Solubility enhancement, formulation development, characterization and IVIVC of sustained release tablet of ketoprofen

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CERTIFICATE OF APPROVAL

The thesis entitled "**Solubility enhancement, formulation development, characterization and IVIVC of sustained release tablet of ketoprofen"** prepared by Rahat Shamim under my guidance in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmacy (Pharmaceutics) is hereby approved for submission.

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ABSTRACT

Ketoprofen granules were prepared by a relatively newer method, namely surfactant-assisted wet granulation (SAWG) approach using different concentrations (1-5%) of Soluplus®, polyethylene glycol (PEG) 6000, PEG 4000, poloxamers L6200 and L3100. The developed granule formulations were characterized for physicochemical and dissolution characteristics. FTIR examined for any drug-excipients interactions. Granule size, percent yield, bulk and tap density, Hausner's ratio, and angle of repose of granules were found to be 571 ±0.81 µm, 93.1 ±0.84 %, 0.223 ± 0.01g/ml, 0.231 ± 0.002 g/ml, 1.098 ± 0.005, 33.81 ± 0.23º, respectively. Granules with 1% Soluplus® revealed highest solubility (3.09 mg/ml), but with 58.3% ketoprofen release until 12 h. Granules containing 5% PEG-6K demonstrated improved solubility of ketoprofen as compared to pure drug, i.e. 2.81 mg/ml vs 0.010 mg/ml. This increase in solubility was observed to be due to micellar solubilization, complex formation, or hydrogen bonding, which was supported by FTIR. All the formulations exhibited release comparable to that of USP-stipulated sustained release pattern following Weibull model (β=1.08) and showing erosion-controlled release. FTIR indicated no chemical interaction between ketoprofen and excipient in granule formulation. The SAWG successfully ameliorated ketoprofen solubility and sustained its release as well. To develop the single unit dose to achieve the better bioavailability of sustained release ketoprofen granules, matrix tablets were prepared using the defined concentration, i.e., 1%, 3% and 5% of Soluplus®, PEG-6K, PEG-4K, L6200 and L3100. The prepared ketoprofen tablets were characterized for physicochemical, *in vitro* dissolution, FTIR and *in-vivo* (human pharmacokinetic study) parameters. FTIR studies were carried out to ensure any possible

interactions among active and other excipients. Friability, thickness, hardness, weight variation, drug content and swelling index were found to be 0.14%, 4.77mm, 7.5 $kgcm^{-1}$, 401mg, 99.95% and 29.38% respectively. *In-vitro* dissolution revealed 60-102% release till 8h. MT2, MT13 depicted the ideal sustained release pattern till 8h, i.e., > 80% which was fitted to Weibull release model with ß value 2.57 and 1.06 respectively, indicating the complex release mechanism. FTIR evident the compatibilities of drug-excipient during compression. A tablet formulation with similar profile to that of the sustained release, MT2 was selected for pharmacokinetic study. MT16 was also selected as a control for PK parameters evaluation. MT16 promptly attained the plasma peak within 1h after administration and followed a rapid exponential decrease till 12h. MT2 as a sustained release tablet showed delayed peak plasma concentration which was maintained above 0.7-1.0 $\mu g.ml^{-1}$ till 24h with a gradual decline. The maximum plasma concentration (C_{max}) resulting from administration of 200 mg of MT2 was statistically lower than that of the MT16 (5.19 ± 0.66 vs 9.62±0.76 $\mu g.ml^{-1}$, $P<0.05$). The time to reach C_{max} (T_{max}) from MT2 was delayed to 5.56±0.30 h as compared to 1.15±0.11 h of MT16. The $AUC_{0-\infty}$, 78.65±7.64 $\mu g.h.ml^{-1}$, of MT2 was higher than 34.39±3.06 $\mu g.h.ml^{-1}$ of MT16. Considering MT2 as Test and MT16 as Reference formulation, the relative bioavailability was found to be 228.89±12.578%. Clearance of drug was observed to be declined for MT2 compared to MT16 (5.855±0.539 to 2.561±0.235 L/h), a reason for sustaining drug concentration in blood beyond 24 h instead of 12 h for MT16. The delayed T_{max}, decreased C_{max}, increased AUC and decreased Cl_T, MT2 exhibited an *in-vivo* behavior corresponding to the sustained drug delivery system. MT2 sustained release matrix tablets depicted the therapeutic ketoprofen plasma level effectively even beyond 24h. Thus, there is a likelihood of administration of once-a-day single dose without plasma fluctuations which were expected from the administration of two doses in a day of MT16. The *in-vitro* characteristics, i.e., the dissolution of MT2 were predictive of the

in-vivo profile of the drug. The IVIVC evaluation indicated a good correlation for the drug releases *in-vitro* and absorbed *in-vivo* at predicated time intervals. The SAWG successfully helped developing the swellable-erodible sustained release matrix tablet formulation of ketoprofen with desired *in-vitro*, biopharmaceutical and pharmacokinetics properties without incorporation of any special ingredients or major manipulation of the formulation ingredients. The desired features in the present dosage form were accomplished just by surfactant-assisted granulation, thus SAWG was regarded as the simpler approach.

Keywords: Surfactant-assisted wet granulation, Soluplus®, Polyethylene glycol, Poloxamers, L6200, L3100, Solubility, Dissolution, Ketoprofen, Matrix tablet.

DEDICATION

PARENTS, FAMILY, FRIENDS AND TEACHERS

Especially to my sister,

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Rahat Shamim

(M.Phil. Pharmaceutics)

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LIST OF ABBREVIATIONS

Abbreviations	Description
AIC	Akaike information criterion
ATR	Attenuated total reflectance
AUC	Area under the curve
AUMC	Area under the first moment curve
B-cell	Bone marrow cells
BCS	Biopharmaceutical classification system
BID	Twice a day
Cl_T	Total clearance
C_{max}	Peak plasma concentration
C_{ss}	Steady state concentration
COX	Cyclooxygenase
DSC	Differential scanning calorimetry
EO	Ethylene oxide
K_3/EDTA	Potassium salt / Ethylene diamine tetra acetic acid
FTIR	Fourier transform infrared spectroscopy
G	Granules
GIT	Gastrointestinal tract
HLB	Hydrophilic Lyophilic balance
HPMC	Hydroxypropyl methyl cellulose
HPLC	High performance liquid chromatography
IR	Infrared
IVIVC	*In-vitro - in-vivo* correlation
K	Slope
Ka	Rate of absorption
K_{el}	Rate of elimination
L3100	Poloxamer/Pluronics®
L6200	Poloxamer/ Pluronics®
LOD	Limit of detection
LOQ	Limit of quantification

List of abbreviations *(Continued….)*

Abbreviations	Description
MRT	Mean residence time
MEC	Minimum effective concentration
MTC	Minimum toxic concentration
N	Diffusion exponential in Korsmeyer-Peppas model
NSAIDs	Non-steroidal anti-inflammatory drugs
OA	Osteoarthritis
OH	Hydroxyl group
P	Compaction pressure
PAMAM	Polyamidoamine
PC	Phosphatidylcholine
PEG	Polyethylene glycol
PO	Propylene oxide
PPO	Poly propylene oxide
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
P_y	Mean yield pressure
R^2	Coefficient of determinants
RA	Rheumatoid arthritis
SAWG	Surfactant-assisted wet granulation
SEDDS	Self-Emulsifying Drug Delivery System
SUPAC	Scale up post approval changes
TID	Thrice a day
$t_{1/2}$	Half life
$t_{1/2\ ab}$	Absorption half life
$t_{1/2\ el}$	Elimination half life
T-cell	Thymus
TGA	Thermo gravimetric analysis
T_{max}	Time to reach peak plasma concentration
USP	United States Pharmacopoeia

List of abbreviations *(Continued….)*

Abbreviations	Description
Tn%	Time to dissolve/absorb n% of drug
UV	Ultraviolet
Vd	Volume of distribution
V_o	Apparent volume

Chapter 1

Introduction

1.1 Solubility – a persistent problem

Solubility is one of the major challenges and a persistent problem to develop the definitive formulation strategies for poorly soluble drugs (Stegemann *et al.*, 2007). It is an important entity of drug to which influences the desired therapeutic concentration in blood and pharmacological retaliation (Krishnaiah, 2010). The drugs having solubility <1µg/ml are categorized as poorly aqueous soluble. A drug having solubility less than 10 mg/ml over pH 1-7 is potentially with absorption problems. The absorption of drug having solubility below 1 mg/ml results in the dissolution rate limited absorption owing to the inter-relationship of the solubility and dissolution (Shekhawat *et al.*, 2017). About 60% of drugs, despite having high permeability are poorly water soluble and are placed in BCS class II of biopharmaceutical classification system (Amidon *et al.*, 1995). The poor solubility of such drugs limits their bioavailability, a pre-requisite for therapeutic activity (Shargel *et al.*, 2016). With relation to BCS class II and IV, there is an intensified need to develop new drug design technologies for enhancing dissolution and absorption (Singh *et al.*, 2011). For such drugs, the main thrust is to enhance their solubility and dissolution (Fahr *et al.*, 2007) in order to improve bioavailability. Ketoprofen, the model drug in this study is one of the poorly soluble drugs which belongs to BCS Class II drugs.

1.2 Ketoprofen - the candidate drug

Ketoprofen has been categorized in the nonsteroidal anti-inflammatory drugs (NSAIDs) and is intended to reduce pain, inflammation and stiffness caused by different conditions including rheumatoid arthritis (RA), osteoarthritis (OA) and menstruation-caused ankylosing

spondylitis. It exerts its acts by inhibiting the synthesis of prostaglandins. Ketoprofen formulation mostly contained a racemic mixture but S (+)-enantiomer is active than its counterpart (Jamali *et al.*, 1990; Solınıs *et al.*, 2002).

1.2.1 Physical and chemical properties of ketoprofen

Ketoprofen is an odorless white to off-white crystalline powder. The melting point of ketoprofen ranges from 92 °C to 95 °C (197.6 °F). It is easily soluble in acetone, diethyl ether and freely soluble in chloroform, dichloromethane, ethanol and, soluble in benzene, strong alkali and methanol but practically insoluble in water. Chemically it is 2-(3-benzoylphenyl) propionic acid with a molecular formula, $C_{12}H_{14}O_3$, and molecular weight of 254.30 g/mol. The chemical structure of ketoprofen is given in Figure 1.1.

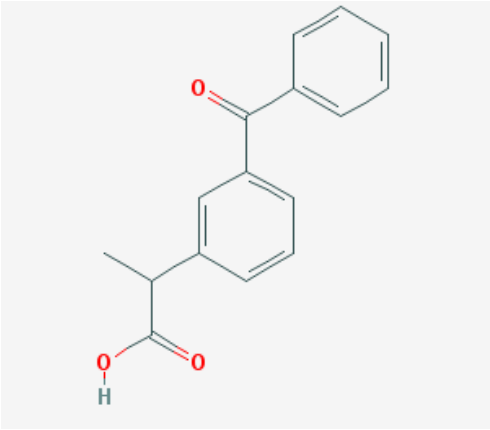


Figure 1.1: Chemical structure of ketoprofen

1.2.2 Pharmacodynamics of ketoprofen

Ketoprofen primarily inhibits the cyclooxygenase isoforms (COX), i.e., COX-1 and COX-2, thus blocks prostaglandin biosynthesis from arachidonic acid (Vane, 1971). However, it is reported to be a COX-1-selective agent (Brideau *et al.*, 2001; Streppa *et al.*, 2002).

S-ketoprofen has been considered as a more potent COX inhibitor as compared to R-ketoprofen (Cabré *et al.*, 1998; Iniguez *et al.*, 1998). The COX-2 inhibition is almost specifically ascribed to S-ketoprofen (Suesa *et al.*, 1993). Pro-inflammatory prostaglandins are involved in inflammation, induction of fever and hyperalgesia, and vasodilatation, thus aggravating the essential signs of inflammation, i.e., redness, swelling, heat, pain and loss of body function. Increases in sensitivity to pain, is a phenomenon which is called hyperalgesia. The prostaglandins are also considered to be involved in the allodynia process where a non-painful stimulus causes pain in the damaged tissues (Hersh *et al.*, 2000). An enzyme, lipoxygenase that blocks bradykinin production is also inhibited by ketoprofen (Lees *et al.*, 2004). Ketoprofen penetrates the blood-brain barrier where a central analgesic activity is started (de Beaurepaire *et al.*, 1990; Díaz-Reval *et al.*, 2004).

1.2.3 Indications of ketoprofen

Ketoprofen is used to relieve pain and inflammation. It is usually indicated for the treatment of rheumatoid arthritis, osteoarthritis and in menstrual pains. Ketoprofen was first approved in 1973 for clinical use in France and United Kingdom and in the United Stated in 1986 (Kantor, 1986).

1.2.4 Osteoarthritis

Osteoarthritis is a chronic disease of old age. Above 50 year, women are most susceptible to it although young age women and men are also affected. At the age of 65 years one third of old people develop osteoarthritis and 80% of them experience disease of joints and degenerative changes (Creamer *et al.*, 1998). OA embroils degeneration of joints cartilage leading to stiffness

of joints with pain along limited mobility resulting in disability particularly in women (Theis *et al.*, 2007). Most affected sites of the body are hands, knee, hip and spine (Yohannes *et al.*, 2010). Acetaminophen and NSAIDs, including the selective cyclooxygenase enzyme COX-2 inhibitors are used for the pharmacological management of disease. At present there is no curative treatments for OA is available; therefore, aims of treatment emphasis is on the reduction of symptoms (pain and stiffness), and diminishing the body disability and functional limitations (Caldwell *et al.*, 2002).

1.2.5 Rheumatoid arthritis

Rheumatoid arthritis mostly affects the small joints of hands and feet, namely the diarthrodial joints. In addition to inflammation of the joint lining (synovium), the forceful tissue front, pannus enters and damages the local articular structures. In RA, thymus (T) and bone marrow (B) cells and macrophages infiltrate the synovium and sometimes organize into discrete lymphoid aggregates with germinal centers. A remarkable increase in macrophage-like and fibroblast-like synoviocytes leads to hyperplasia of the intimal lining. The degenerative enzymes which are locally expressed (Metalloproteinases, serine proteases and aggrecanases) causes the digestion of the extracellular matrix to damage the articular structures. Worldwide, the RA occurs in 0.5–1.0% of the adult population (Firestein, 2003).

1.2.6 Side effects of ketoprofen

The more common allergic and serious side effects that occur with use of ketoprofen along with the body systems involved, have been enlisted in the Table 1.1.

Table 1.1: The side effects of ketoprofen and their symptoms

Type of side effect	System involved	Symptoms
Common	Miscellaneous	Stomach upset, diarrhea, nausea, headache, dizziness and drowsiness
Allergy	Miscellaneous	Shortness of breath, swelling of face, lips, or throat
Serious side effect	Stomach	Ulcers or bleeding with symptoms like stomach pain or upset stomach, black, tarry stools, vomiting up blood
	Heart	Heart attack/Stroke (chest pain, shortness of breath, weakness on one side of body, and slurred speech)
		Heart failure, unusual weight gain, swelling in arms, legs, hands, or feet
	Kidney	Kidney damage (if used for a long time). Symptoms may be decreased urination, swelling in arms, legs, hands, or feet
	Liver	Liver symptoms may include yellowing of skin or the whites of your eyes, flu-like symptoms, such as body aches, fever, nausea, and vomiting, tiredness, pain in the upper part of stomach area, itching

1.2.7 Pharmacokinetics of ketoprofen

In humans, orally administered ketoprofen is rapidly absorbed, metabolized, and excreted. Absorption is more than 90% and peak plasma level is reached within 1-2 hours (Blanco *et al.*, 2003). Total bioavailability is dose proportional in the range of 75-200 mg of dose. The plasma half-life is approximately 2 hours in healthy young. Ketoprofen is 99% bound to plasma protein, mostly albumin (Vergote *et al.*, 2002). The above and other pharmacokinetic parameters have been presented in Table 1.2. Ketoprofen concentration in the synovial fluid

peaks approximately 2 hours after achieving the peak plasma levels and decreases more slowly, so that synovial fluid levels exceed plasma levels from 4 hours after dosing (de la Lastra *et al.*, 2000).

The drug primarily metabolized by glucuronidation to an unstable glucuronic ester, acyl-glucuronide by hepatic microsomal enzymes and is excreted in the urine (Sheng *et al.*, 2006). A little quantity of unchanged ketoprofen is also found in urine and bile regardless of age and kidney function. Up to 50% of the given dose is excreted in urine unchanged. According to other reports, up to 80% of the ketoprofen dose is recovered in the form of glucuro-conjugated metabolite. These glucuronide acyl-conjugates are readily susceptible to *in-vitro* hydrolysis to the parent compound (Kantor, 1986; Miles, 2007; Tettey-Amlalo, 2005).

In elderly subjects of age 65 years or above , conjugation and renal excretion are delayed somewhat (Advenier *et al.*, 1983) causing an increased elimination half-life to 3-5 h. Due to this short half-life of ketoprofen, it does not show accumulation after multiple dosing in elderly patients with RA thus, the drug is not toxic. Thus, no routine dosage adjustment seems to be necessary in these patients. However, the half life of ketoprofen is bit increased in the patients with impaired renal function (creatinine clearance 20-60 ml /min) (Stafanger *et al.*, 1981) and patients with alcoholic cirrhosis. A close correlation between clearance of creatinine and of ketoprofen has been reported in renal dysfunction which does not lead to the risk of excessive accumulation of drug in the body (Kantor, 1986).

Table 1.2: Human pharmacokinetic parameters (Average Values) of ketoprofen and its dosage forms

Parameters	Units	Drug/Dosage form	Dose (mg)	Values	Reference
Plasma protein binding	%	Ketoprofen		About 99%	(Ishizaki *et al.*, 1980)
Bioavailability	%	Ketoprofen		90%	(Ishizaki *et al.*, 1980)
Renal excretion	% as conjugate	Ketoprofen		Up to 65	(Ishizaki *et al.*, 1980)
Peak plasma concentration (C_{max})	µg/ml	Conventional	50	10 µg/ml	(Ishizaki *et al.*, 1980)
		Prompt release capsule	100	10.52±1.43	(Roda *et al.*, 2002)
		Sustained release pellets filled in capsule	200	3.5±1.0	(Houghton *et al.*, 1984)
		Sustained release coated granules filled in capsules	200	23.0±11	(Houghton *et al.*, 1984)
		Sustained release coated granules filled in capsules	200	5.91±0.66	(Roda *et al.*, 2002)
		Sustained release coated tablet	200	4.51±0.65	(Roda *et al.*, 2002)
Time to C_{max} (T_{max})	H	Conventional	50	1-2 hours	(Ishizaki *et al.*, 1980)
		Prompt release capsule	100	1.38±0.48	(Roda *et al.*, 2002)
		Sustained release pellets filled in capsule	200	4.9±1.0	(Houghton *et al.*, 1984)
		Sustained release coated granules filled in capsules	200	0.82±0.18	(Houghton *et al.*, 1984)
		Sustained release coated granules filled in capsules	200	4.17±0.42	(Roda *et al.*, 2002)
		Sustained release coated tablet	200	2.28±0.32	(Roda *et al.*, 2002)

Table 1.2: *(Continued…)*

Parameters	Units	Drug/Dosage form	Dose (mg)	Values	Reference
Area under the curve (AUC)	µg.h/ml	Prompt release capsule	100	66.33±11.78*	(Roda *et al.*, 2002)
		Sustained release pellets filled in capsule	200	40.11±11	(Houghton *et al.*, 1984)
		Sustained release coated granules filled in capsules	200	42.0±13	(Houghton *et al.*, 1984)
		Sustained release coated granules filled in capsules	200	60.25±9.82	(Roda *et al.*, 2002)
		Sustained release coated tablet	200	62.64±12.67	(Roda *et al.*, 2002)
Volume of distribution (V_d)	% of body weight	Conventional	50	10-15	(Ishizaki *et al.*, 1980)
Half-life ($t_{1/2}$)	h	Conventional	50	1-3 hours	(Ishizaki *et al.*, 1980)
		Sustained release pellets filled in capsule	200	8.4±3.4	(Houghton *et al.*, 1984)
		Sustained release coated granules filled in capsules	200	3.3±1.2	(Houghton *et al.*, 1984)

*After two administrations in 24 h.

1.2.8 Available tablet dosage forms

Both the immediate and the modified release dosage form are available in the market for ketoprofen. The available dosage forms for ketoprofen are given in Table 1.3.

Table 1.3: Available dosage forms for ketoprofen

Type of dosage form	Brand	Pharmaceutical Company
Immediate release Tablets	Ticon	Amarant Pharmaceuticals LTD, Pakistan
	Silofen	Silver Oak Corporation, Pakistan
	Profenid	Sanofi Aventis LTD, France
	Ketonel	Saydan Pharmaceutical Industries LTD, Pakistan
	Inflo ket	Valor Pharmaceuticals, Pakistan
	Etopar	Rakaposhi Pharmaceuticals LTD, Pakistan
	Alprof	Alson pharmaceuticals, Pakistan
	Alket	Alson pharmaceuticals, Pakistan
Modified release Tablets	Stayfen SR	Siza International LTD, Pakistan
	Protifen EC	Gray's Pharmaceuticals, Pakistan
	Profenid SR	Sanofi Aventis LTD, France
Sustained release Tablets	Ibifen	Istituto Biochimico Italiano, Italy
Sustained release Capsules	Orudis Retard	Rhone-Poulenc Rorer, Germany

1.2.9 Dose and indications of ketoprofen

The typical oral dose of ketoprofen is 50–100 mg twice daily (BID) to be taken with food. However, controlled release preparations are administered once a day. Table 1.4 shows the different dosage schedule for different conditions (Kantor, 1986).

Table 1.4: Dosage schedule for ketoprofen

Condition	Dosage form	Oral dose	
		Adult	Geriatric
Rheumatoid arthritis Osteoarthritis	Immediate release	Initially 50 mg orally 4 times a day or 75 mg orally 3 times a day Maximum 300 mg /day	Initially,50 mg orally 3 times a day or 75 mg orally twice a day Maximum dose 300 mg/day
	Extended release	200 mg od Maximum 200 mg/day.	Initially,100−150 mg od Maximum200 mg/day
Ankylosing spondylitis	Immediate release	100 mg thrice daily (TID). Maximum 300 mg/day	Initiate at reduced dosage. Maximum 300 mg/day.
Acute gouty arthritis	Immediate release	100 mg per oral (p o) thrice daily (TID).	Initiate at reduced dosage Maximum 300 mg/day
Juvenile rheumatoid arthritis		100-200 mg/m^2/day	
Maximum dose	Immediate-release	300 mg/day	300 mg/day
	Extended-release	200 mg/day	200 mg/day

1.2.10 Contraindications of ketoprofen

Ketoprofen is contraindicated in the following medical conditions:

1.2.10.1 Bronchospasm

A cross sensitivity with ketoprofen is expected in the patients with asthma, nasal polyps and rhinitis which has been due to use of aspirin like with other NSAIDs. The lungs may be affected by prostaglandins inhibition and increased levels of leukotrienes, causing asthma exacerbation (Green, 2001).

1.2.10.2 Peptic ulceration

To the patients with chronic dyspepsia, active or history of recurrent peptic ulceration should not be given ketoprofen. Ketoprofen causes the irritation in gastrointestinal tract (GIT)

through due to its local and systemic actions. While theoretically, it has been possible to prevent the local damage of upper GIT caused by ketoprofen, the systemic consequences of ketoprofen, by inhibiting the protective prostaglandins are still responsible for gastric damage (Green, 2001).

1.2.10.3 Severe renal insufficiency

Being potent vasodilators, the prostaglandins of kidneys counteract effects of vasoconstriction stimulated from the norepinephrine, renin and angiotensin II on renal blood flow. Therefore, prevention of their synthesis can influence renal function in some conditions. The presence of pathologic conditions such as cirrhosis, renal disease, and congestive heart failure, predisposition and renal ischemia can be seen on the patients during ketoprofen treatment (Green, 2001; Kantor, 1986). Thus, a patient with the above risks is greatly dependent on prostaglandins for the normal renal flow. The ketoprofen induces change in renal function which may be asymptomatic or accompanied by edema, are reversed with withdrawal of ketoprofen (Kantor, 1986).

1.2.11 Adverse reactions of ketoprofen

Most common adverse reactions of ketoprofen are: mild upper gastrointestinal complaints such as nausea, dyspepsia or epigastric discomfort. Less common are nervous system symptoms like headache, drowsiness and dizziness and complaints related to the lower gastrointestinal like diarrhea, gastritis, ulcerations, abdominal burning, constipation and flatulence. The central nervous system related side effects are the headache, vertigo, dizziness, nervousness, tinnitus, depression, drowsiness and insomnia.

Ketoprofen rarely causes hypersensitivity reactions such as fever, angio-edema, bronchospasm and rashes may occur rarely. The possible hematological adverse effects of ketoprofen may include agranulocytosis, anemias, neutropenia, thrombocytopenia, and eosinophilia. Ketoprofen is considered to be linked with nephrotoxicity which includes interstitial nephritis and nephrotic syndrome. Ketoprofen may also aggravate renal failure particularly in patients with the existing renal impairments. Use of ketoprofen may lead to retention of fluid, less commonly worsening heart failure in geriatrics. Other adverse effects may include photosensitivity, eczema, alveolitis and pancreatitis (Williams *et al.*, 1988).

1.2.12 Drug interactions of ketoprofen

Despite being 99% protein bound (Williams *et al.*, 1981), ketoprofen does not alter the pharmacokinetics of highly protein-bound drugs like the oral antidiabetic agents or anticoagulant. Aspirin reduces protein binding of ketoprofen which is counterbalanced by increased ketoprofen plasma clearance. Though these effects do not cause any net change in the free ketoprofen concentrations in plasma, the unpredictable individual variations might be expected in plasma concentration due to the complex nature of kinetic interactions. Therefore, co-administration of ketoprofen with aspirin is not recommended.

Probenecid reduces both protein binding and clearance of ketoprofen. The reduction in ketoprofen clearance is due to the inhibition of glucuronidation of ketoprofen since both drugs compete for the same biochemical pathway. As a result, the combined treatment with ketoprofen and probenecid should be avoided. Reduced methotrexate clearance and the serious toxic which may even be fatal results after co-administration of methotrexate with ketoprofen or diclofenac have been reported (Thyss *et al.*, 1986). With increased use of methotrexate in clinical practice

for managing pain in rheumatoid arthritis, the risk of above potentially life-threatening drug-drug interaction should be well recognized (Kantor, 1986).

1.2.13 Issues with ketoprofen

Ketoprofen has shown the highest risk for the gastrointestinal (GI) side effects among NSAIDs (Lazzaroni *et al.*, 2007). The higher gastric concentration and plasma peak of ketoprofen due to rapid absorption have been associated with the use of conventional dosage forms which increases the chances and risks of side effects requiring multiple daily dosing (Abdallah *et al.*, 2012; Roda *et al.*, 2002). This problem inclined to be serious for ketoprofen owing to its half-life (Yamada *et al.*, 2001). Being poorly aqueous soluble (0.010 mg/ml), ketoprofen belongs to BCS Class II drug (Khan *et al.*, 2011). It has a reduced bioavailability secondary to its poor solubility, particularly in acidic pH (Vergote *et al.*, 2002).

A drug with poor solubility demonstrates slow drug absorption which leads to inadequate and variable absorption and GIT mucosal toxicity. For orally administered drugs, solubility is the critical rate limiting parameter to achieve the desired blood concentrations in systemic circulation for pharmacological response (Sharma *et al.*, 2009). Ketoprofen is readily cleared from the blood after administration thus, requires frequent administrations to maintain therapeutic levels in plasma, without excessive fluctuations between the peak and trough levels. Frequent dose administration is one of the possible causes of patient non-compliance.

1.3 Addressing issues associated with ketoprofen

For drugs with lower solubility, the rate limiting step is the drug release from the dosage form and solubility in the gastric fluid and not the absorption, therefore enhancing the solubility

in turn increases the bioavailability for BCS class II drugs. The bioavailability of BCS class II (low solubility and high permeability) substances can be maximized by enhancing solubility and drug dissolution rate in gastrointestinal tract (Krishnaiah, 2010; Kumar *et al.*, 2011; Sharma *et al.*, 2009). To address the solubility issue, different techniques (Savjani *et al.*, 2012) are used which can be grouped in different categories as given in Figure 1.2.

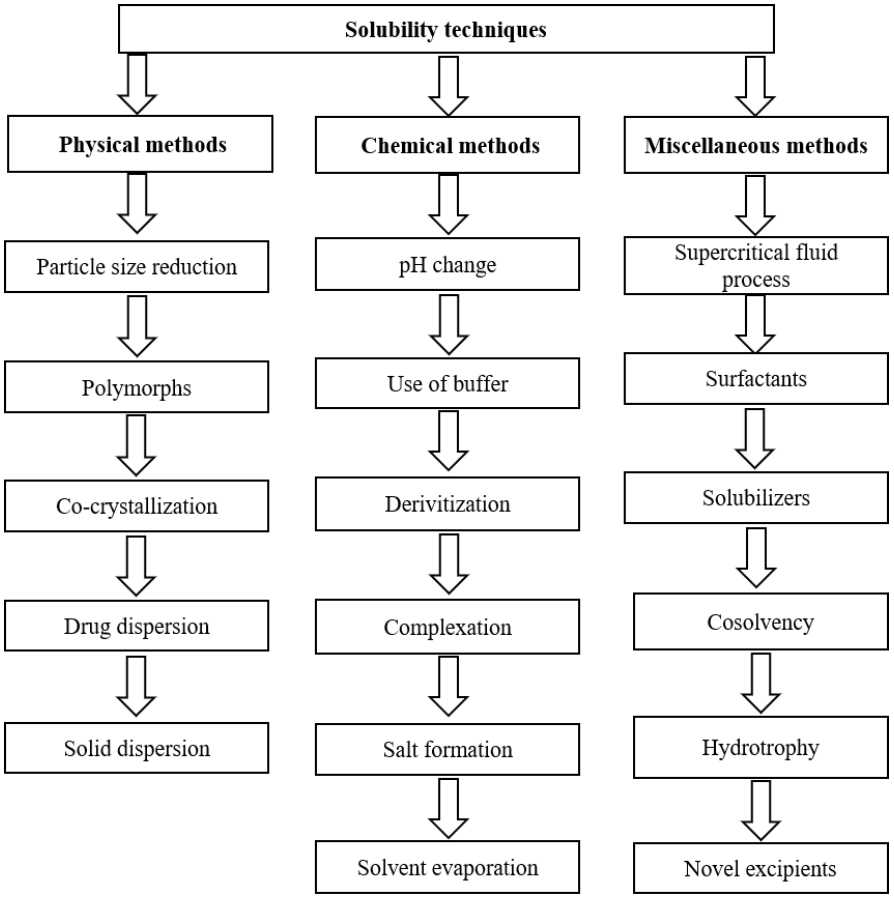


Figure 1.2: Approaches for solubility enhancement of poorly soluble drugs

1.3.1 Physical modifications

Physical modification method for solubility enhancement are particle size reduction like micronization (Blagden *et al.*, 2007) and nanosuspension, modification of crystal habit like

polymorphs (Muller, 2000), amorphous form and co-crystallization (Chowdary *et al.*, 2005), drug dispersion in carriers like eutectic mixtures (Sekiguchi *et al.*, 1961), solid dispersions (Abdul-Fattah *et al.*, 2002), solid solutions and cryogenic techniques (Leuenberger, 2002).

1.3.2 Chemical modifications

Change of pH, use of buffer, derivatization, complexation (Uekama *et al.*, 1998), and salt formation are the chemical modification techniques for enhancing solubility (Rasool *et al.*, 1991).

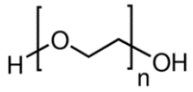
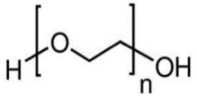
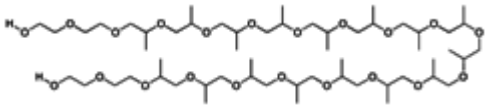
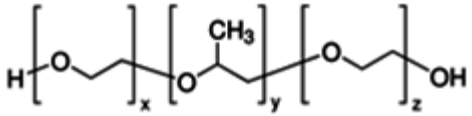
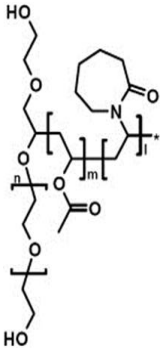
1.3.3 Miscellaneous methods

The miscellaneous methods for improvement of solubility includes supercritical fluid process (Manna *et al.*, 2007), use of adjuvant like surfactant, solubilizers (Sobol, 2018), cosolvency, hydrotrophy (Rasool *et al.*, 1991), and novel excipients (Kohli *et al.*, 2010). Detailed literature on the techniques dedicated for improving the solubility and dissolution ketoprofen has been given in Chapter 2.

1.4 Candidate polymers

In this work five polymers from three major classes of surfactants were used in different concentrations for the amelioration of solubility and the development of sustained released ketoprofen. These included Soluplus®, different grades of polyethylene glycol and Pluronics® (Table 1.5).

Table 1.5: Hydrophilic-lyophilic balance and functionality of polymers under study

Polymer	Structures	HLB	Functionality	References
PEG-4K		19.8	Biodegradable polymeric matrices in controlled release systems, Plasticizer, Tablet lubricant	(Rowe *et al.*, 2006)
PEG-6K		19.1	Biodegradable polymeric matrices in controlled release systems, Plasticizer, Tablet lubricant	(Rowe *et al.*, 2006)
L3100		3.2	Block copolymer, Micellar solubilization, Surfactant	(Kabanov *et al.*, 2002)
L6200		4.0	Block copolymer, Micellar solubilization, Surfactant	(Kabanov *et al.*, 2002)
Soluplus		26.0	Graft copolymer, Polymeric solubilizer to enhance solubility and stability, Binder in wet granulation, Emulsifier	(BASF, 2010)

1.4.1 Soluplus®

Soluplus®, a relatively new polymer, is a polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer having amphiphilic properties. Soluplus®, is different from the classical solubilizers in that it has a bifunctional character where it acts as a matrix polymer for solid solutions and an active solubilizer through micelle formation in water (Nagy *et al.*, 2012). It is used as a carrier for the formulation of solid dispersions. Due to the hydrophilic and nonionic nature, its solubility is not affected along the gastrointestinal tract. Due to its slightly surface-active property, poorly soluble drugs maintain supersaturation in the gastrointestinal tract. Soluplus® shows excellent solubilizing properties for BCS class II drugs (Hardung *et al.*, 2010).

1.4.2 Polyethylene glycols

Polyethylene glycols (PEGs) are the synthetic hydrophilic polymers that swell, disperse or dissolve in water and thus, alter the physical properties of aqueous systems as gelation, thickening or emulsification/stabilization. These polymers usually have repeating or blocks of monomers form the chains of the polymer which may contain the hydrophilic groups that are substituents or are incorporated into the backbone of the polymer. The hydrophilic groups may be nonionic, anionic, cationic or amphoteric (Erothu *et al.*, 2017).

The aqueous solubility and low intrinsic toxicity have made PEGs suitable for biological applications. PEGs increase the solubility of the poorly soluble or hydrophobic drugs when conjugated with PEGs. Furthermore, PEGs improve the drug physical and chemical stability and restricts aggregation of drug molecules during storage as well as in *in-vivo* due to steric

hindrance and/or masking of charges provided through formation of a conformational cloud (Knop *et al.*, 2010).

PEG, derived from ethylene oxide has certain physicochemical properties which enable it to achieve solid dispersions. Its hydroxyl groups (OH) are able to donate electron which are responsible for the interaction with hydrophobic drugs through formation of hydrogen bonds. The above are also responsible for biocompatibility, being odorless, neutrality; nonirritating; and solubility in many organic solvents and in water, and providing a quick release of the dispersed drug (Liao *et al.*, 2009; Ma *et al.*, 2009; Papadimitriou *et al.*, 2012; Patil *et al.*, 2009).

1.4.3 Pluronics®

Pluronics® (Poloxamers) consist of various ethylene oxide/propylene oxide EO/PO blocks, with widely varied properties due to its EO/PO constitutes ratio and their chain lengths (Almgren *et al.*, 1995; Kozlov *et al.*, 2000). Pluronics® form stable micellar systems thermodynamically, in water that are able to solubilize the insoluble drugs (Alexandridis, 1997; Chiappetta *et al.*, 2007). In drug delivery systems, the Pluronics® are employed to improve bioavailability, metabolic stability and drug blood circulation time (Alakhov *et al.*, 1996; Kabanov *et al.*, 2002). The compounds which are well solubilized within the polypropylene oxide (PPO) core of Pluronic micelles trigger formation of micelles aggregates at much lower concentration or temperature (Patel *et al.*, 2013; Sharp *et al.*, 2010).

1.5 Safety data for the surfactants under study

The safety of the surfactants used in this study have been given in Table 1.6.

Table 1.6: Safety data for the surfactants under study

Surfactant	Toxicity	LD_{50} (mg/kg)	Acceptable Daily intake	Reference
Soluplus®	Non-toxic after single ingestion	> 5000	-	(OECDguidelines, 2018c)
PEGs*	Data not available	-	10mg/kg	(Rowe *et al.*, 2006)
L6200	Data not available	>2000	-	(OECDguidelines, 2018b)
L3100	Non-toxic after single ingestion	>2000	-	(OECDguidelines, 2018a)

**both for PEG 4000 and PEG 6000; - Not found from the searched literature*

1.6 *In-vitro* characterization of delivery system

A newly developed delivery system is characterized for its physical and chemical parameters. For instance, the granules are examined for flow properties using compressibility index, angle of repose and Hausner's ratio. For proper processing and uniform ingredient mixing, a good flowability of granules is required (Sinka *et al.*, 2004). Tablets are evaluated for parameters including thickness, friability, weight variation and hardness (Takeuchi *et al.*, 2005). To compute dissolution rate, the percent of drug released is calculated from *in-vitro* drug release data. Kinetic modeling of drug *in-vitro* dug release is performed by DD solver. Different kinetic models including first order, zero order, Higuchi, Hixson Crowell, and Weibull, are employed to define drug release pattern. Drug release mechanism from matrices is computed by n value of Korsmeyer-Peppas model (Dash *et al.*, 2010a).

Drug component interaction of formulations are evaluated by differential scanning calorimetry (DSC) and Fourier transformed infrared spectroscopy (FTIR) (Hussain *et al.*, 2013). To test whether the aim of developing a dosage form has met or not *in-vitro* characterization is necessary. Moreover, it is a prerequisite for selection of the dosage form for the further *in-vivo* evaluation.

1.7 *In-vivo* characterization of dosage forms

Selected formulation based upon *in-vitro* data is further evaluated for *in-vivo* characteristics. The *in-vivo* evaluation is mostly the bioavailability and pharmacokinetic studies. The pharmacokinetic studies can be carried out using compartmental or non-compartmental approaches. Such studies ensure that the developed formulations have the appropriate *in-vivo* features and to determine whether it meets desired goal of the formulation. Furthermore, information on bioavailability and drug clearance could also be obtained. The important pharmacokinetics parameters computed include peak plasma concentration (C_{max}), the time to reach peak plasma concentration (T_{max}), area under the curve (AUC), absorption rate (K_a), elimination rate (K_{el}), half-life ($t_{1/2}$), clearance (Cl_T), and the apparent volume of distribution (V_d), mean residence time (MRT) (Shargel *et al.*, 2015).

1.8 In-vitro in-vivo correlation

Under IVIVC, *in-vitro* release and dissolution data are correlated to the pharmacokinetics parameters with the aim to use *in-vitro* dissolution as a representative of pharmacokinetics of drug (Shargel *et al.*, 2016). A proper dissolution method helps correlate the dissolution and pharmacokinetics parameters in the body. The most commonly used dissolution parameters in establishing IVIVC are percent release or dissolved, rate of dissolution, time to release certain amount of drug, i.e., $T_{60\%}$, and $T_{90\%}$. The *in-vivo* pharmacokinetic parameters are C_{max}, AUC, MRT and Ka (Emami, 2006).

FDA classifies IVIVC into levels A, B, C and multiple-level C (Cardot *et al.*, 2007). Level A is designated as a point to point comparison between *in-vitro* drug release and *in-vivo* fraction drug absorbed and plasma drug concentration. Level B correlation is between the

respective dissolution parameters and the pharmacokinetic parameters computed using the statistical moment theory, such as MRT. The level C defines single point correlation between dissolution and pharmacokinetic parameter (Volpato *et al.*, 2004).

1.9 Problem statement

The conventional dosage forms, after administration demonstrate higher GIT concentrations thus, have the potential of increased chances of side effects. Therefor such delivery system demand the multiple daily administrations (Roda *et al.*, 2002). This problem is expected to be worsen for the model drug, ketoprofen due to its short, 1-3 h, half-life (Yamada *et al.*, 2001). Presenting a drug as single unit dosage form is usually requires incorporation of a high dose in the formulation. Since ketoprofen has a low solubility, particularly in acidic conditions, short half-life, and potential to cause local or systemic disorders in the GIT (Liversidge, 1981; Reynolds *et al.*, 1998; Vergote *et al.*, 2002), reasons for its discontinuation has made ketoprofen a candidate to present as sustained release dosage form (Habib *et al.*, 1995; Palmieri *et al.*, 2002; Parejo *et al.*, 1998).

To overcome the above problems associated with ketoprofen, the focus of several studies has been the preparation of modified drug delivery systems such as granules and microcapsules (Hassan *et al.*, 1995). The ketoprofen could also be an appropriate model drug for improvement of solubility by employing a suitable approach and then development of the sustained release drug delivery systems (Habib *et al.*, 1995; Palmieri *et al.*, 2002). An approach may also be used which simultaneously enhances the solubility and also sustains the release of the drug. The modified release dosage forms enable to achieve and maintain therapeutic levels with one daily dose which also improves the patient convenience (Roda *et al.*, 2002). Thus, it was expected that

with the improvement of the solubility and presenting ketoprofen as sustained release dosage form would help improving the bioavailability, reduce the gastrointestinal side effects, and reduce the frequency and/or the daily dose of the drug.

1.10 Aim and objectives

This study was aimed to formulate a sustained release oral swellable-erodible matrix-based dosage form for ketoprofen to address the problems associated with immediate release dosage form through enhancement of the aqueous solubility, dissolution and bioavailability using variable concentrations of different surfactants.

The objectives of the study were:

1) To improve solubility of ketoprofen using a simpler surfactant-based a granulation approach and to estimate drug solubility as compared to pure drug and evaluation of drug dissolution of granules prepared with surfactants in comparison to the granules prepared by water (Chapter 3).
2) To develop swellable-erodible matrix based sustained release tablet dosage form and to characterize *in-vitro* features of dosage form (Chapter 4).
3) To study the *in-vivo* pharmacokinetics of the newly developed matrix tablet dosage form (Chapter 5).
4) To assess *in-vitro-in-vivo* correlation (IVIVC) of the dosage form (Chapter 6).

Chapter 2

Literature review

2.1 Reported approaches for addressing issue of lower solubility of ketoprofen

Following are the different approaches to address the issue of lower solubility and bioavailability of ketoprofen. The summary of approaches has also been given in Figure 1.2.

2.2 Cosolvency of mixed solvent methods

Cosolvency involves using more than one solubilizers, in smaller amounts (which act synergistically) instead of larger amount of a single solubilizer to enhance solubility of practically insoluble drugs like ketoprofen. Mixed solvency is a simple, accurate, non-toxic and cost-effective method as compared to the conventional method of using organic solvents. The method has been reported to increase ketoprofen solubility up to 30 folds (Vittal *et al.*, 2012).

2.3 Dendrimers

The solubility of ketoprofen and other NSAIDs (ibuprofen and diflunisal) has been reported to be increased remarkably in the presence of poly propylene oxide cored polyamidoamine (PAMAM) dendrimers. The enhancement of solubility was proportional to the dendrimeric concentration, generation and core size (Koç *et al.*, 2013). In another study (Yiyun *et al.*, 2005), further factors for ketoprofen solubility using PAMAM dendrimers was studied. The pH of the solution, concentration and generations of dendrimers (G2-G5) affected to solubility of ketoprofen. Similar findings were also reported for s-triazine based dendrimer prepared using by divergent method (Patel *et al.*, 2014). Synthesized triazine based dendrimers

(G1-G3) enhanced solubility of NSAIDs which was increased with increase in concentration of the dendrimer, pH and dendrimer generation.

2.4 Solid dispersion by solvent evaporation

Ketoprofen solid dispersion was prepared by solvent-evaporation method utilizing polyvinyl alcohol (PVA), or hydrolyzed PVA both with mixture of polyvinylpyrrolidone (PVP) K-25 with lactose. The solid dispersions were then incorporated to tablets by wet granulation and direct compression methods. The highest dissolution rate was shown by the solid dispersion made with hydrolyzed PVA (Rachmat *et al.*, 2011). In another study (Khaleel *et al.*, 2011) the solubility and dissolution of ketoprofen was enhanced using solid dispersion, using solvent evaporation in polymer alone or in combination with Tween 80 as surfactant. The study concluded that the binary solid dispersion in PVP was effective in improving the dissolution properties. The addition of surfactant to the binary solid dispersion improved dissolution properties of ketoprofen.

The solubility of ketoprofen incorporated into chitosan, polymer carrier by solid dispersions using solvent evaporation with different drug to polymer ratios (1:9. 3:7, and 5:5) was reported to be enhanced (Grimling *et al.*, 2014). This study reported the highest solubility up to 32-times with a drug-polymer ratio of 1:9 in the presence of chitosan B. Thus, chitosan was reported to be a useful excipient for enhancing the bioavailability of poorly water-soluble drugs.

