4. RESULTS AND DISCUSSION

4.1. Solubility studies of Etodolac

Saturation solubility of ETO in 0.1N HCL, water, phosphate buffer pH 6.8, PG, PEG 400 and soya bean oil was demonstrated in (Figure 9).

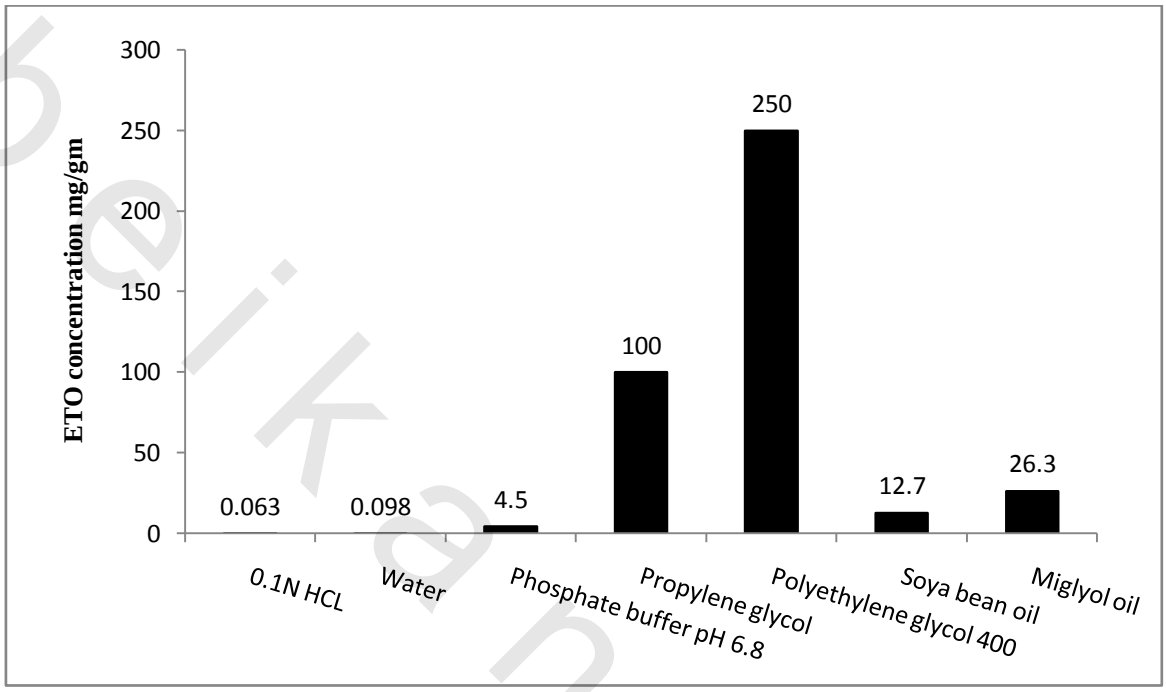


Figure 9: Saturation solubility of ETO in different vehicles.

Results revealed an increase in ETO solubility as the pH was shifted from acidic range (pH 1.2) to neutral range (pH 6.8). The highest solubilizing capacity of ETO was manifested in phosphate buffer pH 6.8 (4.5 mg/ gm), ETO is a weak acidic drug having pKa of 4.65 so its solubility tend to be increased rapidly with pH value above pKa of the drug. This result supports the selection of phosphate buffer pH 6.8 as a suitable dissolution medium achieving sink conditions to perform a suitable dissolution profile for ETO where 1000 ml of the buffer medium able to solubilize 4.5 gm of ETO[92].

In case of PG and PEG 400, the results showed that the saturated solubility of ETO was 100mg/gm and 250 mg/gm respectively. Although the high solubility capacity of ETO in PG (100 mg/gm), it was excluded to formulate a solubilized form of ETO in a hydrophilic base because weight of the fill content in such case is more than the capacity of available softgels, i.e. (fill weight = 3300 mg) and due to the restriction for using PG in softgel formulations to less than 10% of the total fill formulation, as PG migrates to gelatin shell and acts as a plasticizer[100], leading to deformation of the softgel shell.

