3. METHODOLOGY

3.1. Solubility study of Etodolac

Saturation solubility of ETO in 0.1N HCL, water, phosphate buffer (pH 6.8), PG, PEG 400 and soya bean oil was determined using standard shake flask method. An excess quantity of ETO was added to 100 gm of solvent in a tightly capped conical flask. To achieve uniform mixing, samples were constantly agitated at conditions (100 rpm, 37℃ and 24 h) in a reciprocating water bath.

Samples were centrifuged at (4000 rpm for 15 min) after 24h equilibrium where aliquots of supernatant of PG and PEG 400 were diluted to appropriate concentrations with Ethanol 95% however, warmed Ethanol 95% (45℃) was used with the aliquot of soya bean oil. The rest of samples were filtered through filter paper after centrifugation process and diluted with the same previous solvent. The samples were analyzed using spectrophotometer at wavelength 278 nm [89] using ethanol 95% as a blank.

3.2. Preparation of Etodolac fill formulations

3.2.1. Preparation of Lipid based formulations (LBFs) of Etodolac

3.2.1.1. Determination of Required HLB value for oily mixtures

Griffen[90] stated the determination of required HLB value for oily mixture showed in Table 3 experimentally by preparation of emulsifier blends of Span 80/Tween 80 in different ratios shown in table 4 by the following equation[18]:

- $\%\text{Tween 80} = \frac{(\text{HLB value of OM}) - (\text{HLB value of span 80})}{(\text{HLB value of Tween 80}) - (\text{HLB value of span 80})} \times 100$
- % Span 80 = 100 - %Tween 80

Table 3: Composition of the lipid ingredients used in lipid based formulations of ETO

Lipid ingredient	Percentage/100 gm
Bees wax	1 %
Hydrogenated vegetable oil Type I	1.33%
Hydrogenated vegetable oil Type II	5.66%
Soya bean oil	92%

Then oily dispersion medium ingredients were completely melted at 50℃- 60℃. Four grams of emulsifier blend was added to 38 gm of oil in a glass bottle then the mixture was agitated well until the ingredients were well mixed. At the same temperature 38 gm of water was added at one time and the glass bottle was well closed and shaken. After a few minutes the emulsion was re-shaken and then stored overnight in a controlled temperature water bath at 37℃- 40℃.

Table 4: Composition of the emulsifier blend containing Span 80 and Tween 80

Mixture No.	Total HLB value	Span 80	Tween 80
OM 1	5.0	93.45%	6.54%
OM 2	5.5	88.80%	11.20%
OM 3	6.0	84.10%	15.90%
OM 4	6.5	79.40%	20.60%
OM 5	7.0	74.80%	25.20%
OM 6	7.5	70.00%	30.00%
OM 7	8.0	65.40%	34.60%

3.2.1.2. Composition of lipid based formulations (LBFs) of Etodolac

Lipid fill formulations were prepared according lipid formulation classification system (LFCS) proposed by Pouton [55, 56] and Porter *et al* [44]. It was shown experimentally that the appropriate ratio between ETO powder and oily mixture to prepare a palatable suspension able to be proceed to encapsulation process via softgel encapsulation machine was 1:2. Compositions of softgel fill formulations were illustrated in Table 5.

Dispersion method[91] was used to prepare solid-in-liquid suspension for lipid based fill formulations to be encapsulated into softgels[21]. Liquid phase was prepared by melting of excipients involved in lipid fill formulations at 70℃in a reciprocating water bath., ETO was dispersed mechanically by a magnetic stirrer with application of a homogenizer to disintegrate aggregates of ETO into smaller particles in the slurry[91] till obtaining a homogenize dispersion of ETO in the molten mass.

For formulations containing PLX 407 and co-solvents, PLX 407 was molten with its co-solvent at 52-57℃, and then added to the prepared oily molten mass with continuous stirring before ETO dispersion in the prepared liquid vehicle.

3.2.2. Preparation of hydrophilic formulation of Etodolac

Hydrophilic fill formulation of ETO (i.e. F_{10}) was prepared in a solubilized form [21]. 300 mg of ETO was mixed with a magnetic stirrer with 1200 mg of PEG 400 till obtaining a solubilized form of ETO in a hydrophilic vehicle as shown in Table 5

3.3. Characterization of Etodolac fills formulations

3.3.1. *In vitro* dissolution study

The dissolution studies were carried out by using a pharmacopeial dissolution test for ETO capsules supplied with USP apparatus I in a phosphate buffer pH 6.8 ± 0.1.