2.5 Solid dispersion by solvent evaporation followed by kneading and melting

Another study reported the enhancement of solubility of ketoprofen using the solid dispersion with PVP K 30 using solvent evaporation technique followed by kneading and

melting with d-mannitol in different drug to carrier ratios (Yadav *et al.*, 2013). Both dispersions showed improved dissolution rate in contrast to pure ketoprofen. Solid dispersion with PVP K30 showed the highest improvement of dissolution which was attributed to increased wettability and dispersibility, decreased crystallinity and increased amorphous fraction of ketoprofen.

2.6 Pharmacosomes

Pharmacosomes of ketoprofen has been formulated using different drug to phosphatidylcholine (PC) ratios (1:1 and 1:2) with conventional solvent evaporation technique. Drug loading was found to be 93.28% and 85.44% (w/w), respectively for ketoprofen phospholipid complex (1:1) and (1:2). Ketoprofen phospholipid complex (1:1) showed better solubility profile of 93.28% (w/w) than ketoprofen phospholipid complex (1:2). Solubility of ketoprofen in pH 6.8 was improved from 8.741 mg/ml to 18.232 mg/ml using pharmacosomes of ketoprofen (Kamalesh *et al.*, 2014).

2.7 Surface solid dispersion (adsorbate) by surface deposition

The surface solid dispersion (adsorbate) of ketoprofen prepared by surface deposition of ketoprofen from alcoholic solution on the surface of colloidal silicon dioxide (Aerosil 200), insoluble carrier has been reported to enhance dissolution rate of the drug. This increase in dissolution rate of the adsorbate was due to the conversion of drug form the crystalline state to the highly energetic amorphous state. Improving the dissolution of drug was also reported to be resulted in the rapid absorption, enhanced biological activity and reduced side effects (Zayed, 2014).

2.8 Freeze drying of ketoprofen

Dixit and coworkers developed a tablet of freeze dried ketoprofen that showed enhanced solubility and dissolution rate of ketoprofen. This enhanced solubility was attributed to the formation of an amorphous state of ketoprofen freeze dried tablets and particle size reduction (Dixit *et al.*, 2011).

2.9 Addition of excipients

Addition of lyophilized milk along with 3% solubilizer dramatically improved the solubility and dissolution of ketoprofen and ibuprofen (Nafady, 2014). Another study supported the enhancement of ketoprofen solubility (four folds) and significant enhancement of dissolution rate by preparing inclusion complex of ketoprofen with lyophilized skimmed milk (Topaloğlu *et al.*, 1999).

2.10 Particulate systems

To overcome problems associated with ketoprofen, the focus of several studies were the preparation of particulate delivery systems including granules and microcapsules (Hassan *et al.*, 1995). Solubility and dissolution rate of ketoprofen was enhanced using chitosan in presence of salting out agents by formulating microparticles of ketoprofen (Amit *et al.*, 2011).

2.11 Self-emulsifying drug delivery system (SEDDS)

Currently, several formulations have been developed to produce modified emulsified formulations as alternatives to conventional SEDDS for improvement of ketoprofen solubility (Roopesh *et al.*, 2016). Therefore, various formulation strategies have been investigated to

improve the solubility and the rate of dissolution to enhance the oral bioavailability of lipophilic drugs.

2.12 Addition of bio-surfactants

Solubility and dissolution of ketoprofen was enhanced using natural solubilizers such as bile salts of shark and fresh water fish, polysorbate 80 and sodium lauryl sulfate. A physical mix and lyophilized solid dispersion prepared was reported to enhance solubility with shark bile comparable to a synthetic solubilizer, polysorbate 80. The mechanism revealed to enhance the solubility and dissolution was micellar solubilization (Nafady *et al.*, 2013).

2.13 Liqui-solid technique

Liqui-solid technique is majorly employed for lipophilic drugs, poorly soluble or insoluble drugs with the objective to enhance solubility and dissolution rate (Nagabandi *et al.*, 2011). Various carriers have been utilized followed by coating materials exhibiting good micromeritics properties and increased drug release. The enhanced dissolution rate indicates a better bioavailability as a result of higher wetting and surface area available for dissolution.

2.14 Miscellaneous methods

Solubility enhancement with crystallization by use of different crystallization techniques has been reported. The spherical agglomeration, spray drying, freeze drying techniques and cyclodextrin inclusion complexation were shown as suitable methods for regenerating ketoprofen with more solubility, faster dissolution rate and higher bioavailability after direct compression of the crystallized ketoprofen (Dixit *et al.*, 2013; Gil *et al.*, 2004).

Chapter 3

Enhancement of ketoprofen solubility using surfactant-assisted wet granulation (SAWG) technique and characterization of resultant granules

3.1 Introduction

Approaches employed for poorly soluble drugs to increase their solubility and dissolution include micronization (Thanos *et al.*, 2003), solid dispersion (Leuner *et al.*, 2000; Serajuddin, 1999), cyclodextrin inclusion complexation (Gil *et al.*, 2004), steam granulation (Cavallari *et al.*, 2002), co-precipitation (Tommasini *et al.*, 2004), freeze drying (Guyot *et al.*, 1995), spray drying (Villaverde *et al.*, 2004) and hot melt extrusion (Samy, 2012). The above approaches have their own pros and cons. For instance, in micronization, high surface charge on small particles may cause particle agglomeration which presents technical challenges (Gupta *et al.*, 2003). Cyclodextrins inclusion complexes lead to dilution of system which may cause precipitation (Cavallari *et al.*, 2002). Amorphization (Vandelli *et al.*, 1995) results in degradation of milled drug surfaces and subsequent suspension contamination (Chaudhary *et al.*, 2012). Solid dispersions may cause fast and instant drug dissolution due to enhanced wettability and dispersibility of drug particles (Ambike *et al.*, 2004; Das *et al.*, 2011).

Granulation, has been reported for enhancing solubility and in handling of bioavailability issues of BCS Class II drugs (Hassan *et al.*, 1995; Shanmugam, 2015). Enhancement of dissolution with simultaneous control over release of poorly soluble drugs may demonstrate improved bioavailability, fluctuation-free plasma drug concentration, reduced dosing frequency, better drug efficacy, reduced gastrointestinal irritation/other dose-related side effects and improved compliance (Getsios *et al.*, 2004; Qiu, 2009; Qiu Y., 2000).

Ketoprofen, a potent non-steroidal anti-inflammatory drug is a BCS class II drug. (Abdallah *et al.*, 2012; Khan *et al.*, 2011). Low bioavailability, briefer half life of 0.5-2h (Gauri *et al.*, 2011), association with gastric irritation (Abdallah *et al.*, 2012) have made ketoprofen, a candidate drug to present it as a controlled release dosage form. Henceforth, this study was aimed to prepare ketoprofen granules with improved solubility and sustained release profile with a relatively simpler and newer approach of surfactant-assisted wet granulation (SAWG) employing Soluplus®, polyethylene glycol 6000 (PEG-6K), polyethylene glycol 4000 (PEG-4K), poloxamer L6200 and L3100.

3.2 Material and methods

3.2.1 Chemicals

Ketoprofen (Gufic Biosciences Limited) and crospovidone were gifted from Mega Pharmaceuticals LTD Lahore, Pakistan. Hydroxypropylmethyl cellulose K4M (HPMC) (NSF chemicals) were gifted from Sharooq Pharmaceuticals, Lahore Pakistan. PEG-4K and PEG-6K (BDH Chemical limited UK), Avicel PH 102, Lactose DC, Soluplus®, Poloxamer L6200, Poloxamer L3100, sodium chloride, hydrochloric acid, sodium hydroxide and potassium dihydrogen phosphate was purchased from Sigma-Aldrich Company, UK.

3.3 Methods

3.3.1 Preparation of ketoprofen granules

Formulations G1-G15 were prepared by surfactant-assisted wet granulation (SAWG) method according to study design shown in Figure 3.1. For granulation, the respective surfactants were prepared as 1, 3 and 5% (w/v) aqueous solution. Fixed amounts of ketoprofen

(50 %), Avicel® (7 %), lactose (12.5 %), crospovidone (6%), HPMC-K4M (24%) were dry mixed for 10 minutes. To this dry mix, 10 ml separately of Soluplus®, PEG-4K, PEG-6K, L6200 and L3100 (Table 3.1.) were mixed to formulate wet mass.

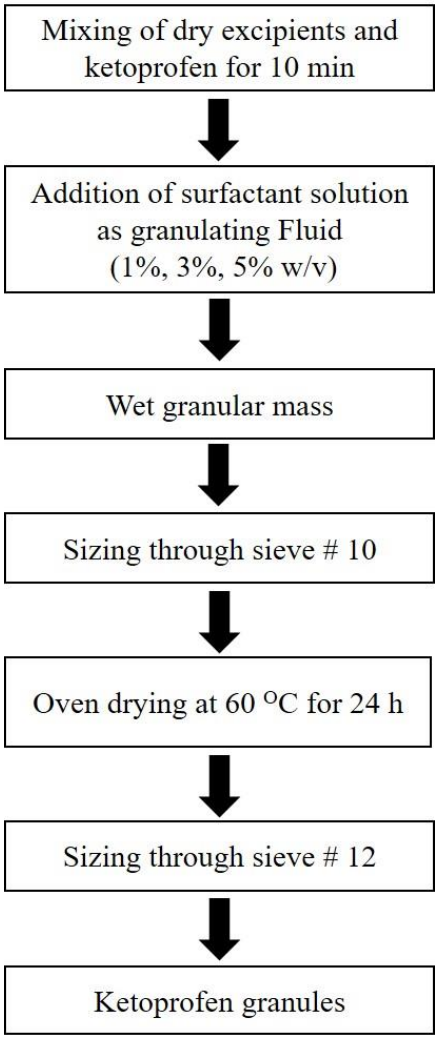


Figure 3.1: Study design for preparation of ketoprofen granule formulations

For G16, a control granule formulation wet mass was prepared using the same scheme (Figure 3.1), but without addition of surfactant at 2nd step, using only water as wetting agent. The resultant mass of all formulations was passed through sieve # 10, dried for 24h at 60 °C (WTC, binder 78532 Tuttlingen, Germany) and passed through sieve # 12 to get uniform granules. The

granules were stored in an airtight container till further studies. The prepared granules were subjected to assessment of solubility and other characterization such as yield, flow properties, compressional behaviour, *in-vitro* release and FTIR analysis.

3.3.2 Measurement of ketoprofen solubility

An excess quantity of test granules was placed in separate glass-stoppered flasks having 25 ml of distilled water and were shaken in an orbital shaker (GFL 3015, Germany) at 37 °C at 50 rpm for 48h. The supernatant was filtered through Whatman No. 41 filter paper and then concentration of drug was determined employing spectrophotometer (Shimadzu UV-2550, Kyoto, Japan) at 258 nm (Nagabandi *et al.*, 2011).

3.3.3 Characterization of granules

3.3.3.1 Yield of granules

The yield of ketoprofen granules was obtained on the basis of amount of the prepared granules in comparison to the weighed amount of solid powder materials used (Abdallah *et al.*, 2012) using Equation 1 (Khan *et al.*, 2010a).

$$\text{Yield (\%)} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100 \text{ [1]}$$

3.3.3.2 Size analysis of granules

Granules were separated into different size fractions using a mechanical sieve shaker (Sieve Shaker AS-200, Retsch, Germany) having six sieves arranged one-on-other in such a way that lower sieve was with smaller sieve size than the sieve on it. The sieves were shaken for about 10 minutes and the particles remained on each sieve were weighed in three repeating

cycles of sieve shaking to obtain the average granule size and was calculated using Equation 2 (Ansel *et al.*, 1995; Wu *et al.*, 2003).

$$\text{Average particle size} = \frac{\%\ \text{Weight retained} \times\ \text{Mean size}}{100} \quad [2]$$

Table 3.1: Formulations composition of different ketoprofen granule formulations

Code	Ketoprofen (%)	Avicel-102 (%)	Lactose (%)	Crospovidone (%)	HPMC-4K (%)	Soluplus (%)	PEG-6K (%)	PEG-4K (%)	L-6200 (%)	L3100 (%)	Water (%)
G1	50	7	12.5	6	24	1	-	-	-	-	-
G2	50	7	12.5	6	24	3	-	-	-	-	-
G3	50	7	12.5	6	24	5	-	-	-	-	-
G4	50	7	12.5	6	24	-	1	-	-	-	-
G5	50	7	12.5	6	24	-	3	-	-	-	-
G6	50	7	12.5	6	24	-	5	-	-	-	-
G7	50	7	12.5	6	24	-	-	1	-	-	-
G8	50	7	12.5	6	24	-	-	3	-	-	-
G9	50	7	12.5	6	24	-	-	5	-	-	-
G10	50	7	12.5	6	24	-	-	-	1	-	
G11	50	7	12.5	6	24	-	-	-	3	-	-
G12	50	7	12.5	6	24	-	-	-	5	-	-
G13	50	7	12.5	6	24	-	-	-		1	-
G14	50	7	12.5	6	24	-	-	-	-	3	-
G15	50	7	12.5	6	24	-	-	-	-	5	-
G16	50	7	12.5	6	24	-	-	-	-		q.s.-

3.3.3.3 Flowability of granule formulations

To ensure good flow properties of the prepared ketoprofen granule formulation, various tests were performed, i.e., bulk density, tapped density, angle of repose, Carr's index and Hausner's ratio were conducted (Rauf *et al.*, 2018).

A- Bulk density

Bulk density of ketoprofen granules was measured by taking sample in 10ml graduated cylinder, without tapping, and apparent volume (V_0) was recorded for unsettled granules. Bulk density in g/ml was calculated (Vercruysse *et al.*, 2012) using Equation 3.

$$\text{Bulk density} = \frac{\text{Mass of granules (m)}}{\text{Initial volume } (V_0)} \quad [3]$$

B- Tap density

Ketoprofen granules were poured into 10ml graduated cylinder and tapped for 20 times. The volume was labeled as bulk volume before tapping and as tapped volume after 20 taps and was calculated (Sahoo *et al.*, 2015) using Equation 4.

$$\text{Tap density} = \frac{\text{Mass of granules (m)}}{\text{Tapped volume (vt)}} \quad [4]$$

C- Angle of repose

Ketoprofen granules were allowed to fall from a funnel through its diameter of 0.9 cm from an elevation of 10 cm. Determined the tangent of repose angle (α) from granule cone (Robert *et al.*, 1965) using Equation 5.

$$\text{Tan}\ (\alpha) = \frac{\text{h}}{\text{r}}\ [5]$$

Where h= height of cone, and r = radius of cone.

D- Carr's index

The compressibility of granules was measured (Robert *et al.*, 1965) by Equation 6.

$$\text{Carr}'\text{s index} = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \times 100\ [6]$$

E- Hausner's ratio

Hausner's ratio of ketoprofen granules was calculated (Robert *et al.*, 1965) using Equation 7.

$$\text{Hausner}'\text{s ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}\ [7]$$

3.3.3.4 Compressional behavior

In-die Heckel Model was employed to assess compressional behavior of granules for deformation under pressure (Equation 8**).** For each formulation, a fixed amount of granules was compacted, using a hydraulic press with flat faced dies, 13mm in diameter, under 5 to 15 MPa compressional force. At each compaction pressure, the reduction in volume was measured using Equation 8.

$$\frac{\text{dD}}{\text{dP}} = \text{k}\ (1 - \text{D})\ [8]$$

Where D is the relative density at pressure applied, P and k (constant) is the measure of plasticity for a test material compressed and reflects the reciprocal of the mean yield pressure, Py. Integration of Equation 8 gives Equation 9.

$$\ln \frac{(1)}{(1-\mathrm{D})} = \mathrm{KP} + \mathrm{A}\ [9]$$

A plot between ln (1)/(1-D) and P (compaction pressure) was plotted. The extrapolation of curve, A, gives the resistance to deformation of a material, known as mean yield pressure (Py) and was calculated by the reciprocal of the slope (k) (Hersey *et al.*, 1971).

3.3.4 *In-vitro* dissolution study

3.3.4.1 Calibration curve of ketoprofen

The calibration curve for ketoprofen was developed in both acidic and basic buffer solution pH 1.2 and pH 6.8, respectively, corresponding to pH of gastric and intestinal simulated media without enzymes. For this purpose, stock solutions and working solutions were prepared.

For the calibration curve in acidic buffer, pH 1.2, stock solutions (A) was prepared by accurately measured 100 mg of ketoprofen dissolved in 100 ml of acidic buffer, pH 1.2 with concentration of 1000µg/ml. For the preparation of working solution (B), 10 ml of solution A was taken and made up volume up to 100 ml with concentration of 100µg/ml. Parallel dilutions were prepared by taking 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml from the solution B and made up the volume up to 10 ml in respective test tubes using acidic buffer (pH 1.2) containing concentration ranges from 2-14µg/ml. The absorbance of each diluted samples was noted at the predetermined λ_{max} (258nm) of ketoprofen using UV-Spectrophotometer (T70UV/VIS PG Instrument). A calibration curve was constructed by plotting the resultant concentrations (2-14µg/ml) verses respective absorbance.

3.3.4.2 Calibration curve in phosphate buffer pH 6.8

Calibration curve in phosphate buffer (pH 6.8) was constructed using the procedure explained in Section 4.6.8.1 except the buffer replacement of acidic pH 1.2 with the phosphate buffer of pH 6.8.

3.3.4.3 *In-vitro* drug release

The *in-vitro* drug release was studied in United States Pharmacopoeia (USP) Dissolution Apparatus I (Basket-Rack Assembly) at 50 rpm. Two buffer media of pH 1.2 and pH 6.8, respectively mimicking gastric and intestinal media were used to obtain the release profiles, respectively (Monographs, 2002) for 2 and 10 h. Samples of 10 ml were taken at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0,10.0, 11 and 12.0 h while maintaining a constant volume (900ml) in the dissolution vessel by replenishing an equal amount of respective medium already maintained at 37 ± 0.1 °C (Bonferoni *et al.*, 2004). The samples were filtered, suitably diluted, and analyzed for ketoprofen at 258 nm, with UV spectrophotometer (UV-2550 Shimadzu, Japan). The percentage of drug released was calculated by the standard calibration curve of pure drug in defined concentration regions, separately for both dissolution medium.

3.3.4.4 Release kinetics

The release kinetics was studied by fitting percent release data to zero order (Kumar *et al.*, 2008), first order (Shoaib *et al.*, 2006), Higuchi, Hixson-Crowell (Kalam *et al.*, 2007), Korsmeyer-Peppas (Khan *et al.*, 2007) and Weibull models (Kalam *et al.*, 2007) using DDSolver Ver 1.0, an extension to Microsoft excel. The highest square correlation coefficient of determinants (R^2) and lowest Akaike information criterion (AIC) was used for the selection of

kinetic model (Nath *et al.,* 2001). The diffusion exponential (n) resulting from Korsmeyer-Peppas model and β values derived from Weibull model was used to understand release mechanism of ketoprofen from granule formulations.

3.3.5 Fourier transform infrared spectroscopy (FTIR)

Infra-Red (IR) spectra of drug, excipients and formulations were recorded on FTIR spectrophotometer (MB 3000, ABB Inc. Canada) using attenuated total reflectance (ATR) attached with FTIR to get the resultant transmittance (Elbary *et al.,* 2011). The FTIR spectrum was taken from the wavelength of 500-4000 cm^{-1}.

3.4 Results and Discussion

Literature screening revealed the use of surfactants for enhancing the solubility of ketoprofen, however using approaches other than the current technique, namely surfactant-assisted wet granulation (SAWG). The motives behind employing SAWG for ameliorating the solubility of model drug in this study was use of the *in-vitro* dissolution studies with surfactant-added media to facilitate the determination of poorly soluble drugs in the samples (Patel *et al.,* 2008; Sheng *et al.,* 2006). These studies led the concept of adding the surfactants in the formulation itself for this study. A previous study which reports the improvement of solubility of ketoprofen with addition of biosurfactants in ketoprofen solid dispersions (Nafady *et al.,* 2013) supported the concept further. The nearest competing literature-cited approach to this study was the liqui-solid technique which requires mixing surfactant and drug, both in solution form followed by their transformation into solid dispersion (Nagabandi *et al.,* 2011). In terms of the nature of the process, kneading was somewhat a competitive approach also. In the afore-said

approach the polymers are added in drug mixture, kneaded to form a paste, and dried under vacuum followed by pulverization to solid dispersion. Contrarily, this current study employed a relatively simpler and novel "surfactant-assisted wet granulation (SAWG)" approach to enhance the solubility of the ketoprofen.

Soluplus® (polyethylene glycol-polyvinyl-caprolactam-polyvinyl-acetate graft copolymer), polyethylene glycols (PEGs) are amphiphilic and hydrophilic polymers (Rowe *et al.*, 2006), and Pluronics® consisting of ethylene oxide/propylene oxide EO/PO blocks respectively (Alexandridis, 1997; Chiappetta *et al.*, 2007), act as solubilizers and surfactants (Tang *et al.*, 2013), and to improve dissolution of BCS class II drugs (Linn *et al.*, 2012). The reason for the selection of Soluplus®, polyethylene glycol and poloxamers (L6200 and L3100) was the fact that these have not been employed for solubility enhancement of ketoprofen. Soluplus® is used to enhance solubility of poorly water soluble but other than ketoprofen or other insoluble drugs, in the form of nanocrystals employing evaporating precipitation aqueous solution method (Gattani *et al.*, 2018) and solid dispersions by hot melt extrusion (Lu *et al.*, 2016), spray dried method (Lavra *et al.*, 2017), β cyclodextrin inclusion complexes with Soluplus® by kneading method (Shankar *et al.*, 2013) or other related methods and so is for the poloxamers used in this study. Whereas polyethylene glycol have rarely been used for ketoprofen to study the dissolution kinetics but by employing in solid dispersion techniques like solvent evaporation method (Margarit *et al.*, 1994), melting method and solvent method (Jachowicz *et al.*, 2000) etc. The present study utilized the surfactants using a simplest method for solubility enhancement of ketoprofen.

In this study a total of 16 ketoprofen granule formulations (G1 to G16) were prepared. The aqueous solutions of the respective surfactants were prepared as granulating solutions with

varied amounts (1%, 3% and 5%) (Table 3.1). The G1-G15 formulations were prepared with required percent of surfactant solutions, i.e., of Soluplus® (G1-G3), PEG-6K (G4-G6), PEG-4K (G7-G9), L6200 (G10-G12) and L3100 (G13-G15) with fixed amounts of ketoprofen and other ingredients. These ingredients included HPMC-4K, Avicel 102, Crospovidone while Lactose was used as the bulking agent. Soluplus®, PEG-6K, PEG-4K, Pluronics® L6200 and L3100 have been employed to enhance solubility of the poorly soluble drugs (Chiappetta *et al.*, 2007; Knop *et al.*, 2010; Lavra *et al.*, 2017), specifically categorized in BCS class II (Khadka *et al.*, 2014; Linn *et al.*, 2012). The G16 (control) was prepared employing only water as granulating agent according to composition given in Table 3.1. The characterization of the prepared ketoprofen granule formulations included solubility measurement, % yield, granule size, flow properties, compressional behavior, and *in-vitro* dissolution.

3.4.1 Solubility of ketoprofen

The saturation solubility of ketoprofen made with different concentrations of surfactants in granules has been shown in Table 3.2 and Figure 3.2. the granulation approach has already been reported to increase solubility of ketoprofen which was regarded as particulate delivery system (Hassan *et al.*, 1995; Shanmugam, 2015). In this present study the solubility of ketoprofen was increased by all the surfactants at all concentrations. However, the magnitude of this enhancement was lesser when the concentration of surfactants was increased from 1-5% (Soluplus®, PEG-6K, PEG-4K and L6200), except in L3100 as there was an increase in solubility with increase in surfactant concentration. This has been shown in Figure 3.3. The granules made with 1% Soluplus®, 1% L6200, and 5% L3100, i.e., G1, G10 and G15 showed the highest solubility given in Figure 3.2. The Figures 3.3 and 3.4 have been given to get insight

of the effect of both, type of surfactants and the concentrations of these surfactants in this study. The Figure 3.4 clearly indicates the highest solubility was shown by 1% Soluplus® while at 5% Soluplus, it was the lowest. At 5%, only the PEG-6K and L3100 showed higher solubility of the ketoprofen. Figure 3.4 further elaborated that the variation in solubility enhancement was lesser when the surfactants were used at 3% concentration.

The enhancement of solubility of ketoprofen was lesser with the increase in surfactant concentrations except in L3100 formulations. The higher solubility might be due to a better wettability of fine granules presenting larger surface area for adsorption (Nagabandi *et al.*, 2011). Addition of surfactant enhances solubility of the APIs by certain mechanisms, i.e., solubilization, enhanced wettability, micronization, crystallization, hydrotrophy, cosolvency, co-grinding and inclusion complexes (Rasool *et al.*, 1991; Sobol, 2018). The present findings were in concordance with the previous studies wherein, the presence of surfactants enhanced the solubility due to formation of water soluble carriers, formation of complex, enclosing hydrophobic drug in the core, simple and micellar solubilization, and facilitation of wettability of poorly soluble drugs have been reported as the reasons of enhanced solubility (Ahuja *et al.*, 2007). G1, G10. G15, G5 and G14 showed highest category of solubility of ketoprofen narrates that the concentration of surfactant was more critical than the type of the surfactants. Since the highest solubility of ketoprofen was noted with the 1% of Soluplus® in this study, hence the role of Soluplus® is worth explaining. There are certain features of Soluplus® which are expected to be related with the enhancement of solubility of drugs, inclusive of the present model drug, ketoprofen.

Table 3.2: Solubility profile of ketoprofen granule formulations G1-G16

Code	Solubility (mg/ml)
G1	3.09
G2	2.61
G3	1.69
G4	2.70
G5	2.92
G6	2.81
G7	2.73
G8	2.50
G9	1.87
G10	3.02
G11	2.86
G12	2.65
G13	2.84
G14	2.92
G15	2.99
G16	0.23

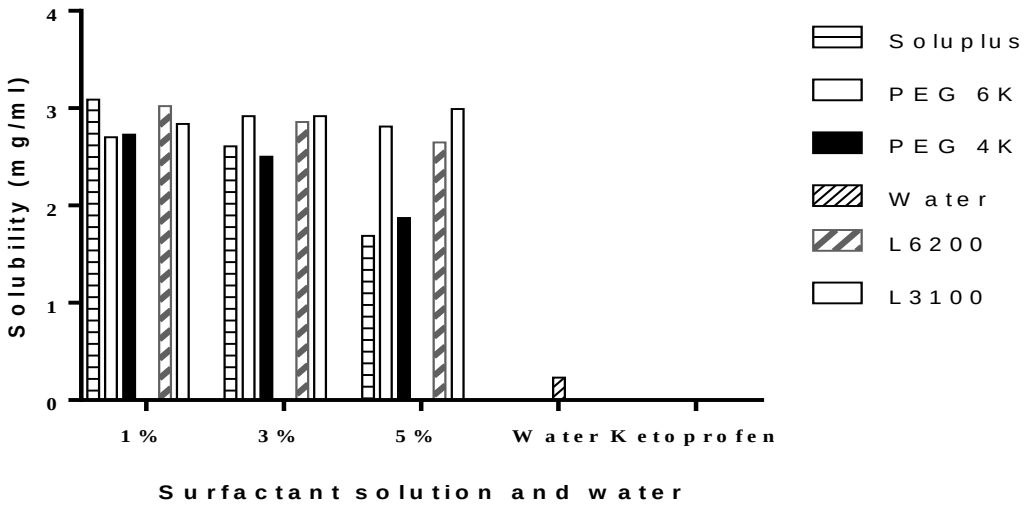


Figure 3.2: Solubility of ketoprofen granule formulations G1-G16 granules

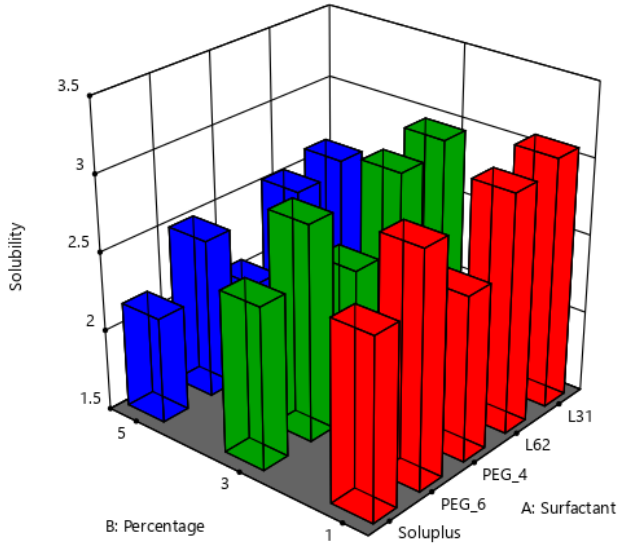
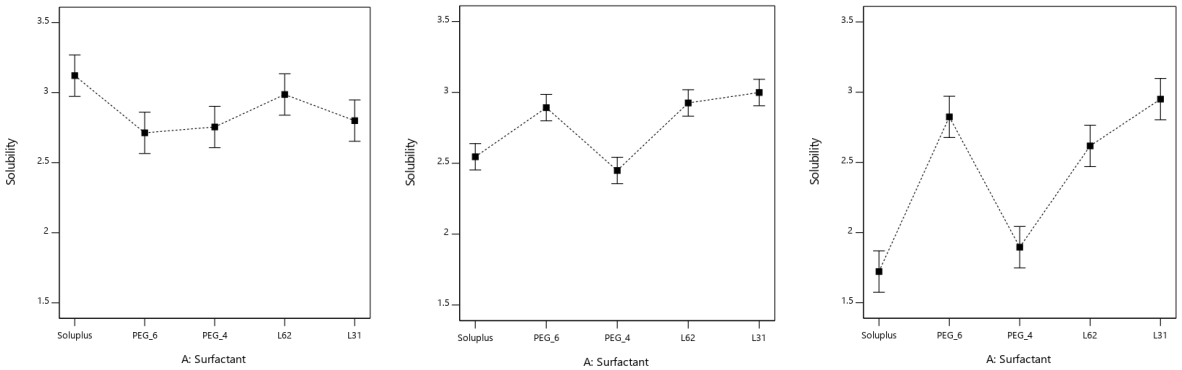


Figure 3.3: Combined effect of percentage and type of surfactant on the solubility of ketoprofen from all granule formulations G1-G15



1% of surfactants 3% of surfactants 5% of surfactants

Figure 3.4: Effect of percentage and types of surfactants on the solubility of ketoprofen

Soluplus®, a triblock-copolymer of polyvinyl caprolactam, polyvinyl acetate, and polyethylene glycol has average molecular weight of 118,000 g/mol. Being amphiphilic, this polymer comprises hydrophilic and hydrophobic blocks, thus self-assembles in the aqueous media resulting into micelle with core-shell structure due to its low CMC, 7.6mg/L (Compendium, 2011). The micellar hydrophilic shell solubilizes and stabilizes micelles in aqueous ambiance while, the hydrophobic inner incorporates and protects hydrophobic drugs (Mondon *et al.*, 2011). Soluplus is a matrix former, as reported in solid dispersion can also be a reason for the enhancement of solubility of ketoprofen in this study literature supports the above as matrix formation, Soluplus® enhances the water solubility of drugs belonging to BCS classes, II and IV (Linn *et al.*, 2012). The present finding of enhanced solubility of ketoprofen has been further supported by previous study where the itraconazole-Soluplus® tablet showed improved solubility (Zhang *et al.*, 2013). Soluplus®, in this study indicated the highest solvation power among the studied surfactants, probably due to its higher HLB values Table 1.5.

The contribution of ketoprofen itself in the improvement of solubility could not be ignored. The drug has certain features which seemed to be involved in prompting interactions

with surfactants that could lead into the enhanced solubility. According to a report, the ketoprofen bears carboxylic acid functions which enables ketoprofen species to exist as or transform into monomer, linear dimer, and cyclic dimer depending upon its environment (Champeau *et al.*, 2016). This oligomeric (being multi-dimeric) form is due to the association of monomers by strong or weak, covalent or intermolecular bonds which may lead to form a homodimer (linking the same molecules) or heterodimer (joining with the different molecules) depending upon the ambience of the drug. Two oppositely charged ions may also be linked to form dimers, called as Bjerrum pairs (Adar *et al.*, 2017). In case of ketoprofen, the hydrogen bonding due to the presence of carboxylic acid group is probably involved in the homo- or Bjerrum dimerisation of ketoprofen with surfactants. The molecule-molecule interaction between drug and surfactant might also be operative in solubility enhancement. In this study the FTIR analysis indicated the involvement of hydrogen bonding and appearance of new bonds in the presence of Soluplus®. More detail of this interaction has been given under FTIR (Section 3.4.7). The thermogravimetric analysis supported formation of hydrogen bonding indicated by the enhanced thermal stability along with possibility of micellization (Section 4.8).

3.4.2 Characteristics of ketoprofen granules

The characteristics of the ketoprofen granules are given in Table 3.3 and have been discussed in the proceeding text.

3.4.2.1 Yield of granules

In this study, the yield of granules was found to be between 73.55 to 96.00% (Figures 3.5 and 3.6). The higher concentration (5%) of PEG-6K (G6) produced the maximum yield as compared to the lowest concentration (1%) of surfactant PEG-6K (G4). Literature reports that

the yield above 50% is considered appropriate while below 40% as the poor yield (Furniss *et al.*, 2006). In this regards, ketoprofen granules, G6, G8, G9) and G13-G16 showed excellent (>90%) while G1-G3, G5, G7 and G10-G12 showed lesser but appropriate yield (>80%). In general, the yield was greater in granules prepared using 5% surfactant. According to the Figure 3.6, highest yield was observed in G15.

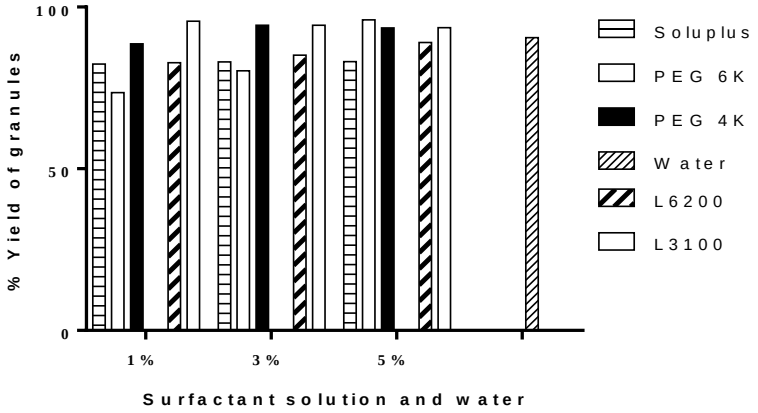


Figure 3.5: Percent yield of ketoprofen granule formulations G1-G16

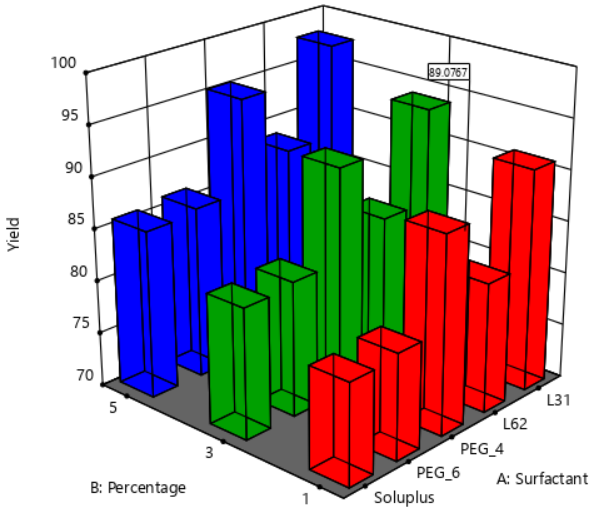


Figure 3.6: Combined effect of percentage and type of surfactant on the yield of ketoprofen granule formulations G1-G15

Table 3.3: Physical characteristics of ketoprofen granules

Code	Yield (%)	Granule size (µm)	Flowability properties				
			Bulk density (g/ml)	Tap density (g/ml)	Angle of repose (θ)	Hausner's ratio	Compressibility index
G1	82.4	571.88	0.222	0.276	11.39	1.241	19.44
G2	83.05	570.49	0.190	0.250	17.44	1.313	23.80
G3	83.15	567.99	0.211	0.282	15.94	1.338	25.26
G4	73.55	571.04	0.171	0.184	22.11	1.078	7.26
G5	80.30	559.52	0.233	0.245	31.38	1.055	5.23
G6	96.00	563.47	0.184	0.203	25.86	1.102	9.21
G7	88.65	568.95	0.247	0.280	24.37	1.133	11.72
G8	94.35	563.28	0.245	0.272	34.06	1.109	9.81
G9	93.50	572.21	0.209	0.230	34.06	1.098	8.90
G10	82.8	570.97	0.500	0.521	20.71	1.042	4.00
G11	85.1	560.75	0.431	0.463	20.71	1.074	6.90
G12	89	560.91	0.481	0.521	19.59	1.083	7.69
G13	95.65	555.87	0.446	0.481	20.41	1.077	7.14
G14	94.4	551.81	0.446	0.481	21.6	1.077	7.14
G15	93.6	584.13	0.463	0.521	20.76	1.125	11.11
G16	90.50	564.47	0.248	0.284	24.62	1.142	12.42

3.4.2.2 Size of ketoprofen granule formulations

The size for ketoprofen granules was ranged between 551.81 and 584.13 µm shown in Table 3.3 and Figures 3.7 and 3.8. As indicated in Figure 3.8, in general the size of ketoprofen granules were smaller at 3% of all surfactants and highest for the 5% surfactants. Among all the granules with 3%, PEG-6K were the smallest while those with 5%, Soluplus® were the largest particles. The size of granules frequently used in pharmaceutical industry is ranged from 0.2 to 5.0 mm which are dispensed as dosage form or may be mixed with excipients before capsule

filling and tablet formation (Shanmugam, 2015) With this reference, all the ketoprofen granules prepared (G1-G16) were appropriate for a dosage form as such, and also for either to be filled in capsule or developed as tablet dosage form (Jannat *et al.*, 2016).

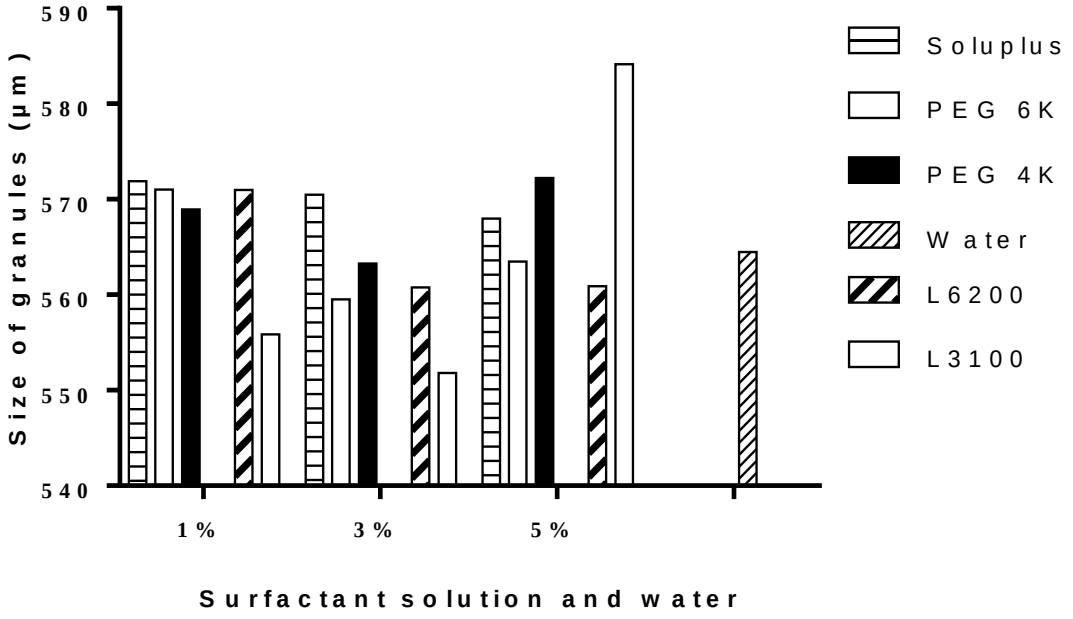


Figure 3.7. Granule size of ketoprofen granule formulations G1-G16

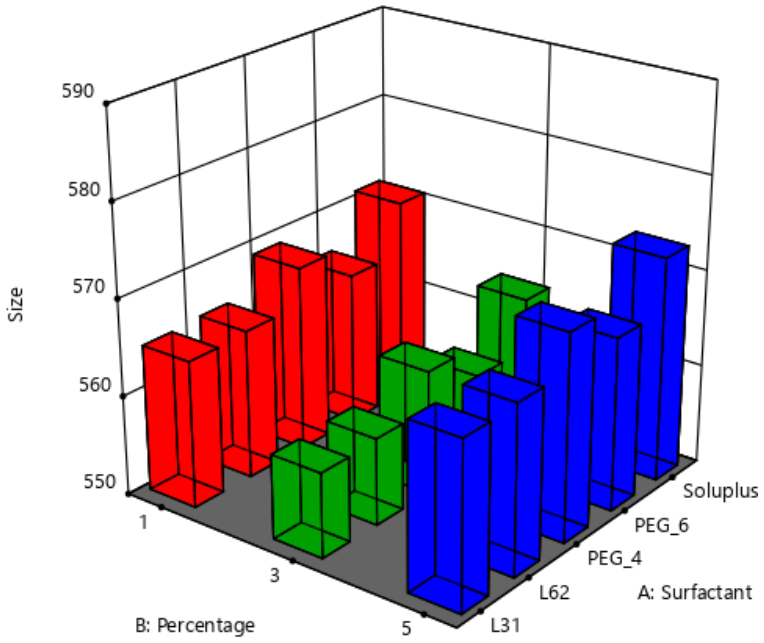


Figure 3.8: Combined effect of percentage and type of surfactant on the size of ketoprofen granule formulations G1-G15

3.4.3 Flowability of ketoprofen granules

3.4.3.1 Bulk and tap densities of ketoprofen granules

Bulk and tap densities are commonly employed to characterize the flow behavior of the granules and also provide information regarding the compressibility of the materials (Jannat *et al.*, 2016). The bulk density of ketoprofen granules was found to be ranged between 0.171 - 0.5 g/ml, being lowest for G4 and highest for G10. The tap density was between 0.184 g/ml (G4) to 0.521g/ml (G10, G12 and G15) as shown in Table 3.3 and Figures 3.9-3.11. Bulk density and tap density were maximum for granules prepared with L6200 at all concentrations shown by Figure 3.11(A) and (B), respectively. All the granules showed appropriate flow and compressibility properties (Rauf *et al.*, 2018).

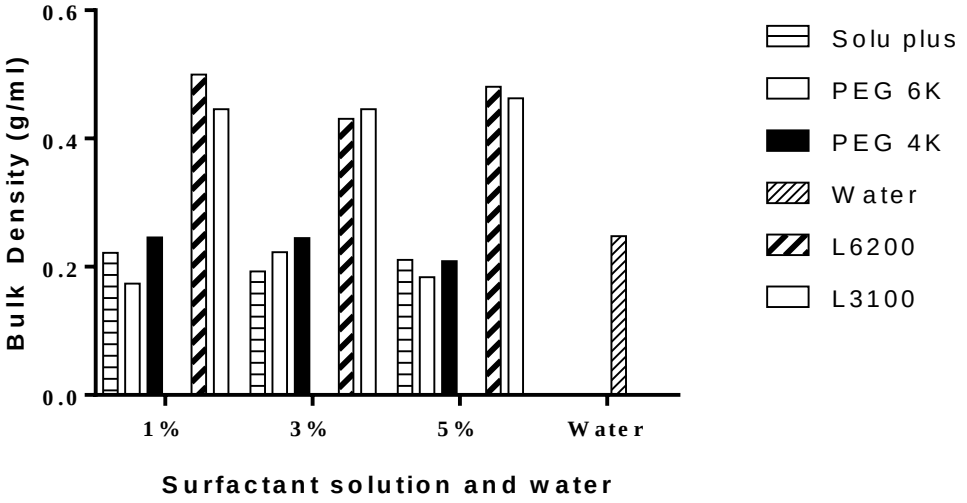


Figure 3.9: Bulk density of ketoprofen granule formulations G1-G16

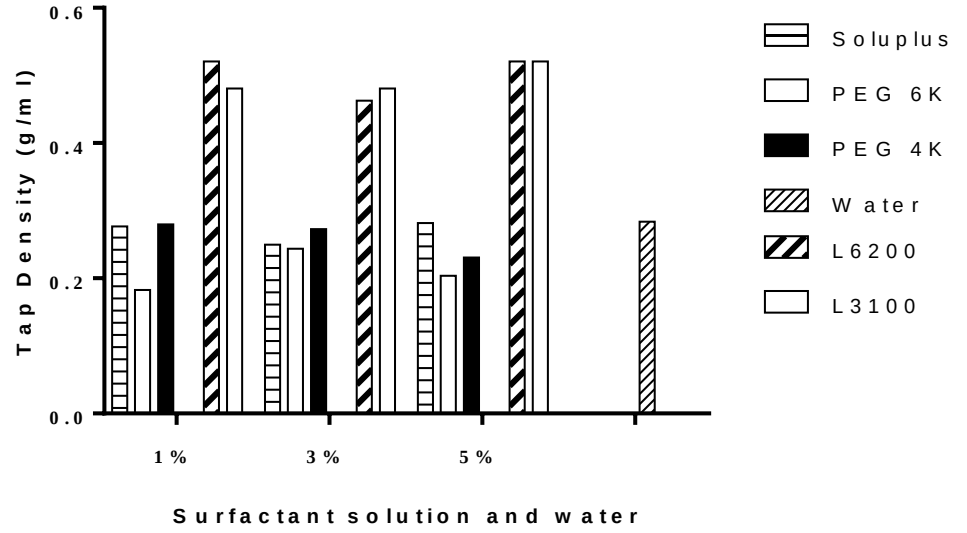
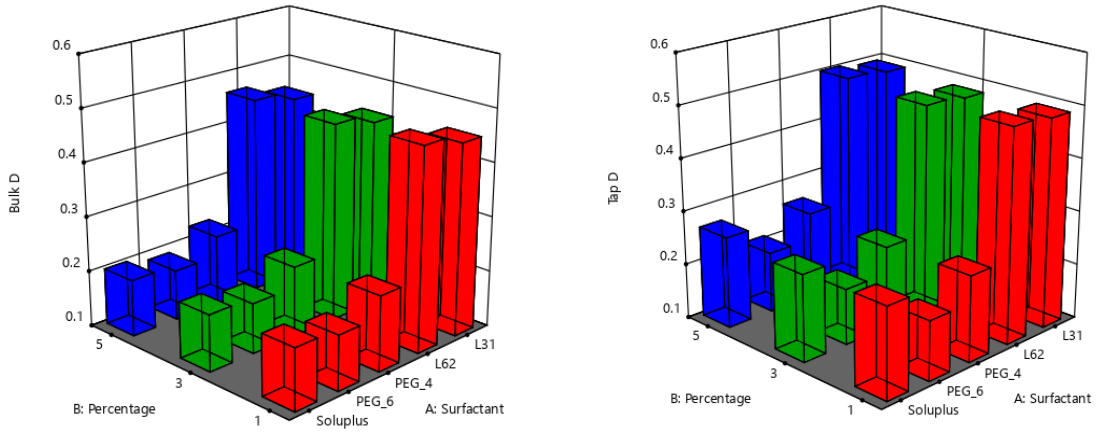


Figure 3.10: Tap density of ketoprofen granule formulations G1-G16



A **B**

Figure 3.11: Combined effect of percentage and type of surfactant on the (A) bulk density and (B) tap density of ketoprofen granule formulations G1-G15

3.4.3.2 Angle of repose

The angle of repose ketoprofen granule formulations ranged between 11.39 to 34.06° as shown in Table 3.3 and Figures 3.12 and 3.13. If angle of repose is greater than 45°, it is considered as poor flowing. Since the values of the angle of repose for all ketoprofen granules were within 34.06 to 11.39° (i.e., lesser than 50), thus each granule formulation demonstrated appropriate flowability. Various factors that affect the angle of repose are granule size, shape, nature of material and methods used for calculation (Alfred *et al.*, 1991). In current research granules were less cohesive and free of moisture, reasons for free flowing (Geldart *et al.*, 2006).