In case of PEG, it showed high solubility of ETO (250 mg/gm) so the weight of the fill content in this case is compatible with the capacity of softgels which can be filled in softgel. Concerning the saturated solubility of ETO in long chain triglyceride (LCT), Results revealed limited solubility of ETO in soya bean oil (12.7 mg / gm) so 300 mg ETO was formulated as suspension not in liquefied form in lipid based fill formulations.

4.2. Preparation of lipid based fill formulations of Etodolac

4.2.1. Determination of the required HLB value of oily mixtures

Upon dispersion of ETO with LBFs, it showed good physical stability with LBFs containing oil only or with lipophilic surfactants while it showed bad physical stability (i.e. Aggregates formation) with LBFs containing hydrophilic surfactants, this may be attributed to a difference between HLB value of these hydrophilic surfactants and the required HLB value of the dispersion medium. To enhance the compatibility between the two phases, required HLB of the dispersion phase was determined experimentally to determine the most appropriate HLB value of hydrophilic surfactant mixture to line with the required HLB of the dispersion phase to avoid bad stability of LBFs of ETO.

Griffen[90] stated that the required HLB values for blends of oils could be determined in a manner similar to that for blends of emulsifiers and he utilized the following equation to calculate the required HLB for oil blends:

- $HLB_{required} = \Sigma\ HLB_i\ X\ f_i$ *where (f_i) is the mass fraction of the oil (i)*

Where Griffen determined the HLB value for an oil (i) experimentally by using non-ionic surfactants then each value was multiplied with its fraction in the oily mixture, then $HLB_{required}$ for oily mixture could be calculated by addition of the resulted value for each oil.

Pasquali *et al*[101] stated that the equation used by Griffin to calculate required HLB for oils had no theortical nor experimental support as it was affected by noticeable error. This noticeable error was appeared in calculation of required HLB of many oils which were listed by Griffin, due to using of temperature dependant non-ionic surfactants in his experiments at temperature 60-70℃ which introduced an errors in such determinations. This source of error could be avoided by using a solution at room temperature as the oily phase. For this reason, the results obtained in the calculation should be used as a first approach; the definite required HLB should be determined experimentally.

For oily mixture, it was shown that at HLB value = 6, the prepared emulsion was physically stable shown no creaming or segregation between the oily and aqueous phase as shown in Figure 10.

4.2.2. Composition of Lipid based formulations (LBFs) of Etodolac

A. Type I and II (F1, F2, F3)

Upon preparation of theses formulas, ETO was well dispersed in the dispersion phase where there was no sedimentation or aggregation formed of ETO during the preparation. This was because of the compatibility of lipophilic surfactants with the low required HLB value of the oily mixture as shown in Figure 11.

B. Type III_a (F4, F5, F8)

On dispersion of ETO in the dispersion phase of F4 small aggregates of ETO were formed upon the preparation process. These aggregates were formed due to the incompatibility of CRM RH 40 and the oily mixture where its HLB value was slightly higher than the required HLB value of the oily mixture.

In case of F5, on dispersion of ETO in the dispersion phase which was composed of 20% PLX 407 and oily mixture, large aggregates of ETO were formed which were completely separated from the oily mixture. This may be attributed due to high difference between HLB value of PLX 407 (18-23)[102] and the required HLB of the oily mixture, this incompatibility was shown in Figure 12.

In case of F8, the hydrophilic surfactant was composed of a mixture of CRM EL(i.e. HLB value = 12-14) and PLX 407 where the total emulsifier represented 20% of the oily mixture, although CRM EL resembled CRM RH 40 in the effect on the solubility of ETO in aqueous medium, CRM EL was selected due to its relatively low HLB value if compared with CRM RH 40 to approach the required HLB value of oily mixture.

The advantage of using a mixture of PLX 407 and CRM EL was to get the best results of the *in vitro* dissolution profile of ETO with a small concentration of PLX 407 with better physical properties if compared to F5 via reducing the formation of large rigid flakes of ETO. Upon dispersion of ETO in the mentioned mixture, no flakes of ETO were seen but there was a little segregation in the formed system due to the total HLB value of emulsifier = 13.8 which leads to the incompatibility between the mixture of emulsifiers and the oily mixture.

C. Type III_b (F6, F7)

Due to formation of large flakes in the case of F5, co-solvents as PG and PEG 400 were used to solubilize the hydrophilic surfactant PLX 407 in F6 and F7 respectively. It was shown that the compatibility of oily mixture was enhanced with hydrophilic emulsifier; it may be attributed to an increase in the concentration of hydrophilic co-solvent more than the concentration of the oily mixture in the fill content as shown in Figure 13.