The dissolution medium (1000 ml) was continuously operated at 100 rpm at 37℃ ± 0.5℃ for 60 minutes. Fill formulations were manually filled in hard gelatin capsule (size 0) equivalent to 300 mg ETO was added to the stirred dissolution medium at zero time[92]. At different time intervals (15,30,45,60 minutes), samples of 10 ml each, were withdrawn and filtered using 0.45µm Millipore filter, followed by compensation with the same volume of fresh dissolution medium. 5 ml of filtrate was diluted with 50 ml of phosphate buffer solution pH 6.8; samples (in triplicates) were then measured spectrophotometrically at wave length 278 nm using buffer solution as a blank.

Table 5: Composition of ETO fill formulations

Class Type	**I**	**II**		**III_a**		**III_b**		**III_a**	**IV**	**-**
Material name (mg)	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**	**F_6**	**F_7**	**F_8**	**F_9**	**F_{10}**
Drug	300	300	300	300	300	300	300	300	300	300
Soya lecithin oil	-	60	-	-	-	-	-	-	-	
Span 85	-	-	60	-	-	-	-	-	-	
Cremophore EL (CRM EL)	-	-	-	-	-	-	-.	106.68	-	
Cremophore RH 40 (CRM RH 40)	-	-	-	120	-	-	-	-	600	
Polyethylene glycol 400 (PEG 400)	-	-	-	-	-.	-	360	-	-	1200
Propylene glycol (PG)	-	-	-	-	-	360	-	-	-	-
Poloxamer 407 (PLX 407)	-	-	-	-	120	120	120	13.32	-	-
Bees wax	6	5.4	5.4	4.8	4.8	1.2	1.2	4.8	-.	-
Hydrogenated vegetable oil-Type I	7.99	7.18	7.18	6.4	6.4	1.6	1.6	6.4	-	-
Hydrogenated vegetable oil-Type II	33.99	30.56	30.56	27.2	27.2	6.8	6.8	27.2	-	-
Soya bean oil	552	496.8	496.8	441.6	441.6	110.4	110.4	441.6	-	-

3.3.2. Viscosity measurement

The viscosity of lipid based formulations were measured by using Brookfield type rotary viscometer where different spindles and at different shear rates in a controlled temperature water bath. Spindle number 96 with shear rate 30 rpm was selected to measure the viscosity of lipid fill formulations at 35- 37℃. Apparent viscosity is an exponential term and therefore the log apparent viscosity is an appropriate way of reporting the results [93].

3.3.3. Drug content analysis

For each formula, one manually filled hard gelatin capsule where the average capsule weight was (900 mg ± 5%) was placed with 20 ml of distilled water in a Sonicator for 15 minutes at room temperature, and then diluted to 100 ml with ethanol 95% in a tightly capped volumetric flask. Samples (in triplicates) were filtered using 0.45 µm Millipore filter then assayed for their drug content spectrophotometrically at wavelength 278 nm using ethanol 95% as a blank[94].

3.4. Characterization of Etodolac Softgels

3.4.1. Rupture test Etodolac softgels

Air filled soft gels containing fill formulations were subjected to rupture test (i.e. a pharmacopeial official test for softgels) in triplicates by using USP apparatus II operated at 50 rpm in 500 ml water for 15 minutes where each capsule was fitted in a sinker due to presence of air bubbles inside the capsule leading to floating of capsule at the surface of the medium[92] till ruptured. In case of unruptured capsule, the test may be proceeded to 30 min till capsule rupture

3.4.2. Water migration study of Etodolac softgels

In order to study the effect of core fill composition on the water sorption behavior of the softgels, they were subjected to water migration studies[94];, air filled softgels were prepared according to the composition shown in Table 6.

Table 6: Shell composition of air filled soft capsule

Material Name	Concentration
Gelatin	49.85%
Glycerin	10.7%
Sorbitol	9.2%
Purified water	29.62%

Three air filled softgels were injected manually by syringe with the fill formulations of ETO and the orifice was sealed with molten gelatin. Injected air filled capsules were weighed and placed in a controlled temperature room (21-24℃) and controlled relative humidity (20-30%) to be permitted to come to moisture equilibrium under these conditions[27]. The capsules were weighed for successive 7 days until it became constant indicating that equilibrated moisture absorption had been achieved[94].

After equilibrium, the moisture content of the fill in a softgels (in triplicates) was measured using a karl-Fisher apparatus[21, 94], where the softgel was cut open at the seam with a knife and the fill was collected into a syringe. The syringe was capped tightly to prevent any moisture transfer between the fill and the surroundings until measurements were completed[21] where the fills were inserted into the titration vessel containing dried methanol (Karl-Fisher grade) and titrated with pyridine after stirring for 2 min.