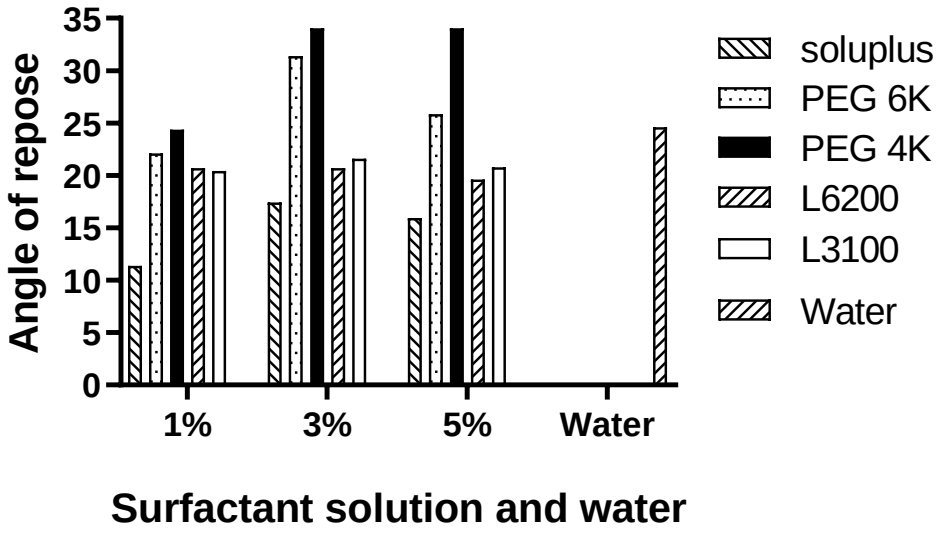
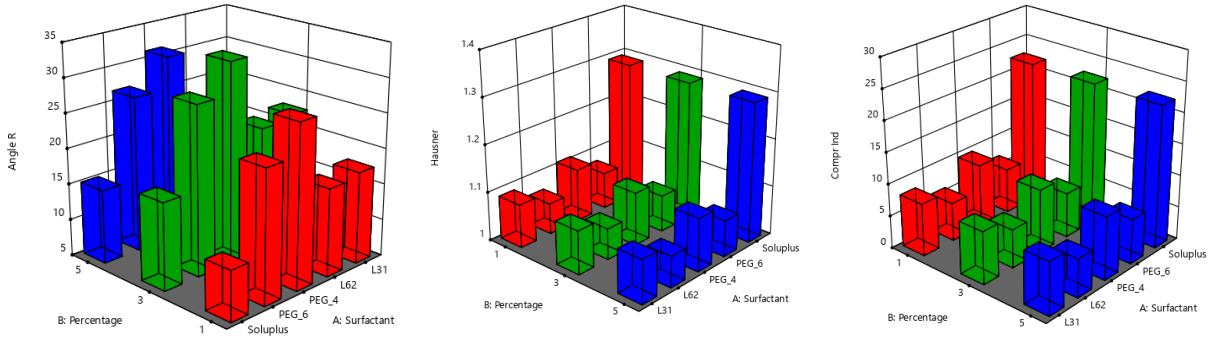


Figure 3.12: Angle of repose of ketoprofen granule formulations G1-G16



A B C

Figure 3.13: Combined effect of percentage and type of surfactant on the (A) Angle of repose, (B) Hausner's ratio and (C) Compressibility index of ketoprofen granule formulations G1-G15

3.4.3.3 Hausner's ratio

Hausner's ratio of all ketoprofen formulations (Table 3.3 and Figure 3.12 and 3.13) varied between 1.042 and 1.338. The formulations with Hausner's ratio greater than 1.25 show

poor flow properties, thus a value less than 1.25 is preferable while values close to 1 indicate good flow properties (Amidon *et al.*, 2007). All the formulations, except G2 and G3 revealed free flowing properties based on Hausner's ratio (Table 3.4). However, based on the values of angle of repose, the above formulations inclusive of G2 and G3 exhibited good flowability which was not supported by the Hausner's index (Table 3.3). This discrepancy might be attributed to use of different methods of calculation for flowability as described in USP30-NF25 (Robert *et al.*, 1965).

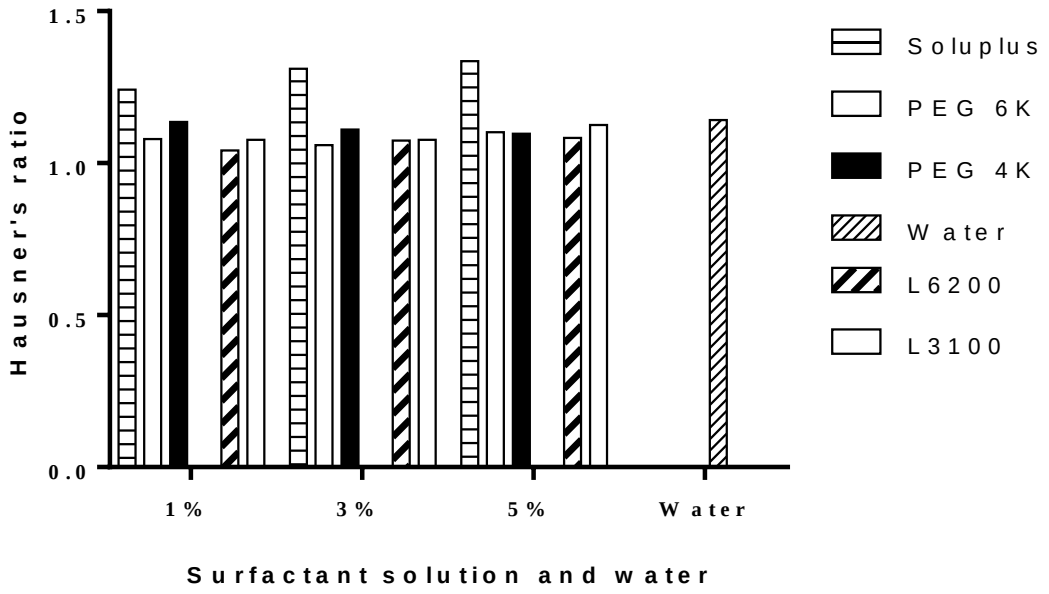


Figure 3.14: Hausner's ratio of ketoprofen granule formulations G1-G16

3.4.3.4 *C*arr's (compressibility) index

Carr's index for all formulations was ranged between 25.26 and 4.00 as shown in Table 3.3 and Figures 3.12 and 3.14. As indicated in Table 3.4, the formulation with Carr's index greater than 25 shows poor characteristics while values less than 15 indicate good flow

properties (Amidon *et al.*, 2007). The formulations G4-G6 and G8-G14 showed excellent, and G7-G15 and G16 showed good flowability according to the Carr's index while the formulations G1 to G3 showed fair flowability (Figure 3.13C). The formulations G2 and G3 had already been shown to have poor flowability on ground of the Hausner's ratio.

Table 3.4: Reference values of powder flow (USP30-NF25)

Flow property	Compressibility index	Hausner's index	Angle of repose
Excellent	<10	1.0-1.11	25-30
Good	11>16	1.12-1.18	31-35
Fair	16-20	1.19-1.25	36-40
Pourable	21-25	1.26-1.34	41-45
Poor	26-31	1.35-1.45	46-55
Very poor	32-37	1.46-1.59	56-65
V. Very poor	>38	>1.60	>66

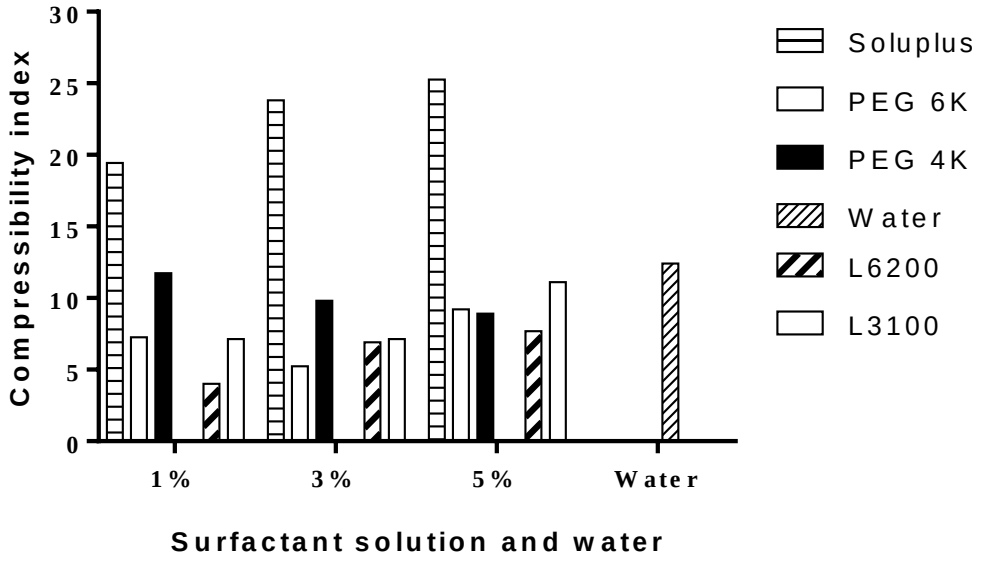


Figure 3.15: Compressibility index of ketoprofen granule formulations G1-G16

3.4.4 Compressional behavior

The Heckel equation represents the indicators for compressibility, hardness or the softness, plasticity and the capacity to deform of the materials and includes mean yield pressure (Py) and constant (k). The parameters of compression of all ketoprofen granule formulations computed with Heckel equation have been given in Figure 3.16. In this study, reduction in volume of compacts was used rather employing increase in density under pressure in the Heckel equation. Low Py and high k values of granule formulations indicate the material under test is softer, more plastic and readily deformable. Contrarily, the granules with low k and high Py values indicate that the granules are hard, less plastic in nature and may need more force to deform them. Slow packing rate and granules of different size may be a reason for high Py values.

In this study, the physical mix of excipients showed higher Py values than that of the pure drug indicating that the physical mix was less plastic and would require more force for their deformation. Granules formulation G8 made up of 3% PEG-4K showed almost the same Py value as that of the pure drug while granules G1and G3 made of 1% and 5% Soluplus® ,G6 made of 5% PEG-6K, G10 and G11 made of 1% and 3% of L6200 and G13 made of 1% of L3100 respectively showed lesser Py values than that of the pure drug indicating more suitability of compaction and high plasticity after presenting them as the granules (Hersey *et al.*, 1971). Rest of the formulations showed higher Py values than that of the pure drug representing less plasticity for deformation.

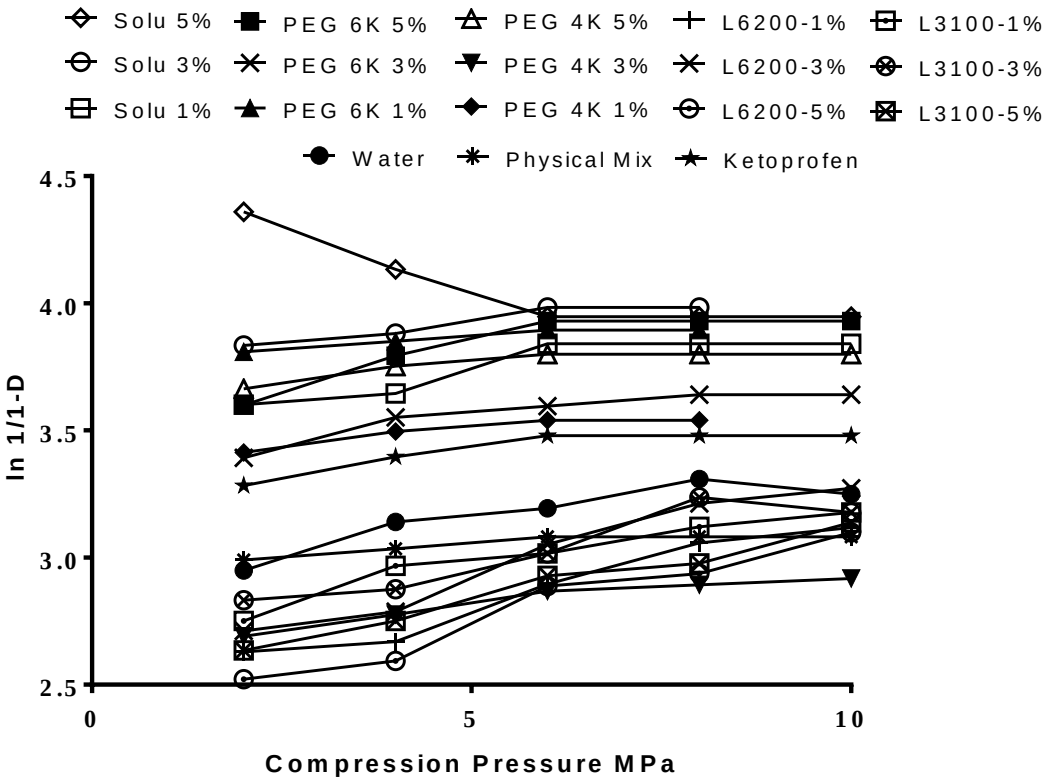


Figure 3.16: Heckel Analysis of granule formulations G1-G16, ketoprofen and physical mix of excipients

3.4.5 *In-vitro* release of ketoprofen granules

3.4.5.1 Calibration curve

The calibration curves in acidic as well as in basic media, pH 1.2 and 6.8, respectively have been given in Figure 3.17 and 3.18.

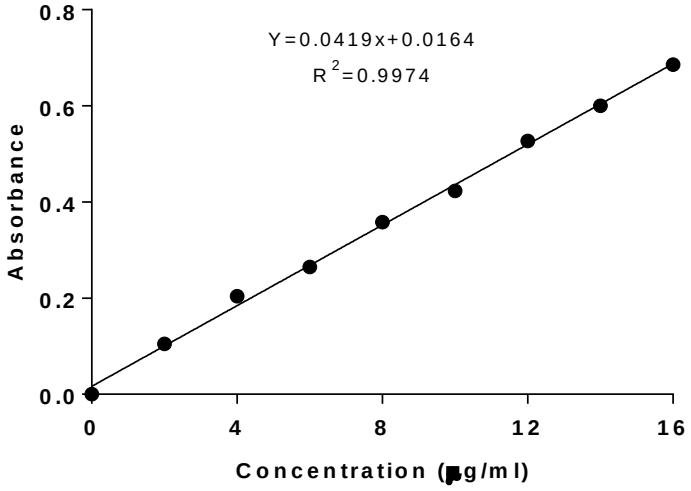


Figure 3.17: Calibration curve for ketoprofen in acidic medium, pH 1.2

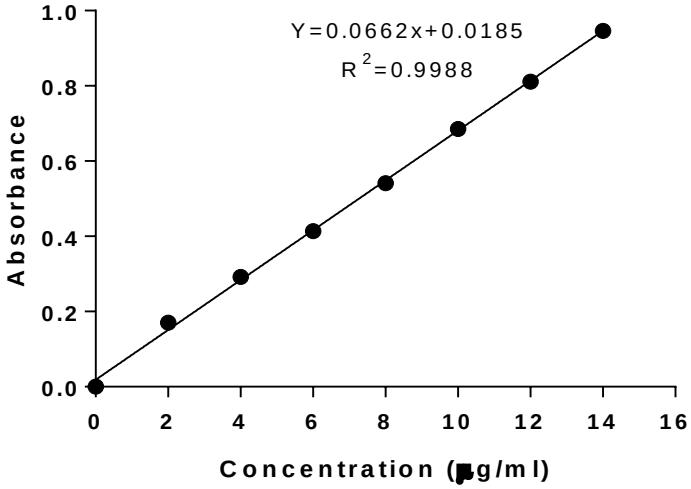


Figure 3.18: Calibration curve for ketoprofen in basic medium, pH 6.8

In-vitro dissolution of ketoprofen granules was first analyzed in simulated gastric fluid for initial 2h at pH 1.2 followed by intestinal media, pH 6.8 for 10h. The release data have been shown in Table 3.5 and that of the release profiles in Figure 3.19-3.25. Ketoprofen release for

1%, 3% and 5% surfactants have been demonstrated in Figures 3.19-3.21. All the release profiles from G1-G16 have been compared in Figure 3.22. Figure 3.23 shows the fast release granule formulations while Figure 3.24 exhibits the slow release formulations. The combined effect of surfactants and their percentages are given in Figure 3.25. The percent drug release from the granules was calculated using absorbance values in spectrophotometer. Formulations showed a lesser release in simulated gastric fluid except the G16 which was prepared without any surfactant and prepared employing only water as granulating agent. Release profile of ketoprofen from the granule formulations showed the fast as well as the slow release of ketoprofen in the intestinal media.

The G3 showed the lowest percent release, i.e., 27% till 12h while G15 gave the fastest and complete release within 3h. From G6, ketoprofen release of 19.42%, 32.68%, 59.76% and 78.20% were within 15-40%, 25-60%, 35-75% and >70%, respectively, at 1h, 2h, 4h and 8h which USP has specified for sustained release formulations (Monographs, 2002). The rest of the granule formulations (G4 and G5) prepared using PEG 6K at all concentrations showed more or less the sustained release pattern though it is reported to release drug quickly (Ma *et al.*, 2009; Papadimitriou *et al.*, 2012; Patil *et al.*, 2009). However, the inhibition of release may be ascribed to other ingredients, i.e., HPMC, Avicel, etc.

The Table 3.6 relates the solubility and the dissolution at specified interval. The G6 also showed convincingly enhanced solubility up to 2.81 mg mL^{-1} as compared to ketoprofen pure drug solubility at ambient temperature, 0.010mg mL^{-1}, reported in literature (Shohin *et al.*, 2012). Drug release pattern of G4-G6 (PEG-6K), G10-G12 (L6200) and G13-G15 (L3100) showed an increase in release as the percent concentration of surfactant was increased but all

other formulations (G1-G3 and G7-G9) showed decrease in drug release behavior with increased percent concentrations of surfactants (Figure 3.25).

Table 3.5: Percent drug release of all ketoprofen granule formulations

Time (h)	Drug release (%) from granule formulations															
	G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**	**G9**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**
0.5	3.83	3.75	3.32	3.83	4.44	12.36	3.83	3.75	3.32	12.5	26.23	15.35	18.34	12.87	15.89	3.32
1	4.35	5.21	4.52	4.95	4.95	19.42	4.35	5.21	4.52	22.53	37.57	34.39	36.80	30.21	24.91	4.52
1.5	5.56	6.24	5.38	6.33	6.85	27.00	5.56	6.24	5.38	30.1	40.24	39.46	43.68	36.22	35.98	5.38
2	6.16	7.19	5.99	27.34	31.13	32.68	6.16	7.19	5.99	36.22	43.23	42.55	54.87	41.42	44.89	52.84
3	9.93	10.36	7.85	37.26	44.40	54.17	11.14	10.36	10.04	65.24	81.60	80.18	84.88	65.00	106.52	83.58
4	12.24	12.24	9.06	42.64	55.81	59.76	15.53	17.73	12.24	72.41	83.03	81.60	88.91	74.34		99.30
5	22.12	16.63	10.37	51.97	60.31	65.80	26.51	23.21	14.43	78.67	88.45	84.46	92.91	85.22		
6	25.41	20.91	14.43	57.02	69.31	76.23	33.09	26.07	19.70	83.87	75.93	85.34	90.99	92.65		
7	28.32	23.32	16.30	62.80	72.67	77.02	41.64	34.63	23.87	87.21	94.99	90.29	100.81	104.48		
8	30.90	26.18	17.29	65.69	76.23	78.20	45.17	46.04	29.36	92.44	101.69	97.00				
9	37.04	30.02	17.95	70.63	81.60	84.02	53.95	49.12	34.74	99.89	103.66	99.16				
10	42.97	32.98	20.91	73.48	91.37	88.85	64.92	55.59	44.07							
11	53.95	38.77	22.45	89.40	100	97.52	69.31	59.43	49.56							
12	58.34	46.00	27.60	95.10	101	100	78.09	63.82	53.95							

Blank box shows that the drug release has already been completed to 100%

Drug release of ketoprofen from different granules showed the following order L3100 > L6200 > PEG-6K > PEG-4K > Soluplus®. Relatively faster release of drug from matrix containing L3100 in this study is in concordance with the previous report (Sultana *et al.*, 2017). This depicted that the surfactants prominently influence the drug solubility, drug dissolution that is translated into the same effect on the residence time in intestinal fluid (Singh *et al.*, 2000).

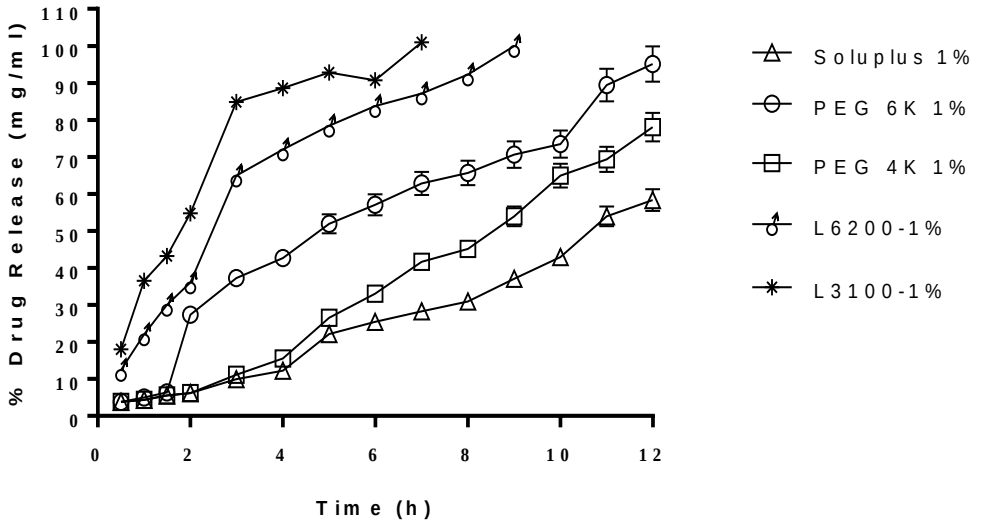


Figure 3.19: Comparative dissolution of ketoprofen from 1% of surfactants, G1 (Soluplus), G4 (PEG 6K), G7 (PEG 4K), G10 (L6200) and G13 (L3100)

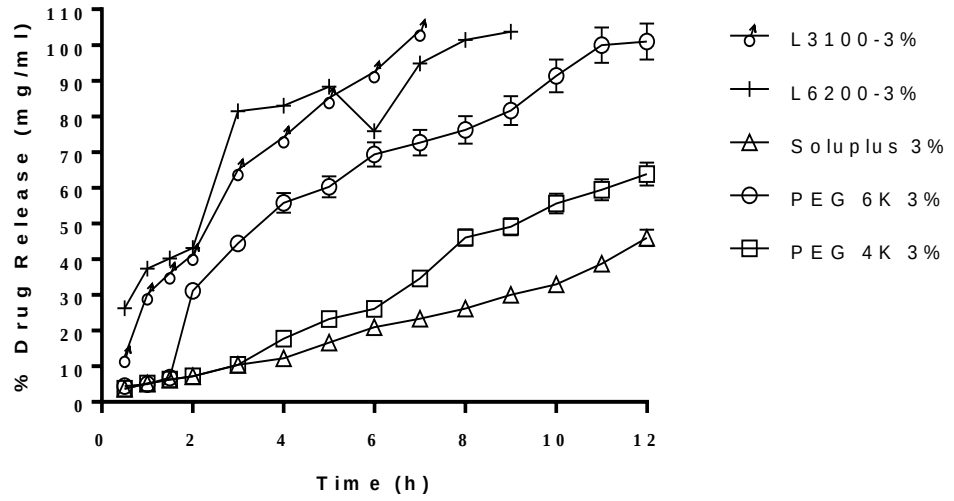


Figure 3.20: Comparative dissolution of ketoprofen from 3% of surfactants, G2 (Soluplus), G5 (PEG 6K) G8 (PEG 4K) G11 (L6200) and G14 (L3100)

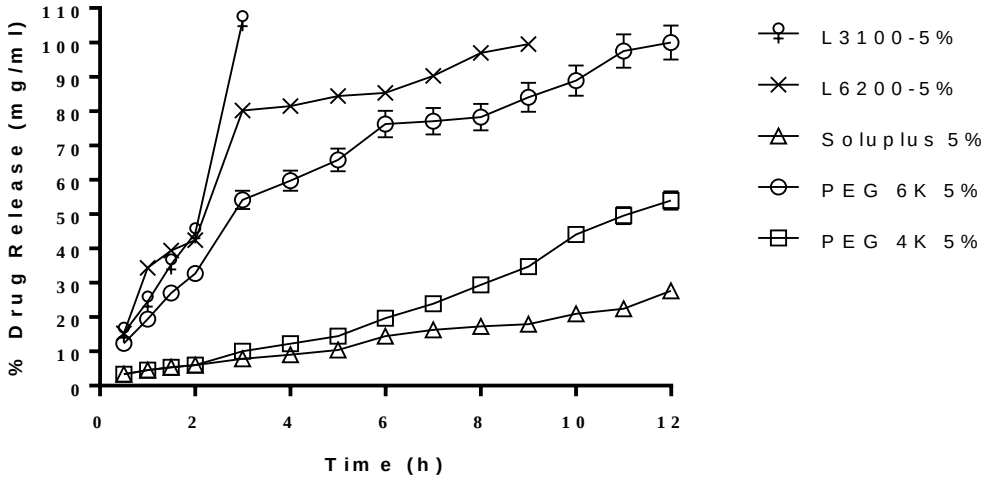


Figure 3.21: Comparative dissolution of ketoprofen from 5% of surfactants, G3 (Soluplus), G6 (PEG 6K) and G9 (PEG 4K), G12 (L6200) and G15 (L3100)

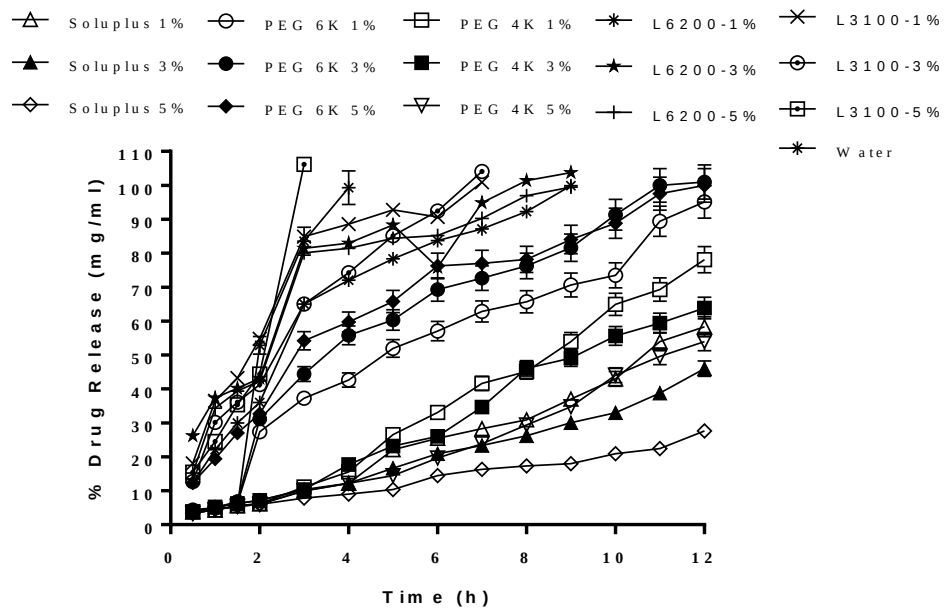


Figure 3.22: Comparative dissolution of ketoprofen form surfactant-based granule formulations containing 1, 3 and 5% of surfactants (G1-G15) and water-based granule (G16).

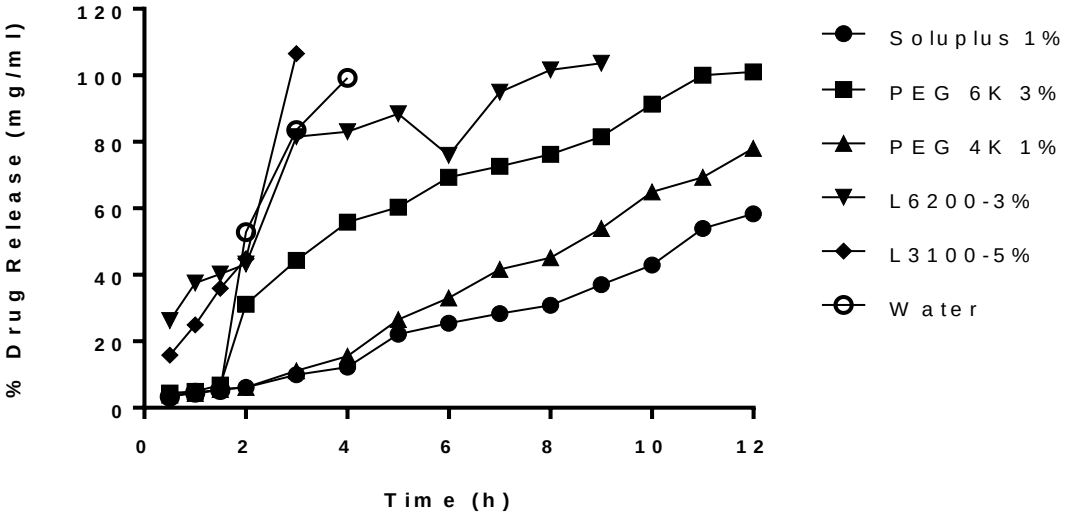


Figure 3.23: The ketoprofen granules showing highest release among the different categories of surfactants

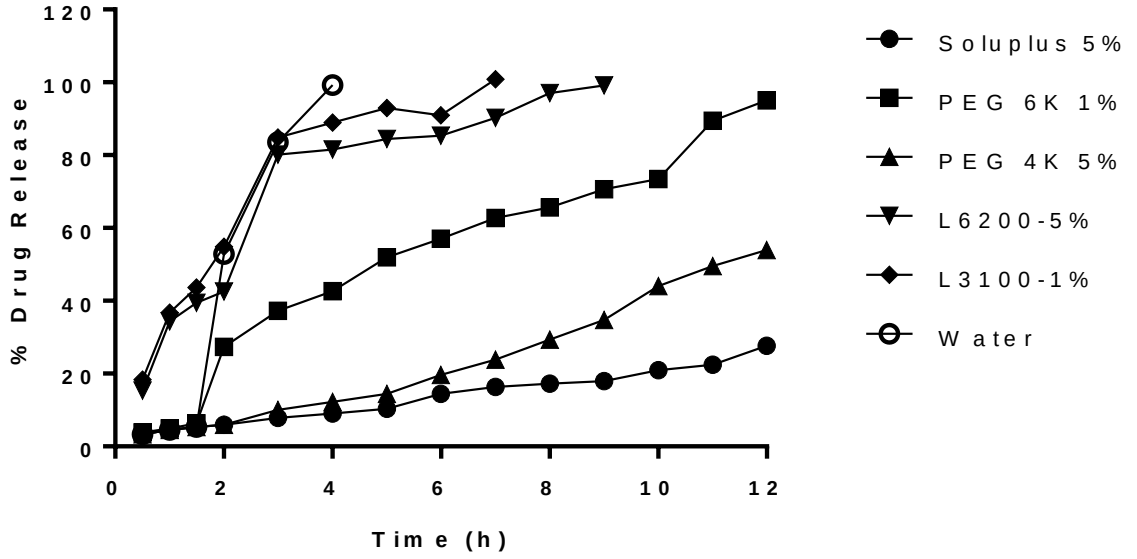


Figure 3.24: The ketoprofen granules showing the lower release among the different categories of surfactants

All the Figures 3.19-3.25 report that the surfactant-based ketoprofen granules endured release of ketoprofen in the acidic media followed by a gradual release of drug in a controlled manner at the basic pH. The above corresponds that the granules would exhibit lesser and then sustained release in the acidic and basic media, respectively.

This release inhibition could be assigned to the hydrogen bonding between surfactant-drug and their interaction, entrapment of drug in micelle core from where it was released as supported by FTIR and DSC (Section 3.4.7 and 4.8).

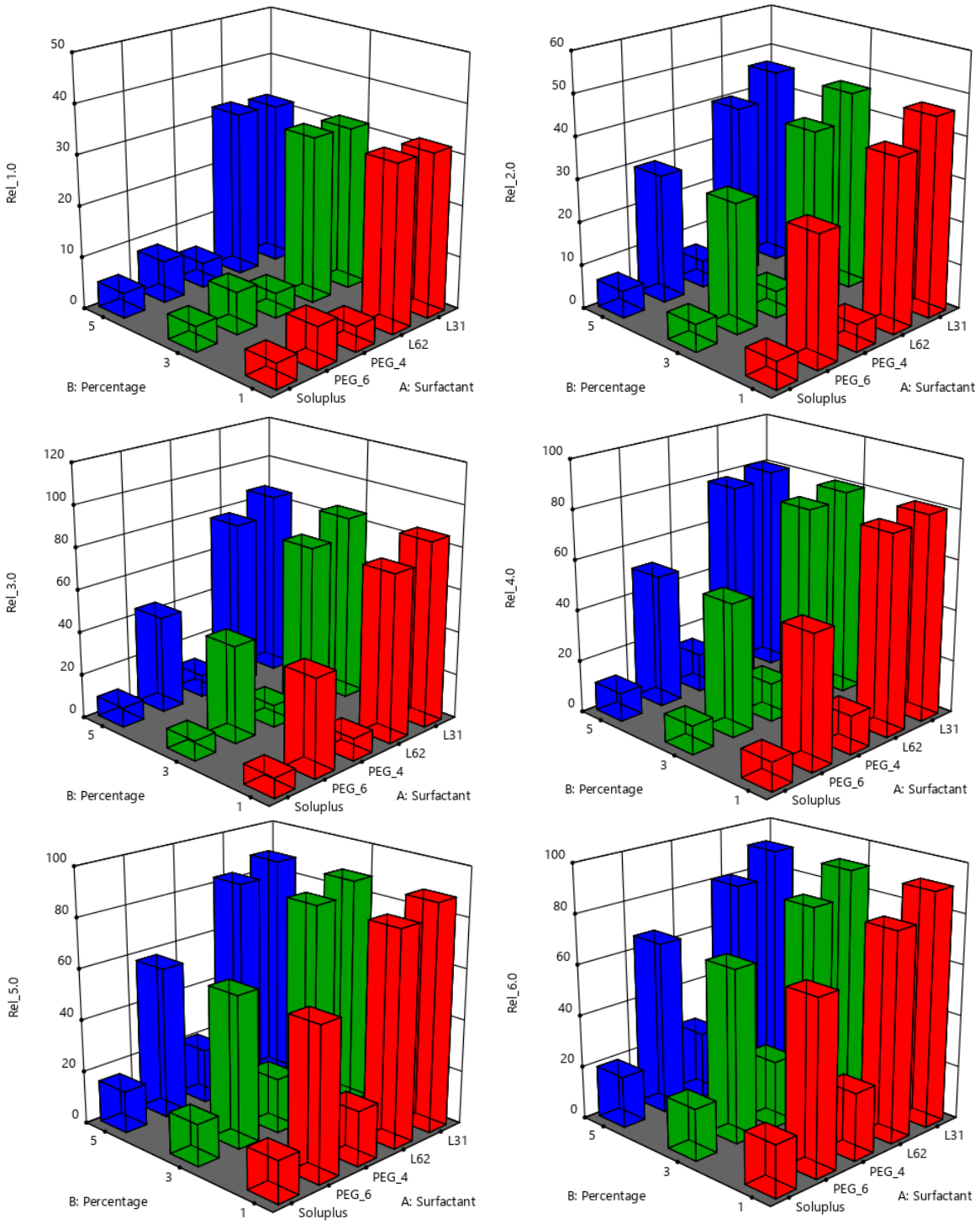


Figure 3.25: Combined effect of percentage and type of surfactant on the release of ketoprofen from granules at different time intervals

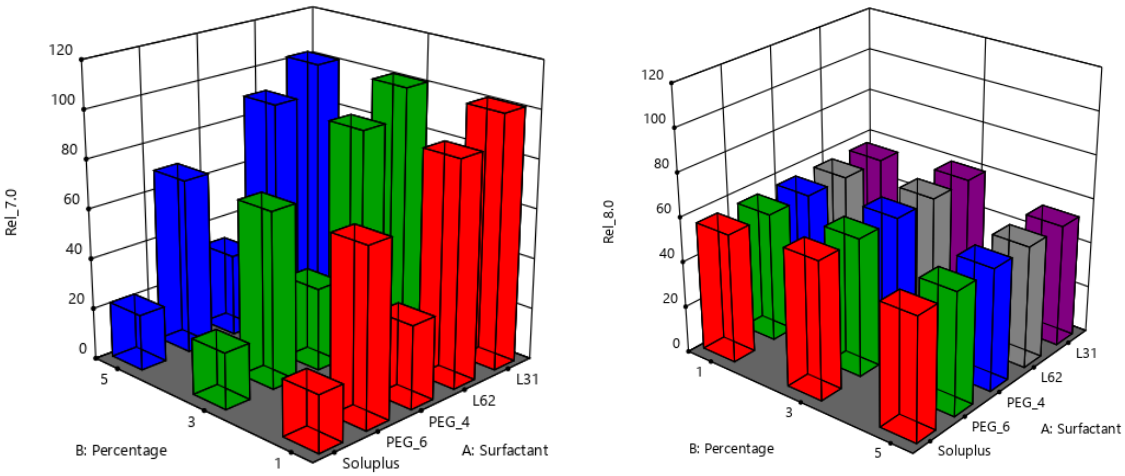


Figure 3.25: *Continued…*

Table 3.6: Solubility and dissolution profile of ketoprofen granule formulations G1-G16

Code	Solubility (mg/ml)	Highest Drug dissolution (%)	At time (h)
G1	3.09	58.335	12
G2	2.61	46.000	12
G3	1.69	27.604	12
G4	2.70	95.104	12
G5	2.92	101.000	12
G6	2.81	100.000	12
G7	2.73	78.091	12
G8	2.50	63.823	12
G9	1.87	53.945	12
G10	3.02	99.89	9
G11	2.86	103.66	9
G12	2.65	99.16	9
G13	2.84	100.81	7
G14	2.92	104.48	7
G15	2.99	106.2	3
G16	0.23	99.30	4

The G15 (2.99 mg/ml), solubility co-related with higher release, i.e., 106.5% at 3h fulfilled the general trend of increase in solubility resulted in increase in dissolution. But this behavior of G15 The G1 and G10 showed enhanced solubility (3.09 and 3.02 mg/ml respectively) as compared to other surfactant-based formulations (1.69 to 2.99 mg/ml), G16 (0.23 mg/ml) and pure drug (0.010 mg/ml) as reported (Shohin *et al.*, 2012). Release deviated the desired sustained release profile which was our ultimate study aim. Furthermore, despite the highest solubility of G1 (3.02 mg/ml) showed the 58.3% release at 12h. This trend of enhanced solubility and decreased release support the unusual relationship of dissolution and solubility, i.e., increased solubility results in decreased dissolution.

The solubility and dissolution rate, both are different phenomenon (Remington, 2006) the former being a static while the latter is a dynamic phenomenon (Shargel *et al.*, 2016). Thus, despite a drug have poor solubility, its dissolution rate may be rapid and vice versa (Remington, 2006). G16 despite its lowest solubility (0.23mg/ml) showed almost highest release (99%) even at earlier time point, i.e., at 4h. Higher solubility and higher drug release are due to good plasticity, mechanical strength and porosity of the granules as compared to the pure drug (Katikaneni *et al.*, 1995; Obeidat *et al.*, 2014).

3.4.6 Release kinetics

The release data associated with highest R^2 and lowest AIC among all kinetics models indicated that the release of the most of the formulations were best fitted to the Weibull model, as shown in Table 3.7. Weibull model is useful in comparing the release profiles of matrix type delivery systems that has been described for different types of dissolution processes (Dash *et al.*, 2010a).

Table 3.7: Dissolution kinetics modeling of all ketoprofen granule formulations G1-G16

Code	zero order		First order		Higuchi		Hixon-Crowell		Korsmeyer Peppas			Weibull		
	R^2	AIC	R^2	AIC	R^2	AIC	R^2	AIC	R^2	AIC	n	R^2	AIC	ß
G1	0.97	71.66	0.93	83.38	0.75	100.08	0.94	80.18	0.99	62.17	1.26	0.99	61.80	2.56
G2	0.98	53.15	0.97	63.74	0.81	87.25	0.97	60.45	0.99	53.97	1.06	0.99	44.58	2.31
G3	0.96	49.90	0.96	47.90	0.88	65.32	0.96	48.34	0.97	46.14	0.84	0.99	39.31	1.6
G4	0.94	94.14	0.95	90.28	0.88	103.40	0.97	85.76	0.97	88.17	0.79	0.97	89.05	1.1
G5	0.91	103.04	0.95	96.07	0.89	105.77	0.97	89.61	0.96	94.93	0.74	0.98	89.14	1.0
G6	0.76	112.51	0.98	74.22	0.97	84.51	0.98	80.47	0.98	82.51	0.57	0.99	77.80	1.08
G7	0.97	80.29	0.90	97.46	0.74	110.55	0.92	93.32	1.00	57.42	1.31	1.00	55.44	2.5
G8	0.98	70.22	0.93	86.50	0.77	103.78	0.95	82.06	0.99	62.34	1.18	0.99	57.51	1.94
G9	0.96	74.74	0.91	84.52	0.73	99.94	0.93	81.83	0.99	57.44	1.39	1.00	44.35	2.82
G10	0.78	86.17	0.98	61.25	0.94	72.06	0.98	57.61	0.96	70.13	0.60	0.99	59.46	1.21
G11	0.40	95.32	0.91	74.09	0.90	75.48	0.89	76.14	0.91	76.95	0.46	0.92	76.93	1.39
G12	0.49	94.19	0.96	66.05	0.91	74.67	0.95	68.92	0.91	76.60	0.49	0.96	69.72	1.04
G13	0.55	74.76	0.97	50.99	0.92	58.77	0.98	48.40	0.92	60.70	0.51	0.98	50.94	1.44
G14	0.89	63.07	0.96	53.77	0.94	57.45	0.98	45.33	0.99	44.03	0.68	0.98	49.30	2.01
G15	0.88	34.06	0.69	38.79	0.60	40.17	0.75	37.84	0.95	31.42	1.52	0.93	35.66	9.53
G16	0.83	46.10	0.68	49.95	0.55	52.03	0.73	48.96	0.90	45.01	1.49	1.00	29.60	0.94

The value of ß derived from Weibull model is indicative of drug release, progression and shape of dissolution curve. The value of ß > 1 explains the sigmoidal release curve with a lag time in the initial phase, ß < 1 gives steeper increase and ß = 1 represents exponential rise in the drug dissolution curve. The ß value of the ketoprofen from surfactant-based granules were above 1 thus, their release yielded sigmoidal curves which also indicated that the drug release was through a matrix. This showed further that the surfactant-based (G1 to G15) and non-surfactant-based granules (G16) acted as the matrix for the drug owing to the composition of the granules. The diffusional value (n) calculated from Korsmeyer-Peppas model for all, except G3-G6 and G10-G14 were found to be greater than 1, which also supported the above release mechanism of ketoprofen from the granules as super case II transport (coupled diffusion and erosion). The n value for G3-G6 and G10-G14 was between 0.46 and 0.84 which showed the anomalous transport (Arora *et al.*, 2011).