Different concentrations of co-solvents were examined with the PLX 407 and the dispersion of ETO in the dispersion medium. It was observed that at concentration less 40%, aggregates of ETO were formed in addition that the viscosity of the formed fill was more than 5000 cP. In this case the optimum concentration of co-solvents was 60%. Upon dispersion of ETO in the formed mixture, no aggregates of ETO were formed and the formed system had a good physical properties.

D. Type IV (F9)

CRM RH 40 was warmed at 40℃ to a liquefied form and then ETO was dispersed in the formed dispersion medium. No aggregates or separation were seen upon the preparation of ETO in F9.

4.3. Preparation of hydrophilic formulation of Etodolac

Depending on the saturated solubility of ETO in PEG 400, PEG 400 was selected to formulate the solubilized form of ETO in softgel. 300 mg of ETO was solubilized in 1.200 gm of PEG 400 by preparation of a solution of ETO in PEG 400 at 37℃ in a controlled temperature water bath.

4.4. *In vitro* dissolution study

According to USP requirements for ETO dissolution test; not less than 75% of the labeled amount of ETO is dissolved in 30 mins[92]A comparative dissolution profiles among different lipid based formulations of ETO in comparison with hydrophilic based formulation were took place to show the effect of lipid based classification system and hydrophilic based formulation on the dissolution behavior of ETO in phosphate buffer pH 6.8.

Figure 10: Required HLB value of oily mixture at different HLB values.

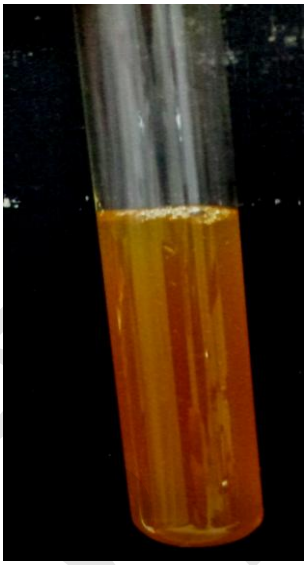


Figure 11: Miscibility of soya lecithin oil with oily mixture.

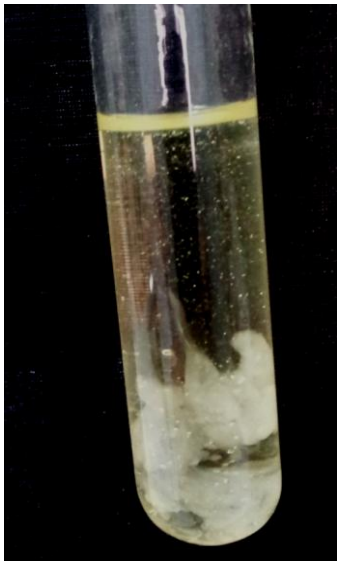


Figure 12: Immiscibility of poloxamer 407 with oily mixture.

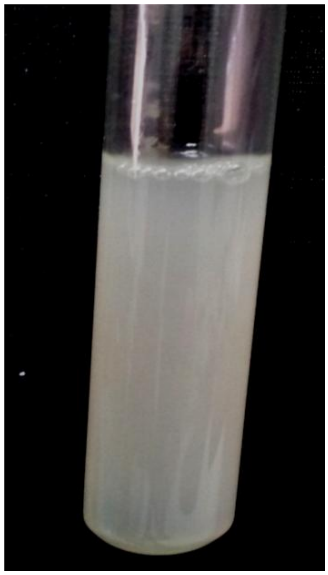


Figure 13: Miscibility of poloxamer 407 in propylene glycol with oily mixture.

4.4.1. Dissolution profile of fill formulations of ETO

Dissolution profiles of formulated ETO in Type I (F1) was compared to different dissolution profiles of lipid based fill formulations of ETO represented in Type II (F2, F3), Type III_a (F4, F5, F8), Type III_b (F6, F7), Type IV (F9) and hydrophilic based fill formulation F10.