The moisture content of the shell was measured using a loss on drying method (LOD)[21, 26, 95], . The softgels (in triplicates) were cut open at the seam with a knife and its fill contents were drained. The shell was then given a quick wash in isopropyl alcohol and wiped clean from any remaining fill contents with a paper towel before the initiation of measurements[21]. The weight of the softgel was recorded before placing in an electric oven at 105℃ for 24 h[26, 95], then the weight of the shell was recorded again and the percentage of moisture content was calculated by the following formula:

$$(W_0 - W) / W_0 \quad X\ 100$$

Where W_0 is the weight of the shell before drying
W is the weight of the shell after drying

For comparison, before the study took place the moisture content of empty air filled softgels (in triplicates) were measured using LOD method, also the moisture content of the fill compositions were measured (in triplicates) by using Karl-Fisher instrument.

3.4.3. Evaluation of the mechanical properties of Etodolac softgels

Rectangular strips (2.5 cm x 2 cm) were prepared by cutting the softgels vertically at the lower seam with a knife and cleaned from the fill contents by isopropyl alcohol and a dry towel. The thickness of each strip was measured at six different points by using a micrometer to calculate the average thickness which was (0.7 - 0.8 mm). The locally made tensile strength tester was constructed from a small balance fixed at the top of wooden plate for reading the stress needed to cut the strip in kilograms (Kgs), a ruler fixed at the middle of the plate for reading the elongation length after the stress application and a plastic wheel fixed at the bottom of the plate for stretching the strip as shown in (Figure 7).

The tested strip was hanged in the tensile strength tester between the two jaws of the device and keeping 1 cm length of the strip between them (Figure 8). The strip was then stretched by rolling the plastic wheel of the device. The weight in kilograms (breaking load) and the length of the elongated strips at which the strip cuts, were recorded. The average of at least four tested strip was calculated for each formula in softgels[96].

Puncture force (PF), tensile strength (TS), modulus of elasticity (young's modulus) and percentage of elongation (%PE) were calculated using the following equations:

PF (N) = PF (Kg) x 9.8[97]
TS (MPa) = PF (N) / cross sectional area (mm^2)[96]
%PE = $(L - L_0 / L_0) \times 100$[96], *Where L and L_0 are the film length at certain stress and the original film length (cm), respectively*.
E (MPa) = TS / ($L - L_o$) / L_o, *Where E is young's modulus (modulus of elasticity)*[96].

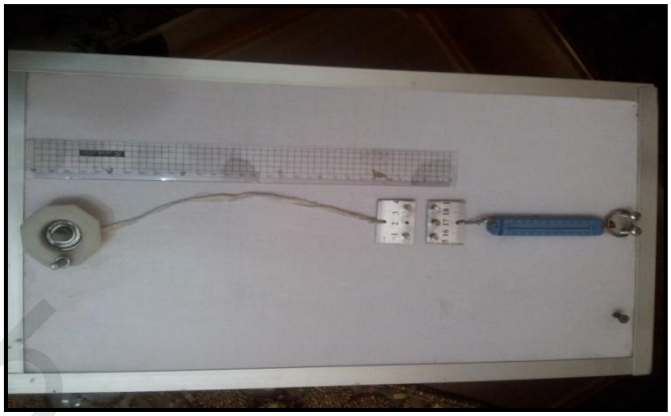

Figure 7: Locally made tensile strength tester.

Figure 8: Fixed shell strip between two jaws.

3.5. Stability studies on ETO softgels

Accelerated stability study was carried out on filled soft gelatin capsules containing selected formulations of ETO stored in a stability cabinet (Vötsch, Germany) for 3 months at controlled relative humidity (RH 75%) and controlled temperature 40℃[98]. In addition, shelf stability was carried out on filled capsules stored in a room with RH (60-65%) and temperature (20-25℃) [99] where different samples were taken at different time intervals and subjected to following experiments.

3.5.1. High performance liquid chromatography chemical stability test

Standard ETO and test samples of F 1 and F 10 fill contents were diluted with Ethanol 95% to appropriate concentration and their peak areas were measured by using slightly modified USP HPLC method for ETO analysis. The HPLC instrument (Agilent 1200, USA) was equipped with a reversed-phase C18 (25 cm x 4.6 mm; particle size = 5 µm). The filtered and degassed isocratic mobile phase (Acetonitrile: water: phosphoric acid ; 600 ml : 400 ml : 0.4 gm, with pH adjustment = 3) was run at a flow rate 1.5 ml per minute at room temperature and the column effluent was monitored by an UV detector set at 225 nm. Samples (20 µL) were automatically injected into the analytical column concurrently within standard solution injections [92].

3.5.2. *In vitro* dissolution test

Three soft gelatin capsules from each formula were chosen randomly, were cut at the seam with a knife and its fill contents were filled manually in a hard gelatin capsule (Size 0) to be equivalent to 300 mg ETO. The test was carried out with the same procedures in experiment number 3.3.1.

3.5. 3. Rupture test for Etodolac softgels

The test was carried out on triplicate samples of fill formulations and the procedures were similar to experiment 3.4.1.