcer, the adjacent squamous epithelium shows regenerative changes. On the other hand, the mucosal ulceration in groups treated with PM₄ (Figure 9C) was with a partial loss of mucosa with fibrin deposition and mild neutrophil infiltration. The specimens from rats treated with IC₄ (Figure 9D) revealed no significant ulcerations and the tissues were almost intact.

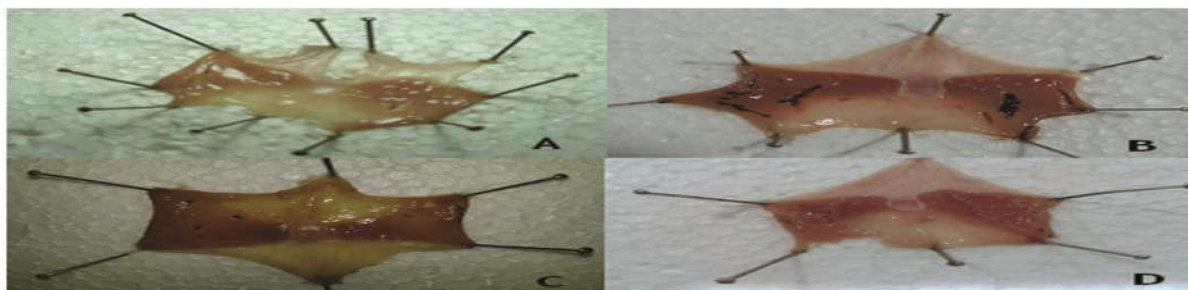


Figure 12: Macroscopic damage in stomach mucosa of rats of (A) normal control, (B) free ACECLOFENAC, (C) PM and (D) IC with skimmed milk.

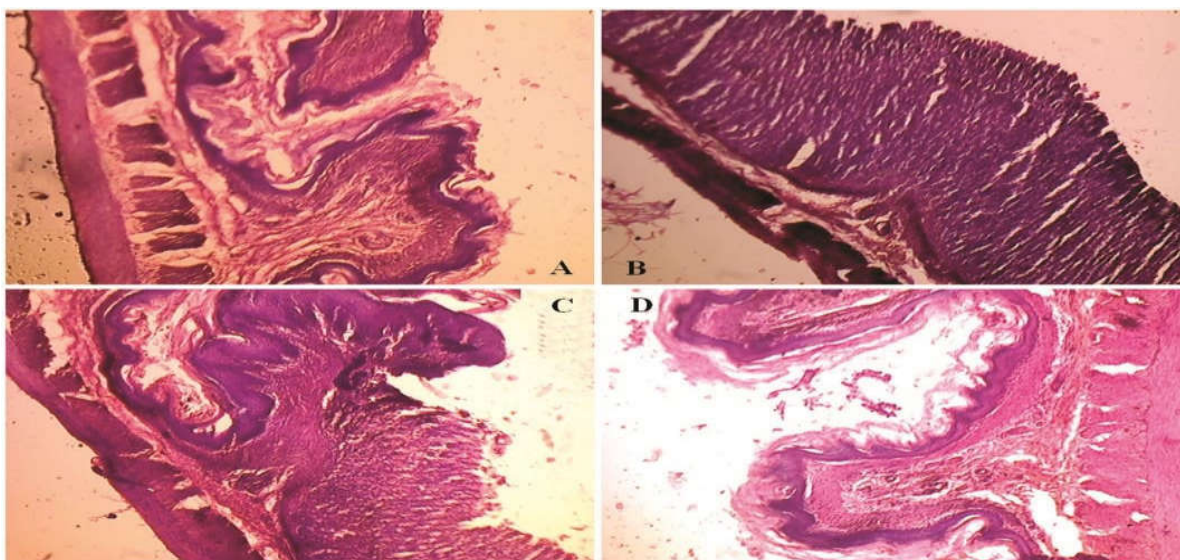


Figure 13: Histopathological photographs of rat stomach specimens stained with hematoxylin and eosin A: from rats untreated; B: from rats treated with free ACECLOFENAC; C: from rats treated with PM and D: from rats treated with IC with skimmed milk.

DISCUSSION:

Aceclofenac, a non-steroidal anti-inflammatory drug which is poorly soluble in water and ulcerogenic. Milk has been used against the gastric disturbances caused by non-steroidal anti-inflammatory drugs. In this study, skimmed milk (SKM) is used as the carrier for inclusion complex (IC) due to its surface active agent and amino acid content. This is done to enhance the solubility, dissolution rate and prevent ulcerogenicity of Aceclofenac though IC with SKM.

Firstly, IC of Aceclofenac were prepared with SKM by solvent evaporation method using rota evaporator and were evaluated for solubility, dissolution, solid state characterization, drug excipient interaction, rat intestinal permeation, ulcerogenicity and histopathological studies.

Thus, the results showed that the dissolution release and amount of aceclofenac permeated

through rat small intestine was enhanced significantly with IC. Decrease in the gastric lesion index values of IC were observed than physical mixture (PM) and free Aceclofenac. The histopathological studies revealed significant reduction in ulceration in rat stomach after treatment with IC.

CONCLUSION:

In conclusion, the present investigations suggest that the IC of Aceclofenac with SKM is useful carrier for oral administration of Aceclofenac with improved solubility, dissolution release rate and reduced gastro-intestinal complications.

However, in the present study performed by us we have not evaluated which component of SKM is responsible for formation of IC, we expect that casein and whey proteins are majorly concerned with it though because of their significant surface activity.

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