Type II formulations (F2, F3) in comparable with Type I formulation (F_1) demonstrated PD 30 of 17.53%, 21.56% and 7% respectively as shown in Figure 14. Results revealed that the dissolution of formulated ETO in Type I (i.e. oil only) was below the USP requirements for ETO dissolution; this was due to absence of any surfactant in Type I systems. Thus, they had very limited ability to self-disperse in water. They were mainly depend on digestion to facilitate colloidal dispersion by solubilization of digestion products in mixed micelles[57].

On other hand, results revealed that the dissolution of formulated ETO in Type II systems (i.e. formed of LCT and water insoluble surfactants) were below the USP requirements for ETO dissolution test because of poor hydrophilicity of the surfactant to be dissolved and form micelles in aqueous solution and promote the emulsification of glycerides in the aqueous phase, then it exist itself as a dispersed phase, either with or separated from the oily components[56, 57]. Due to dissolution results of F2 and F3 were beyond USP requirements for etodolac dissolution, F2 was only selected for characterization of Type II.

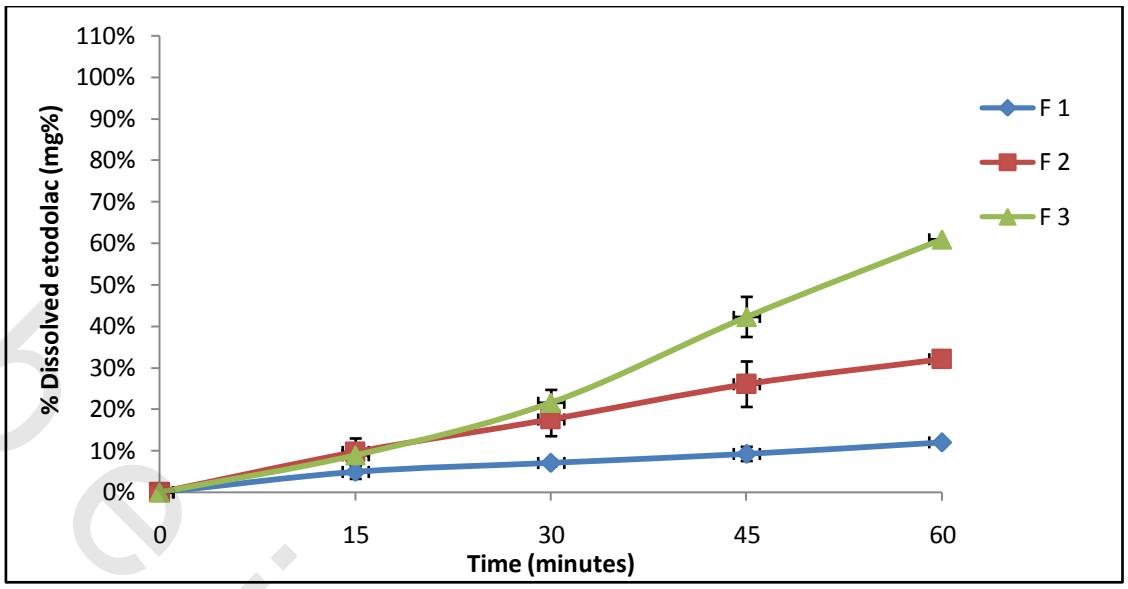


Figure 14: **Comparative dissolution pattern between Type I and Type II.**

Dissolution results of Type III_a (F4, F5, F8) and Type III_b (F6, F7) were compared to the results of Type I formulation demonstrated PD 30 76.27%, 75.43%, 93.77%,103.4%, 94% respectively as shown in Figure 15 and Figure 16. Results revealed that F4, F5, F8, F6 and F7 achieved the USP requirement for ETO dissolution. Pouton[56] speculated that the water-soluble components will tend to part from the oil during dispersion and become dissolved in the aqueous phase, the result of this phase separation, which may in fact be the driving force for emulsification.

It was observed that the combination between two hydrophilic surfactants (F8) (i.e. composed of PLX 407 and CRM EL) showed better results than using single hydrophilic surfactant. Dissolution results ETO formulated as Type III_b showed better results than Type IIIa formulations as shown in figure 16. The reason behind these results was due to inclusion of co-solvents like PEG 400 or PG. Pouton *et al*[57] stated that inclusion of co-solvents in lipid based fill formulations will lead to increase the solvent capacity of the formulation for drugs which dissolve freely in co-solvents, however to enhance the solvent capacity significantly the co-solvent must be present at high concentration and this was associated with the risk of drug precipitation when the formulation was dispersed in water. Moreover, inclusions of co-solvents aid the dispersion of systems which contain a high proportion of water soluble surfactants. Also, inclusion of co-solvents were useful in reduction variability and irritancy caused by high local concentrations of surfactants[55]. On other hand, Cole *et al* [9] illustrated the practical limits on using concentration of co-solvents with oil components and also possible incompatibilities of low molecular weight co-solvents with capsule shell.