The release impedance was expected from the release retarding ingredients, such as HPMC (Huang *et al.*, 2005; Nokhodchi *et al.*, 2010) added in granules. HPMC is non-ionic in nature and is derived from cellulose ether which remains stable at pH 3.0-11 (Kamel *et al.*, 2008; Lee *et al.*, 2005). HPMC is commonly preferred in hydrophilic matrix formulation to provide a control over release of drugs. The HPMC-based hydrophilic matrix formulation is affected by the grades of HPMC due to difference in gel layer viscosity (Alderman, 1984; Colombo, 1993; Lee *et al.*, 1987). In this study, HPMC K4M was employed which and K100M are considered more effective for sustained release system (Nokhodchi *et al.*, 1995). On hydration, HPMC chains are separated from the matrix (Kamel *et al.*, 2008), developing a transitory interfacial front resulting into semisolid core that gradually dissociates and surrounded by a gel border that allows diffusion of drug through. HPMC controls this gel layer and so that

the drug release. Furthermore, the rate of penetration of fluid into the HPMC-matrix and its erosion are the controlling factors for HPMC matrix systems (Tahara *et al.*, 1995). Such systems are regarded as the swelling controlled systems (Alderman, 1984). Its rapid hydration, low toxicity, good gelling and compression properties have made it a preferred polymer for prolonged release formulations.

Avicel is used as a tablet disintegrant but helps to retard drug release rate as well, particularly when concomitantly used with HPMC in a formulation. Avicel possess low disintegrating forces and exhibits low swelling properties thus, retards the swelling and hydration of HPMC resulting in slow drug release. The swelling and erosion of HPMC matrix tablet may be affected by the proper tablet hardness that is evident with the addition of Avicel (Cao *et al.*, 2005; Lee *et al.*, 1999).

3.4.7 Fourier transform infrared spectroscopy (FTIR)

The spectra of pure drug presented characteristic peaks at 1689 cm^{-1} (C=O stretching of acid), 1650 cm^{-1} (C=O stretching of ketone), 1596 cm^{-1}, 1581 cm^{-1} (C=C stretching of aromatic ring), 1442 cm^{-1} (C-H deformation of CH_3 asymmetrical), 1373 cm^{-1} (C-H deformation of CH_3 symmetrical) respectively. The Soluplus® spectra showed a characteristic peak at 2923 cm^{-1}, (aliphatic C-H stretching), 1740 cm^{-1} and 1627 cm^{-1}(C=O stretching) while PEG revealed its characteristic peaks at 2877 cm^{-1}(aliphatic C-H stretching), 1280 cm^{-1}(C-O stretching), 1095-1141 cm^{-1}(C-O-C) of ether and O-H stretching at 3400 cm^{-1}. The poloxamer spectra showed its characteristic peak at 1373 cm^{-1} and 1498 cm^{-1}(CH bending vibrations), 2869 cm^{-1} and 2970 cm^{-1} (CH stretching vibrations). The characteristic peaks of ketoprofen functional groups were found

in all granule's formulations. The resultant FTIR spectrum (Figure 3.21) neither showed any drug-excipient incompatibility nor denaturation of active ketoprofen in granules.

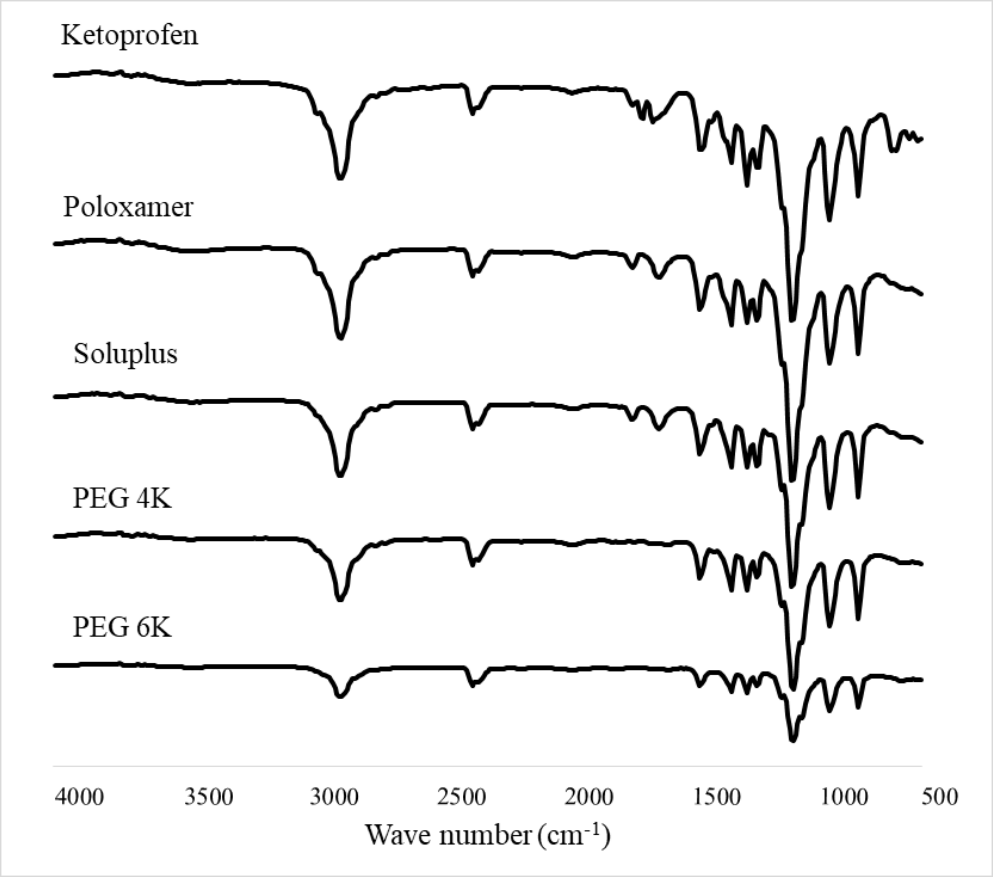


Figure 3.26: FTIR of ketoprofen; Soluplus®, PEG-6K and PEG-4K

The FTIR supports the interaction between ketoprofen and surfactant (Soluplus®) through hydrogen bonding, a reason for solvation power of Soluplus® (Varshosaz *et al.*, 2017). FTIR spectra of ketoprofen, surfactant (Soluplus®) and formulations supported the involvement of hydrogen bonding in the enhanced solubility of the ketoprofen, as indicated by the disappearance of –OH band (3500 to 3400 cm^{-1}) from spectrum of formulation that was evident in the spectrum of the surfactant. The surfactant has several –OH groups which, due to polarity can easily form

hydrogen bonding with the polar part of the ketoprofen. Ketoprofen has a carbonyl functional group (between phenyl rings) and carboxylic acid group it is further confirmed by the fact that band of –COOH (1693.1 cm^{-1}) of the ketoprofen was decreased in the formulation. However, the peak intensity corresponding to –C=O group remained intact in the formulation. Therefore, there is ample evidence that the carboxylic group of the ketoprofen is involved in the hydrogen bonding with –OH groups of the surfactant.

The surfactant-based granulation successfully improved the solubility of ketoprofen. Granules in this study were appropriate in terms of yield, size, flowability, compressional behavior and improving solubility of ketoprofen. The release of the ketoprofen from granules was also shown to be sustained. The increase in solubility and being sustained release could be translated into an oral solid unit dosage form, i.e., the ketoprofen granules could be filled into capsule or compressed into tablet. In pursuance of the enhanced compliance during multiple administration, tablet could be preferred pharmaceutical form as they could be swallowed easily hence, the granules prepared in the present study were considered to be compressed into matrix tablet dosage form. The effect of compressed dosage form on the release and other features of the tablet dosage form could be investigated to develop a viable oral dosage form of ketoprofen.

3.5 Conclusion

Solubility of ketoprofen was enhanced with use of surfactants, particularly at their lower concentrations. The release profile of ketoprofen could be controlled with the use of surfactants. Ketoprofen-PEG 6K 5% granules showed sustained release pattern mostly, following the Weibull model. FTIR showed no interaction among excipients and active ingredient except hydrogen bonding between drug and the surfactant. The current SAWG approach simultaneously

improved the solubility and also sustained the release of ketoprofen from the granules. The granules could be further processed to develop unit dosage form, such as capsule or tablet formulation.

Chapter 4

Development and characterization of sustained release matrix tablets of ketoprofen

4.1 Introduction

An ideal drug delivery system should be administered in single dose and should deliver drug directly at the specific site. Conventional drug therapy involves repeated dosing of drug to ensure its *in-vivo* stability, bioavailability and pharmacological activity (Venkateswarlu, 2008). But some drugs are unstable and toxic, exhibit extreme solubility problems, narrow therapeutic range or require long-term use (Pundir *et al.*, 2017). In such cases, multiple doses per day of the drug is needed to maintain plasma drug levels (Shargel *et al.*, 2016). The oral sustained drug delivery system is aimed to modify and enhance the drug performance by decreasing the dose, frequency of dose, and increasing duration of drug action providing uniform drug delivery over a period of time (Venkateswarlu, 2008). Unlike the conventional drug delivery system, these systems sustain the release of drug and help to maintain plasma drug concentration in therapeutic window and enhance the therapeutic efficacy of drug. These systems offer benefits like patient compliance, increase in plasma drug concentration, avoid side effects and multiple dosing to overcome the problems related to the use of the conventional system.

Variety of sustained release systems based upon mechanism are ion exchange controlled, dissolution controlled, diffusion controlled, erosion controlled and transport controlled (Figure 4.1 (Aulton *et al.*, 2002). For sustained release dosage forms matrix systems based upon diffusion controlled release are employed (Colombo *et al.*, 2000). These matrix systems are further divided into different types on the basis of their mechanisms to prolong the drug release, i.e., monolithic matrix systems, reservoir matrix systems and osmotic pump systems. Drug is

encapsulated or dispersed in hydrophilic or hydrophobic matrix in monolithic matrix systems to control pattern of drug release (Colombo, 1993; Kim, 1999; Nerurkar *et al.*, 2005; Phaechamud, 2008; Varshosaz *et al.*, 2006). Classification of these systems include hydrophilic/soluble matrix that release drug after getting swell upon hydration and hydrophobic/insoluble matrix systems that release drug after getting dissolved with solvent (Nokhodchi *et al.*, 2012).

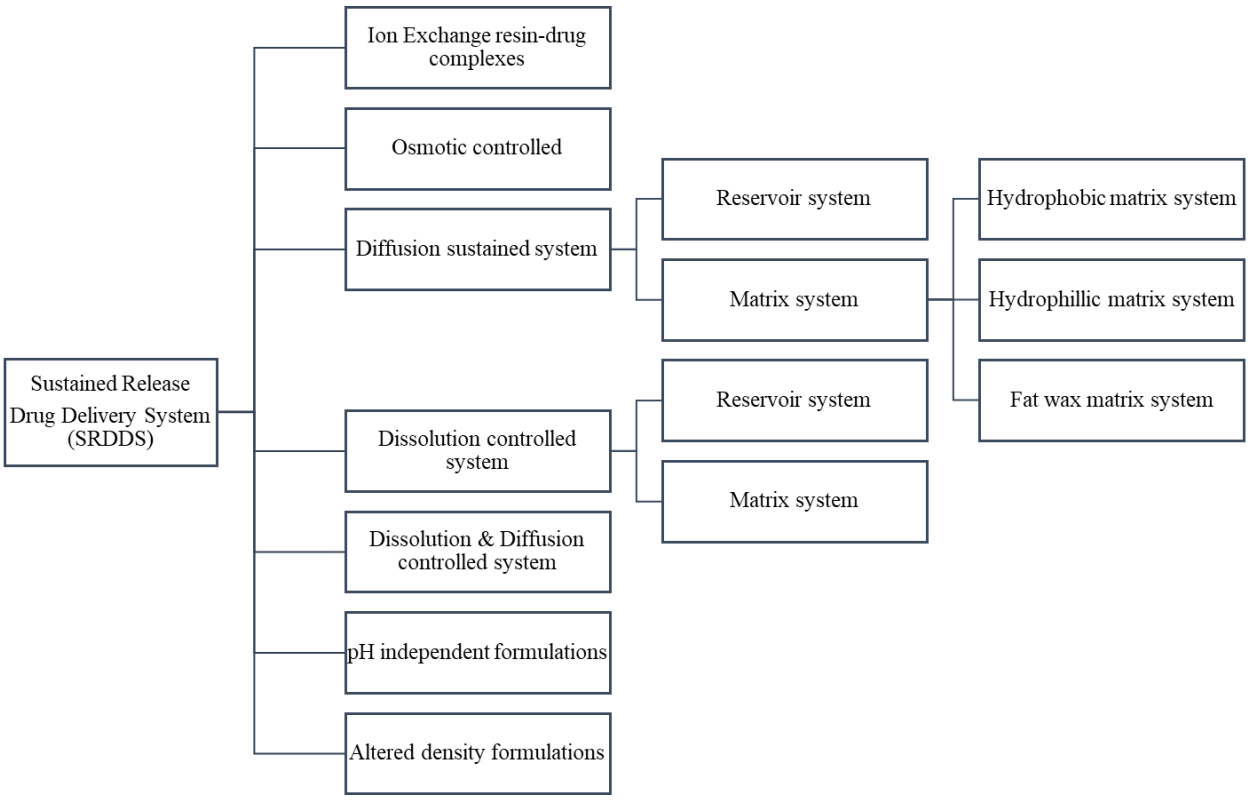


Figure 4.1: Types of sustained release systems

For highly soluble drugs, hydrophobic matrix are used (Tiwari *et al.*, 2003). For oral controlled drug delivery hydrophilic matrix systems are mostly used (Colombo, 1993; Prajapati *et al.*, 2010; Siepmann *et al.*, 2012; Tiwari *et al.*, 2008). Soluble drugs release primarily by

diffusion through the gel layer whereas insoluble and/or poorly soluble drugs release primarily by tablet erosion (Johnson *et al.*, 1993; Skoug *et al.*, 1993).

4.2 Selection criterion for oral sustained release drug delivery

A number of physicochemical and pharmacokinetic parameters for selection of drug to be formulated as sustained release dosage form have been exhibited in Table 4.1.

The features of ketoprofen making it favorable for sustained release delivery system include its low molecular weight (254.3 g/mol), low solubility, (0.010 mg/ml), and short half-life (1-3h) (Shohin *et al.*, 2012).

However, the release of ketoprofen is dependent on pH, contrary to the requirement for the sustained release dosage form. Nevertheless, this feature could be translated into a benefit of making the drug release at the pH of small intestine and by accomplishing this, the gastrointestinal risk in stomach could be avoided.

The aim of this part of study was to develop the sustained release matrix tablets of ketoprofen from the compression of granules prepared in Chapter 3. The developed tablets were characterized in terms of physicochemical and the *in-vitro* dissolution characteristics of the tablet. The further aim of this study was to select an appropriate sustained matrix tablet formulation suitable for the pharmacokinetic study.

Table 4.1: Physicochemical and pharmacokinetic parameters for drug selection as candidate for sustained release

Parameter		Preference
Physicochemical	Molecular weight/size	<1000
	Solubility	>0.1 µg/ml for pH 1 to pH 7.8
	Pka	Non-ionized moiety > 0.1 % at pH 1 to pH 7.8
	Apparent partition coefficient	High
	Absorption mechanism	Diffusion
	General absorbability	From all GI segments
	Release	Should not be influenced by pH and enzymes
Pharmacokinetics	Total clearance	Should not be dose dependent
	Elimination rate constant	Required for design
	Elimination half life	Preferably between 0.5 and 8 h
	Apparent volume of distribution Vd	Larger Vd and MEC, the larger will be the required dose size
	Intrinsic absorption rate	Must be greater than release rate
	Therapeutic concentration	The lower $C_{ss\ av}$ and smaller Vd
	Toxic concentration	Wide values of MTC and MEC or safer drugs

4.3 Materials and methods

Magnesium stearate was gifted by Mega Pharmaceuticals Ltd. Lahore, Pakistan. Other materials were purchased and received as described in Section 3.2.1.

4.4 Preparation of granules using SAWG technique

The granules were prepared using wet granulation technique described in Section 3.3.1

4.5 Compression of granules into tablets

The prepared fine granules were dried mixed with magnesium stearate as lubricant and compressed, using a single punch rotary machine (Brook motors BS170) to form tablets of nearly uniform size, thickness and hardness. A total of 16 formulations using different concentrations of surfactants were prepared as mentioned in Table 4.2. The formulation, MT16 was prepared using water as granulating agent and considered as control. The prepared tablets were then stored at room temperature for further testing.

4.6 *In-vitro* characterization of matrix tablets

The prepared tablets were characterized for physical parameters such as friability, hardness, content uniformity, thickness and swelling behavior, dissolution study and release kinetics.

4.6.1 Friability

Friability of twenty tablets from each formulation was measured by using Roche Friabilator (Curio, Pakistan). The Friabilator was set on rotation for 4min at 25rpm to complete 100 revolutions and calculated the friability by measuring the percentage weight loss in the tablets.

Table 4.2: Formulations prepared by wet granulation method with different surfactants percent compositions

Code	Ketoprofen (mg)	Avicel-102 (mg)	Lactose (mg)	Crospovidone (mg)	HPMC-4K (mg)	Mg-stearate (mg)	Soluplus (ml)	PEG-6K (ml)	PEG-4K (ml)	L6200 (ml)	L3100 (ml)	Water (ml)	Total weight (mg)
MT1	200	28	50	24	96	2	10	-	-	-	-	-	400
MT2	200	28	50	24	96	2	10	-	-	-	-	-	400
MT3	200	28	50	24	96	2	10	-	-	-	-	-	400
MT4	200	28	50	24	96	2	-	10	-	-	-	-	400
MT5	200	28	50	24	96	2	-	10	-	-	-	-	400
MT6	200	28	50	24	96	2	-	10	-	-	-	-	400
MT7	200	28	50	24	96	2	-	-	10	-	-	-	400
MT8	200	28	50	24	96	2	-	-	10	-	-	-	400
MT9	200	28	50	24	96	2	-	-	10	-	-	-	400
MT10	200	28	50	24	96	2	-	-	-	10	-	-	400
MT11	200	28	50	24	96	2	-	-	-	10	-	-	400
MT12	200	28	50	24	96	2	-	-	-	10	-	-	400
MT13	200	28	50	24	96	2	-	-	-	-	10	-	400
MT14	200	28	50	24	96	2	-	-	-	-	10	-	400
MT15	200	28	50	24	96	2	-	-	-	-	10	-	400
MT16	200	28	50	24	96	2	-	-	-	-	-	10	400

4.6.2 Hardness

The hardness of 10 tablets was measured by determining the compressive hardness of tablets using digital hardness tester (Erweka, Germany). Tablets were placed between the open jaws of the hardness tester and an average force required to crush them was obtained and recorded.

4.6.3 Content uniformity

Drug content uniformity was determined for each formulation by crushing ten tablets to powder using pestle and mortar. The aliquot part of 200mg powder was weighed from the crushed mixture and dissolved in freshly prepared 100 ml phosphate buffer having pH 6.8. The resultant solution was diluted with 1 to 100 ml of same buffer (pH 6.8) and determined using UV-Visible spectrophotometer at predetermined λ_{max} of ketoprofen (258nm). The absorbance of unknown drug concentration in solution was analyzed on the basis of already prepared standard calibration curve.

4.6.4 Weight variation

Twenty tablets from each formulation were selected to determine the weight variation using an electronic balance (Shimadzu, Japan). The average weight obtained from each formulation was used for calculation of percent variation among the prepared tablets.

4.6.5 Thickness

Ten tablets were taken and the thickness, in mm was noted using the Vernier caliper.

4.6.6 Swelling behavior of ketoprofen matrix tablets

The swelling behavior for all tablet formulations was observed by placing the accurately weighed tablet (initial weight, W1) in respective petri dishes, each filled with 10 ml phosphate buffer, pH 6.8. Each petri dish was placed under the stereo zoom microscope lens and images were captured with the help of stereo camera (Olympus model SZ2-IIST; Tokyo, Japan) to observe the swelling behavior. After 6 h, increase in weight of the tablets (final weight, W2) was measured for individual tablets after removing from the fluid and wiping off water with absorbent paper. The tablets' ability to uptake water, measured as increase in weight (W_U) was determined by using equation.

$$\% W_U = \left[{}^{(W_2 - W_1)}/_{W_1} \right] \times 100 \qquad [1]$$

4.6.7 Dissolution studies

The dissolution studies were carried out for six matrix tablets as described in Section 3.3.4.3 with one exception. For dissolution study of matrix tablet of ketoprofen, USP dissolution test apparatus-II (Erweka, Germany) was used to determine percent drug release after pre-defined time intervals, i.e., 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0h. The filtered samples were diluted to acquired proper absorbance and were analyzed at λmax of 258nm using UV spectrophotometer to determine the release of ketoprofen.

4.6.8 Kinetics of drug release

Model dependent and independent approaches of release kinetics were applied to all ketoprofen formulations using DDSolver Ver 1.0, an add-in extension to MS Excel® (Zhang *et al.*, 2010). Release data of tablets were applied to the following kinetic models:

Zero Order: $Q_t = K_0 t$ [2]

First Order: $Q_t = \ln Q_0 - K_1 t$ [3]

Higuchi: $M_t = K_H t^{1/2}$ [4]

Hixson Crowell: $Q_0^{1/3} - Q_t^{1/3} = K_{HC} t$ [5]

Korsmeyer-Peppas: $M_t / M_\infty = K t^n$ [6]

Here Q_0 indicates drug amount in tablets at time zero, Q_t reflects the released amount of drug at time specified, M_t and M_∞ are the amount of drug considered at a definite and infinite times, respectively. K_0, K_1, K_H, K_{HC} and K are respective constants of release kinetic obtained from the linear curves, respectively for zero-, first-order, and Higuchi, Hixson Crowell, and Korsmeyer-Peppas models. Criteria of model selection for the formulations was described earlier in Section 3.3.4.4, i.e., highest R^2 and lowest AIC.

4.6.9 Determination of similarity and dissimilarity of release profile

The model independent approach explained in terms of dissimilarity factor (f_1) and similarity factor (f_2) were assessed between the pair of the test tablet formulation and reference stipulated (desired) sustained release criteria, given in Table 4.3 was adopted from literature (Hussain, 2018; O'hara *et al.*, 1998).

$$f_1 = \left[\frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t}\right] x100 \quad [7]$$

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} x100\right\} \quad [8]$$

Where n is sample numbers, t is sample time, T_t and R_t represent the mean percent drug released at specified time, respectively for test and reference dissolution curves. The selection criteria in this kinetic release modeling is having dissimilarity (f_2) and similarity (f_1) factor values <15 and >50 respectively.

Table 4.3: The stipulated release criteria used as reference

Time (h)	1	2	3	4	5	6	7	8
R_v (%)	15	20	35	45	55	65	75	85

4.6.10 Fourier transform infrared spectroscopy (FTIR)

The drug and the excipients interaction were already studied for all excipients expect that of the magnesium stearate using Fourier transform infrared spectroscopy as explained in Section 3.4.7. In this part of study, the FTIR was obtained for tablet dosage form containing all the ingredients inclusive of magnesium stearate according to the setting given for ketoprofen granule formulation (Section 3.3.7).

4.6.11 Differential Scanning calorimetry (DSC)

DSC model (SDTQ600, TA Instrument, USA) was used to assess heat capacity and enthalpy of ketoprofen melting from the crushed tablet to understand energy transformation and transition of states-led change in heat capacity of ketoprofen. Alumina crucible used with ramp rate of 10 °C and temperature range of 25-300 °C, provided nitrogen atmosphere with flow rate of 100 ml/min.

4.7 Results and discussion

Sixteen formulations of ketoprofen matrix tablets (MT1 to MT16) were prepared including a control tablet formulation (MT16) which was labelled as a surfactant free or water-based formulation (Table 4.2). The sustained release matrix tablets of ketoprofen were prepared from the compression of granules. Five different surfactants, i.e., Soluplus® (MT1-MT3), PEG-6K (MT4-MT6), PEG-4K (MT7-MT9), L-6200 (MT10-F12) and L3100 (MT13-F15) were used in varied concentration (1%, 3% and 5%) while keeping ketoprofen and other ingredients fixed as explained in Section 3.1 with addition of one step of compression in order to prepare matrix tablets. Ketoprofen tablets were assessed on the basis of physicochemical characteristics including friability, hardness, content uniformity, weight variation, thickness and swelling behavior. The *in-vitro* dissolution testing was performed to evaluate specifically the sustained release characteristics of the sustained release matrix tablet of ketoprofen. The FTIR and DSC was also tested for the selected formulation. Based on the physicochemical characteristics and the *in-vitro* dissolution characteristics of the tablet, a matrix tablet was selected to carry out for the pharmacokinetic study.

4.7.1 Physicochemical properties of matrix ketoprofen tablets

4.7.1.1 Friability

The physicochemical properties of matrix ketoprofen tablets have been given in Table 4.4. Friability is normally confined to uncoated tablets to check the tendency to crumble, cracked or cleaved during transportation and storage. In the friability testing, the weight loss found for all ketoprofen formulation was in a range of 0.06-0.41% (Table 4.4) which followed the USP specification of tablet friability, i.e., not more than 1% (Brown, 2006).

Table 4.4: Physicochemical characteristics of ketoprofen sustained release tablets

Code	Friability (%)	Hardness (kg/cm2)	Content Uniformity (%)	Weight Variation (mg)	Thickness (mm)	Swelling Index (%)
MT1	0.11±0.02	8.17±1.18	100.35±1.20	399±2.96	4.75±0.08	15.98±1.05
MT2	0.13±0.02	8.71±0.63	99.33±1.24	401±2.52	4.86±0.05	51.60±1.24
MT3	0.14±0.02	8.75±1.60	101±1.65	400±2.95	4.87±0.06	45.00±1.15
MT4	0.41±0.01	8.31±1.46	98.95±2.05	402±2.27	4.93±0.04	25.63±0.98
MT5	0.06±0.02	8.69±1.67	100.45±2.86	402±2.99	4.87±0.08	17.04±0.75
MT6	0.07±0.03	6.36±1.16	99.75±2.44	399±3.76	4.77±0.07	18.87±1.20
MT7	0.14±0.04	7.83±1.97	101.55±2.55	402±2.18	4.62±0.07	30.59±1.65
MT8	0.06±0.03	6.89±1.17	100.25±1.69	400±3.49	4.79±0.08	24.17±1.11
MT9	0.08±0.02	6.03±0.81	98.85±1.05	399±2.57	4.83±0.06	32.99±0.85
MT10	0.16±0.02	7.80±0.55	100.40±1.55	402±3.78	4.74±0.08	24.50±0.56
MT11	0.16±0.03	7.96±1.15	99.66±0.96	401±3.05	4.82±0.7	20.75±0.45
MT12	0.18±0.02	6.48±1.65	101±1.86	403±2.77	4.69±0.03	22.28±0.18
MT13	0.14±0.02	7.90±0.83	98.75±2.65	402±3.12	4.74±0.02	40.70±1.40
MT14	0.23±0.04	8.46±0.13	101±0.95	399±3.48	4.65±0.04	19.01±1.25
MT15	0.10±0.03	6.62±0.55	98.25±1.95	399±2.55	4.7±0.04	22.39±0.95
MT16	0.12±0.01	6.46±1.08	99.55±1.75	400±3.48	4.74±0.11	58.54±1.98

Figure 4.2 did not show any specific trend, yet the friability was higher in case of tablet formulation MT4, which was prepared using 1% PEG-6K. The next highest friability was noted for the 3% PEG-6K. Nevertheless, with reference to the compendial acceptance, the friability of the newly developed matrix tablet was not dependent on the type or the amount of surfactant and other excipients used in the development of the tablet formulations. The friability was lowest particularly in case of 3% Soluplus®, PEG-6K and PEG-4K (Figure 4.3).

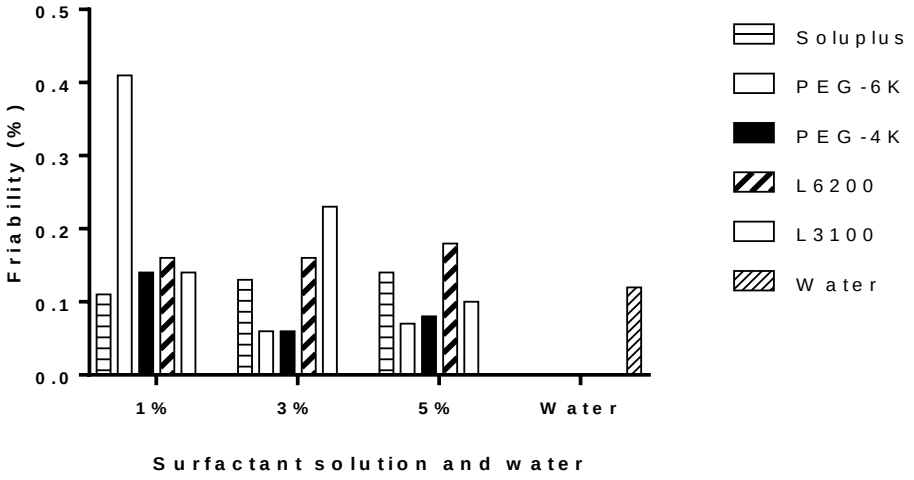
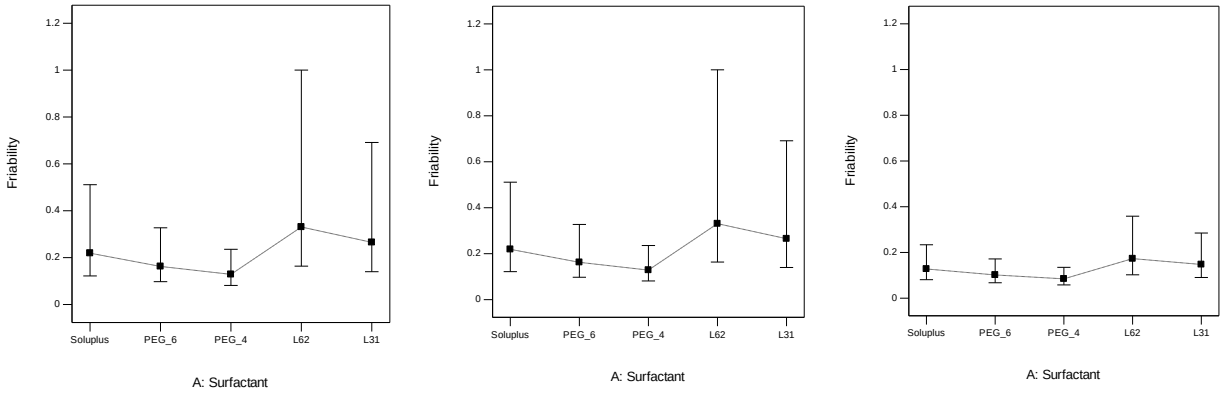


Figure 4.2: Friability of ketoprofen sustained release tablets



1% of surfactants 3% of surfactants 5% of surfactants

Figure 4.3: Effect of percentage and types of surfactants on the friability of ketoprofen matrix tablets

4.7.1.2 Hardness

The crushing strength of all ketoprofen matrix tablet formulations, showed in Table 4.4 and Figure 4.4 were found within the range of (6.03 -8.75kg/cm). MT3 and MT9 showed the maximum (8.75kg/cm) and minimum (6.03kg/cm) hardness, respectively. Except MT6-MT9, MT12 and MT15-MT16, all formulations met the compendial criteria of hardness 7-10 $kgcm^{-1}$

laid down by USP (Halaçoglu *et al.*, 2015). Formulations prepared with Soluplus®, PEG-6K, L6200 and L3100 almost gave an appropriate and specified crushing strength to the tablets. The highest hardness was found in case of 3% Soluplus®, the Figure 4.5 showed.

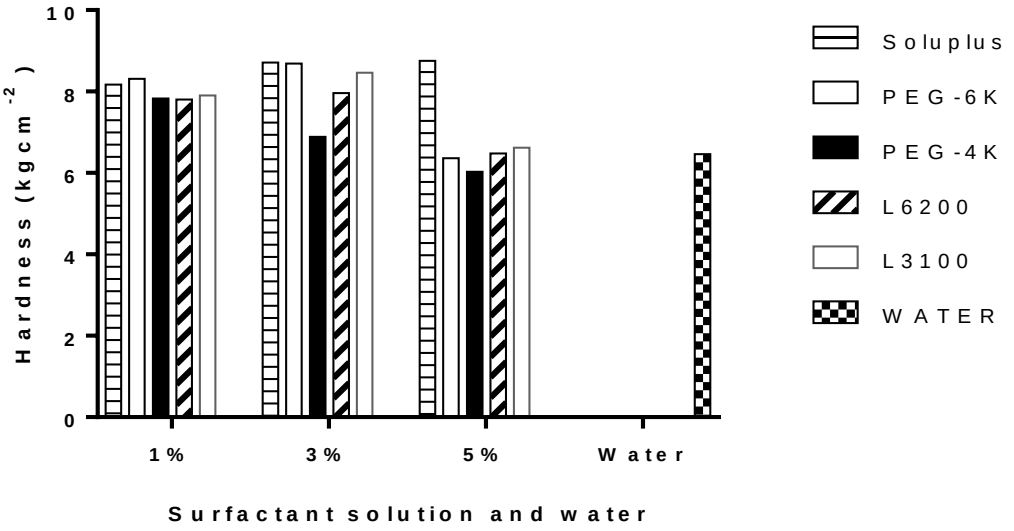
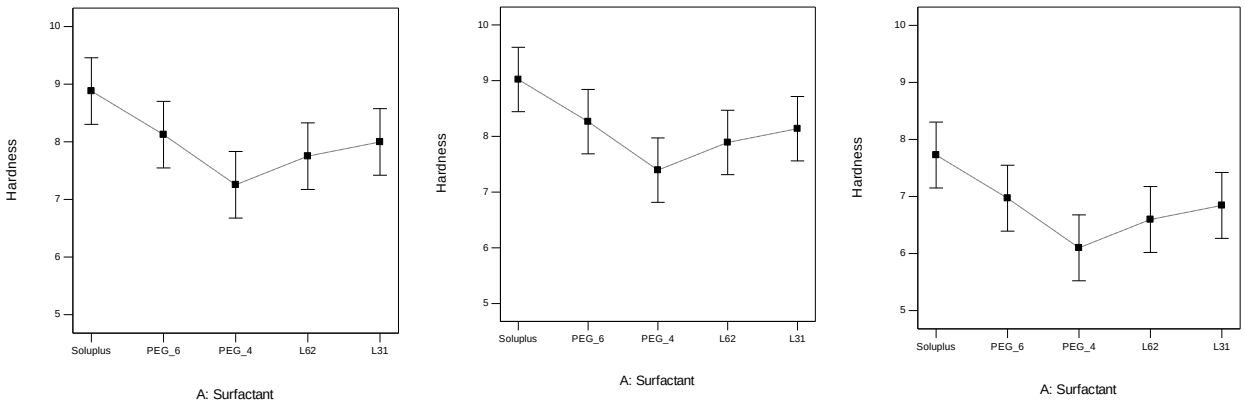


Figure 4.4: Hardness of ketoprofen sustained release tablets



1% of surfactants 3% of surfactants 5% of surfactants

Figure 4.5: Effect of percentage and types of surfactants on the and hardness of ketoprofen matrix tablets

4.7.1.3 Content uniformity

Uniformity of drug content was obtained in a range 98.25-101.55% in all formulations and follow ketoprofen monograph specification mentioned in USP-35 (Roukville, 2012). The content uniformity data have been given in Table 4.4 and Figures 4.6-4.7. This test ensure the consistency of unit dosage form in each formulation batch, if the assay content fall under the acceptable range which is usually specified in particular drug monograph (USP28-NF23, 2006). If the assay of the selected formulation showed results greater than or lesser than acceptable assay range, the batch categorized as cancelled.

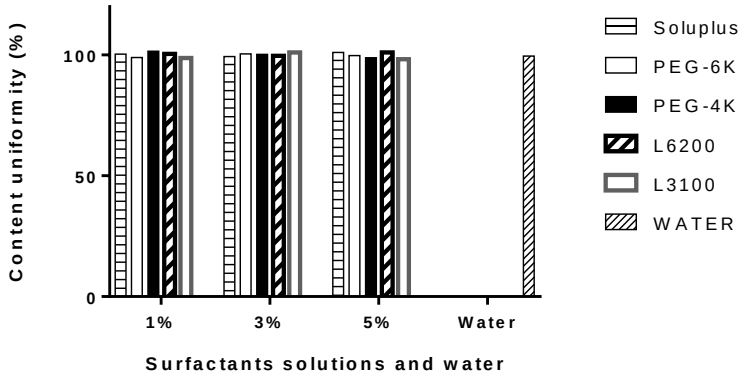


Figure 4.6: Content uniformity of ketoprofen sustained release tablets

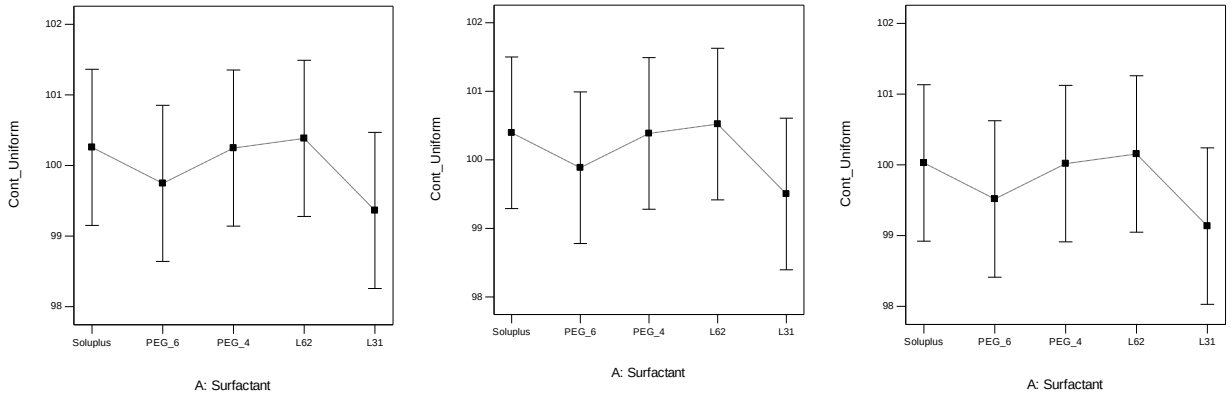


Figure 4.7: Effect of percentage and types of surfactants on the content uniformity of ketoprofen matrix tablets

4.7.1.4 Weight variation

Weight variation is one of the determinants of uniformity of drug dose in the dosage units. Tablets possessing weight more than 325mg should meet the specification of variance of not more than 5% (Lachman *et al.*, 1976). The weight of tablet obtained for all formulations was in a range 399-403mg for which the variation was found to be 3.78%, as shown in Table 4.4 and Figure 4.8.

The type of surfactants did not influence the weight variation of the ketoprofen matrix tablets however, the concentration of the surfactant has a dominant effect on the weight variation of the tablets, Figure 4.9 A and B show, respectively. The lowest weight variation was observed with the surfactant's concentration of 5%, as shown in Figure 4.9B.

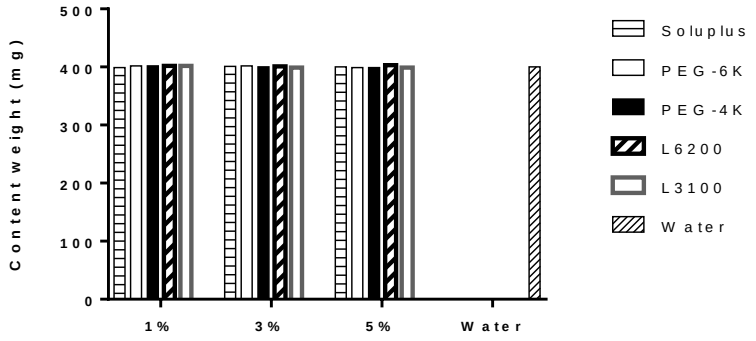
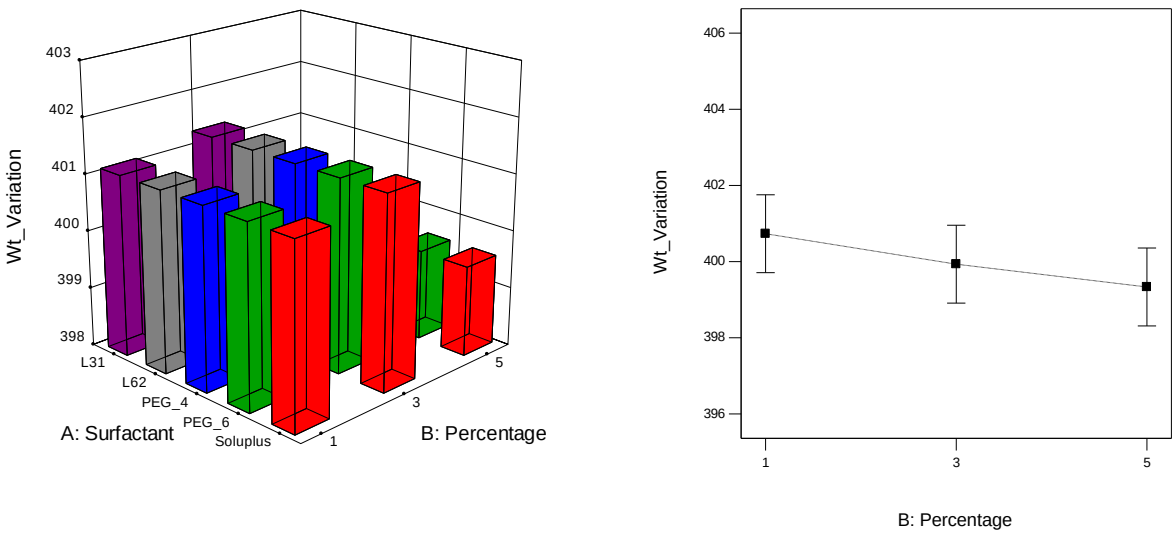


Figure 4.8: Content uniformity of ketoprofen matrix tablet formulations



A B

Figure 4.9: Combined effect, on the weight variation of ketoprofen matrix tablets of (A) types of surfactants as 3-dimentional plot and (B) percentage of surfactants as one factor plot

4.7.1.5 Thickness

Table 4.4 shows the data on the thickness of ketoprofen matrix table formulations. The value of tablet thickness was obtained in a range of 4.62-4.93mm (Figure 4.10). Thickness is important and dimensional variable entity in formulation development used to hold the pressure applied during compression. Minimum variation in the thickness data of ketoprofen matrix tablet formulations was found appropriate since it was within the specified acceptable value, i.e., within the ±5%. If thickness of tablets is not in the acceptable range, this indicates a problem of fill levels in container during compression (Liberman *et al.*, 1987).

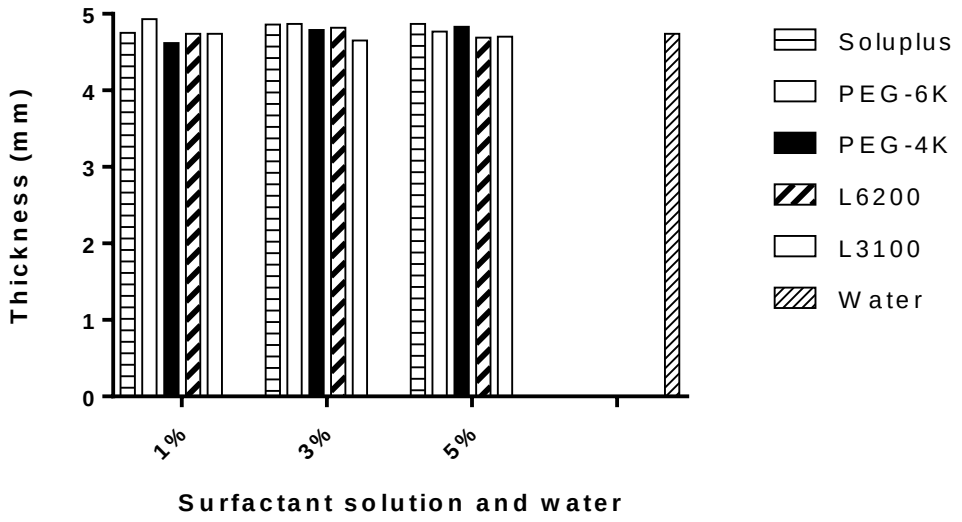
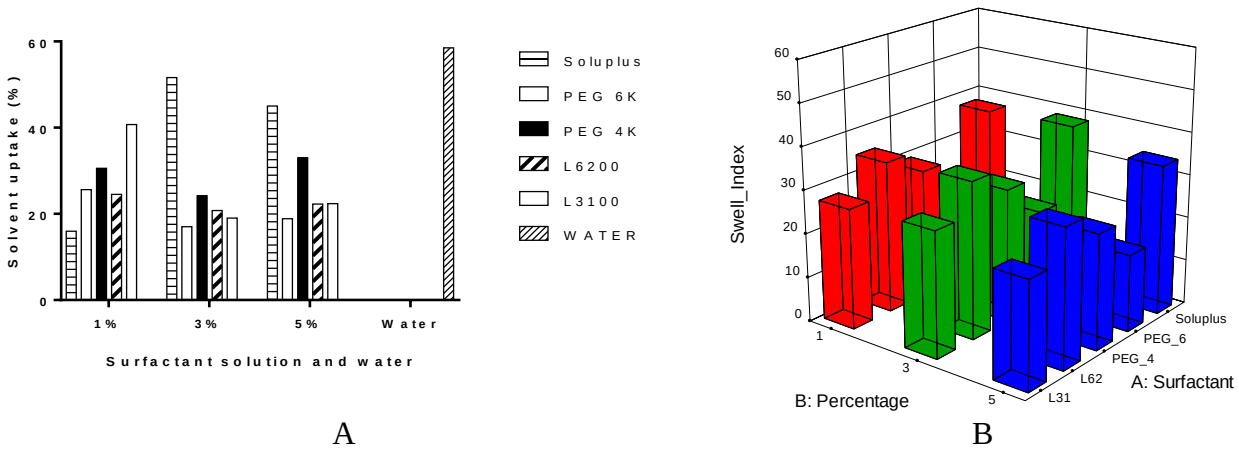


Figure 4.10: Thickness of ketoprofen matrix tablet formulations

4.7.1.6 Swelling index and behavior

Swelling index and the behavior of the ketoprofen matrix tablets, studied under Stereo zoom microscope from 0 to 6 h has been presented in Table 4.4 and Figure 4.11. The solvent uptake values found for all formulations ranged between a low value of 15.98% to higher values of 58.54 % for MT1 and MT16, respectively.