Although relative good dissolution results of F5 as in figure 15, it was excluded from the chosen formulations in the study due to difficulty to inject softgels with F_5 due to its large rigid flakes.

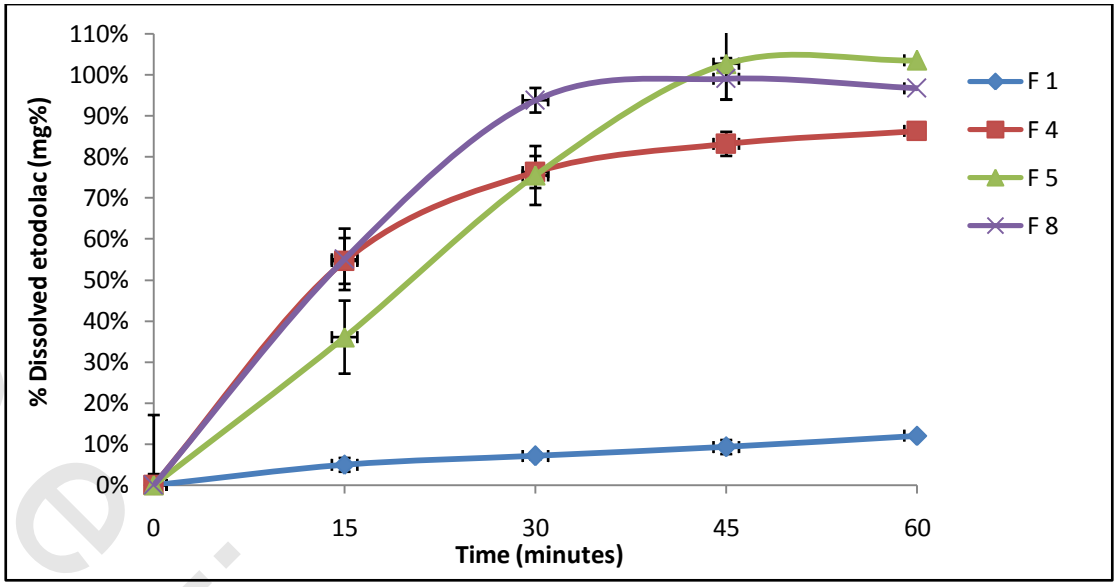


Figure 15: Comparative dissolution pattern between Type I and Type III_b

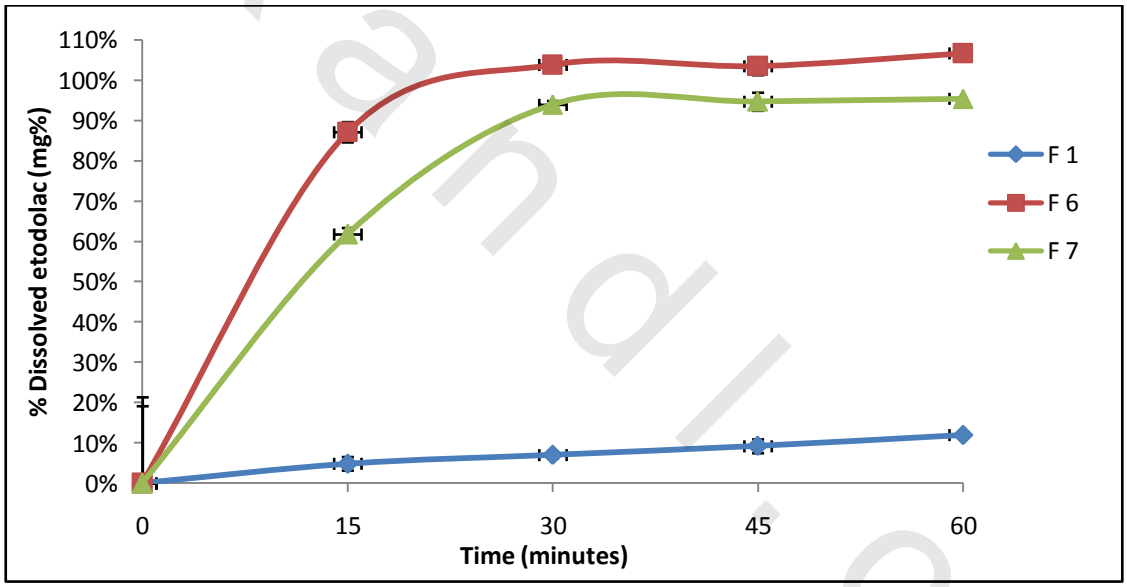


Figure 16: **Comparative dissolution pattern between Type I and Type III_b**

Dissolution results of Type IV (F 9) and hydrophilic fill formulation of ETO (F10) show better results than Type I formulation demonstrated PD 30 94%, 81% respectively as shown in Figure 17.

The typical relationship between concentration of solubilizing agent and total drug solubility for a co-solvent and a micellar system was studied by Pouton[57]. It is reported that drug will precipitate from the co-solvent solution but remain in solution when the micellar system is diluted which is in agreement with our results. Formulation of ETO in a pure water soluble surfactant (F9) show better dissolution results than pure co-solvent system (F10).