As shown in Figure 4.11B, the highest solvent uptake was seen in the Soluplus® at all the concentration, though it was slightly higher in case of 3% Soluplus®. Figure 4.12 reports that the ketoprofen tablets prepared using the all concentrations of PEG-6K, showed lesser solvent uptake capacity. For the rest of all surfactants, particularly at 1 and 3% concentrations showed increases swelling of the matrix tablets (Figure 4.12).



A B

Figure 4.11: Solvent uptake of ketoprofen matrix tablet formulations, as indicated by: (A) linear graph of solvent uptake and (B) 3-dimentional surface plot

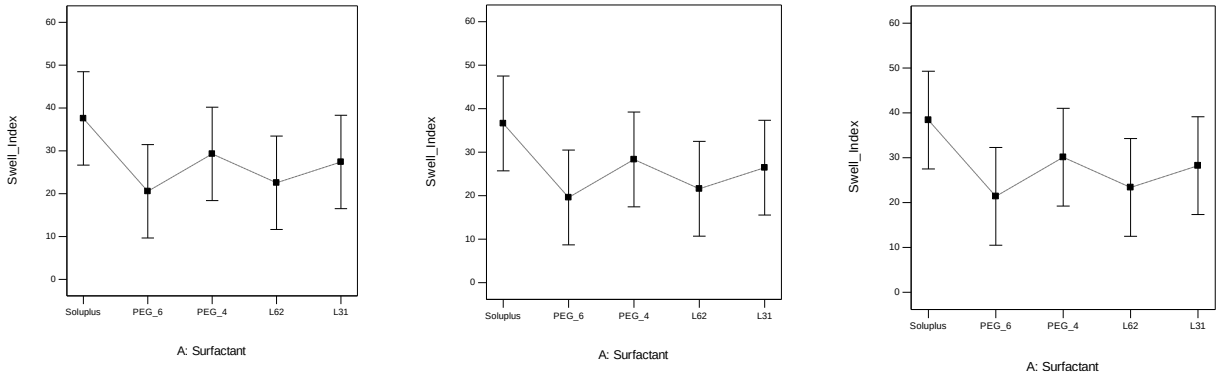
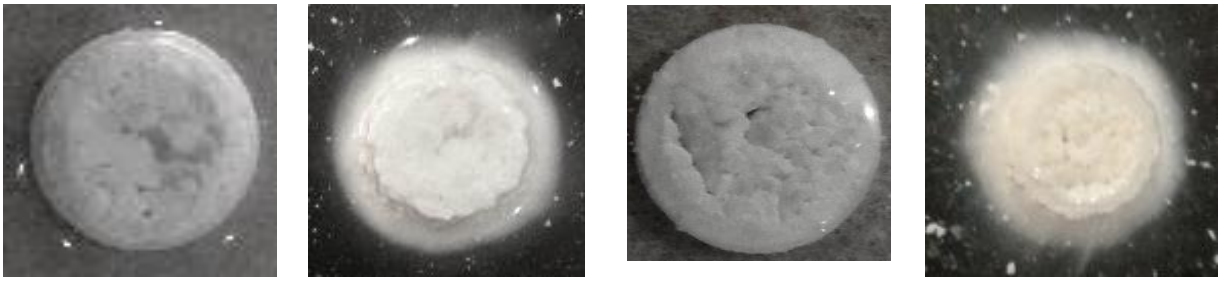


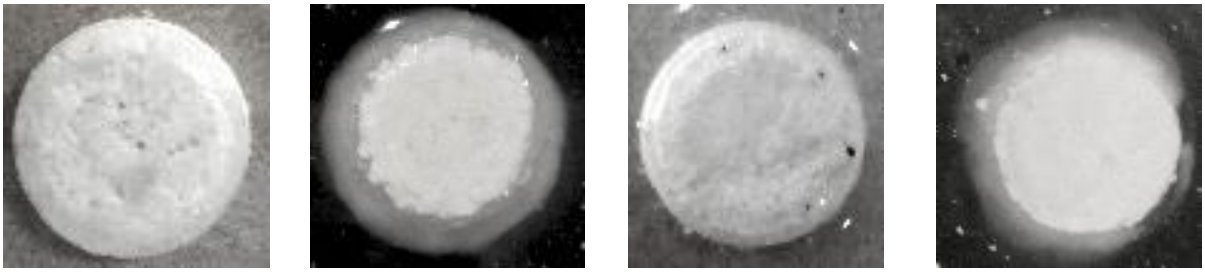
Figure 4.12: Effect of percentage and types of surfactants on the swelling index of ketoprofen matrix tablets

Change in physical appearance and weight of matrix tablets was noted at 6 h and has been given in Figure 4.13. The Figure showed gel formation, swelling and erosion. The erosion is triggered by the hydrolysis-led break down of cross-links, solubilization of insoluble polymer or degradation of water-labile bonds in the polymeric back bone. The HPMC-based hydrophilic intra-granular matrix in aqueous media forms a gel layer around the tablet followed by hydration-led swelling of the polymer which results into increased size due to the penetration of media in the matrix (Johnson *et al.*, 1993; Lee *et al.*, 1987; Lindner *et al.*, 1995; Peppas *et al.*,

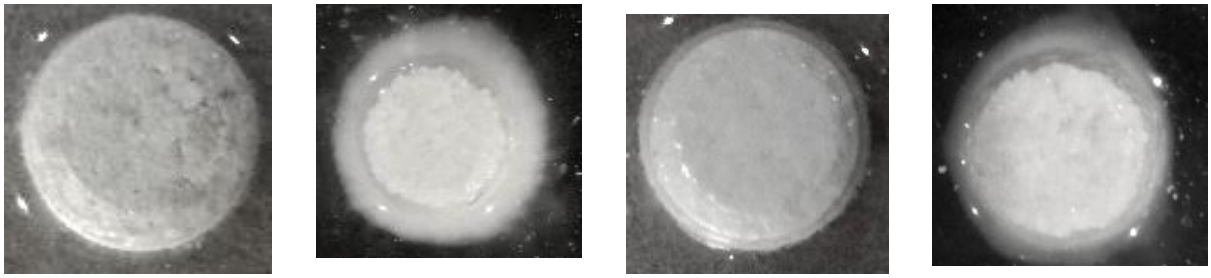
1980; Skoug *et al.*, 1993). The presence of intragranular HPMC in the tablet was shown to be effective as matrix which made the matrix tablet formulations as swellable as well as erodible for release of ketoprofen (Figure 4.13). Avicel® and Crospovidone® also facilitate swellability, inhibit penetration of dissolution media and reduced wettability of tablets (Lee *et al.*, 1999). Apparent change in tablets from smooth appearance to a rough or erosive tablet corner was noted in all formulations which was found almost the same but with different extent of erosion. Most of the formulations showed erosion (Figure 4.13) coupled with a diffusion mechanism to release ketoprofen. Formulation prepared as control (MT16) showed maximum solvent uptake and showed rapid release of drug (Figure 4.10A). Swelling behavior of most of the matrix formulations indicates their release mechanism as diffusion which serves as rate determining step with parallel erosion from the smooth surface of tablets. To support the release mechanism of drug from the matrix tablets, it has been related to the n values computed from Korsmeyer-Peppas release model and other factors in Section 4.7.1.8.



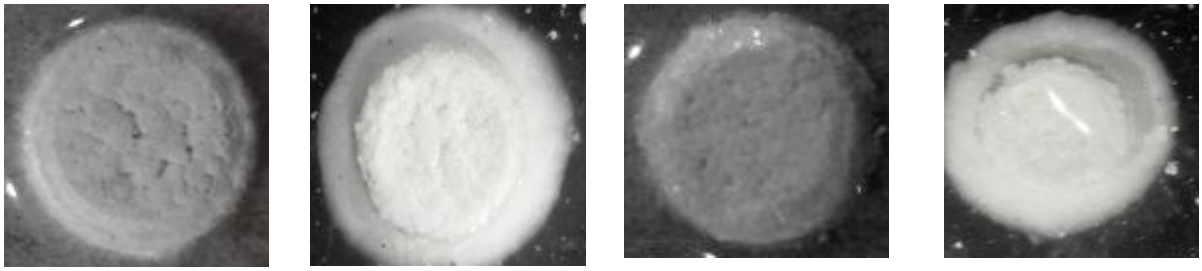
MT1 MT2



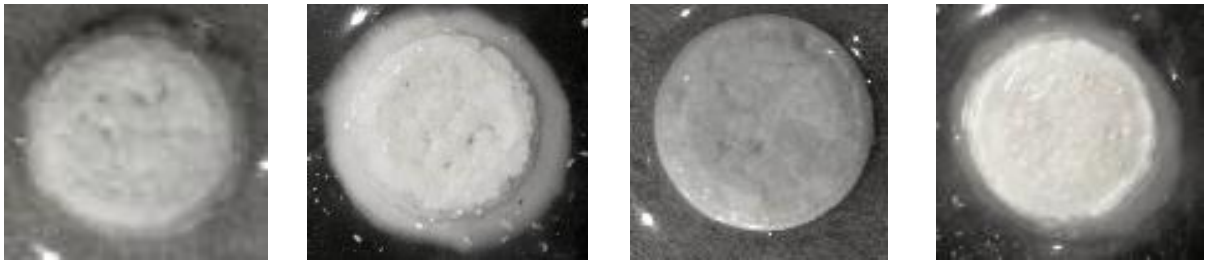
MT3 MT4



MT5 MT6



MT7 MT8



MT9 MT10

Figure 4.13: *Continued…*

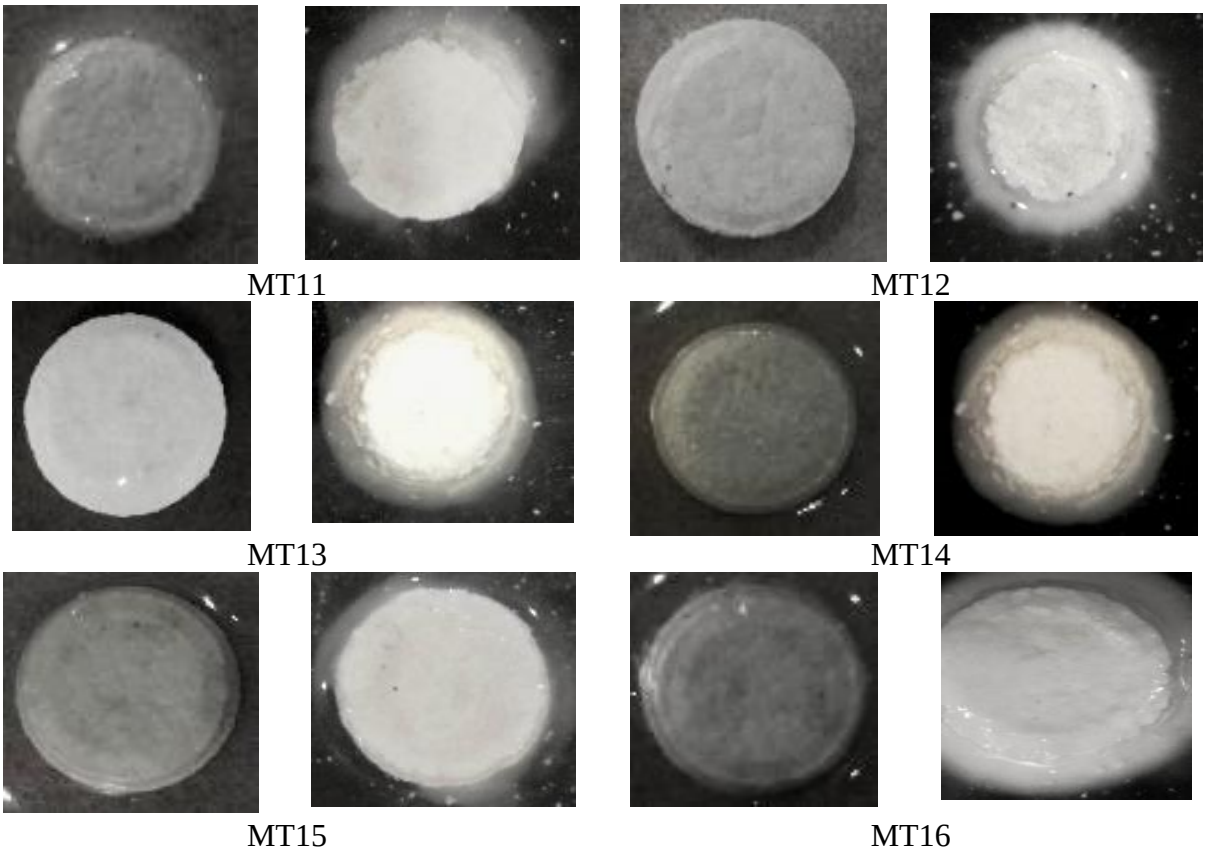


Figure 4.13: Swelling behaviour of mattix tablets of ketoprofen at 0 to 6 h

4.7.1.7 Dissolution studies

In-vitro dissolution study of ketoprofen formulations were performed for 8h using buffer solutions with respective time frame, i.e., 2h in simulated gastric media (pH 1.2) followed 6h in simulated gastrointestinal media (pH 6.8), both were without the enzymes. The comparative release of ketoprofen from all the matrix tablet has been given in Table 4.5 and Figure 4.14. For the better understanding of the release, the release has been categorized as ketoprofen release from tablets prepared using 1%, 3% and 5% of surfactants in Figure 4.15A-4.15C, Figure 4.16A-4.16C show the matrix table formulations with highest, lowest and sustained release profiles, respectively. Figure 4.16 demonstrates the combined effect of surfactant types and concentrations on the ketoprofen release from tablets.

Release of the tablet formulations shown to be with varied magnitudes of release inhibition which was dependent on the concentration and the type of surfactants (Table 4.5). Formulations prepared using 1% surfactants showed release in range of 60-90%, 3% surfactant showed 60-100% and 5% surfactant concentrations showed almost 100% at earlier times (Figure 4.14) as compared to 1% and 3% concentrations. This trend showed that as the surfactant concentration increased, the percent drug release was also increased and completed in early time.

Release specifications for sustained release formulations, specified according to USP are 15-40%, 25-60%, 35-75% and >70%, at 1h, 2h, 4h and 8h, respectively as have been shown in Section 3.4.5.1. Release of almost four formulations out of sixteen, i.e., MT2, MT3, MT6 and MT13 met the USP specifications for sustained release formulations.

The control formulation MT16 showed an entirely different release profile, it released ketoprofen greater than 50% in the acidic media, showing an uncontrolled release (Figure 4.14). However, the release in acidic media was almost controlled in all other formulations prepared by using the surfactant which remained about 20%. Soluplus® matrix tablets (MT2 and MT3) showed ideal sustained release in respective buffer media, i.e., < 20% in acidic media and > 80% till 8h. PEG-6K formulation (MT4 and MT5) showed > 60% release in basic media till 8h. Although these specified formulations showed appropriate release in acidic media, i.e., < 20% but could not meet the release specifications stipulated for the sustained release dosage forms (Section 3.4.5). MT6 showed almost 98% ketoprofen release in 7h. PEG-4K formulation (MT7) showed slowest release among all formulations, i.e., 59.9% until 8 h. Its 3% (MT8) and 5% (MT9) formulations exhibited complete release in 6h and 3h, respectively. MT4 (1% PEG-6K), MT7 (1% PEG-4K) and MT10 (1% L6200) inhibited ketoprofen release to an extent which led it varied from the specifications of desired release pattern.

Table 4.5: Percent drug release (Mean±S.D) of ketoprofen sustained release tablets

Formulation Code	Percent drug release (Mean±S.D) at Time (h)							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
MT1	6.86±0.06	7.03±0.01	31.6±0.02	41.17±0.03	47.88±0.03	53.86±0.04	61.6±0.02	65.87±0.04
MT2	10.55±0.04	14.74±0.04	27.15±0.03	44.42±0.03	62.48±0.02	62.82±0.04	85.86±0.10	87.2±0.08
MT3	4.97±0.08	9.49±0.02	43.81±0.01	47.95±0.02	51.18±0.06	59.82±0.02	71.06±0.08	91.43±0.06
MT4	6.40±0.03	8.97±0.08	30.98±0.06	40.62±0.04	42.37±0.08	45.13±0.04	58.2±0.10	60.17±0.06
MT5	6.67±0.02	17.13±0.02	44.56±0.02	53.04±0.01	54.88±0.02	57.79±0.02	62.95±0.06	64.68±0.08
MT6	8.04±0.03	19.60±0.1	53.47±0.03	65.85±0.06	68.38±0.06	69.11±0.08	98.91±0.04	102.4
MT7	5.96±0.05	18.20±0.04	38.93±0.01	45.58±0.04	62.61±0.08	51.96±0.10	55.15±0.08	59.9±0.10
MT8	8.57±0.04	18.45±0.02	40.55±0.01	63.02±0.02	65.8±0.02	106.76±0.08		
MT9	10.24±0.02	20.46±0.01	116±0.09					
MT10	9.91±0.08	13.42±0.02	37.23±0.02	41.5±0.05	46.73±0.02	49.69±0.04	54.2±0.05	60.15
MT11	9.85±0.06	19.20±0.04	35.28±0.03	51.82±0.02	65.94±0.04	114.91±0.06		
MT12	6.54±0.04	9.75±0.01	30.81±0.08	50.53±0.04	51.48±0.06	106.9±0.08		
MT13	8.04±0.04	11.87±0.03	39.13±0.06	53.18±0.01	61.53±0.02	62.82±0.04	67.61±0.08	79.56±0.08
MT14	8.15±0.02	14.47±0.02	35.75±0.07	57.25±0.06	64.85±0.01	119.12±0.02		
MT15	7.99±0.04	13.34±0.01	32.28±0.01	47.21±0.02	58.88±0.04	84.23±0.06	101.73±0.06	
MT16	25.98±0.05	53.56±0.06	83.6± 0.04	101.5±0.05				

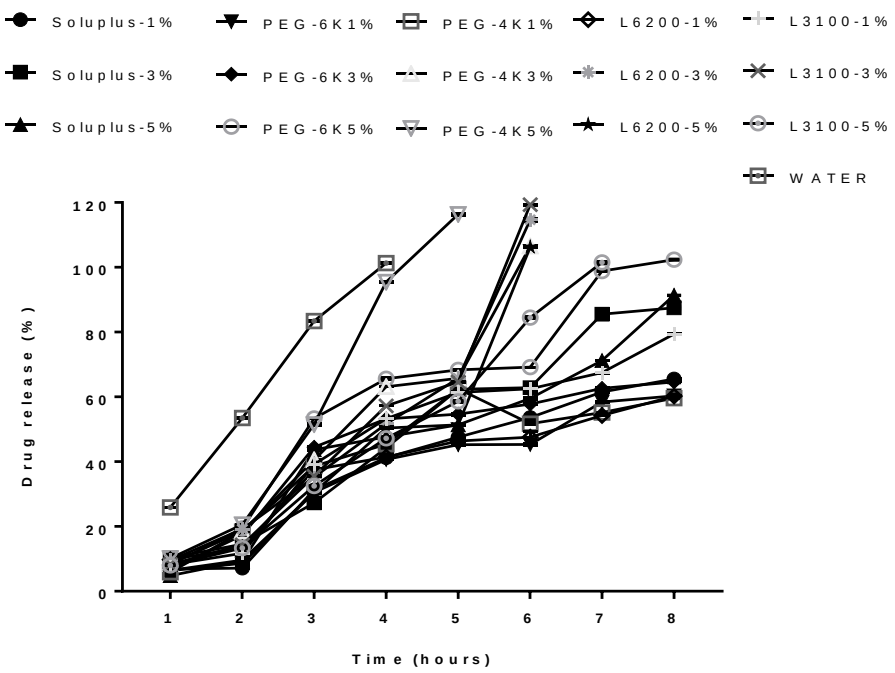
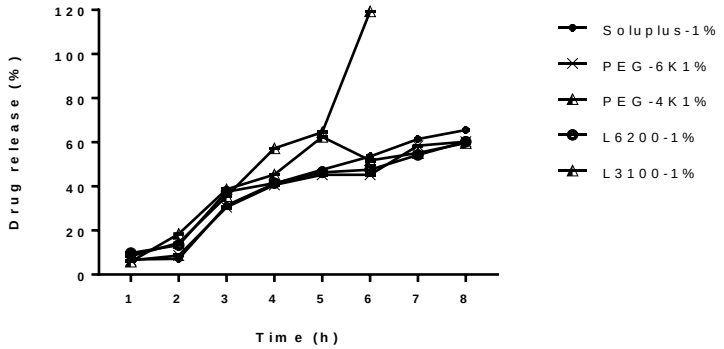


Figure 4.14: Comparative release (%) of ketoprofen from granules prepared by 1-5% surfactants

The formulation was also HPMC-matrix-based and in this respect, it was expected to release ketoprofen in a controlled manner. Converse was true for this formulation, which was indicative of the effect of surfactants in sustaining the release exerted through hydrogen bonding and other drug-surfactant interactions within granules carried on in the tablet formulations as well (Section 3.4.5.1).

The Figure 4.17 shows the combined effect of types and concentrations of surfactants on the release of ketoprofen at different time intervals. This Figure helped to observe which surfactant and concentration caused the release of ketoprofen within the stipulated % release specifications of the sustained release systems.



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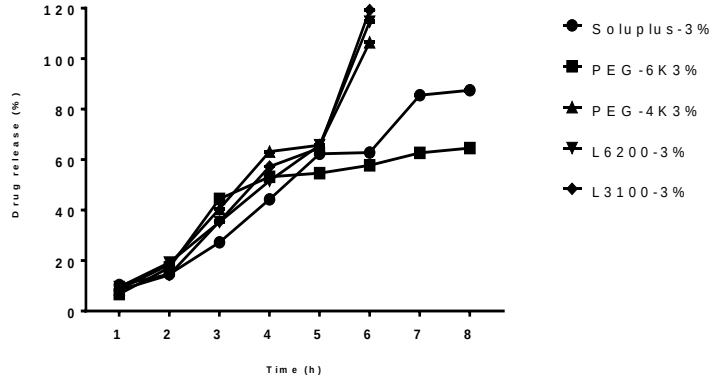

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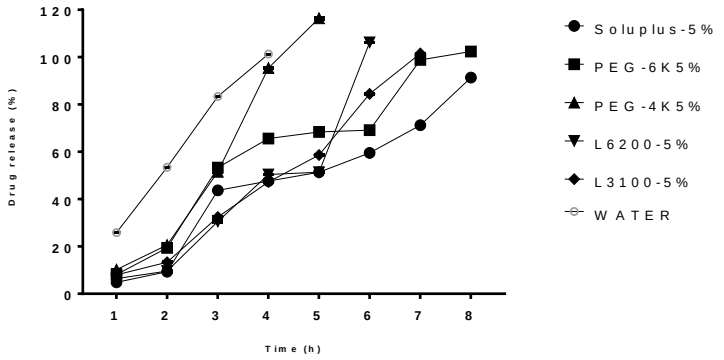

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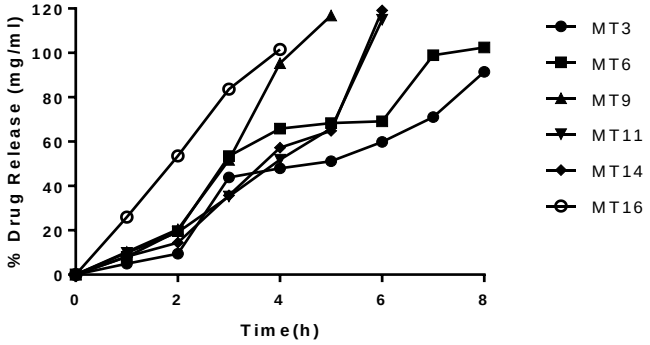
Figure 4.15: Percent release of ketoprofen from matrix tablets prepared by surfactants: (A) 1%, (B) 3% and (C) 5%



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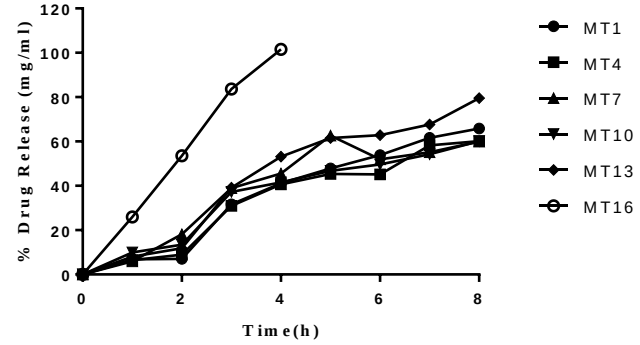

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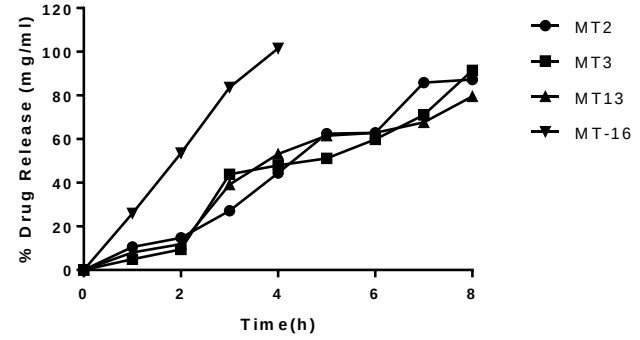


C

Figure 4.16: Ketoprofen matrix tablets showing: (A) highest drug release (B) slowest drug release and (C) sustained release pattern among the categories of the study surfactants as compared to the control

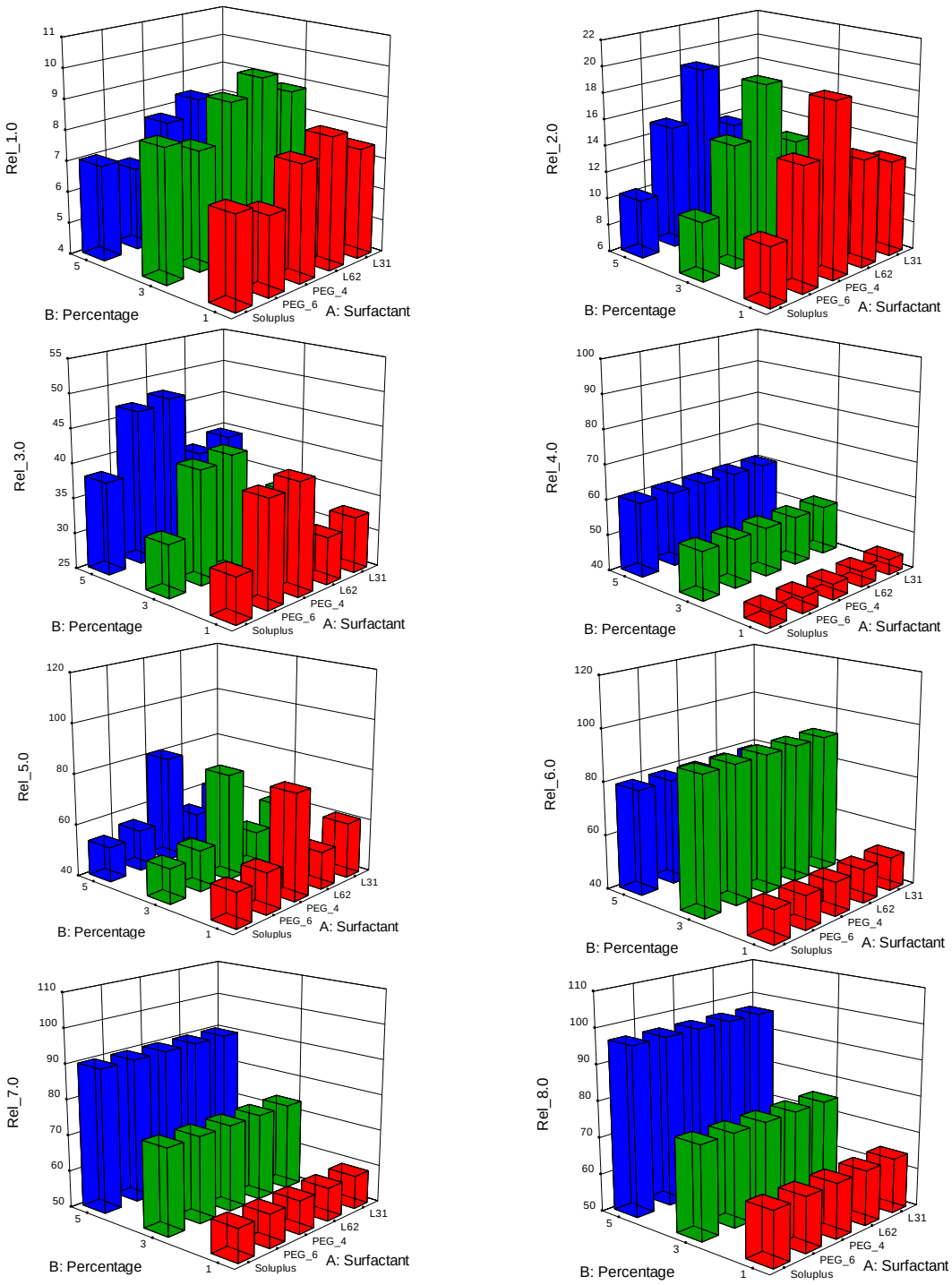


Figure 4.17: Combined effect of the types and percentage of surfactants on the release of ketoprofen from matrix tablets at different time intervals

The hardness of MT 8 and MT 9 was 6.89 and 6.03 $Kg.cm^{-2}$, respectively. Similarly, the other formulations with hardness values closer to the above formulations, MT6, MT12 and MT15 with hardness of 6.36, 6.48 and 6.62 $Kg.cm^{-2}$, respectively released more than 98% ketoprofen earlier than 8 h, i.e., at 7, 6, and 7h, respectively. The effect of hardness looks operative in the early release of ketoprofen from the above matrix tablet formulations.

An early release could also be related to the type and the amount of the surfactant. The L6200 matrix tablets (MT10-MT12) also showed the increased release trend when surfactant concentration in the formulation was increased from lowest to highest. MT11 and MT12 released the entire (100%) ketoprofen within 6h. However, MT10 withheld ketoprofen and released up to 60% ketoprofen in 8h. The ketoprofen release from the tablet formulation prepared using L3100, formulation MT13 (1%) met the desired sustained release characteristics (Farheen *et al.*, 2017) at every specified time interval. The MT14 and MT15 showed more than 100% release at 6 and 7h respectively. The formulations which followed sustained release pattern were MT2, MT3, MT6 and MT13.

4.7.1.8 Release kinetics

The release kinetics data and respective coefficients of correlation (R^2), calculated using DDSolver ver 1.0 have been given in Table 4.6. All formulations specifically prepared with 1% surfactant concentration followed Weibull release model which supports matrix type release as explained in Section 3.3.6. The Soluplus® has been reported to serve as a matrix of solid dispersions to assist dissolution of drugs (Bhuptani *et al.*, 2016; Nagy *et al.*, 2012) hence could be implied that in this study, the use of surfactants in granulation have resulted into a matrix type delivery system.

Table 4.6: Release kinetics of ketoprofen sustained release tablets

Formulation	Zero Order		First Order		Higuchi		Hixon Crowell		Weibull			Korsmeyer Peppas		
Code	R^2	AIC	R^2	AIC	R^2	AIC	R^2	AIC	R^2	AIC	ß	R^2	AIC	n
MT1	0.94	44.95	0.92	46.96	0.78	55.65	0.94	45.19	0.97	44.47	1.08	0.94	46.86	0.97
MT2	0.96	45.27	0.86	56.11	0.75	61.02	0.91	53.17	0.98	44.74	2.57	0.97	45.91	1.11
MT3	0.93	50.16	0.86	55.91	0.74	60.78	0.89	53.90	0.93	54.27	1.30	0.93	51.97	1.06
MT4	0.92	45.37	0.93	44.51	0.79	53.22	0.94	43.76	0.95	45.34	0.93	0.93	46.66	0.90
MT5	0.79	54.26	0.91	47.63	0.82	53.10	0.89	48.96	0.95	47.44	0.69	0.87	52.23	0.70
MT6	0.93	52.97	0.85	58.67	0.79	61.37	0.89	55.85	0.93	56.13	1.43	0.93	54.49	0.91
MT7	0.74	55.13	0.87	49.73	0.79	53.38	0.84	51.01	0.91	50.37	0.63	0.83	53.49	0.68
MT8	0.91	40.00	0.75	46.28	0.65	48.39	0.80	44.88	0.94	42.04	5.22	0.96	36.77	1.43
MT9	0.89	36.38	0.65	42.03	0.60	42.74	0.72	40.96	0.96	35.19	8.85	0.98	30.24	1.62
MT10	0.87	48.01	0.94	41.50	0.85	48.87	0.93	42.87	0.95	44.10	0.78	0.93	45.39	0.74
MT11	0.86	43.47	0.68	48.48	0.58	50.05	0.73	47.41	0.91	44.68	7.64	0.96	37.23	1.77
MT12	0.80	45.18	0.66	48.44	0.53	50.34	0.70	47.64	0.90	45.20	7.06	0.93	40.56	1.98
MT13	0.91	50.08	0.92	50.07	0.80	57.11	0.93	48.22	0.96	48.07	1.06	0.92	51.12	0.88
MT14	0.84	45.05	0.66	49.70	0.56	51.15	0.71	48.69	0.90	46.33	7.34	0.96	38.90	1.85
MT15	0.94	44.67	0.78	53.79	0.67	56.64	0.83	51.92	0.98	39.77	6.37	0.99	30.58	1.45
MT16	0.99	16.44	0.84	27.08	0.81	27.77	0.91	24.70	1.00	16.30	6.56	0.99	17.60	0.94

Thus, the formulations MT1-MT3 showed ß value >1 which indicated a steeper release while MT4-MT5, MT7 and MT10 exhibited sigmoidal release pattern with the period of initial lag time. All ketoprofen granule (chapter 3) formulations also followed a steeper release mechanism with β value >1. Most of the formulations prepared with 3% (MT8, MT11, and MT14) and 5% (MT9, MT12, MT15) surfactant concentration, followed Korsmeyer-Peppas release, except MT3 and MT6 which followed zero order. Formulation prepared as control (MT16) also followed zero order release. It supported the matrix release system of modified dosage form with the low solubility drug. The value of diffusion coefficient, n computed by Korsmeyer-Peppas model can guide about the diffusional release mechanism from a system. The value of n interprets four variable release mechanisms, i.e., (a) the value of n = 0.45 represents classical Fickian diffusion transport, (b) n value between 0.45 and 0.89, corresponds to Non-Fickian transport or an anomalous (i.e., combined diffusion-relaxation) release, (c) n = 0.89, represents Case II transport (purely matrix relaxation or erosion-mediated release with zero order release) and (d) n > 0.89 indicates super case II release (diffusion-erosion). All the prepared ketoprofen matrix tablets which followed Korsmeyer-Peppas release yielded n value >1 which supported the release mechanism of ketoprofen as diffusion and erosion exponentially entitled as super case II transport (Dash *et al.*, 2010b).

The swelling behavior of ketoprofen matrix tablets in relation with n values derived from Korsmeyer-Peppas model, have been presented in Table 4.7. The drug release could be related with the composition of the matrix, i.e., HPMC, Avicel®, Crospovidone and the surfactants used for development of the granules in SAWG approach. The swelling behavior also dictates the release of the drug from the matrix system. The above may affect the ketoprofen release from matrix tablets in different combinations.

Table 4.7: Swelling index and the release mechanisms of ketoprofen matrix tablet formulations

Code	Swelling Index	n-value	Drug transport mechanism	
MT1	15.98	0.97	Case II transportation	Zero order relaxation
MT2	51.60	1.11	Super case II transportation	Diffusion & erosion
MT3	45.00	1.06	Super case II transportation	Diffusion & erosion
MT4	25.63	0.90	Case II transportation	Zero order relaxation
MT5	17.04	0.70	Non-Fickian	Anomalous
MT6	18.87	0.91	Case II transportation	Zero order relaxation
MT7	30.59	0.68	Non-Fickian	Anomalous
MT8	24.17	1.43	Super case II transportation	Diffusion & erosion
MT9	32.99	1.62	Super case II transportation	Diffusion & erosion
MT10	24.50	0.74	Non-Fickian	Anomalous
MT11	20.75	1.77	Super case II transportation	Diffusion & erosion
MT12	22.28	1.98	Super case II transportation	Diffusion & erosion
MT13	40.70	0.88	Non-Fickian	Anomalous
MT14	19.01	1.85	Super case II transportation	Diffusion & erosion
MT15	22.39	1.45	Super case II transportation	Diffusion & erosion
MT16	58.54	0.94	Case II transportation	Zero order relaxation

As the Avicel® and Crospovidone® facilitate the tablet swellability, retard penetration of dissolution media and hydration of tablets, as stated previously, the drug release is withheld at its initial hours (Lee *et al.*, 1999). On contact with aqueous or GIT media, HPMC forms gel layer around tablet followed by hydration leading to swelling, increase tablet size, dissolution of the soluble and erosion of insoluble portion of drug from matrix and release the drug from tablet (Johnson *et al.*, 1993; Lee *et al.*, 1987; Lindner *et al.*, 1995; Peppas *et al.*, 1980; Skoug *et al.*, 1993). The drug release depends on the thickness of gel layer, and the degree of polymer swelling (Johnson *et al.*, 1993; Skoug *et al.*, 1993; Sujja-

Areevath *et al.*, 1998). Thus, the system becomes swelling control, the release from which is controlled by the penetration rate of media and erosion of matrix (Tahara *et al.*, 1995). Soluplus also serves as a matrix of solid dispersions to assist dissolution of drugs (Bhuptani *et al.*, 2016; Nagy *et al.*, 2012). HPMC and Soluplus® enhanced the tablets capability to resist or retard the drug release for at least 8h.

Soluplus®, PEG-4k-PEG-6k, Pluronics® L6200-L3100 showed enhanced binding ability of matrix tablets which retards the drug release as compared to control (MT16). Surfactants along with the role of high molecular weight excipients, i.e., HPMC-K4M, Avicel and Crospovidone stated previously added up the dominancy of predictive diffusion and cleared surface erosion drug release mechanism. The diffusion and erosion mechanism was supported by their corresponding exponential value 'n' in Korsmeyer-Peppas model. Out of sixteen formulations, eight formulations represented to hold 3% (MT2, MT8, MT 11, MT 14) and 5% (MT3, MT9, MT12, MT15) surfactant concentrations showed $n > 1$ which depicts the super case II transport release entitled to predominate the diffusion and erosion drug transport mechanism. Control (MT16) showed exponential value as $0.89>n<1$ and increased burst release in initial hours i.e., 2h-3h caused the matrix tablets to become more porous which precipitated the factor of rapid dissolution from dosage form. This unusual behavior also showed by MT9 which also unable to retain the drug till late hours and released the drug at 3 h.

Matrix system based on swelling usually pertained numerous pores on the tablet surface which resulted in grooves in delayed hours of dissolution (Peppas *et al.*, 2014). Pore formation were the dominant feature responsible for drug diffusion from the polymeric matrix, enforced to erode and dissolve in particular dissolution media (Nokhodchi *et al.*, 2012).

Formulation prepared as control (MT16) showed maximum solvent uptake capacity and showed rapid release in terms of drug dissolution profile. Surfactants used with 3% and 5% concentrations except in case of PEG-6K, followed super case II transport which supports diffusion and as well as erosion release mechanism. The corresponding n value for MT5 (3% PEG-6K) and MT6 (5% PEG-6K) supports the anomalous or non-Fickian and case II zero order release behavior, respectively. The tablet with 1% surfactants concentrations of PEG-4K, L6200 and L3100 showed non-Fickian release behavior while of Soluplus® and PEG-6K depicts zero order relaxation matrix release system. Swelling of HPMC has been regarded as critical factor for the release of ketoprofen from sustained release systems containing HPMC (Roda *et al.*, 2002).

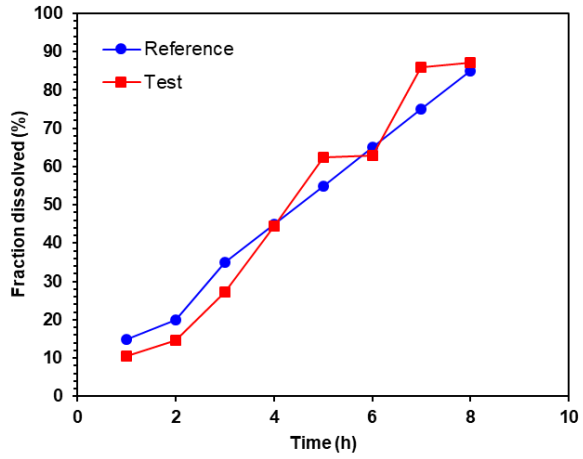
4.7.2 Comparative release of test and reference release specifications

The dissolution profiles of all ketoprofen tablet formulations were compared to the specification of reference release (Table 4.3 and Figure 4.18) using dissimilarity (f_1) and similarity (f_2) factors. The f_1 and f_2 values demonstrate the closeness of the dissolution profile with the desired (reference) release profile. The values of f_1 and f_2 of all formulations (MT1-MT16) have been mentioned in Table 4.9. The profiles of release of MT2, MT3 and MT13 as compared to the reference release profile have been given in Figure 4.11A-C. Formulations MT2, MT3 and MT13 showed dissimilarity and similarity factors within acceptable range of <15 and >50, respectively (Zhang *et al.*, 2010). However, among the above three formulations, only the sustained release formulation, MT2 showed f_1 and f_2 indicating a release profile closer to the reference release profile (Table 4.8).

Table 4.8: The similarity/dissimilarity of the ketoprofen matrix tablets to the stipulated sustained release profile

Code	Dissimilarity Factor	Similarity Factor	Remarks	Model fitted
MT1	20.04	47.69	Dissimilar	Weibull
MT2*	10.34	60.62	Similar	Weibull
MT3*	13.08	57.43	Similar	Zero
MT4	25.10	42.30	Dissimilar	Weibull
MT5	17.34	49.32	Dissimilar	Weibull
MT6	26.70	40.59	Dissimilar	Zero
MT7	20.49	44.23	Dissimilar	Weibull
MT8	35.8	35.57	Dissimilar	Korsmeyer-Peppas
MT9	21.88	78.93	Dissimilar	Korsmeyer-Peppas
MT10	21.93	43.56	Dissimilar	Weibull
MT11	31.44	33.71	Dissimilar	Korsmeyer-Peppas
MT12	31.08	37.31	Dissimilar	Korsmeyer-Peppas
MT13*	12.39	59.35	Similar	Weibull
MT14	38.02	31.62	Dissimilar	Korsmeyer-Peppas
MT15	22.07	44.04	Dissimilar	Korsmeyer-Peppas
MT16	130.14	19.23	Dissimilar	Zero

*Release of ketoprofen showed similarity to that of the desired release characteristics.



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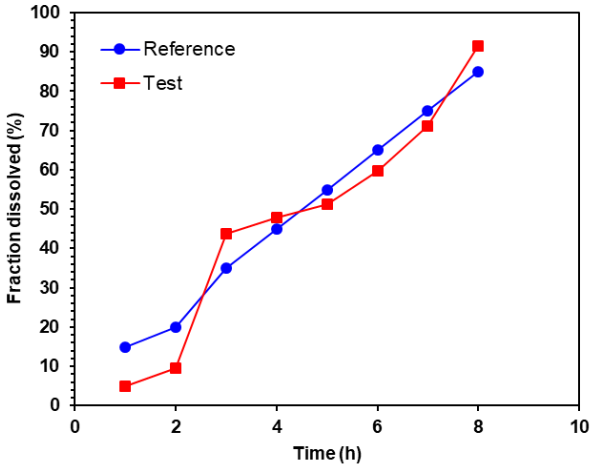

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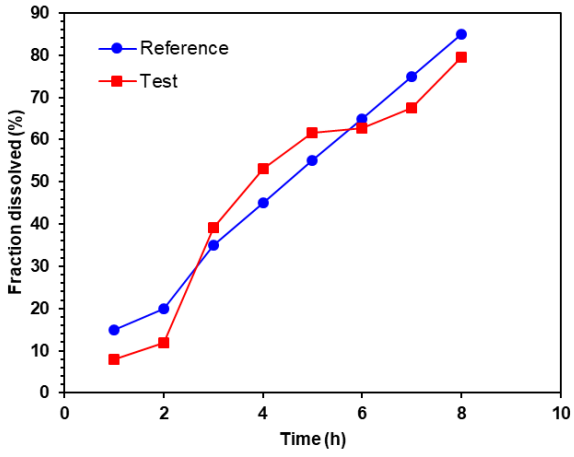

C

Figure 4.18: Release profiles of the reference release as compared to: (A) MT2, (B) MT3 and (C) MT13

4.7.2.1 Fourier Transform Infrared spectroscopy

FTIR spectra of ketoprofen matrix tablet dosage form neither showed any interaction nor depicts any incompatibility among drug and the excipient. All formulations showed characteristic peak of ketoprofen which narrates its compatibility with the incorporated excipients during formulation and especially during compression Figure 4.19.