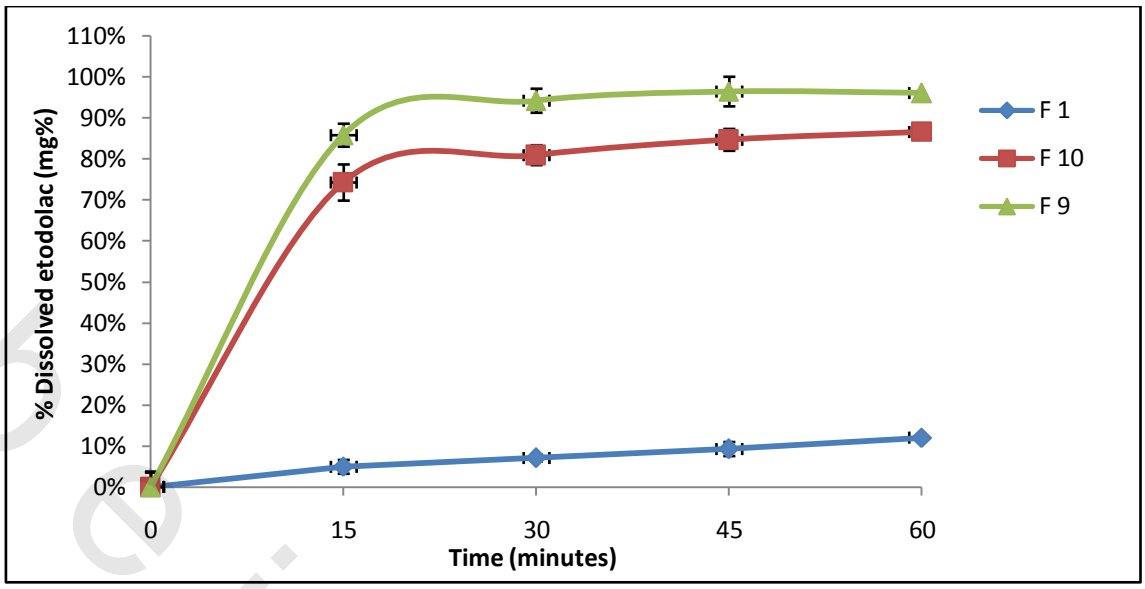


Figure 17: Comparative dissolution pattern among Type I, Type IV and F_{10}

4.5. Viscosity and drug content analysis for Etodolac formulations

Viscosity measurements were carried out at temperature 35-37℃ to simulate the fill formulation temperature at the time of encapsulation process and higher temperatures should be avoided as they could interfere with the sealing of softgels[21]. From previous results shown in Table 7, it was shown that no difference between the measured apparent viscosities between different formulas.

Drug content test was applied to ensure the consistency of dosage units, each unit in a prepared trial should have ETO content within a narrow range around the label claim where the term uniformity of dosage unit[94].

The results demonstrated at Table 7 show that ETO was well dispersed in the dispersion medium for all formulas due to fine particle size of ETO (80 µm) leading to an acceptable blend uniformity during encapsulation and content uniformity in the final softgel product[21].