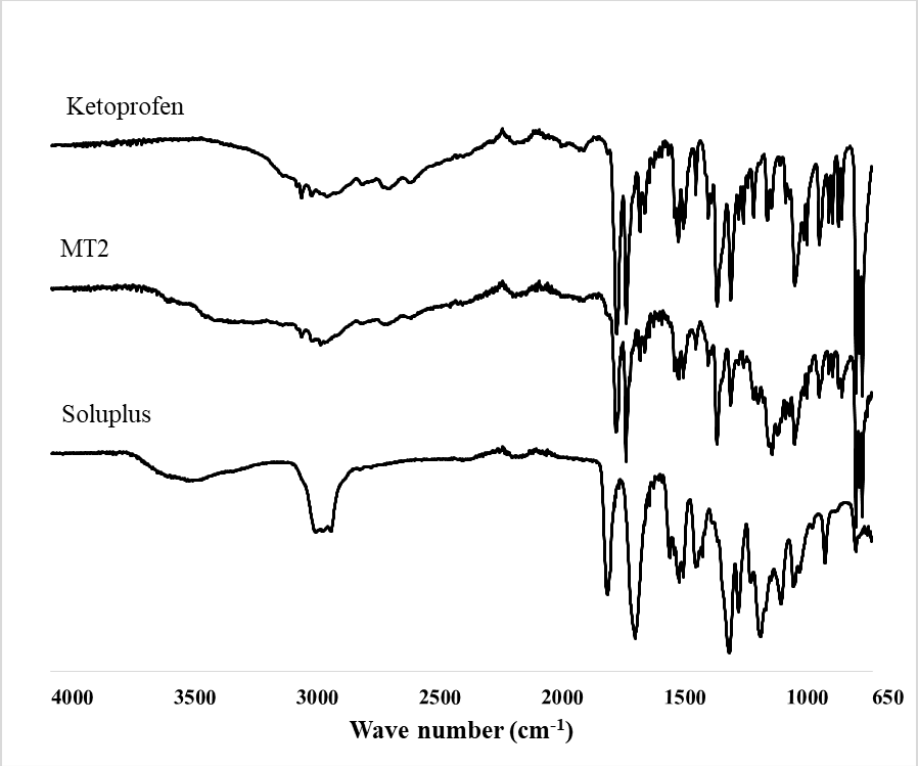

Figure 4.19: The FTIR scan of ketoprofen, Soluplus and MT2.

4.8 Thermo gravimetric analysis (TGA)/Differential scanning calorimetry (DSC)

The selected matrix tablet formulation was evaluated for the purity of the ketoprofen and its compatibility and interaction with excipients in the swellable matrix tablet using DSC model (SDTQ600, TA Instrument, USA). Alumina crucible used with ramp rate of 10°C and temperature range of 25°-300°C, provided nitrogen atmosphere with flow rate of 100ml/min.

Figure 4.20-4.22 show a TGA curves of ketoprofen, Soluplus® and that of the MT2 formulation. Ketoprofen showed thermal stability up to 228^{o} C, the initial weight loss was

due to evaporation of the moisture from the sample. A sharp decline starting from 228 °C represented the fast decomposition of ketoprofen (Yang *et al.*, 2008). TGA Curves of Soluplus® showed a gradual decrease in weight loss up to 200 °C. This represents that both drug and surfactant were stable in the working temperature during drying of the wet granulation process was much lower, i.e., 60 °C for 24 h than that of the temperature causing instability (Gupta *et al.*, 2016). Therefore, selection of surfactant and drug for the method can be justified. The formulation also showed a thermal stability comparable to pure ketoprofen i.e. up to 229 °C. The decomposition temperature of ketoprofen started from 229º C with slow decline up to 300 °C, representing a better stability attributable to the formation of hydrogen bonding between surfactant and drug, also supported by the FTIR.

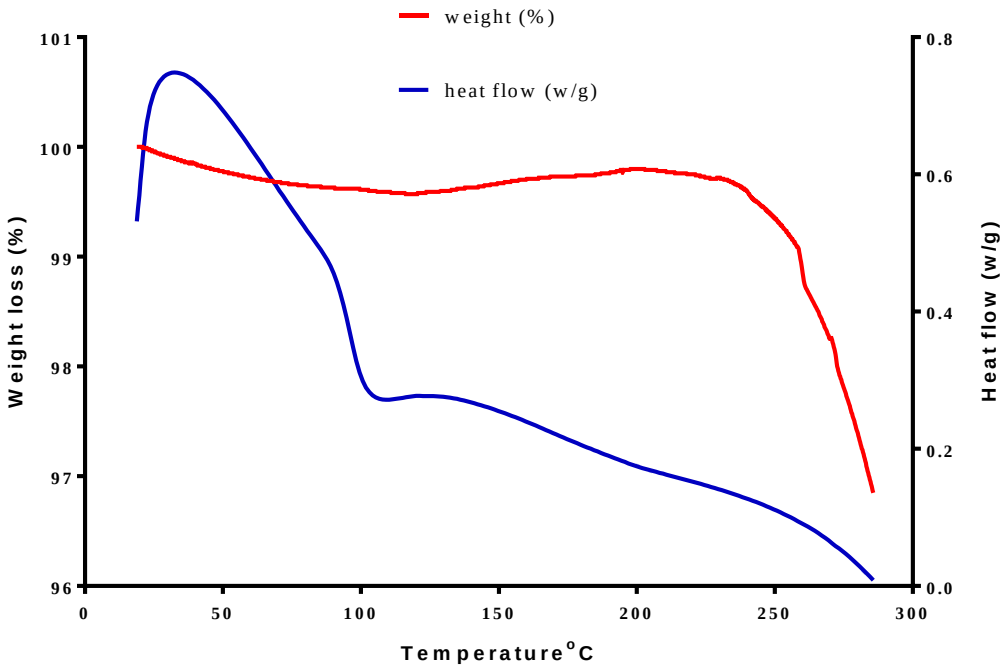

Figure 4.20: TGA and DSC thermogram of ketoprofen

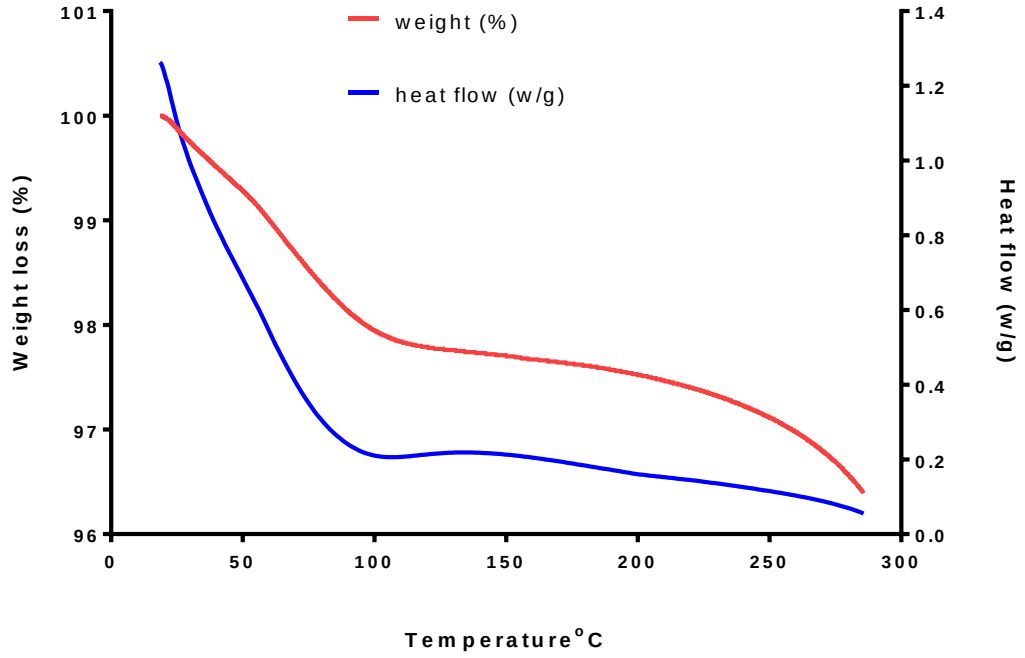

Figure 4.21: TGA and DSC thermogram of Soluplus®

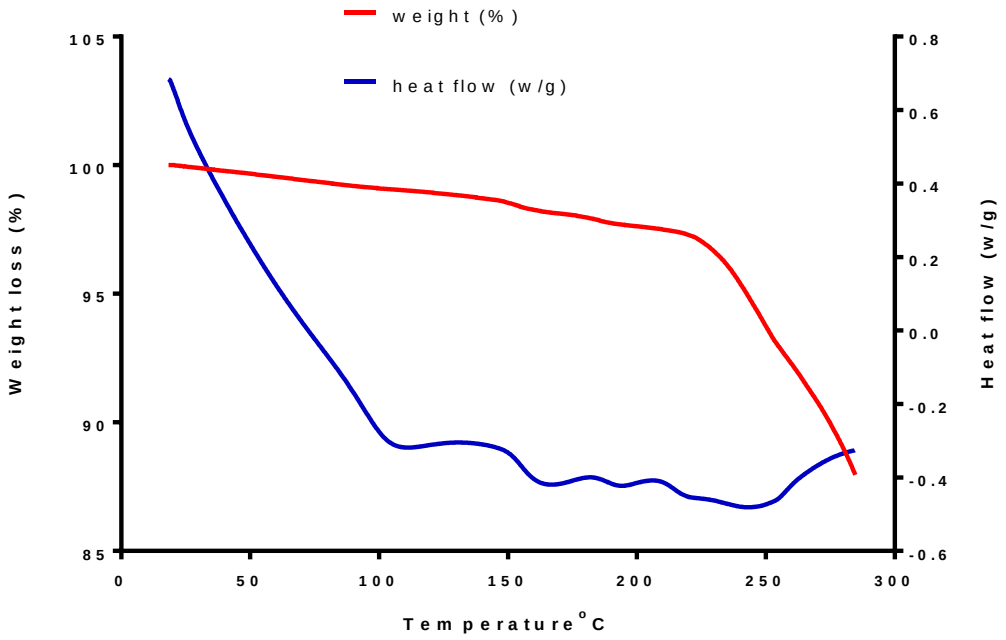

Figure 4.22: TGA and DSC thermogram of formulation MT2

The DSC thermogram of ketoprofen, Soluplus and MT2 formulation are given in Figures 4.20-4.22. The thermograph of drug shows a melting event starting from 92.16°C which was consistent with the literature value of the melting point of ketoprofen. No polymorphic transition was observed in the thermogram of ketoprofen (Üner *et al.*, 2005) The DSC thermograph of Soluplus® showed a broad endotherm starting at 73^{o}C, representing its melting, consistent with the literature values (>58^{o}C). However, the thermogram of formulation showed that the presence of the Soluplus® caused an increase in the melting point of ketoprofen by ~5^{o}C degrees, as shown by endotherm starting at 97°C. Increase in the melting point of ketoprofen reflected the promotion of physicochemical stability of the drug, might be due to a stronger intermolecular interaction. This findings is in line with the previous study where Soluplus® has been reported to increase the physicochemical stability of dutasteride, a poorly soluble drug (Lee *et al.*, 2015). The increase in the melting point of ketoprofen was assignable to hydrogen bonding between surfactant and carboxylic group of ketoprofen as supported by complementary findings of DSC and FTIR. The FTIR spectrum showed the shifting of peaks of functional group. Hydrogen bonding can also be a reason for micellization, as evident from the FTIR and DSC studies.

4.8.1 Selection of the tablet dosage form for further study

The formulation meeting the desired criteria (minimum friability, higher hardness, higher content uniformity, and maximum swelling index with desired release profile) was selected for the further pharmacokinetic study and establishing the IVIVC. With reference to the friability and content uniformity, all the formulations MT1 to MT15 were appropriate. However, the matrix tablet formulations MT6-MT9, MT12 and MT15 did not show the appropriate hardness, thus were excluded on grounds of their non-compliance to the specifications for hardness. Formulations MT1, MT4-MT5, MT7 to MT8, MT10-MT12 and

MT14 to MT15 did not meet the USP specifications for sustained release formulations, so were excluded from any further study.

Only MT2, MT3 and MT13 (Table 4.8) were with minimum friability, higher hardness, higher content uniformity, and the maximum swelling index (Table 4.4) with release profile similar to that of the sustained release dosage form (Figure 4.18A) thus, met the desired criteria. G2, corresponding granules of MT2 (having the similar composition), also showed the desired release criteria met by the MT2 tablet formation. However, the tablet formulation prepared using 3% Soluplus® showed a closer release profile to that of the desired sustained release formulation along with lesser safety risk, due to lesser amount of Soluplus® were the reason to select the MT2 for the pharmacokinetic study and for IVIVC (OECDguidelines, 2018c). Furthermore, compression into tablet yielded improved features in the resultant dosage form. The other features of the MT2 that made it appropriate for further study have been given in Figure 4.23 which also shows the levels of factors required to achieve the above (desired) characteristics of MT2.

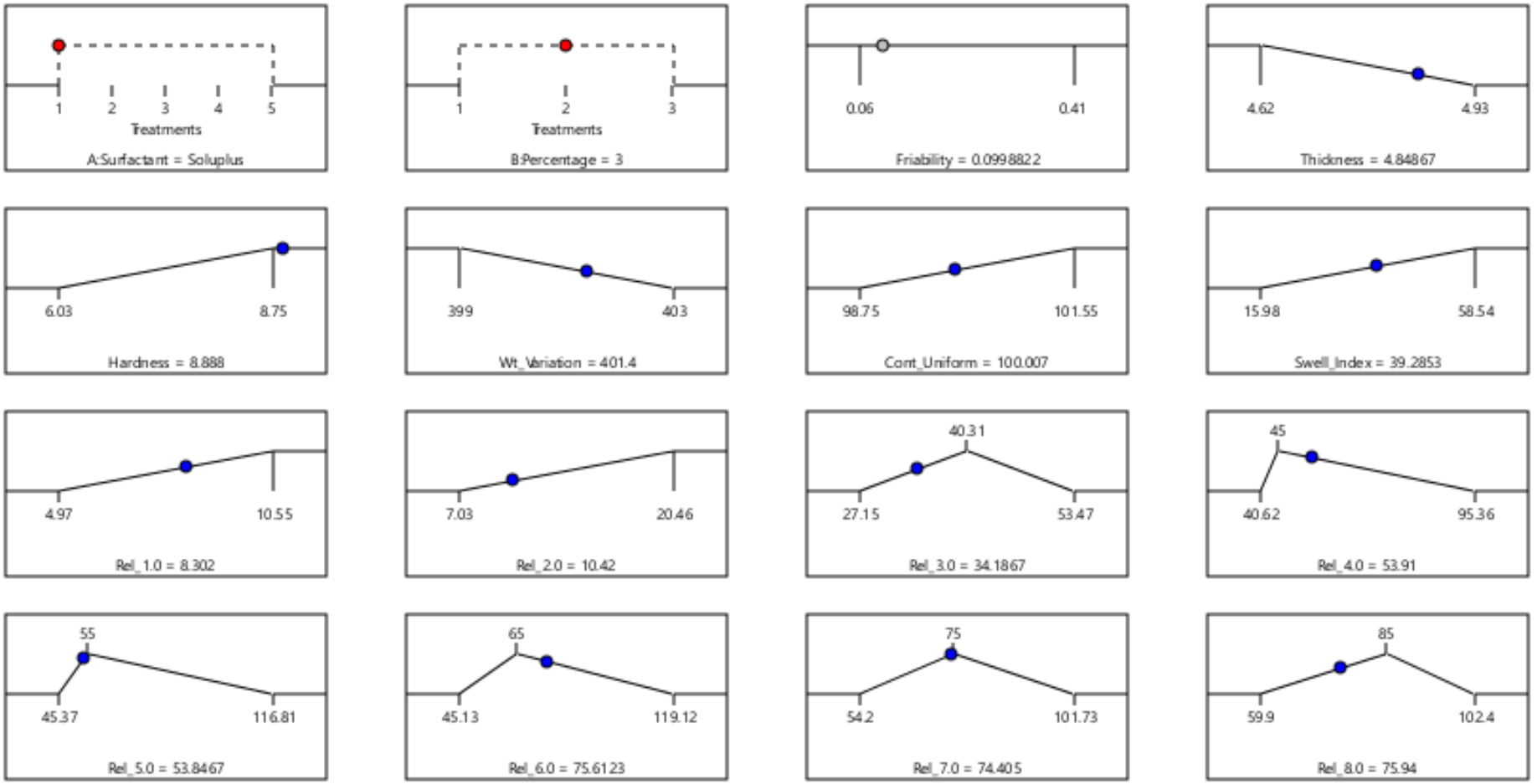

Figure 4.23: Settings of factors' levels for the desired (predicted) properties of MT2

4.9 Conclusion

Matrix tablets were successfully developed using the concentration, i.e., 1%, 3% and 5% of Soluplus®, PEG-6K, PEG-4K, L6200 and L3100. Friability, thickness, hardness, weight variation, drug content and swelling index for majority of the formulation within the aspired specifications. The formulations MT2 and MT13 depicted the ideal sustained release pattern till 8h which showed a complex release mechanism. FTIR indicated lack of chemical interactions between drug and excipient during compression. A tablet formulation with similar profile to that of the sustained release (MT2) was selected for pharmacokinetic study.

Chapter 5

Pharmacokinetic study of ketoprofen swellable-erodible matrix tablet

5.1 Introduction

The pharmacokinetics is the kinetics of drug absorption, distribution, metabolism and excretion following its administration. Each dosage form has its specific developmental aim. The pharmacokinetic assessment helps to ensure accomplishing the aim for the development of a dosage form (Shargel *et al.*, 2016). Pharmacokinetics characterizes the drug absorption, distribution and elimination. Each pharmacokinetics process has certain matrices. For instance, the absorption of a drug could be interpreted using the pharmacokinetic parameters for the absorption, which includes the area under the curve ($AUC_{0-\infty}$), peak blood concentration (C_{max}), time to reach C_{max} (T_{max}), rate of absorption (Ka) and absorption half-life ($t_{1/2}ab$). Volume of distribution (Vd) is the distribution parameter. Elimination process is indicated using the rate of elimination (K_{el}), half-life ($t_{1/2el}$), and clearance (Cl_T). Kinetics of the elimination process helps to find the dose, dose frequency, and also indicates the drug accumulation. In pharmacokinetic study, the above pharmacokinetic parameters are computed using compartmental or non-compartmental approach. In compartmental approach, the body is considered as having compartments where the drug is distributed. This approach gives more pharmacokinetic information, i.e., on drug distribution as compared to the non-compartmental approach (Hutchinson *et al.*, 2014).

The aim of this part of study was to evaluate the pharmacokinetics of the selected ketoprofen matrix tablet, MT2 as well as MT16 tablet prepared using water as granulating solvent. The objectives of this study were:

1. To determine the plasma level time data for MT2 and MT16 and to construct plasma level time curves

2. To compute the absorption, distribution and elimination parameters for the plasma level time data
3. To compare the absorption matrices, i.e., concentration of ketoprofen at different time intervals, Peak plasma concentration (C_{max}), time to C_{max} (T_{max}) and area under curve ($AUC_{0-\infty}$) of the MT2 and MT16
4. To determine the relative bioavailability of MT2 and MT16

5.2 Materials, Human subjects and Methods

For the preparation of matrix tablets in this part of study, the materials used had been given in Section 3.2.1 and 4.3. Acetonitrile (HPLC grade), disodium hydrogen phosphate and glacial acetic acid were purchased from Sigma Aldrich®.

5.2.1 Human volunteers

Six healthy, non-smoker human volunteers comprising male and female of age 25-35 year of age, weighing 55-67 kg were recruited for this study. They were briefed about the purpose, objectives and the way of the study and an informed consent was obtained from the study participants. The medical examination was carried out to ensure that the volunteers were healthy and disease-free. Study was conducted after obtaining an ethical approval from the Human Ethical Committee, Punjab University College of Pharmacy, University of the Punjab, Lahore (HEC/PUCP/1954A, dated 14-03-2018). The volunteers did not take any medication during last 15 days prior to study. Prior to study the volunteers were fasted overnight to avoid any interference of the meal with the absorption of the drug. A standard breakfast and meal were served after 2 and 6 h post drug administration, respectively. The volunteers were remained present at the study site until the completion of the sampling.

5.2.2 Study design

The study was carried out first with the development and validation of HPLC method and then pharmacokinetics of the drug in dosage form with a before-after design where at first session, the six human volunteers received 200 mg of MT16 and in the second session, same dose of MT2 by the volunteers. The both sessions were separated with a washout period of 8 days to avoid any carry-on effect of the drug.

5.2.3 Determination of ketoprofen in human plasma using HPLC method

5.2.3.1 Mobile phase

The mobile phase was made using disodium hydrogen phosphate buffer solution (0.05M) and acetonitrile in ratio of 90:10 V/V. The pH of the mobile phase was adjusted to 6.5 with glacial acetic acid. The mobile phase was filtered using 0.45 μm filter membrane and degassed for 5 min.

5.2.3.2 Preparation of standard solution

A stock solution of ketoprofen was prepared in mobile phase having concentration of 1mg/ml. Then a series of working standard solutions were prepared by diluting the stock solution with mobile phase to obtain 3.9, 7.80, 15.60, 31.20, 62.50, 125.00, 250.00, 500.00, and 750.00 μg/ml.

5.2.3.3 Higher performance liquid chromatographic system

The chromatographic analysis was employed using high performance liquid chromatography (HPLC) as reported (Khan *et al.*, 2011) with slight modifications. HPLC instrument (Agilent Affinity 2 HPLC) with LC-10AT VP pumps, manual injector with loop

volume of 5µl (Rheodyne), and programmable variable wavelength UV detector were used in this study.

5.2.3.4 Chromatographic conditions

A volume of 5 µl was eluted through a column (Zorbax XDB – C8, 150 x 4.6 mm, ID 5 microns having Di-isobutyl n-octadecyl silane supported by porous silica, maintained at 25 °C) at a flow rate of 1.5 ml/min and the detection was carried out at λ_{max} 258nm.

5.2.3.5 Development and validation of HPLC method

Extraction of drug from plasmas

Spiked and mixed 1 ml of human plasma samples with 1 ml of standard drug solution, having concentration 3.9, 15.60 and 62.50 µg/ml. Each spiked plasma was mixed with 1 ml of acetonitrile and centrifuged for 10 min at 3000 rpm. The supernatant was dried and reconstituted with 1 ml of mobile phase (Section 5.2.3.1). The sample was filtered using 0.45 µm nylon membrane syringe filter and was stored in HPLC vials and refrigerated pending analysis (Puozzo *et al.*, 2004).

HPLC analysis

The samples extracted from plasma and standard solution used to spiked plasma analyzed at HPLC conditions mentioned in Section 5.2.3.4 to identify the peak of the drug in plasma samples. The resulting peaks were used to determine the system suitability and recovery. Peak data of the samples were used to determine the drug concentration from the standard curve.

System suitability

The peak resolution, retention time, capacity factor, tailing factor (peak symmetry), number of theoretical plates, height equivalent to theoretical plate and reduced plate height were observed and computed to assess the analytical method suitability.

5.2.3.6 Method validation

The testing method was validated for its linearity, repeatability, precision, robustness and accuracy (Zimmer, 2014). The limit of detection (LOD) and limit of quantification (LOQ) of ketoprofen in plasma samples were also determined. The injection volume was 5µl, flow rate 1.5 min/ml, and the drug was detected by UV detector at 258nm. Sufficient time was allowed for chromatogram to come to stable baseline by running mobile phase. One injection of mobile phase (blank), three injections of standard solution and three injections of sample solutions, each of 5 µl were injected separately or as per the devised sequence were used to get the peak area. The peak area of the samples was converted into the concentration of ketoprofen in plasma samples using a previously constructed calibration curve.

Linearity

In linearity the range over which ketoprofen exhibited linear response was identified. For this purpose, different concentration of drug with the help of mobile phase were prepared as 3.9, 7.80, 15.60, 31.20, 62.50, 125.00, 250.00, 500.00, and 750.00 µg/ml. Peak data were plotted against the respective concentration and linearity was assessed visually and confirmed by the linear regression analysis.

Accuracy

Plasma samples were spiked with standard solution of drug having concentrations of 3.9, 15.60 and 62.50 µg/ml, and extracted as mentioned under Section 5.2.3.5. Each sample was analyzed in triplicate and the amount calculated from the standard curve was compared to that of the true value, amount used to spike to determine accuracy.

Repeatability and reproducibility

Plasma samples were spiked with standard solutions of the drug having concentrations 3.9, 15.60 and 62.50 µg/mL, and extracted as mentioned in Section. The samples were analyzed 6 times in a single day to determine repeatability of test method. The same solutions were analyzed once daily for 6 consecutive days to determine reproducibility.

Precision

Intraday and inter-day analysis data were used to determine respective precision, relative standard deviation (RSD).

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) is the lowest concentration of analyte in a given sample that can be detected but not necessarily quantitated, and is calculated as three times the baseline noise (Pharmacopeia, 2015). The baseline noise was determined from four blank injections. The noise was measured from 0.5-1.5 minutes and 2.0-3.0 minutes (the retention time of drug peak is ~ 1.7 min. Noise was expressed as the average of two measurements based on 0.5-1.5 minutes and 2.0-3.0 minutes. The theoretical limit of quantitation was calculated as 10 X the baseline noise.

Robustness

On a single day, the effects of varying key HPLC parameters on the peak area and retention time of ketoprofen were investigated. The analysis was performed at column temperatures (25 ± 2 ºC), pH of mobile phase (6.5 ± 2) and flow rates (1.5 ± 2 ml/min) to determine the impact of these conditions on robustness. The normal level of studied HPLC parameters was also performed as a control. Triplicate injections of one of drug solution were injected for each condition.

5.2.4 Pharmacokinetics study of ketoprofen

5.2.4.1 Preparation of MT2 and MT16 for pharmacokinetic study

The matrix tablet formulations, MT2 and MT16 for pharmacokinetic study were prepared using method given in Section 3.3.1 and 4.5 according to the guidelines of current good manufacturing practices (Singh *et al.*, 2014). The formulations were tested again according to Section 4.6 and found reproducible.

5.2.4.2 Dosing of MT2 and MT16 tablet formulation

Single dose of the selected surfactant-based and water-based ketoprofen matrix tablets at oral dose of 200 mg with 250 ml water at 8 am was given to individual in before-after design with a washout period of eight days.

5.2.4.3 Blood sampling and processing

For MT16, 3 ml of blood sample was taken from heparinized cannula fitted to the forearm before administration (zero time) and then at predefined time intervals, i.e., 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, and 12.0h in 5 ml potassium ethylene diamine tetracetic acid (K_3EDTA) containing tubes (1.5%, w/v). Since the formulation MT2 showed sustained

release behavior, thus the blood sampling was extended to 24 h by adopting the same sampling intervals of MT16 with additional samples at 16, 20 and 24 h. Samples of blood collected were centrifuged for 10 min at 4000 rpm and plasma layer was immediately transferred to Eppendorf tubes and were stored at -18 ºC till analysis (Hettich Zentrifugen, Japan).

5.2.4.4 Determination of *in-vivo* pharmacokinetic parameters

The peak height area was converted to concentration of ketoprofen using the previously constructed calibration curve against each time point. Using a computer software, PKSolver, an MS Excel add-on different pharmacokinetic parameter was calculated from the plasma level time data of ketoprofen employing compartmental and non-compartmental approaches. The maximum plasma drug concentration (C_{max}), time to reach C_{max} (T_{max}) , rate of absorption (K_a), volume of distribution (V_d), rate of elimination (K_{el}), half-life $t_{1/2}$, and total clearance (Cl_T) were computed in this study (Attia *et al.*, 2007; Pal *et al.*, 2009). The compartmental approach gives the information on the distribution parameters, y-intercept of the extrapolated terminal portion of curve (B), y-intercept of the extrapolated residual curve (A), associated rate constants, such as absorption rate (k_a), distribution rate (α) and elimination rate (β) and corresponding half-life, $t_{1/2\alpha}$ and $t_{1/2\beta}$. It also gives transfer rate of drug from blood to tissues (K_{12}) and from tissues to blood (K_{21}) and from blood to environment (K_{10}).

The non-compartmental approch is used to compute zero moment plasma level time curve (AUC) and the first moment curve, AUMC. From the both of the above parameters, mean residence time of a drug is computed. The above resulting parameters were compared for the surfactant-based tablet and water-base tablet dosage forms. The following equation was used to compute the relative bioavailability.

$$\% \text{ Relative bioavailability (F)} = \frac{\text{AUC}_{0-\infty} \text{ of MT2}}{\text{AUC}_{0-\infty} \text{ MT16}} \text{X } 100 \qquad [1]$$

5.2.5 Statistical data analysis

Non-parametric independent Wilcoxon test was used for assessing the difference between the pharmacokinetics of MT16 and MT2 using SPSS ver. 21. P value less than 0.05 was considered significant difference (Kumar *et al.*, 2009).

5.3 Results and discussion

The aim of developing sustained release drug delivery systems is to control the delivery of drug and/or its release at the intended site (Rao *et al.*, 2013). Such delivery systems appear in the systemic circulation and remain there for longer time as compared to the conventional counterparts (Gwen *et al.*, 1996; Patel *et al.*, 2011). Formulating a sustain release delivery system for a poorly soluble drug is challenging. But designing effective delivery systems for such drugs is a main focus of pharmaceutical formulator, industry, and researchers. The poorly soluble drugs belonging to BCS class II are the candidates for sustained release delivery systems to improve their low bioavailability (Borgquist *et al.*, 2006; Gwen *et al.*, 1996). Sustaining the release of such drugs enables localization of drug at its specific absorption site which results in enhanced absorption even at lesser dose (Kumar *et al.*, 2011). The release of drugs is required to be controlled for longer therapeutic effects (Singh, 2006).

A delivery system may effectively control drug release if it is formulated properly in such a way that the factors controlling the release are tuned appropriately. In the first phase of this study, granules of ketoprofen were developed using different surfactants using SAWG approach. The granules were exhibited to be the matrix system. The granules were compressed into tablet formulations which were characterized and matched with the desired

features of the sustained release delivery system. The selected dosage form was assessed for the *in-vitro* drug release and *in-vivo* bioavailability. *In-vivo* bioavailability is usually studied for only the selected formulation from the several developed formulations. Hence, a selected formulation based on *in-vitro* dissolution characteristics such as MT2 was selected for the pharmacokinetic study using a validated HPLC method.

5.3.1 System suitability

As shown in Figure 5.2, the analyte peak was well resolved. The system suitability factors, such as retention time (T_R), capacity factor (k′), tailing factor (peak symmetry, T), number of theoretical plates (N), height equivalent to theoretical plate (HETP) and reduced plate height were observed to be well within the accepted criteria (Table 5.1).

Table 5.1: **System suitability parameters calculated from the chromatogram of ketoprofen**

System suitability parameters	Values
Retention time (T_R)	6.427
Capacity factor (k′)	5.427
Tailing factor (T)	1.25
Number of theoretical plates (N)	1032
Height equivalent to theoretical plate (HETP)	242.1
Reduced plate height	48.42

5.3.2 Method validation

5.3.2.1 Linearity

Table 5.2 reports the results of linearity of testing method for determination of ketoprofen. The data of ten solutions were utilized for assessment of linearity of the testing method. The linearity of ketoprofen ranging from 0.039-10.0 µg/ml was listed

and given in Figure 5.1. The coefficient of determination (R^2) for drug solutions ranging from 0.0039 mg to 1.0 mg met the required acceptance criteria of R^2> 0.9900 for the coefficient of determination (Pharmacopeia, 2015).

Table 5.2: Linearity of ketoprofen quantitative assay

Target Concentration (mg/mL)	Peak Area
0.0039	79.84
0.0078	161.68
0.0156	319.36
0.0313	630.72
0.0625	1265.44
0.1250	2531.88
0.250	4973.76
0.500	9904.52
0.750	19710.04
1.00	38430.08

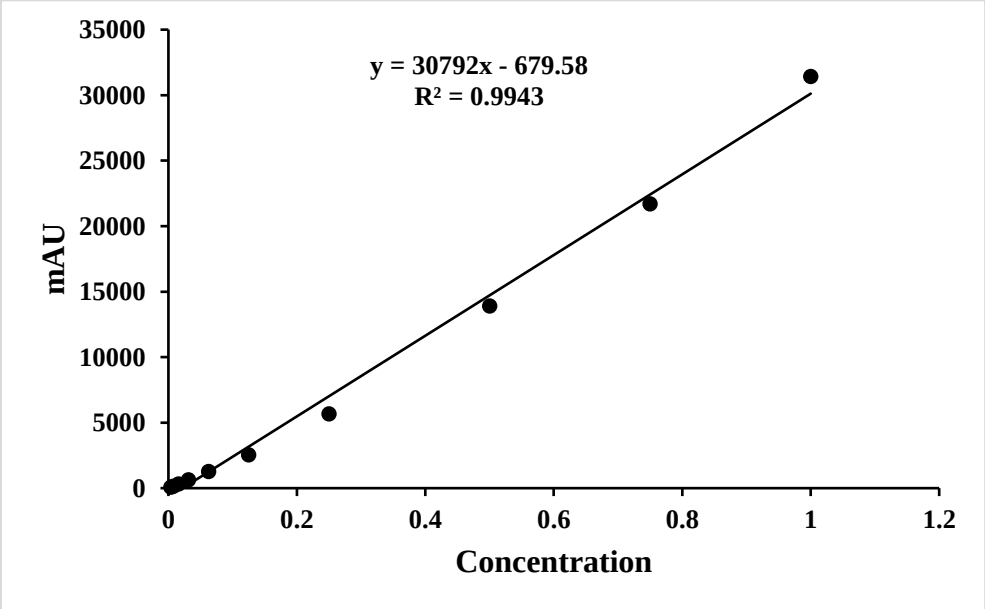

Figure 5.1: Linearity of ketoprofen quantitative assay

5.3.2.2 Accuracy

The sample recovery was ranged from 99 to 100 % which indicated the accuracy of the HPLC method.

5.3.2.3 Repeatability and reproducibility

The results demonstrated the repeatability of the test method. The % CV for retention time and peak area met the required acceptance criteria of (Pharmacopeia, 2015) for repeatability (≤ 5%). The data of repeatability of ketoprofen purity determining method is enlisted in Table 5.3.

Table 5.3: Repeatability of quantitative assay

Injection	Retention time	Peak Area
1	1.682	451.54
2	1.686	450.94
3	1.684	451.15
4	1.701	453.68
5	1.682	450.14
6	1.681	449.97
Average	1.686	451.24
SD	0.008	1.339
% CV	0.45	0.30

5.3.2.4 Precision

The standard deviation and % CV of ketoprofen in the Table 5.4 justifies the precision of the method.

Table 5.4: Precision of ketoprofen quantitative assay

Days	Peak area			Mean	SD
	Inject 1	Inject 2	Inject3		
1	119.02	119.61	120.4	119.677	0.565
2	119.19	119.25	119.38	119.273	0.079
3	119.16	120.3	119.81	119.757	0.467
1	119.63	119.2	119.01	119.280	0.259
2	119.56	119.05	119.44	119.350	0.218
3	119,04	119.84	119.2	119.520	0.320

5.3.2.5 LOQ and LOD

The data of Table justifies that the testing method showed robustness against change of column and age of the column. The LOD was found to be 0.040 µg/ml and thus, the LOQ was 0.132 µg/ml. The retention time was 6.5 min. The HPLC showed a well resolved sharp peak of ketoprofen at retention time of 6.43 min. Figure 5.2 shows a representative HPLC chromatogram of ketoprofen measured in human plasma sample.

5.3.2.6 Robustness

The data of robustness on the basis of age of the column and different lot for purity testing of ketoprofen is enlisted in Table 5.5.

Table 5.5: Robustness (column age wise) of API quantitative assay

Injection	"Used" Column	"New" Column	Mean
Peak Area			
1	356	357	354.50
2	352	359	
3	348	355	

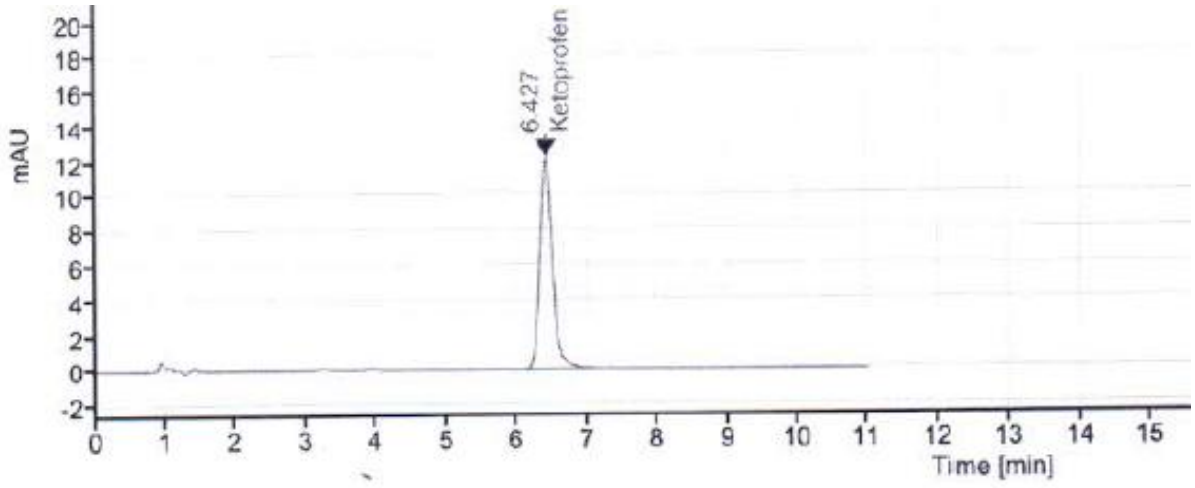

Figure 5.2: Representative chromatogram of ketoprofen showing a well resolved peak at 6.43 min

5.3.3 Plasma level time data of MT16 after oral administration to human volunteers

The control (water based) tablet formulation, MT16, was administered and plasma level time curve for ketoprofen was drawn for individual human volunteers based on the release profile, the sample time interval for pharmacokinetics of MT16 were determined up to 12 h. Table 5.6 shows the concentration of individual human volunteer as well as average ± SD in µg/ml after administering 200mg MT16 tablet formulation to six human subjects. The Figures 5.3 and 5.8 represent the plasma level time curve of ketoprofen after administration of water based ketoprofen tablet. Mean (± SD) concentration of ketoprofen in blood has been given in Figure 5.9. The drug in all the subjects followed 2-compartment model. No drug was detected at time zero, while concentration was detected at 0.5 h which continued until 12 h.

Table 5.6: Plasma level time data of ketoprofen after oral administration of MT16

Time (h)	Concentration (µg/ml) in human volunteer						Mean	SD
	1	2	3	4	5	6		
0.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.5	7.907	6.678	7.124	6.532	5.861	5.214	6.553	0.943
1.0	11.210	10.200	9.360	8.465	12.500	9.957	10.282	1.417
1.5	10.410	9.798	8.308	9.098	10.193	11.275	9.847	1.040
2.0	8.260	7.415	6.885	6.258	6.961	7.029	7.135	0.666
4.0	2.732	3.634	2.462	2.462	4.228	3.760	3.213	0.757
6.0	1.628	1.344	1.239	0.990	1.353	1.871	1.404	0.308
8.0	0.640	0.500	0.577	0.473	0.826	0.573	0.598	0.127
10.0	0.433	0.324	0.299	0.276	0.302	0.232	0.311	0.067
12.0	0.133	0.146	0.165	0.138	0.183	0.146	0.152	0.019

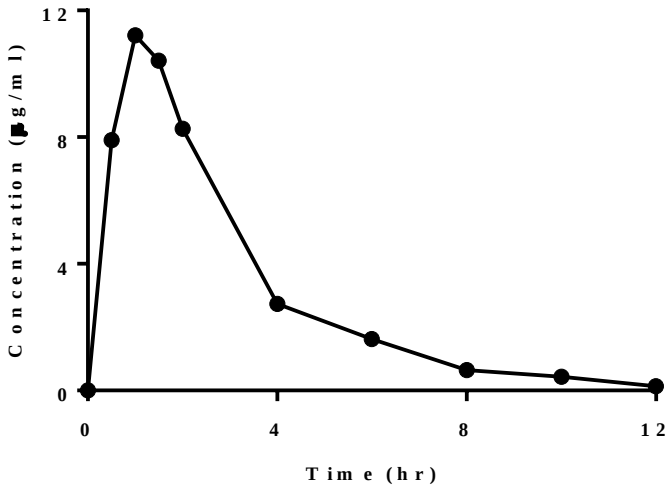

Figure 5.3: Plasma level time curve of ketoprofen for human volunteer 1 after oral administration of MT16.

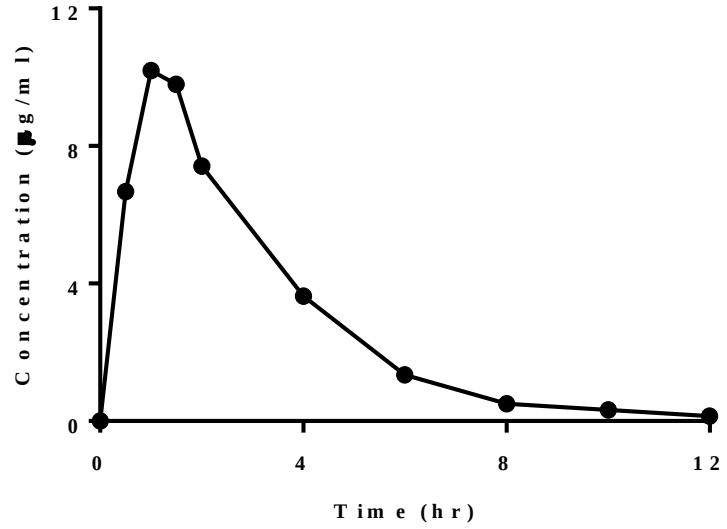

Figure 5.4: Plasma level time curve of ketoprofen for human volunteer 2 after oral administration of MT16

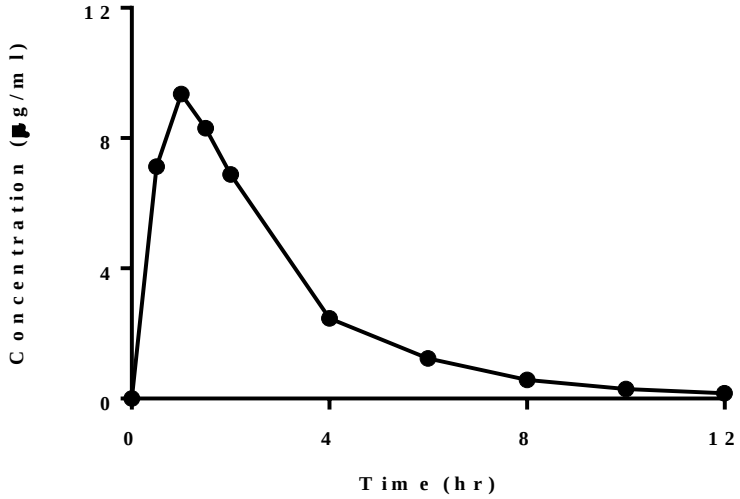

Figure 5.5: Plasma level time curve of ketoprofen for human volunteer 3 after oral administration MT16

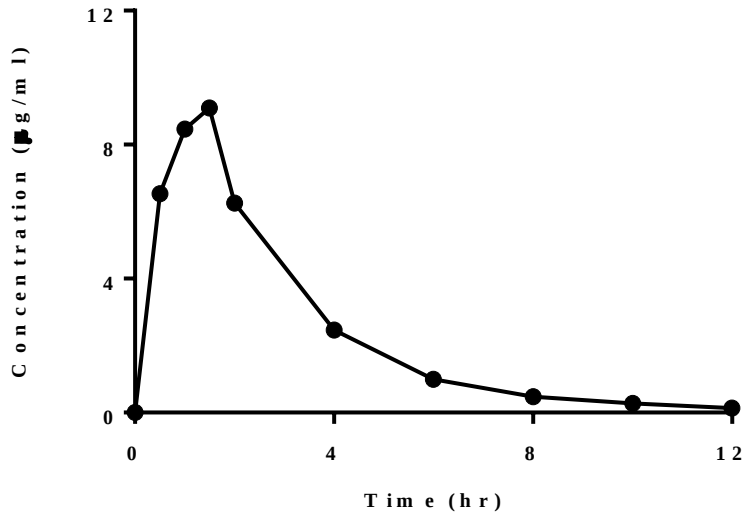

Figure 5.6: Plasma level time curve of ketoprofen for human volunteer 4 after oral administration MT16

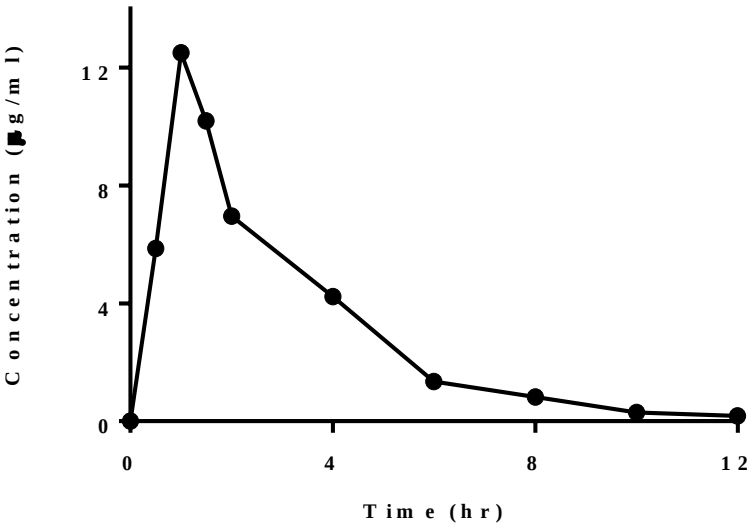

Figure 5.7: Plasma level time curve of ketoprofen for human volunteer 5 after oral administration MT16

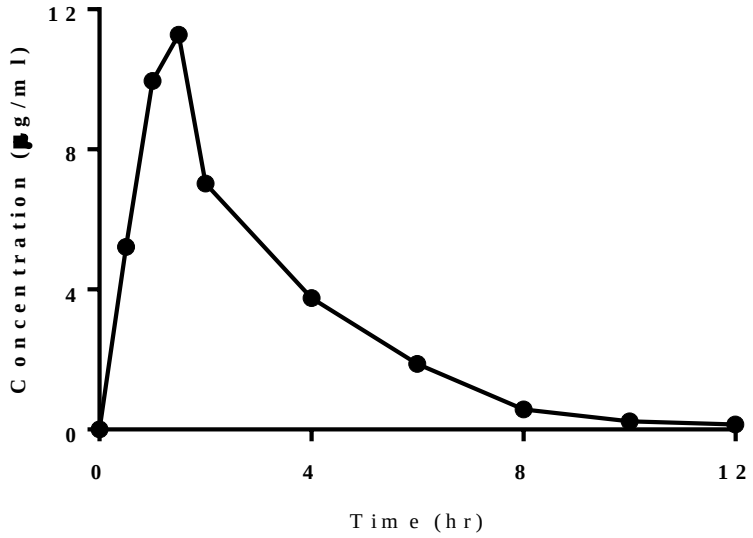

Figure 5.8: Plasma level time curve of ketoprofen for human volunteer 6 after oral administration MT16

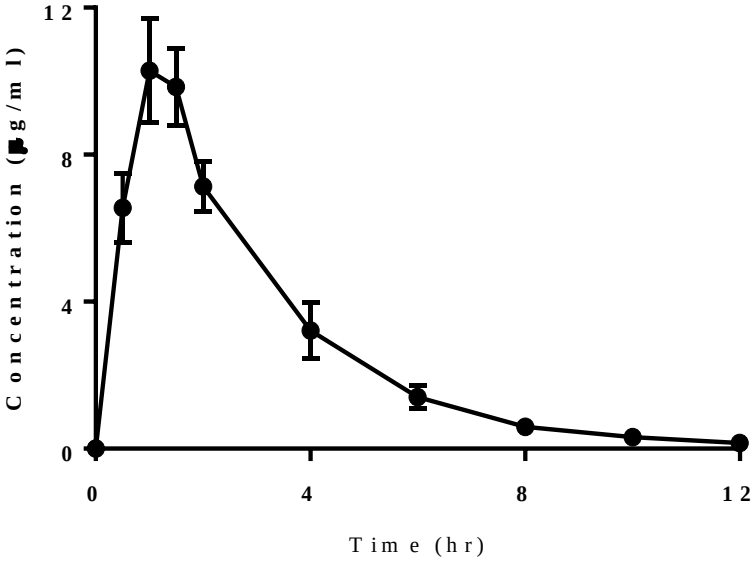

Figure 5.9: Comparative Plasma level time curve of water-based ketoprofen tablet formulation administered to human volunteers 1-6.

5.3.4 Pharmacokinetic parameters of ketoprofen after oral administration of MT16

The plasma level time data was the basis for computation of pharmacokinetic parameters after oral administration of MT16 to six human volunteers following two-compartmental and non-compartmental model approach. The R^2 (regression values) are the effective investigational parameters for selecting the compartmental model which were approaching to 1 for 2-compartment model. Pharmacokinetic parameters such as absorption, distribution and elimination were calculated from the plasma level time curve for each of the six volunteers using non-compartmental and two compartmental approaches using a computer software, PKSolver. The results are summarized in the Table 5.7.

Compartmental analysis gives a detailed information about the drug distribution. This analysis further describes parameters such as, absorption rate (K_a), half life of absorption ($t_{1/2ab}$), micro rate constants (K_{10}, K_{12}, K_{21}), and the extrapolated y-intercepts of absorption and elimination curve (A and B, respectively). The various pharmacokinetic parameters were computed without T_{lag} using two compartment model describing the rate in absorption (Ka) as 1.024 h^{-1} ±0.084, distribution rate (α) as 0.836±0.126 h^{-1} and of rate of elimination (β or K_{el}) as 0.246±0.099 h^{-1}. The absorption and elimination half-life ($t_{1/2}\beta$ or $t_{1/2}Ke$ and $t_{1/2}K_a$, respectively) were computed as 0.681±0.053 h and 3.298±1.629 h, respectively. The $t_{1/2}\beta$ for MT16, equivalent to a prompt release formulation in the present study was comparable to the reported value, 1-3 h for this parameter (Ishizaki *et al.*, 1980). The peak plasma concentration (C_{max}) was found as 9.62±0.76 µg/ml at the T_{max} of 1.15±0.11 h. The C_{max} of ketoprofen after administration of 200 mg MT16 was comparable to 10.52±1.43 µg/ml from 100 mg prompt release capsule formulation (Roda *et al.*, 2002) but was lesser than the reported value, 15-25 µg/ml in another study. T_{max} was within the reported value of 0.5-2h (Upton *et al.*, 1981) and close to 1.38±0.48h (Roda *et al.*, 2002). The area under the curve from time zero to t (AUC_{0-t}) and total area under the curve ($AUC_{0-\infty}$) were observed, respectively as 33.46±3.48 and

34.39±3.06 µg.h/ml. Values of the total area under the moment curve ($AUMC_{0-\infty}$) and mean residence time (MRT), respectively were 108.88±8.93 $\mu g.h^2$/ml and 3.18±0.37 h. Volume of distribution (V_d), volume of the central compartment and the total clearance (Cl_T) were 8.835±0.503 l, 4.180±3.255 and 1.015±0.559 l/h, respectively.

5.3.5 Plasma level time data of ketoprofen after oral administration of MT2

The selected ketoprofen matrix tablet, MT2 was carried forward for the pharmacokinetics study due to its closeness to the USP-stipulated criteria for sustained release profile. An appropriate hardness, least friability and appropriate control over release along with other parameters given in Section 4.8.1 prompted the selection of the ketoprofen tablet formulated using Soluplus®. Table 5.8 shows the concentration of individual human volunteer as well as average ± SD in µg/ml after administering 200 mg of MT2 tablet formulation to six human subjects. The time interval for blood sampling up to 24 h were taken based on the release characteristics of the drug. The Figures 5.10 to 5.15 represent the plasma level time curve of ketoprofen after administration of ketoprofen tablet for six subjects. Mean (± SD) concentration of ketoprofen in blood has been given in Figure 5.16. No drug was detected at time zero, while concentration was detected at 0.5 h which continued until 24 h demonstrating sustaining of the therapeutic blood concentration of drug for 24 h.

Table 5.7: Pharmacokinetic parameters of ketoprofen from MT16

Parameters	Human volunteer						Mean	SD
	1	2	3	4	5	6		
A (µg/ml)	79.323	153.738	173.726	258.389	141.042	55.252	143.578	72.292
α (/h)	0.833	0.847	0.981	0.892	0.859	0.602	0.836	0.126
K_a (/h)	1.146	0.975	1.105	0.972	1.006	0.939	1.024	0.083
$t_{1/2}K_a$	0.605	0.711	0.627	0.713	0.689	0.738	0.681	0.053
C_{max} (µg/ml)	10.70	9.645	9.03	8.62	10.19	9.513	9.62	0.76
T_{max} (h)	1.08	1.176	1.03	1.10	1.16	1.331	1.15	0.11
AUC_{0-t} (µg.h/ml)	35.74	34.353	29.95	28.36	36.96	35.426	33.46	3.48
$AUC_{0-\infty}$ (µg.h/ml)	36.67	34.957	31.01	30.33	37.85	35.526	34.39	3.06
$AUMC_{0-\infty}$ (µg.h^2/ml)	111.37	105.070	100.28	117.27	120.43	98.866	108.88	8.93
MRT (h)	3.04	3.006	3.23	3.87	3.18	2.783	3.18	0.37
Vd (l)	8.329	8.619	9.114	9.071	8.302	9.573	8.835	0.503
Vc (l)	3.477	2.708	5.905	9.640	3.254	0.097	4.180	3.255
$t_{1/2}$α (h)	0.832	0.818	0.707	0.777	0.807	1.151	0.849	0.155
K_{12} (/h)	0.118	0.108	0.191	0.141	0.134	0.004	0.116	0.062
K_{21} (/h)	0.283	0.345	0.295	0.132	0.342	0.421	0.303	0.097
B (µg/ml)	2.948	4.161	2.905	0.775	4.719	1.935	2.907	1.438
K_{el} (/h)	0.222	0.271	0.213	0.108	0.253	0.411	0.246	0.099
K_{10} (/h)	0.655	0.664	0.708	0.727	0.636	0.588	0.663	0.050
$t_{1/2}$β (h)	3.122	2.562	3.258	6.425	2.735	1.685	3.298	1.629
CL_P (l/h)	5.454	5.721	6.449	6.593	5.283	5.629	5.855	0.539
CL_T (l/h)	0.982	0.935	1.741	1.276	1.113	0.040	1.015	0.559

Table 5.8: Plasma level time data of ketoprofen after oral administration of 200 mg of MT2

Time (h)	Concentration (µg/ml) in human volunteer						Mean	SD
	1	2	3	4	5	6		
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.173	0.154	0.146	0.134	0.231	0.136	0.162	0.036
1.0	1.014	0.845	0.726	0.837	0.937	0.658	0.836	0.131
1.5	1.996	2.579	1.867	1.997	1.234	1.128	1.800	0.540
2.0	4.236	3.957	3.759	3.917	4.187	4.957	4.169	0.425
4.0	7.446	6.500	6.175	6.435	6.300	5.981	6.473	0.512
6.0	5.846	4.973	4.995	5.524	5.778	5.100	5.369	0.397
8.0	5.040	4.200	3.990	4.158	5.024	4.256	4.445	0.464
10.0	4.151	3.866	3.487	3.282	4.264	3.775	3.804	0.377
12.0	3.720	3.100	2.945	3.069	3.947	3.214	3.333	0.404
16.0	2.140	2.230	2.119	2.208	2.864	2.380	2.323	0.280
20.0	1.620	1.446	1.374	1.432	1.993	1.268	1.522	0.258
24.0	0.980	0.895	0.639	0.825	0.983	0.820	0.857	0.128

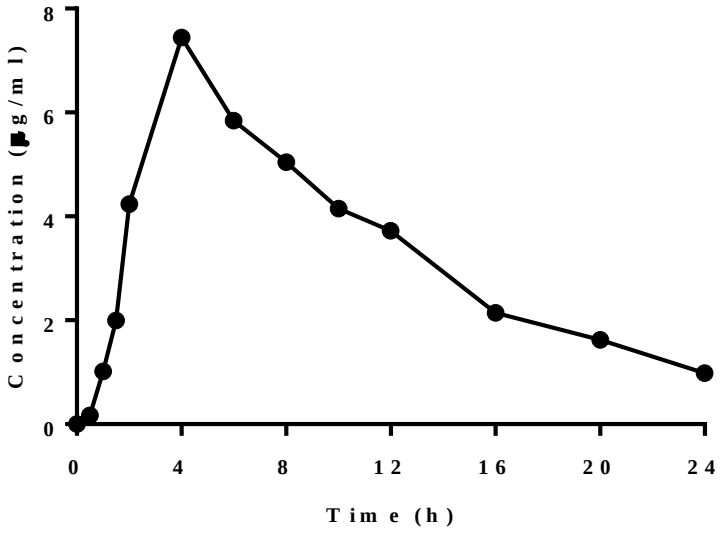

Figure 5.10: Plasma level time curve of ketoprofen for human volunteer 1 after oral administration of MT2

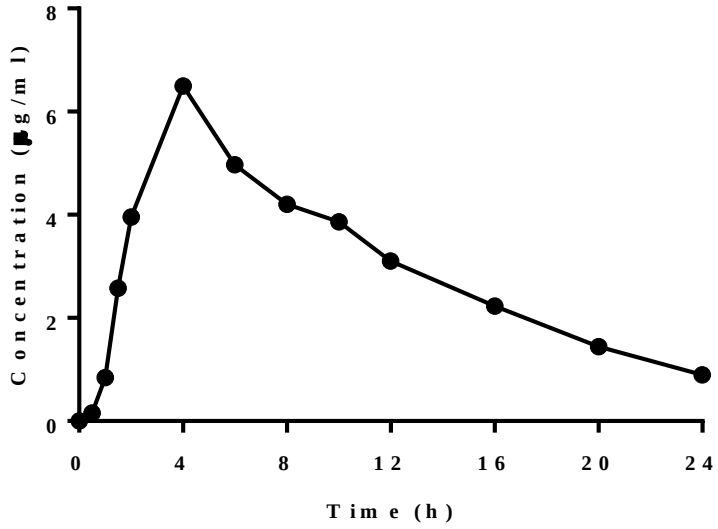

Figure 5.11: Plasma level time curve of Ketoprofen for human volunteer 2 after oral administration of MT2

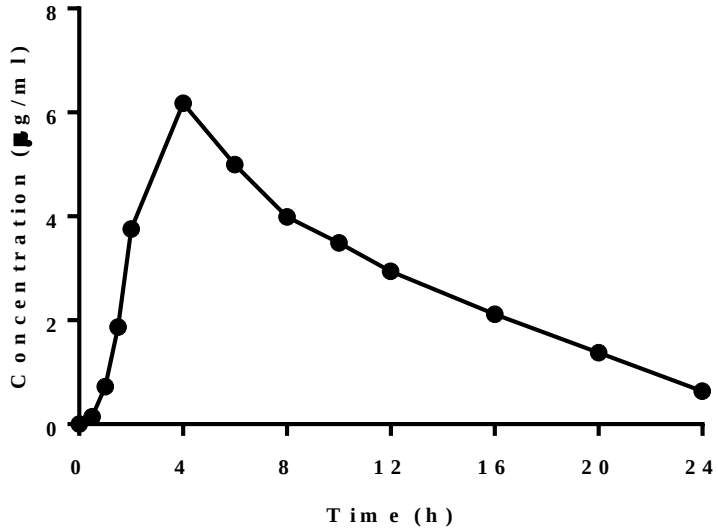

Figure 5.12: Plasma level time curve of Ketoprofen for human volunteer 3 after oral administration of MT2

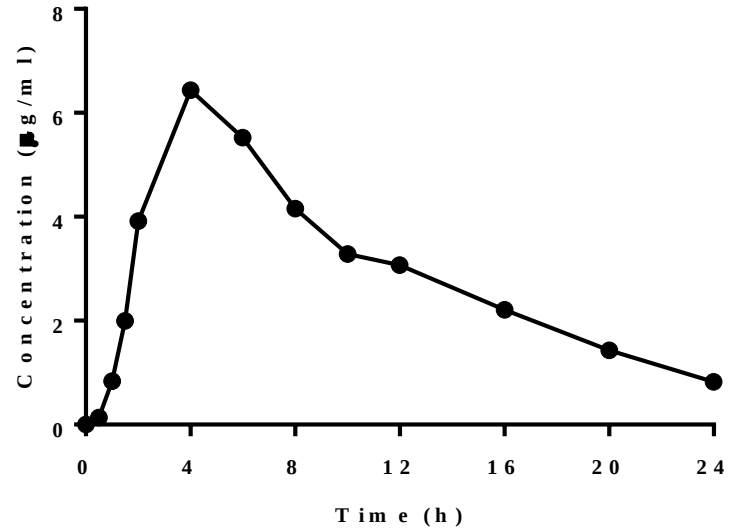

Figure 5.13: Plasma level time curve of Ketoprofen for human volunteer 4 after oral administration of MT2

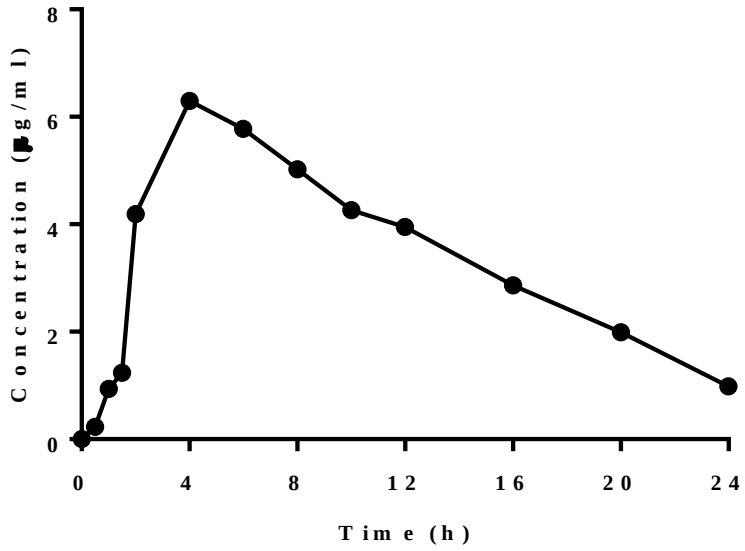

Figure 5.14: Plasma level time curve of Ketoprofen for human volunteer 5 after oral administration of MT2

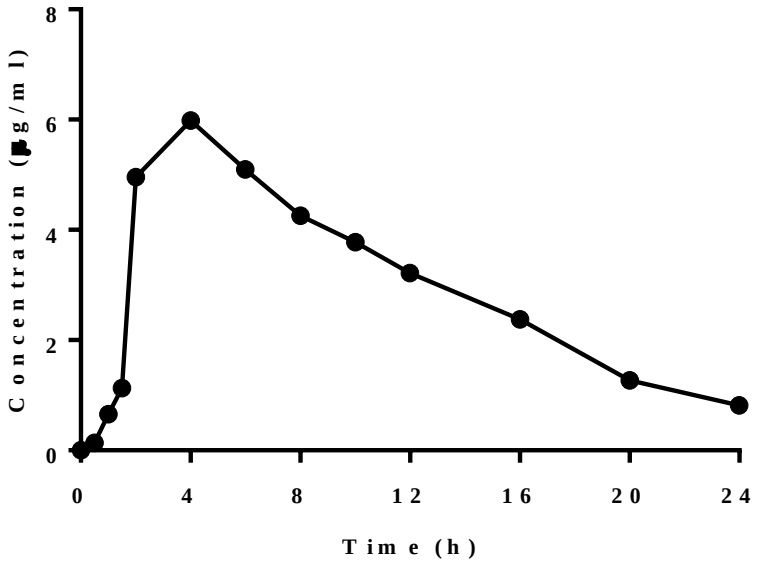

Figure 5.15: Plasma level time curve of Ketoprofen for human volunteer 6 after oral administration of MT2

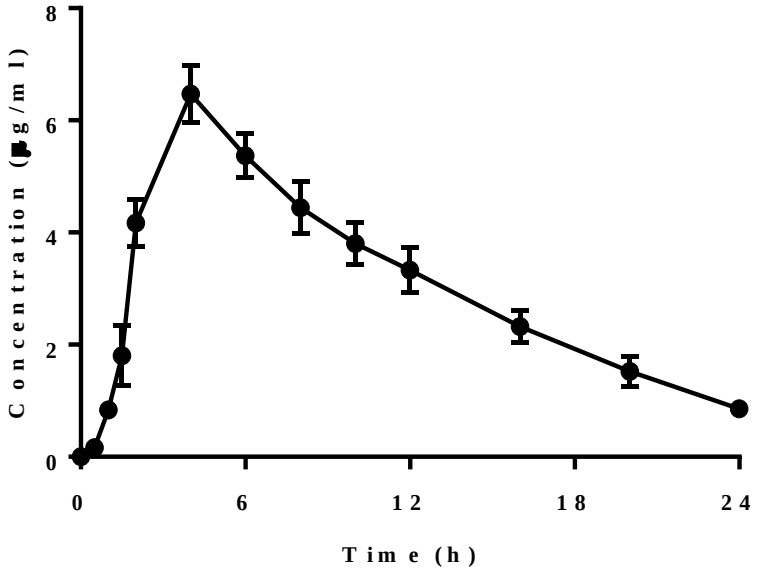

Figure 5.16: Average plasma level time curve of ketoprofen after administration of Soluplus®-based ketoprofen tablet formulation

5.3.6 Pharmacokinetic parameters of ketoprofen after administration of matrix tablet, MT2

The individual plasma level time curve of the six human subjects was the basis for the computation of different pharmacokinetic parameters using compartmental model analysis. Kinetic software PKSolver was adopted for computation of various pharmacokinetic parameters. Two compartment model best fitted to the plasma level time data of ketoprofen in this study. The values of correlation coefficient (R^2) were close to 1 for two compartment model in the individual human volunteers adopted in this study. These results are summarized in the Table 5.9.

According to two compartmental model, the results after oral administration of MT2 tablets to six human volunteers demonstrated C_{max} of 5.19±0.33 µg/ml being attained at the T_{max} of 5.56±0.30 h. The literature cited values of C_{max} were 5.91±0.66, and 4.51±0.65 µg/ml form 200 mg ketoprofen sustained release capsule containing coated granules filled in capsule and sustained release coated tablets, respectively (Roda *et al.*, 2002) and 3.5±1.0 h from 200 mg ketoprofen sustained release pellets filled into capsule (Houghton *et al.*, 1984). The present T_{max} values derived after administration of 200 mg ketoprofen matrix tablets (5.56±0.30 h) was comparable to 4.17±0.42 h from ketoprofen granules in capsule (Roda *et al.*, 2002) and 4.9±1.0 h after ketoprofen pellets in capsule (Houghton *et al.*, 1984) but higher than 2.28±0.32 h from the coated tablets (Roda *et al.*, 2002).

The absorption half-life was found to be 3.614±0.189 h. The distribution half life ($t_{1/2\alpha}$) was observed as 4.116±0.249 h. The extrapolated absorption and elimination curves back to y-axis, A and B, respectively were 98.148±52.232 µg/ml and 21.817±7.339 µg/ml. The elimination half life after administration of 200 mg ketoprofen matrix tablets was found to be 4.105±0.273 h which was comparable to 3.3±1.2 h from ketoprofen granules in capsule but lesser than 8.4 ±3.4 h from pelleted ketoprofen in capsule (Houghton *et al.*, 1984).

Table 5.9: Pharmacokinetic parameters of ketoprofen matrix tablet after oral administration of MT2

Parameters	Human volunteers						Mean	SD
	1	2	3	4	5	6		
A (µg/ml)	196.243	117.395	73.140	62.927	78.295	60.891	98.148	52.232
α (/h)	0.175	0.176	0.171	0.173	0.151	0.167	0.169	0.009
K_a (/h)	0.189	0.196	0.198	0.203	0.175	0.193	0.192	0.010
$t_{1/2}K_a$	3.670	3.545	3.505	3.413	3.958	3.596	3.614	0.189
C_{max} (µg/ml)	5.72	5.13	4.81	5.05	5.41	4.99	5.19	0.33
T_{max} (h)	5.49	5.38	5.43	5.35	6.15	5.56	5.56	0.30
AUC_{0-t} (µg.h/ml)	79.62	70.41	66.48	69.11	81.59	70.14	72.89	6.17
$AUC_{0-\infty}$ (µg.h/ml)	85.43	75.20	71.19	73.80	90.71	75.58	78.65	7.64
$AUMC_{0-\infty}$ (µg.h^2/ml)	938.63	810.85	775.24	794.38	1,120.15	842.76	880.33	130.83
MRT (h)	10.99	10.78	10.89	10.76	12.35	11.15	11.15	0.60
Vd (l)	13.325	15.074	16.387	15.882	14.639	15.779	15.181	1.100
Vc (l)	0.268	0.387	0.354	4.145	0.017	0.516	0.948	1.575
$t_{1/2}\alpha$ (h)	3.950	3.936	4.052	4.008	4.600	4.151	4.116	0.249
K_{12} (/h)*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
K_{21} (/h)	0.178	0.179	0.173	0.168	0.150	0.168	0.169	0.010
B (µg/ml)	19.308	18.583	18.641	18.353	19.238	36.778	21.817	7.339
K_{el} (/h)	0.179	0.179	0.173	0.167	0.150	0.169	0.169	0.011
K_{10} (/h)	0.176	0.176	0.171	0.171	0.151	0.168	0.169	0.009
$t_{1/2}\beta$ (h)	3.881	3.875	4.004	4.158	4.612	4.102	4.105	0.273
CL_P (l/h)	2.340	2.659	2.809	2.709	2.204	2.646	2.561	0.235
CL_T (l/h)	0.048	0.069	0.061	0.698	0.003	0.087	0.161	0.265

*Could not be calculated

The values of absorption rate (K_a), distribution rate (α) and elimination rate (K_{el} or β) were 0.192± 0.010, 0.169±0.009 and 0.169±0.011 h^{-1}, respectively. The AUC_{0-t} and $AUC_{0-\infty}$ of MT2 were 72.89±6.17 µg.h/ml and 78.65±7.64 µg.h/ml, respectively. The observed value

of $AUC_{0-\infty}$ in this study was higher than the literature cited values for 200 mg ketoprofen granules in capsule, coated tablet and pellets filled in capsule, i.e., 60.25±9.82 µg.h/ml, and 62.64±0.65 µg.h/ml (Roda *et al.*, 2002) and 40.0±11.0 µg.h.ml^{-1}, respectively (Houghton *et al.*, 1984). The MRT of tablet formulation MT2 calculated using the values of total $AUC_{0-\infty}$ and $AUMC_{0-\infty}$ (78.65±7.64 and 880.33±130.83 µg.h^2/ml, respectively) was 11.15±0.60 h. The values of V_d and Cl_T after administration of MT2 tablets was 15.181±1.100 l and 0.161±0.265 l/h, respectively (Table 5.9).

5.4 Comparative pharmacokinetics of MT16 and MT2 tablets

Table 5.10 describes the comparative plasma level time profiles after oral administration of MT16 and MT2. The Figure 5.17 compares the plasma level time profile of MT16 and MT2. Table 5.10 shows mean ketoprofen concentrations detected in human subjects after oral administration of MT16 and MT2. The significance levels (p value) computed by non-parametric paired Wilcoxon test statistics have also given against each timer interval. In case of MT16 and MT2, the ketoprofen did not appear in blood at zero time of administration. While for MT16, drug was about to disappear at 12 h post administration, the drug concentration for MT2 remained above detectable levels until 24 h. The ketoprofen from MT16 showed fast absorption with peak concentration accomplished at 1 h post administration followed by a prompt elimination from the human plasma until 12 h as compared to MT2 which attained peak plasma concentration gradually at 5.5 h and, then eliminated with an extended time in blood sampled at 24 h.

Table 5.10: Comparative plasma level time of ketoprofen after administration of MT16 and MT2 analyzed by paired Wilcoxon test

Parameter	Concentration (µg/ml) Mean ±SD		Difference (Sig. p <0.05)
Time (h)	MT16	MT2	
0.5	6.553	0.162	0.028
1.0	10.282	0.836	0.028
1.5	9.847	1.800	0.028
2.0	7.135	4.169	0.028
4.0	3.213	6.473	0.028
6.0	1.404	5.369	0.028
8.0	0.598	4.445	0.028
10.0	0.311	3.804	0.028
12.0	0.152	3.333	0.028
16.0	-	2.323	-
20.0	-	1.522	-
24.0	-	0.857	-

The higher ketoprofen concentrations was observed after administration of MT16 at different time points, for instance, at 0.5 h, 6.553±0.943 µg/ml as compared to 0.162±0.036 µg/ml after MT2 tablets (P=0.004). Similarly, ketoprofen concentration reached to maximum (C_{max}), 10.282±1.417 µg/ml at 1.0 h (T_{max}) for MT16 tablet as compared to 0.8360±0.131 µg/ml of MT2 tablet at same time point (P=0.004). The C_{max} values of MT16 and MT2 have been compared as given in Table 5.10 and Figure 5.18. Application of Wilcoxon test showed statistical difference for the parameter. The peak ketoprofen concentration for MT2 tablets (5.19±0.33 µg/ml) attained at 5.56±0.30 h. In case of MT16, at about this time interval, i.e., at 6 h, drug was almost eliminated as shown by the concentration 1.404±0.308 µg/ml. The C_{max} value of MT16 was significantly higher than that of MT2, i.e., 9.62±0.76 Vs 5.19±0.33µg/ml after administration of equal doses (200 mg) of both formulations.

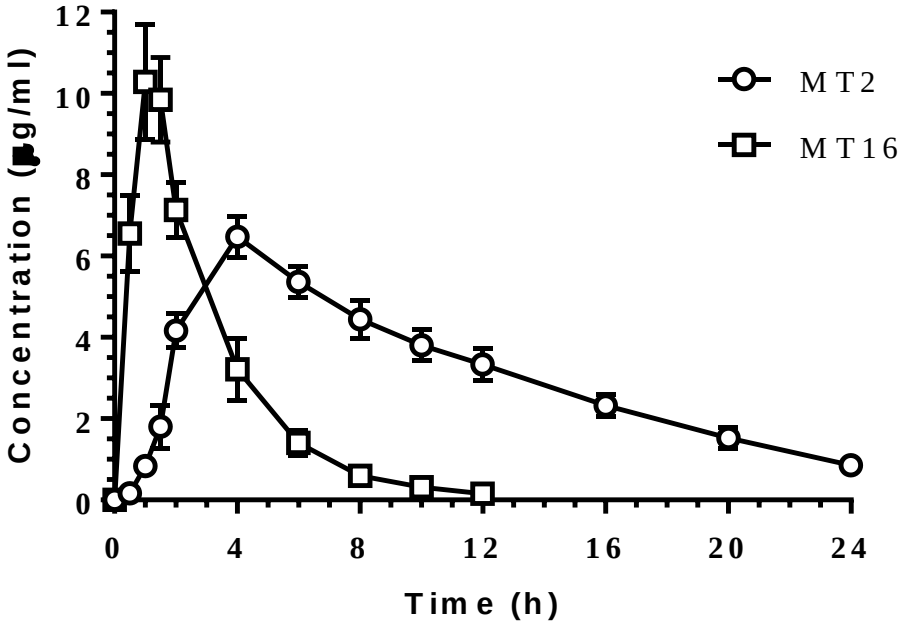

Figure 5.17: Comparative plasma level time curve of ketoprofen after oral adminstration of Soluplus®-based ketoprofen tablet (MT2) and water-based ketoprofen tablet (MT16) in human volunteers (n=6, ± SD).

Though ketoprofen from MT2 showed a delayed absorption, yet its lowest concentrations at extreme sampling time points, i.e., at 0.5 and 24 h, was found to be above therapeutic analgesic threshold concentration reported as 0.7-1.0 µg/ml (Houghton *et al.*, 1984). The persistence of drug above threshold concentration also implies that the analgesic effect of ketoprofen could be maintained even after 24 h. In this study, the standard deviation for MT2 was also noted to be lesser as compared to that of the MT16.

Table 5.11: Comparative peak plasma concentration (C_{max}) of ketoprofen after administration of MT16 and MT2 analyzed by paired Wilcoxon test

Human	C_{max} (µg/ml)		Difference (Sig. p <0.05)
	MT16	MT2	
1	10.70	5.72	0.028
2	9.65	5.13	0.028
3	9.03	4.81	0.028
4	8.62	5.05	0.028
5	10.19	5.41	0.028
6	9.51	4.99	0.028
Average	9.617	5.185	-
Standard Deviation	0.755	0.299	-

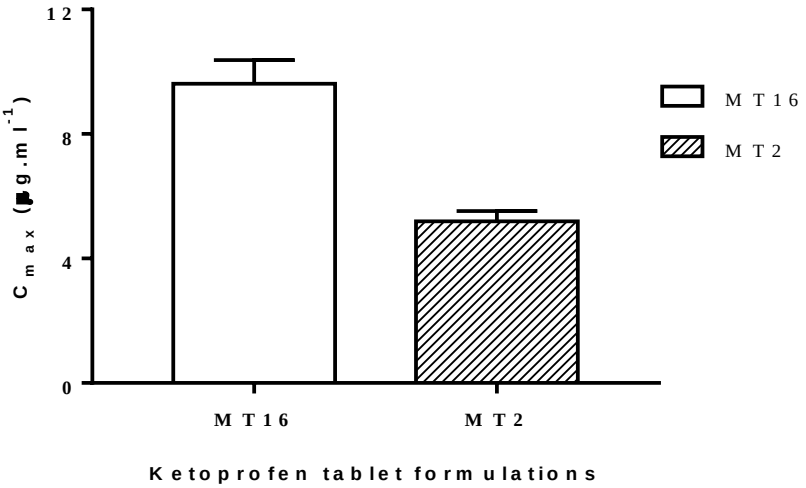

Figure 5.18: Comparative peak plasma concentration (C_{max}) yielded after oral administration of MT16 and MT2

Table 5.11 shows T_{max} of MT16 (1.147±0.105 h) was statistically briefer than that of MT2 (5.560±0.273 h) at P<0.05. The comparison is also given in Figure 5.19. The attainment of the highest ketoprofen concentration after MT2 was delayed in MT2.

Table 5.12: Comparative time to reach peak plasma concentration (T_{max}) yielded after oral administration of MT16 and MT2

Human	T_{max} (h)		Difference (Sig. p <0.05)
	MT16	MT2	
1	1.08	5.49	0.004
2	1.18	5.38	0.004
3	1.03	5.43	0.004
4	1.10	5.35	0.004
5	1.16	6.15	0.004
6	1.33	5.56	0.004
Average	1.147	5.560	-
Standard Deviation	0.105	0.273	-

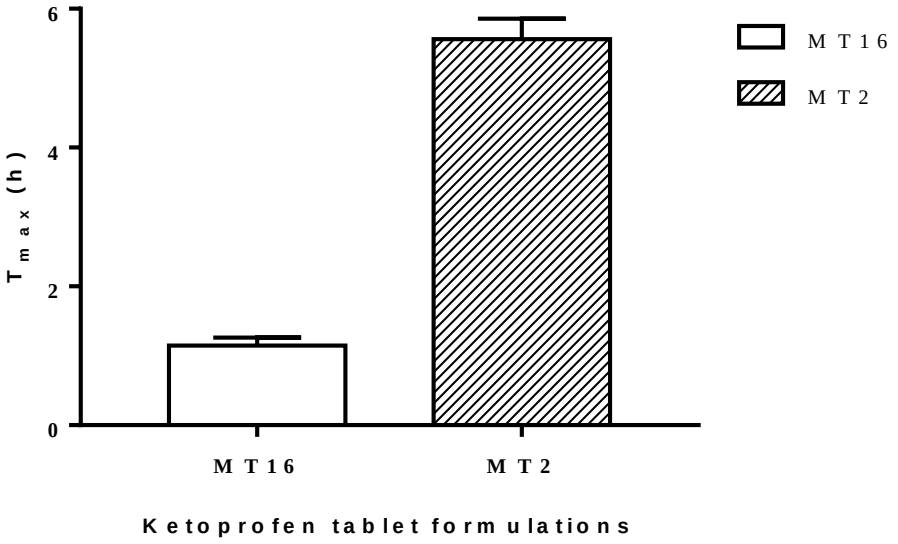

Figure 5.19: Comparative peak plasma concentration (T_{max}) yielded after oral administration of MT16 and MT2

The AUC value of MT16 and MT2 have been compared using the paired Wilcoxon test and the resultant P value have been presented in Table 5.12 and Figure 5.12**.** A major variation was found in the values of total area under the curve ($AUC_{0-\infty}$) for MT16 and MT2 tablets, i.e., 78.65±7.64 µg.h.ml^{-1}for MT2 tablet and 34.39±3.06 µg.h.ml^{-1} for MT16 showing

2.29-fold higher absorption of ketoprofen from tablets over control. The difference in AUC values might be due to difference in dissolution. The sustained release *in-vitro* was reflected by a sustained absorption of drug.

Table 5.13: Comparative area under the curve ($AUC_{0-\infty}$) after oral administration of MT16 and MT2

Human	$AUC_{0-\infty}$(µg.h.ml^{-1})		Difference (Sig. p <0.05)
	MT16	MT2	
1	36.67	85.43	0.004
2	34.957	75.20	0.004
3	31.01	71.19	0.004
4	30.33	73.80	0.004
5	37.85	90.71	0.004
6	35.526	75.58	0.004
Average	34.391	78.652	-
Standard Deviation	3.055	7.640	-

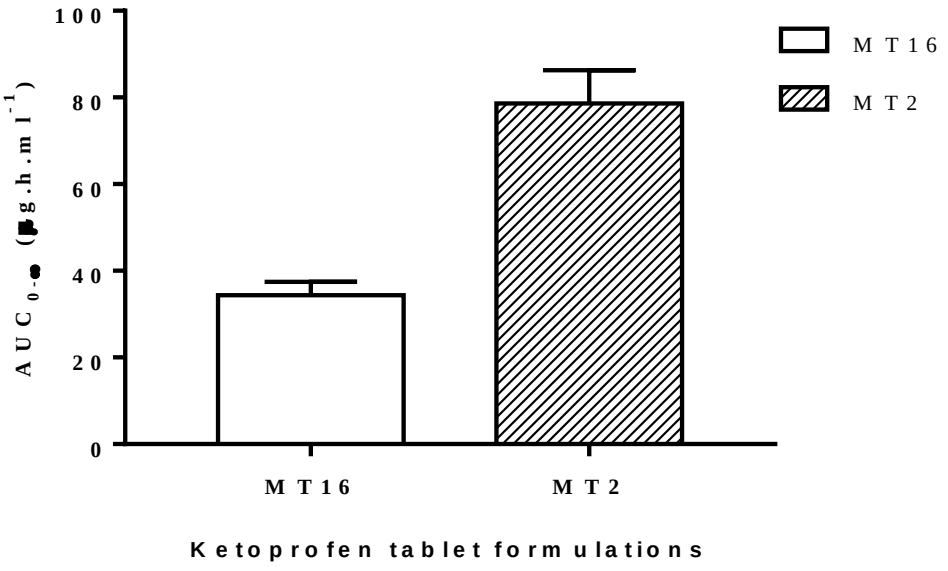

Figure 5.20: Comparative area under the curve resulting after oral administration of MT16 and MT2

The $AUMC_{0-\infty}$ values for the MT16 were far lower (P=0.004), i.e., 108.88±8.93 $\mu g.h^2.ml^{-1}$ as compared to 880.33±130.83 $\mu g.h^2.ml^{-1}$ of MT2 tablets. The mean residence time (MRT) of MT16 was identified as 3.18±0.37 h whereas for MT2 tablets it was increased up to 11.5±0.60 h, showing the ketoprofen stayed longer when it was administered as MT2 as compared to briefer stay of MT16 revealed by the respective MRT values. The volume of distribution was majorly changed for MT2 from MT16 with least value of 8.835±0.503 ml for MT16 as compared to 15.181±1.100 ml for MT2.

The foregoing discussion on C_{max}, T_{max} and AUC revealed the major differences between the MT16 and MT2. The highest plasma concentration was spotted earlier in the case of MT16 administration (T_{max}=1h) and that was extended to (T_{max}) 5.56±0.30 h for MT2 tablets at P=0.004. Likewise, absorption of ketoprofen was steadier, and expanded much in MT2 tablet compared to MT16 tablet on account of sustained ketoprofen release from MT2 tablets. This steady rise in concentration could be ascribed to the release of the drug from the Soluplus® micelle, detachment of drug from Soluplus®-drug or breakdown of hydrogen bonding between drug and the Soluplus®.

5.4.1 Relative bioavailability

Relative bioavailability was computed using the values of total area under the curve for the matrix tablet MT2 (test) and the (MT16) (control, reference). The values of relative bioavailability given as percent are shown Table 5.13 revealed a substantial increase in the percent relative bioavailability of MT2, the selected swellable-erodible matrix tablet formulation, i.e., 228.89±12.574%. The multifold higher $AUC_{0-\infty}$ (average 2.29 time) for the matrix tablet MT2 (78.652±37.640 $\mu g.h.ml^{-1}$) as compared to the MT16 (34.391±3.055 $\mu g.h.ml^{-1}$) exhibited a raised oral bioavailability of ketoprofen for MT2 matrix tablet

formulation. The reported values of $AUC_{0-\infty}$ were: 60.25±9.82 µg.h^2/ml from coated granules filled in capsule, 62.64±12.67 µg.h^2/ml from coated tablets (Roda *et al.*, 2002) and 40.0±11.0 µg.h^2/ml from pellets filled in capsule (Houghton *et al.*, 1984). When the present $AUC_{0-\infty}$ for MT2 was compared to that of the reported sustained release formulations, coated granules filled in capsule, coated tablets and pellet in capsule, 1.31, 1.26- and 1.97-folds higher bioavailability was observed. This higher bioavailability of the ketoprofen for MT2 as compared to reported sustained release formulations was due to the surfactant-based granules incorporated in MT2 which demonstrated higher ketoprofen solubility. The enhanced $AUC_{0-\infty}$ and the value of relative bioavailability supported that the Soluplus® increased the bioavailability of ketoprofen (MT2) as it increased the bioavailability of poorly water-soluble drug, dutasteride, itraconazole, danazol and fenofibrate (Bhuptani *et al.*, 2016; Zhang *et al.*, 2013).

Table 5.14: Percentage relative bioavailability of ketoprofen after administration of MT16 and MT2 matrix tablets

Human	**$AUC_{0-\infty}$(µg.h.ml^{-1})**		**%age relative bioavailability**
	MT16	**MT2**	
1	36.67	85.43	233.0023
2	34.957	75.20	215.1326
3	31.01	71.19	229.5607
4	30.33	73.80	243.3119
5	37.85	90.71	239.617
6	35.526	75.58	212.7382
Average	34.391	78.652	228.89
Standard Deviation	3.055	7.640	12.574

Tablet prepared using Soluplus 3% was selected for the pharmacokinetic study. The pharmacokinetic behavior of the drug was found appropriate. After appropriate

pharmacokinetic characteristics, a formulation is also a candidate for the establishment of IVIVC to determine type of correlation between *in-vitro* dissolution and *in-vivo* pharmacokinetics characteristics.

5.5 Conclusion

The pharmacokinetics of the selected MT2 ketoprofen matrix tablet (containing 3% Soluplus®) in human subjects assumed two compartment model. All the pharmacokinetic parameters of MT2 indicated significantly exhibiting sustained behavior, with a remarkable increase in percent relative bioavailability of ketoprofen after admistration of surfactant-based swellable-erodible matrix tablet as comapred to water-based system.

Chapter 6

In-vitro in-vivo correlation (IVIVC) analysis of surfactant-based swellable-erodible matrix tablet of ketoprofen (MT2)

6.1 Introduction

The IVIVC can be used to derive blood levels of drug from some of pharmacokinetic parameters and *in-vitro* dissolution data (Shargel *et al.*, 2016). IVIVC is a correlation between any of the *in-vitro* dissolution parameter of dosage form with its pharmacokinetic parameters The establishment of IVIVC requires several pharmacokinetic parameters, such as plasma level time curve, AUC, C_{max}, and fraction absorbed and unabsorbed of drug (Hu *et al.*, 2018; Wagner, 1983). In IVIVC, the correlation could be established between the fraction of drug absorbed and time of absorption to the fraction of drug dissolved *in-vitro* and the time of dissolution.

According to the FDA guidelines for IVIVC, newly developed dosage forms are to be evaluated for the four levels of *in-vitro* - *in-vivo* correlation; i.e., Level A, level B, level C, and multiple level C. The level A correlation is ranked the highest level of correlation where an *in-vitro* profile is compared to *in-vivo* profile. This profile to profile comparison is also called as the point to point comparison where level A is established, that *in-vitro* dissolution can predict pharmacokinetics of the drug. The level B correlation is the association of *in-vitro* mean percent dissolution time with the *in-vivo* pharmacokinetic parameters computed using statistical moment theory, i.e., MRT, mean dissolution time (MDT vitro), MAT etc. A single point derived from the whole data, i.e., $T_{10\%}$ vitro Vs AUC, C_{max}, T_{max} etc. Establishment of appropriate relationship can help predicting *in-vivo* equivalence of dosage form just with its dissolution study, without relying on the expensive pharmacokinetic studies in animals or humans (Gonzalez *et al.*, 2015). IVIVC reduces the regulatory burden as it may substitute the additional *in-vivo* studies. IVIVC is applied for optimization of dosage forms, provides

justifications for therapeutically compliant specifications for release from dosage form, and facilitates scale up post approval changes (SUPAC) and line extensions of products. Furthermore, it as the acts as surrogate for bioequivalence and serves as a justification for biowaivers (Mitra *et al.*, 2015).

The Wagner-Nelson method is used to calculate the fraction of drug absorbed and unabsorbed and predicts time to absorb certain percent of drug including mostly $T_{10\%}$, $T_{50\%}$ and $T_{90\%}$. Therefore, in this study the above parameters were computed for assessment of IVIVC in Section 6.3.4. Time to release certain percent of drug, i.e., $T_{25\%}$, $T_{50\%}$, and $T_{80\%}$ were computed from the release kinetics data using DDSolver. The time to release 10% ($T_{10\%}$), 75% ($T_{75\%}$), and 90% ($T_{90\%}$) were additionally calculated for broader comparison. The above parameters were computed from data of the percent drug release with the function of forecast implemented in MS Excel®.

The aim of this study was to establish IVIVC between the *in-vitro* release measured in Section 6.3.4 and *in-vivo* pharmacokinetic parameters of ketoprofen tablets. The objectives of the study were:

1. To determine the fraction of ketoprofen absorbed.
2. To compute additional dissolution and absorption parameters
3. To determine point to point correlation
4. To assess IVIVC

6.2 Materials and Methods

6.2.1 Determination of faction of ketoprofen absorbed

The Wagner-Nelson method was used in Pk-Fit, a pharmacokinetic software, the plasma level time data were entered to calculate fraction of drug absorbed and unabsorbed, along with the time to absorb specific percentage of drug, i.e., $T_{10\%}$, etc. The computation of

fraction drug absorbed requires on the rate of elimination (K_{el}) which was computed with PK-Solver version 1.0, a pharmacokinetic software using Two-compartmental approach (Table 6.1). The other requirement for implementation of Wagner-Nelson method is the F-value which is the fraction of dose absorbed or absolute bioavailability. The reference value of F was taken as 1, as reported (Ishizaki *et al.*, 1980). Following equation was used to compute percentage absorbed and un-absorbed in Wagner-Nelson method:

$$F = [(C_t + k_{el}\, AUC_{0-t}) / k_{el}\, AUC_{0-\infty}] \times 100 \text{ [1]}$$

Where F is the absorbed drug fraction, C_t is the drug plasma concentration at time t, k_{el} is the overall elimination rate, AUC_{0-t} is the area under curve from time zero to t and $AUC_{0-\infty}$ is area under curve from zero time to infinity (Khan *et al.*, 2010b).

Table 6.1: K_{el} values employed for calculation of the fraction absorbed and unabsorbed of ketoprofen after oral administration of 200 mg dose

Human Subject	**1**	**2**	**3**	**4**	**5**	**6**
Estimated K_{el}	0.179	0.179	0.173	0.167	0.150	0.169

6.2.2 Determination of additional release and absorption parameters

Additional release parameters, such as time to release specific percent of drug were calculated to establish IVIVC. The different times to release specific percent of drug ($T_{10\%}$-$T_{90\%}$) were calculated using Korsmeyer-Peppas model and compared to the time to absorb certain percent of ketoprofen as computed using Wegner-Nelson method at the same points. The release parameters were computed using DDSolver form the *in-vitro* dissolution data of drug. These include, the percentage of drug released after maximum dissolution time (8h), $T_{25\%}$, $T_{50\%}$ and $T_{80\%}$ of MT2.

The Pk-Fit produced data for fractions of ketoprofen absorbed and unabsorbed for MT2 as well as the ketoprofen absorption times, i.e., $T_{10\%}$, $T_{50\%}$, and $T_{90\%}$. To correspond the times required to release certain percentages of drug, such as $T_{10\%}$, $T_{25\%}$, $T_{50\%}$, $T_{75\%}$, $T_{80\%}$, and $T_{90\%}$, the time to absorb 25% ($T_{25\%}$), 75% ($T_{75\%}$), and 80% ($T_{80\%}$) were estimated with the forecast function of Microsoft Excel®.

6.2.3 Determination of point to point correlation

The point to point comparisons were made to establish IVIVC for the following *in-vitro* and *in-vivo* parameters:

A. *In-vitro* percent release of ketoprofen and its plasma concentration against various time intervals.
B. Percent ketoprofen released and percent absorbed against time points.
C. Percent ketoprofen unreleased and percent unabsorbed drug against time intervals.
D. Time required to release certain percent of ketoprofen to time required to absorb certain percent of drug

6.2.4 Assessment of IVIVC

The following *in-vitro* and *in-vivo* parameters were correlated:

A. Percent ketoprofen released versus ketoprofen plasma concentration
B. Percent ketoprofen released against ketoprofen $AUC_{0-\infty}$.
C. Percent ketoprofen released versus percent ketoprofen absorbed
D. Percent ketoprofen unreleased against percent ketoprofen unabsorbed.

6.3 Results and Discussion

6.3.1 Fraction of ketoprofen absorbed

The fractions absorbed and unabsorbed of ketoprofen after oral administration of 200 mg of as selected ketoprofen matrix tablet, MT2 in human subjects have been given in Table 6.2-6.7.

Table 6.2: Fractions absorbed and unabsorbed of ketoprofen after oral administration of 200 mg of oral dose ((MT2) in subject 1.

Time (h)	Concentrations (μg.ml^{-1})	Fraction absorption (%age)	Fraction unabsorbed (%age)
0.5	0.173	1.1811	98.819
1	1.014	7.0242	92.976
1.5	1.996	14.322	85.678
2	4.236	30.783	69.217
4	7.446	65.425	34.575
6	5.846	70.518	29.482
8	5.040	77.985	22.016
10	4.151	82.926	17.074
12	3.720	89.317	10.683
16	2.140	92.701	7.2988
20	1.620	98.1	1.9004
24	0.980	100	0

Table 6.3: Fraction ketoprofen absorbed and fraction unabsorbed after oral administration of 200 mg of oral dose ((MT2) in subject 2

Time (h)	Concentrations ($\mu g.ml^{-1}$)	Fraction absorption (%age)	Fraction unabsorbed (%age)
0.5	0.154	1.1733	98.827
1	0.845	6.5384	93.462
1.5	2.579	20.301	79.699
2	3.957	32.483	67.517
4	6.500	64.678	35.322
6	4.973	68.519	31.481
8	4.200	74.856	25.144
10	3.866	82.949	17.051
12	3.100	86.456	13.544
16	2.230	94.027	5.9732
20	1.446	97.907	2.0935
24	0.895	100	0

Table 6.4 Fraction ketoprofen absorbed and fraction unabsorbed after oral administration of 200 mg of oral dose (MT2) in subject 3.

Time (h)	Concentrations ($\mu g.ml^{-1}$)	Fraction absorption (%age)	Fraction unabsorbed (%age)
0.5	0.146	1.2376	98.7624
1	0.726	6.2566	93.7434
1.5	1.867	16.439	83.5613
2	3.759	33.789	66.2113
4	6.175	67.383	32.617
6	4.995	73.497	26.5035
8	3.990	77.961	22.0394
10	3.487	84.384	15.6163
12	2.945	89.021	10.9789
16	2.119	96.546	3.4538
20	1.374	100	0.00
24	0.639	100	0.00

Table 6.5 Fraction of ketoprofen absorbed and fraction unabsorbed after oral administration of 200 mg of oral dose (MT2) in subject 4

Time (h)	Concentrations (μg.ml^{-1})	Fraction absorption (%age)	Fraction unabsorbed (%age)
0.5	0.134	1.1066	98.893
1	0.837	7.001	92.999
1.5	1.997	17.135	82.865
2	3.917	34.313	65.687
4	6.435	67.979	32.021
6	5.524	76.589	23.411
8	4.158	78.578	21.422
10	3.282	81.484	18.516
12	3.069	88.203	11.797
16	2.208	95.35	4.6502
20	1.432	98.836	1.164
24	0.825	100	0

Table 6.6: Fractions of ketoprofen absorbed and fraction unabsorbed after oral administration of 200 mg of oral dose (MT2) in subject 5

Time (h)	Concentrations ($\mu g.ml^{-1}$)	Fraction absorption (%age)	Fraction unabsorbed (%age)
0.5	0.231	1.7961	98.204
1	0.937	7.4154	92.585
1.5	1.234	10.251	89.749
2	4.187	33.906	66.094
4	6.300	61.531	38.469
6	5.778	71.196	28.804
8	5.024	77.689	22.311
10	4.264	82.434	17.566
12	3.947	89.289	10.711
16	2.864	96.486	3.5141
20	1.993	100	0
24	0.983	100	0

Table 6.7: Fraction of ketoprofen absorbed and fraction unabsorbed after oral administration of 200 mg of oral dose (MT2) in subject 6.

Time (h)	Concentrations (µg.ml^{-1})	Fraction absorption (%age)	Fraction unabsorbed (%age)
0.5	0.136	1.102	98.898
1	0.658	5.4213	94.579
1.5	1.128	9.6621	90.338
2	4.957	41.431	58.57
4	5.981	63.764	36.236
6	5.100	71.474	28.526
8	4.256	77.205	22.795
10	3.775	84.018	15.982
12	3.214	88.839	11.161
16	2.380	97.055	2.9448
20	1.268	97.996	2.0039
24	0.820	100	0

6.3.2 Additional release and absorption parameters

6.3.2.1 Additional release parameters

The additional release parameters have been enlisted in table 6.8.

Table 6.8: Highest percent ketoprofen release after 8 h, and dissolution parameters of matrix tablets (Mean ± SD, n=3).

Formulation	% drug released (Max. 8 h)	$T_{25\%}$ (h)	$T_{50\%}$ (h)	$T_{80\%}$ (h)
MT2	87.2	2.83	4.31	6.75

6.3.2.2 Additional absorption parameters

Table 6.9 reports the time necessary to absorb various percentages of ketoprofen after the oral administration of matrix tablet in human subjects 1 to 6, which were compared to the time required to release certain percentages of ketoprofen from the matrix tablets. The release

profile of ketoprofen was compared to that of a reference release profile given as specification for the sustained release delivery system as given in Table 6.10.

Table 6.9: Time required to absorb percent of ketoprofen after administration of MT2 in human subjects 1 to 6.

Animals	**Time for absorption of % dose**		
	10% of dose ($T_{10\%}$)	**50% of dose ($T_{50\%}$)**	**90% of dose ($T_{90\%}$)**
Subject 1	1.204	3.120	12.810
Subject 2	1.126	3.088	13.872
Subject 3	1.184	2.965	12.520
Subject 4	1.148	2.932	13.006
Subject 5	1.456	3.165	12.395
Subject 6	1.505	2.767	12.565
Mean	1.271	3.006	12.861
SD	0.166	0.135	0.542

Table 6.10: *In-vitro* comparison of targeted ketoprofen release against the observed release of MT2 tablets

Time (h)	**% Ketoprofen released**	
	MT2	**Targeted (Reference)**
1	10.55	15.0
2	14.74	20.0
3	27.15	35.0
4	44.42	45.0
5	62.48	55.0
6	62.82	65.0
7	85.86	75.0
8	87.2	85.0

6.3.3 Point to point comparison

The profile to profile comparison of the 8 h *in-vitro* release profile (Table 6.10) to the plasma ketoprofen concentration (Figure 6.1), % release to percentage ketoprofen absorbed at the same points defines highest, the level A correlation. The Wagner-Nelson method was used to compute fraction of ketoprofen absorbed by entering the values of elimination rate constant obtained after two compartmental analysis. The fraction absorbed of ketoprofen was compared to the percent ketoprofen release at same time points as superimposing plot (Figures 6.2 and 6.3). Figure 6.4 shows a plot comparing all the above-mentioned *in-vitro* and *in-vivo* parameters against time.

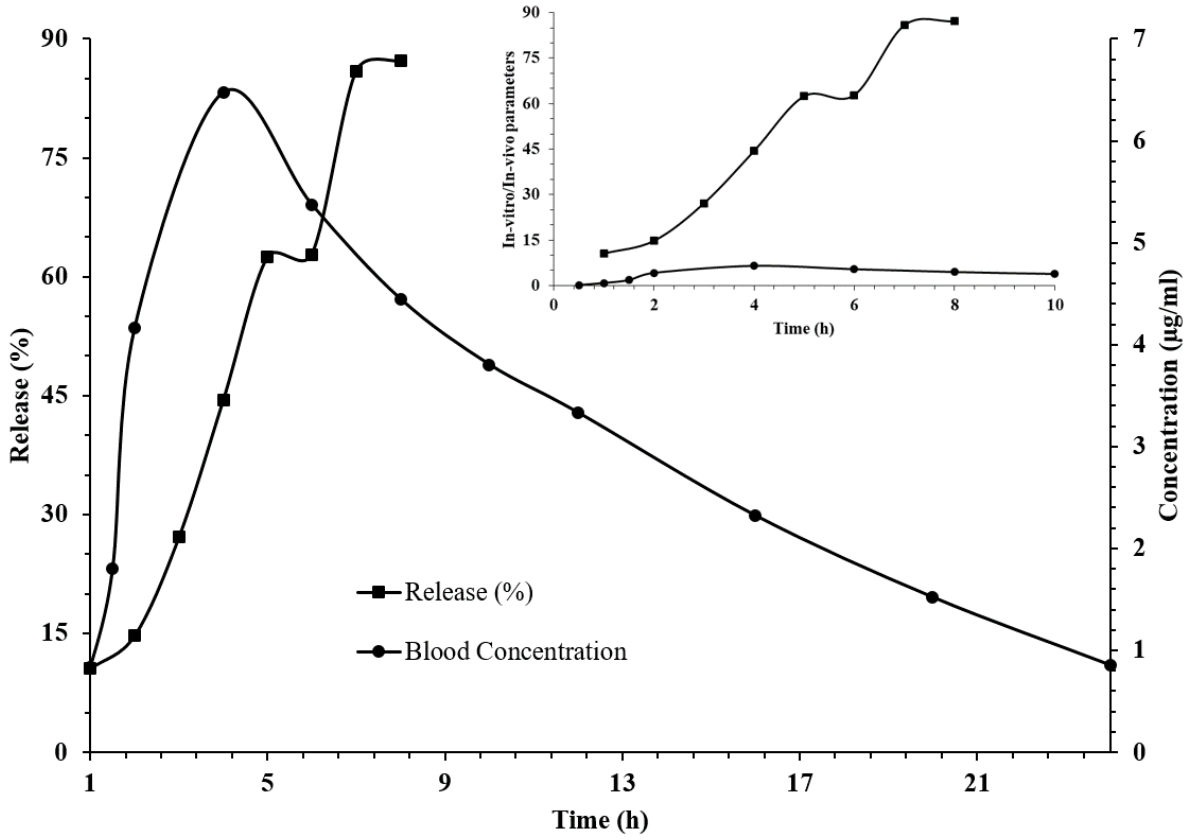

Figure 6.1: Comparison of the *in-vitro* release and blood plasma concentration of ketoprofen after oral administration of 200 mg of MT2 (the Inset is without secondary axis)

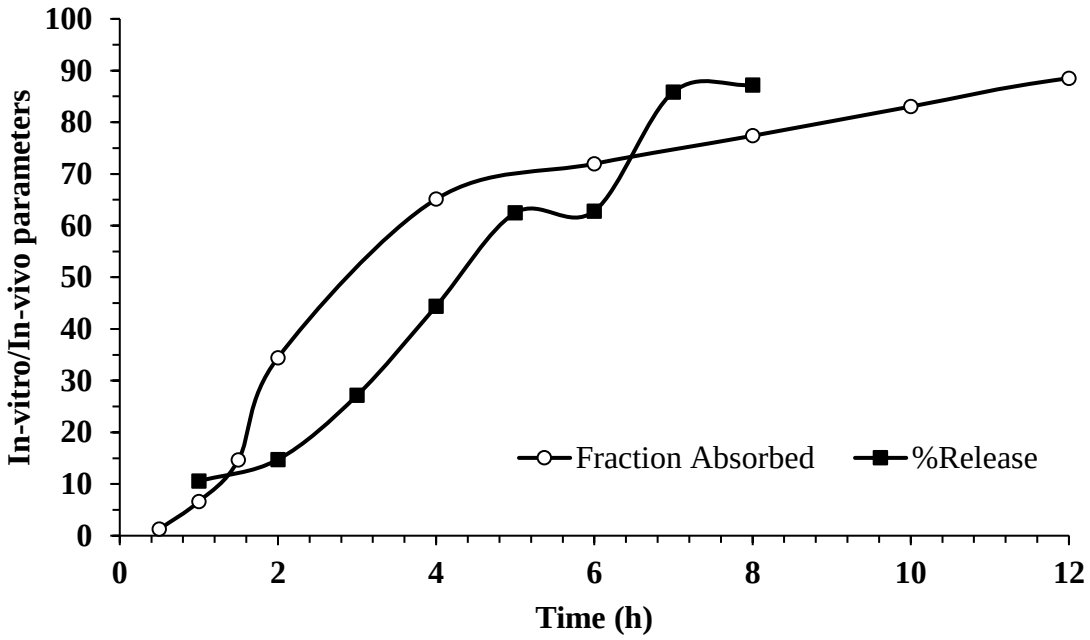

Figure 6.2: Comparison of percent released and fraction absorbed of ketoprofen after administration of 200 mg of MT2

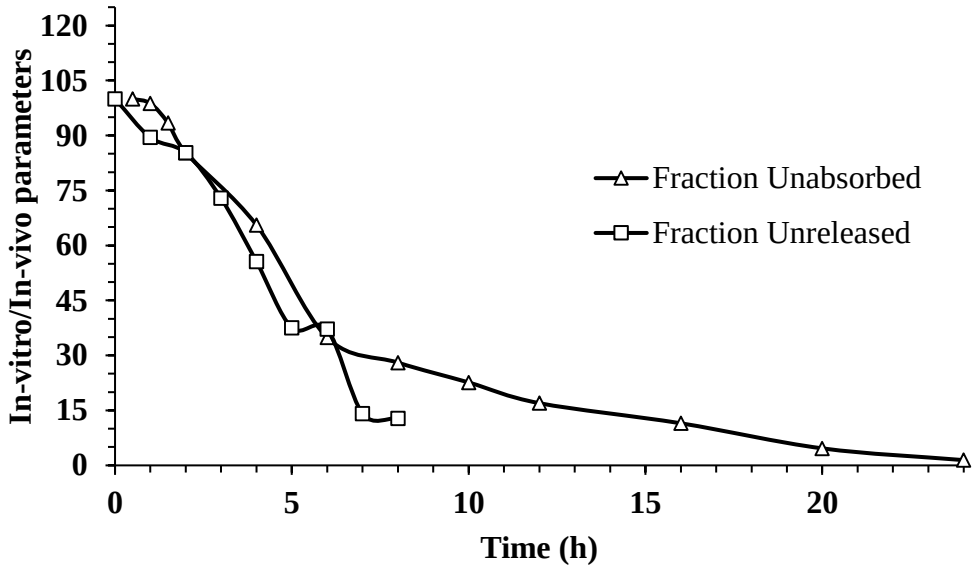

Figure 6.3: Comparison of fraction unreleased and fraction of unabsorbed of ketoprofen at various times after administration of 200 mg of MT2

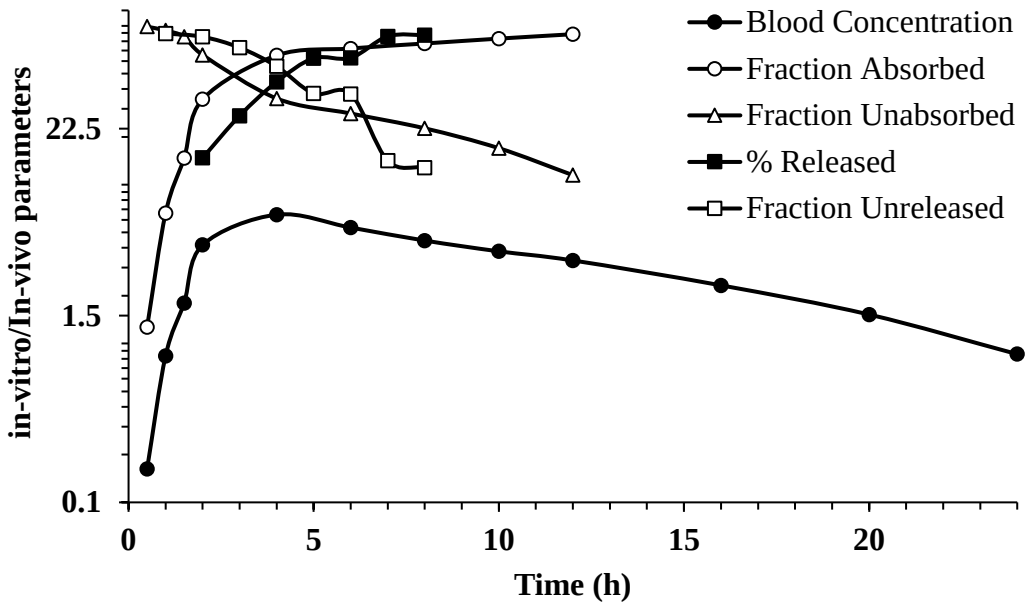

Figure 6.4: Comparison of dissolution and pharmacokinetic parameters of ketoprofen after its oral administration as matrix tablet (MT2) [Semilog graph paper has been used to elaborate the comparison].

The point to point comparison between percent ketoprofen released (Table 6.10) and percent ketoprofen absorbed at different time points generated corresponding profiles. (Figure 6.1). The Figure 6.1 shows the gradual increase in absorption started from 0.5 h until 5 h corresponding to increase in the dissolution started from 1 h till 6 h. The percent ketoprofen absorbed was gradually increased and higher than the percent ketoprofen release until 6 h. After 6 h, the % release of drug was higher. However, both of the profiles were corresponding the rising as well as when becomes at steady state levels (Figure 6.5). The percent ketoprofen absorbed was also compared to the percent release referred by USP for sustained release formulations (Macha *et al.*, 2009). The percent ketoprofen absorbed was shown to be elevated as compared to the observed percent ketoprofen release as well as that of the targeted release in this experimentation (Figure 6.5). Comparable profiles of predicted time for different %ketoprofen dissolved and the time for different percentages of drug absorbed was noted as shown in Figure 6.6.

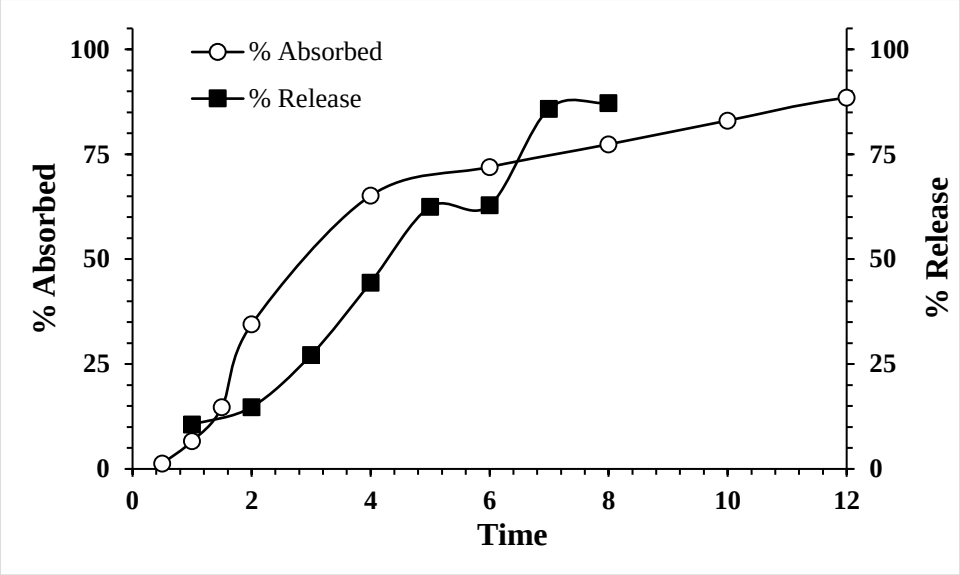

Figure 6.5: Ketoprofen tablet MT2 dissolved and absorbed at pre-defined time intervals

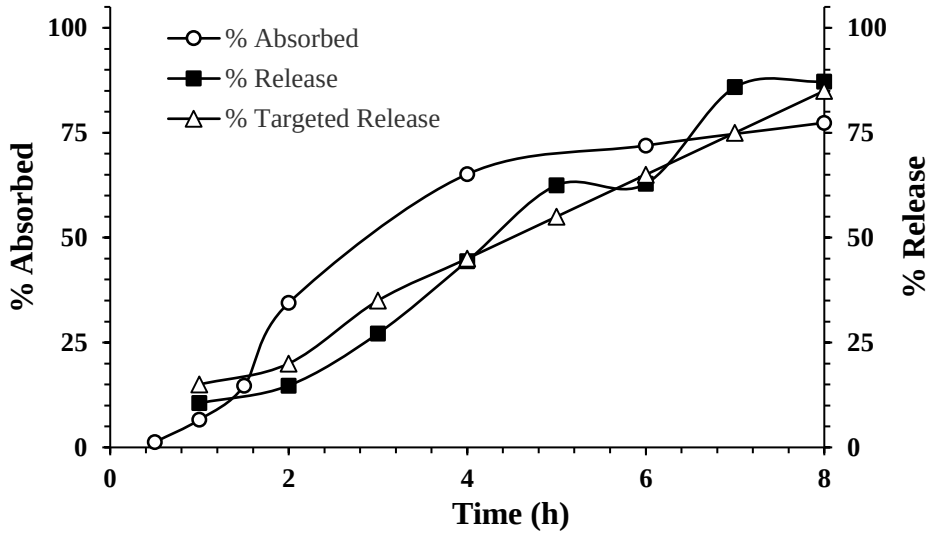

Figure 6.6: Comparative reference (targeted) release and ketoprofen selected tablet (MT2) dissolved and absorbed at predefined time

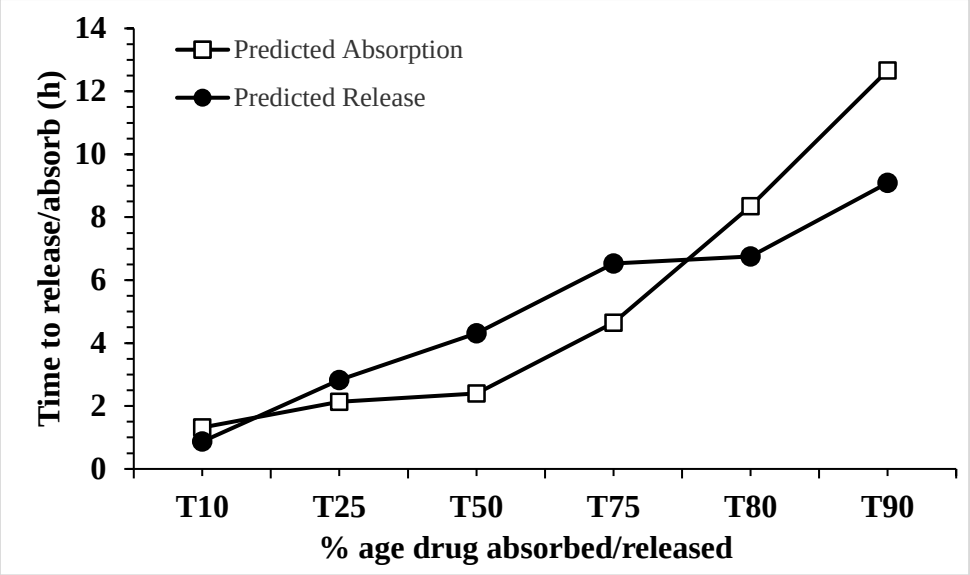

Figure 6.7: Percent ketoprofen released and absorbed at specified time points

6.3.4 IVIVC between release and pharmacokinetic parameters

As shown in Figure 6.8, the percent ketoprofen release from matrix tablet of MT2 showed an appropriate correlation with the plasma concentration as indicated by R^2 value of 0.9356. The correlation between the percent ketoprofen and the total area under the curve ($AUC_{0-\infty}$) was bit weaker indicated by R^2 = 7669 and given in Figure 6.9. Figure 6.10 reported a higher correlation (R^2=0.9613) between the percent ketoprofen release and the percent ketoprofen absorbed. The percentage of ketoprofen unreleased and the fraction of ketoprofen unabsorbed also correlated as shown by the R^2 = 0.9613 (Figure 6.11). The data for the predicted times to release 10%-90% ketoprofen and the corresponding times for percentage ketoprofen absorption, shown in Table 6.11 was good correlated (R^2 = 0.8203) as shown in Figure 6.12.

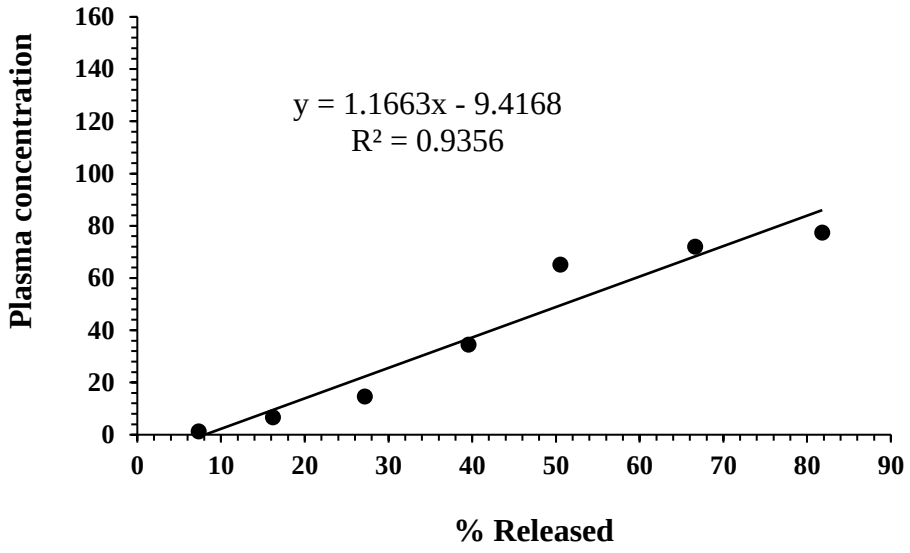

Figure 6.8: Percentage ketoprofen release from MT2 and plasma concentration

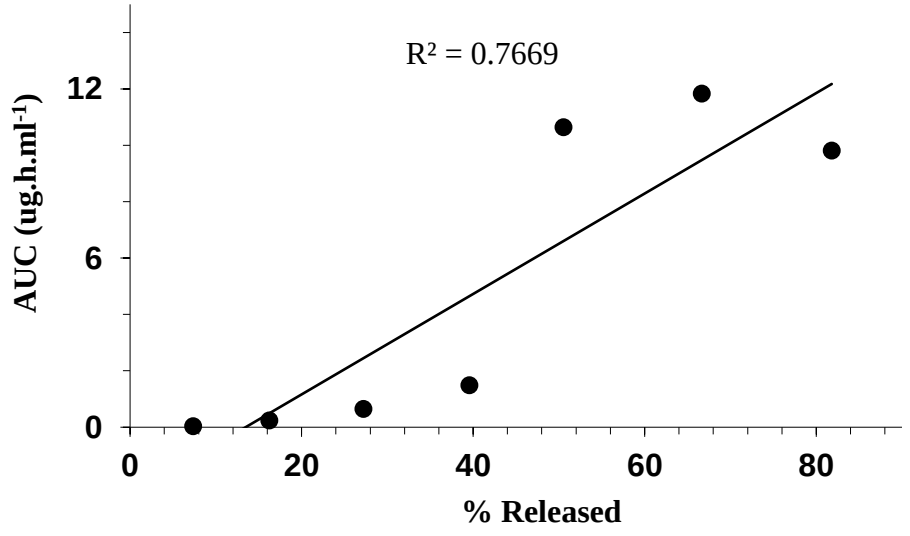

Figure 6.9: Comparative percent ketoprofen release and area under the curve.

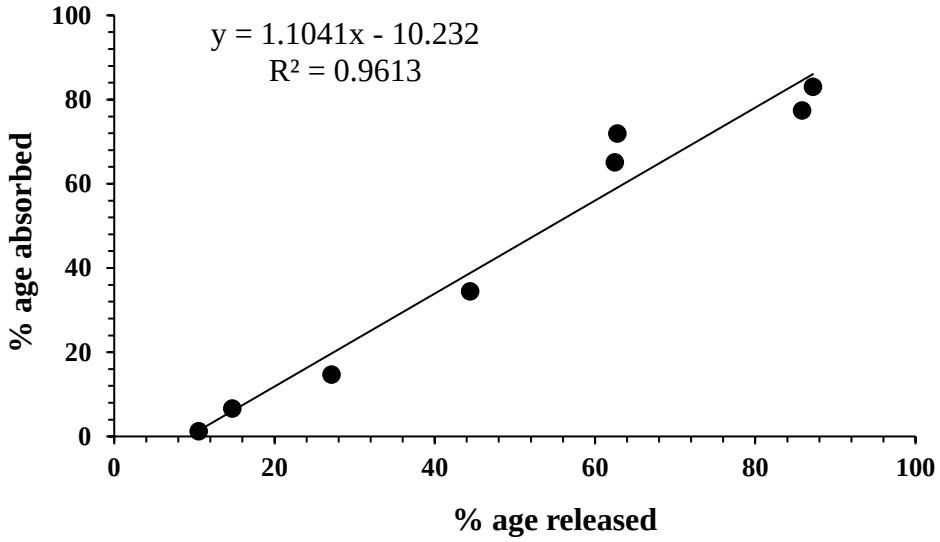

Figure 6.10: Comparison of ketoprofen MT2 tablets *in-vitro* percent dissolved and *in-vivo* percent absorbed

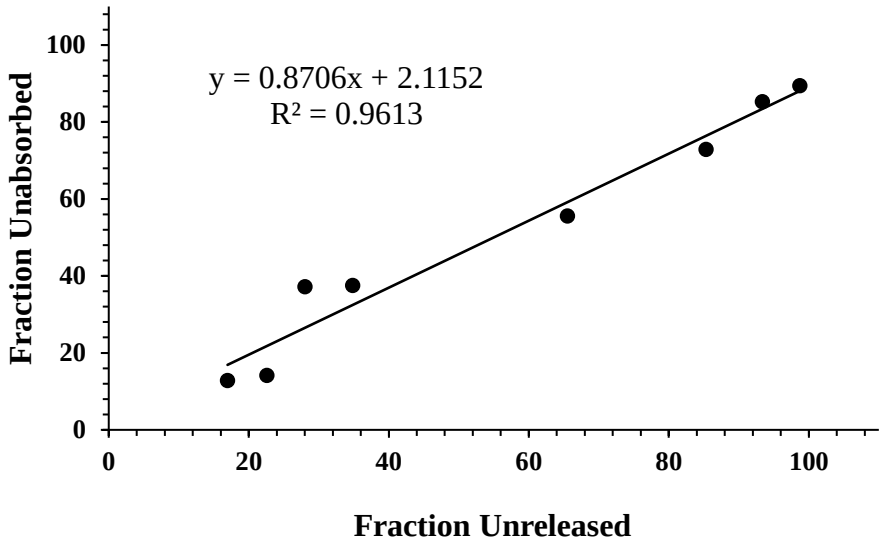

Figure 6.11: Comparison of fraction unreleased drug and fraction of unabsorbed drug

Table 6.11: Percent ketoprofen released or absorbed at predefined times computed using Korsmeyer-Peppas model and Wagner-Nelson method

% drug released/absorbed at time points	Predicted time form ketoprofen	
	% release (Korsmeyer-Peppas Model)	% absorption (Wegner-Nelson method)
T10%	0.87	1.317
T25%	2.83	2.139
T50%	4.31	2.393
T75%	6.53	4.643
T80%	6.75	8.350
T90%	9.09	12.662

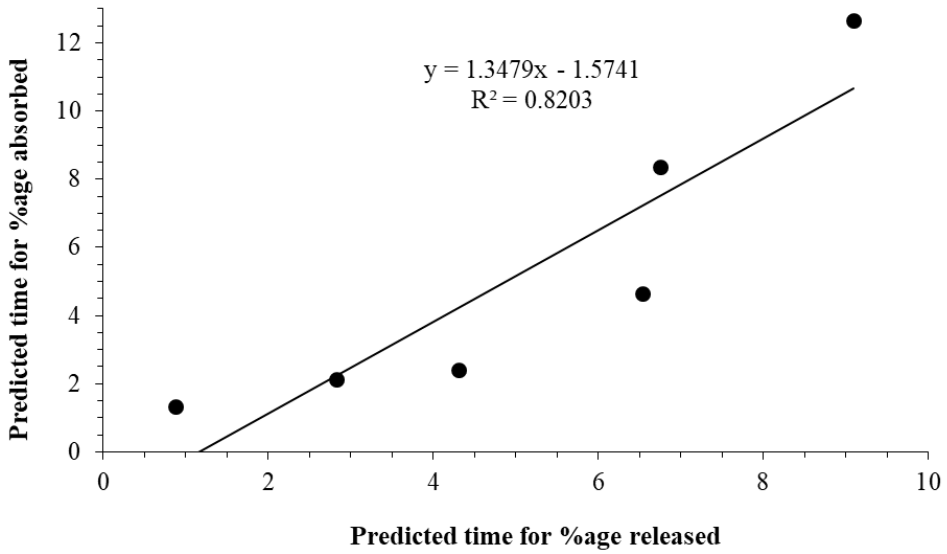

Figure 6.12: Percent ketoprofen absorbed and the release at specified time intervals

Though a release of ketoprofen was not found to be well correlated to the pharmacokinetic parameter was observed (Figure 6.12)which may be attributed to the effect of physiological factors such as pH, intestinal motility, GI transit time, fed and fasting status (Charman *et al.*, 1997; Grundy *et al.*, 1996; Streubel *et al.*, 2000). Level A correlated was indicated by the above findings for MT2 matrix tablets. The level A correlation is the most

preferred level correlation, since being the highest level of correlation (Emami, 2006) and it directly correlates multiple points of *in-vitro* dissolution profile with the points of the *in-vivo* pharmacokinetics. Use of SAWG approach for preparation of granules followed by compression into matrix tablet has facilitated the accomplishment of this highest level of correlation. Achievement of Level A permits biowaiver for minor variation in raw material quality and manufacturing processing (Sakore *et al.*, 2011). This also enables to predict the pharmacokinetics of a drug from its *in-vitro* dissolution assessment. The above possibility also facilitated designing bioavailability and bioequivalence studies for different formulations and also be used lead to SUPAC for a process of new drug development (Cai *et al.*, 2016).

The BCS classifies the drugs according to their solubility and permeability. Ketoprofen has been cited as BCS class II drug which exhibits poor solubility and high permeability (Kaleemullah *et al.*, 2017). According to literature ketoprofen has 100% oral bioavailability (Ishizaki *et al.*, 1980), a reason for considering F as 1 in the computation of fraction ketoprofen absorbed/unabsorbed in Wegner-Nelson method. This is also a probable reason for establishment of level A correlation. Permeability of drug facilitates establishing correlation between plasma and dissolution data which is not a rate limiting step in drug absorption for sustained/controlled DDSs (Lu *et al.*, 2011). IVIVC, i.e., level A correlation is expected for the extended-release dosage form of BCS Class I and Class II drugs with site-independent permeability (Nainar *et al.*, 2012). When drugs belonging to BCS class II, show non-dependent permeation and lower dissolution rate than the absorption rate, level A correlation is achieved (Souliman *et al.*, 2006).

The current formulated ketoprofen delivery system gradually and continuously released the controlled amount of ketoprofen drug that is expected to reach at the site of absorption throughout the release time course, therefore it achieved the Level A correlation.

6.4 Conclusion

With a successful correlation between the pairs of percentage of *in-vitro* drug released vs percent of *in-vivo* drug absorbed and the projected time to absorb percent of ketoprofen against time to release percentage of drug was established which indicated Level A correlation.

Chapter 7

General discussion and conclusion

This work proposed a novel SAWG approach for enhancing the solubility and simultaneously, sustaining release of ketoprofen presented as granules or matrix tablet formulation. The drug was found released by steady diffusion-erosion of matrix. SAWG approach successfully improved solubility of ketoprofen in granules which provided simultaneously, control over release and also provided the possibility of transforming granules into matrix tablets. The tablets demonstrated sustained release of ketoprofen assuming diffusion-erosion of matrix. Weibull model found to be the best kinetic model which could be used to describe the release of ketoprofen from the matrix tablet. The combination of surfactant, Soluplus®, HPMC, Avicel® and Crospovidone® in granules increased the solubility and helped controlling the release of ketoprofen in granules. The release of ketoprofen was close to the desired reference release profile when the granules were compressed into tablets. FTIR indicated no interactions chemically among the API and the ingredients, but supported hydrogen bonding. In this study, a new sustained release ketoprofen matrix tablet has been developed using a simpler approach resulting into a dosage form which has been suitable for the once-daily oral dose.

A couple of sustained release dosage forms for ketoprofen have been reported which have been given in Table 1.2 (Chapter 1). For instance, a sustained release capsule formulation Orudis Retard® which incorporated coated granules prepared, as reported by manufacturer using sucrose, colloidal silica, ethyl cellulose, lactose and talc and filled in capsule (Roda *et al.*, 2002). A sustained release ketoprofen pellets filled into capsule has been reported (Houghton *et al.*, 1984). Gastro-resistant cellulose acetophtalate-dibutyl phthalate-coated tablets with restricted release of drug for 2h has also been cited as Ibifen® (Roda *et al.*, 2002). In this study, the dosage form with desired features could be successfully

developed using SAWG method which did not require incorporation of any special ingredients or major manipulation of formulative ingredients. In this study the granulations for tablets were developed merely by addition of surfactants thus, SAWG was regarded as the simpler approach. The dosage form demonstrated a sustained release pattern showing a gradual fractional release of drug over 8 h contrary to the immediate release dosage forms which released ketoprofen substantially in stomach. The water-based granules containing matrix tablets in this study released > 50% of ketoprofen at pH 1.2 within 2 h. The drug release from the dosage form was restricted at pH 1.2, i.e., in the gastric media. This release inhibition was accomplished by the HPMC as reported (Roda *et al.,* 2002), coupled with the Avicel, and more prominently the surfactants. The bioavailability of the of the newly developed formulations was different from that of the water-based matrix tablet which in turn was like the conventional or immediate release ketoprofen delivery systems.

Tablet prepared using Soluplus 3% was selected for the pharmacokinetic study. The pharmacokinetics of MT2 ketoprofen matrix tablet in human subjects assumed two compartment model. The present developed sustained release tablet showed 90% ketoprofen absorption in 12.662 h after administration showed the duration of action for 05 to 24 h, since it was above drug's MEC (0.7-1.0 µg/ml). The SAWG brought changes in the ketoprofen formulations which demonstrated different release profiles and also accounted for the different extent and rate of absorption and the duration of action. All the pharmacokinetic parameters of MT2 indicated significantly sustained behavior, with a remarkable increase in percent relative bioavailability of ketoprofen after admistration of matrix tablet as comapred to water-based system. The sustained release formulations offer certain pharmacokinetic advantages: produces no or little fluctuations for plasma, lesser dose frequency of administration and this offer optimum patient convenience. After appropriate pharmacokinetic characteristics, a formulation is also subjected to the establishment of

IVIVC to determine type of correlation between *in-vitro* dissolution and *in-vivo* pharmacokinetics characteristics.

The selected tablet formulations ultimately yielded appropriate absorption profile. With a successful correlation between the pair of percent drug released versus percent drug absorbed and the predicted time to absorb percent of drug versus time to release percent drug indicated Level A correlation.

Chapter 8

Envisioned benefits and the prospects of the present study

8.1 Extended study for detailed investigation of release profile of ketoprofen

In this study, the release of the ketoprofen prepared using different types and concentrations of surfactants were studied up to 8 h. The formulation which did not show the 100% release from the matrices, such as MT1-MT5, MT7, MT10, and MT13 should be investigated for more detailed dissolution study by considering 12 h as the last time interval.

8.2 Extended study for detailed investigation of swelling index and behavior of ketoprofen

The swelling index (solvent uptake) and behavior, in this study was investigated at 6 in the distilled water was compared to time 0. To get more insight, the study could be extended observing the swelling index and behavior at different time intervals as well as using two dissolution media, i.e., the acidic and the basic pH. This would help understanding the swelling and release mechanism of the ketoprofen from the matrix tablets.

8.3 Pharmacokinetic study using cross-over design

In this study the pharmacokinetics of MT2 and MT16 was compared in an eight day washout-separated, two-session before-after design. A future study could be planned with the cross over design with appropriate washout period. The cross-over design is better in a way that the individuals act as their own control.

8.4 Comparative pharmacokinetics of BD administration of control

In this study, the control drug was administered as the single dose. A future stud could be extended to the twice per day administration of control (immediate release dosage from) to compare the pharmacokinetics. This could give more insight of the plasma drug concentration.

8.5 Measurement of synovial ketoprofen concentrations

The concentration of ketoprofen decreases more slowly in synovial fluid as compared to the concentration in blood, thus the concentration of ketoprofen after administration of sustained release formulation, should be predicted/studied.

8.6 Use of more surfactants with diverse properties

In this study a couple of surfactants were employed for enhancement of solubility of ketoprofen but a further study could be extended to a greater number of surfactants of different properties or HLB values for the study drug, ketoprofen or for other drugs with same physicochemical and biopharmaceutical properties, such as aspirin.

8.7 Use of different algorithms for establishment of IVIVC

For the establishment of IVIVC, the Wagner-Nelson method was employed, the study could be extended with Loo-Reigelman and convolution and deconvolution techniques. A further investigation could carry out to by using the convolution method to simulate the pharmacokinetic parameters directly from the *in-vitro* dissolution parameters. The simulated pharmacokinetics could be validated by computing the prediction error for reliability of the predicted pharmacokinetic parameters.

8.8 Scale down of the procedure to prepare 100 mg of ketoprofen tablets

The same procedure could be employed to prepare a 100mg tablet using the same approach and Soluplus®. This scaling down of the ketoprofen comes under the definition of scale up and post approval changes (SUPAC) for new drug development. However, since IVIVC has already been established for ketoprofen 200 mg tablet in this study, it could be possible to predict the pharmacokinetics of drugs with the above correlation.

8.9 Comparison to the commercially available modified release dosage forms

In this study, the newly developed formulations was compared to the control (prepared using water during granulation) and to the reference-specifications for the sustained release formulations. A study could be planned to compare the current selected dosage from to the commercially available dosage forms of ketoprofen.

8.10 Histopathological examination of upper GIT damage

Theoretically the upper GIT damage led by ketoprofen could be preventable. A histopathological investigation might help to prove the prevention local damage due to lesser drug release in acidic media (upper GIT), lower absorption and the newly developed sustained drug delivery of ketoprofen.

8.11 Envisioned applications of the study

The newly developed delivery system may lead an early availability of drug in plasma and warrant a constant (steady state) blood concentration of ketoprofen within the therapeutic window for about 24 h. The drug maintained a reasonable concentration above the minimum effective concentration of 0.7-1.0 µg/ml as reported (Houghton *et al.*, 1984) through this dosage form up till 24 h, i.e., before reaching to below detection limit (disappearance of drug

from blood). This will give an analgesic effect for 24 h. Thus, there is a possibility of once-daily oral administration of the ketoprofen without resulting into the fluctuations in plasma concentration. Such type of dosage form is supposed to enhance the patient compliance.

8.12 Applications of approach used in this study to other drugs

The effect of surfactants on the highly soluble drug but with limited penetration could also be investigated. The findings of SAWG approach may not be specific for particular surfactants or for a drug thus, could be employed for applications to a wider range of drugs.

Chapter 9

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