4.6. Rupture test for Etodolac softgels

ETO softgels were subjected to rupture test to observe the effect of ETO fill content on the dissolution of the softgel shell. From this point F1, F2, F4, F6, F7, F 8, F9 and F10 softgels were subjected for this test. Results illustrated in table 8 revealed that the fill content of F1, F2, F4, F6, F7, F8, F9 and F10 were not affected on the dissolution of softgel shell.

Table 7: Viscosity measurements in centipoises and drug content

Formula	Log apparent viscosity results	Drug content
F_1	3.167 – 3.184	101.4% ± 0.03
F_2	3.117 – 3.130	113.43% ± 0.048439
F_4	3.577 – 3.602	107.47% ± 0.035796
F_6	3.531 – 3.563	103.90% ± 0.064133
F_7	3.698 - 3.707	108.23% ± 0.007234
F_8	3.301 – 3.342	107.60% ± 0.046184
F_9	3.477 – 3.602	106.13% ± 0.003055
F_{10}	3.000 – 3.114	111.70% ± 0.033601

Table 8: Rupture test results for ETO softgels using apparatus II at 50 rpm in 500ml water

Formula	After 15 mins
F 1	Ruptured
F 2	Ruptured
F 4	Ruptured
F 6	Ruptured
F 7	Ruptured
F 8	Ruptured
F 9	Ruptured
F 10	Ruptured

4.7. Water migration study for Etodolac softgels

Results of water migration study of ETO fill formulations were presented as schematic diagram in Figure 18.

The shell material of such "dry" softgels usually contains 10 -15 % w/w water depending on the specific gelatin shell formulation used[(34)]. It was stated that water content in gelatin was hypothesized to correspond to the water sorbed by the polar groups in gelatin or the structural water, which is bound with the proteins by hydrogen bonding both inside and outside the helical fragments[(24, 103)].

Results revealed that water migration among the fill, the shell and the surrounding atmospheric humidity (20-30%) depends on the fill composition of ETO formulation.

In case of F1, F2 and F9; fill formulations were not affected on the water migration process between the shell and the surrounding medium where the shell was equilibrated with the surrounding medium by decreasing the percentage of the water content to reach 7-9%.

Water migrated from the shell to fill content in F 4 and F 8 leading to increase in a water content to reach 1.8-2.5%. On other hand, water migrated from the shell to the surrounding medium to reach 7.8-8.8%. This may be due to presence of a relative low concentration of CRM RH 40 and CRM EL in the presence of high concentration of oil (i.e. 80%) which tends to increase the water uptake of the fill components due to their dehydrating effect[(104)].

Propylene glycol in F 6 is more than 10% of the total fill formulation leading to migration of a portion of propylene glycol to the shell to act as a plasticizer. Water migration from the shell to the fill may be explained by hygroscopicity of propylene glycol leading to raising in the water content inside to reach 4.1%. On the other hand, moisture is absorbed by the shell from the surrounding medium leading to increase in moisture content in the shell to 18.3% and this in accordance with the results of shelf stability which will be mentioned in the following tests.

Both F7 and F10 contain high concentrations of PEG 400; 60%, 100% respectively where PEG 400 has a higher affinity for water used in shell formulation that may lead to water migration from the shell components into the polyethylene glycol fill to raise the water content to 3.8%, 6.3% respectively. On other hand there is no reported data about migration of hygroscopic PEG 400 from the fill to the shell, so there is no increase in water content in the shell.

4.8. Evaluation of mechanical properties of Etodolac softgels

Empty softgels were applied in this test to figure out the effect of the fill content of ETO formulations on the mechanical strength of shell of soft gelatin capsule. Mechanical properties of ETO softgels were presented in Table 9.

Although water content in the shell capsule of F1 was decreased in comparison with the water content in the empty capsule, there was no sharp difference in the mechanical properties of the shell of F1 capsule and empty capsule; this may be attributed due to the change in the shell thickness between each capsule.

It was shown from the previous mentioned water migration study results that the water content of the shell of the softgel capsules; F2, F4, F8 and F9 was decreased leading to an increase in the mechanical properties of the shell leading to loss of the elastic properties of the shell of softgel on storing at a normal room conditions. This was referred due to previous mentioned reasons in water migration study for F2, F4, F8 and F9.

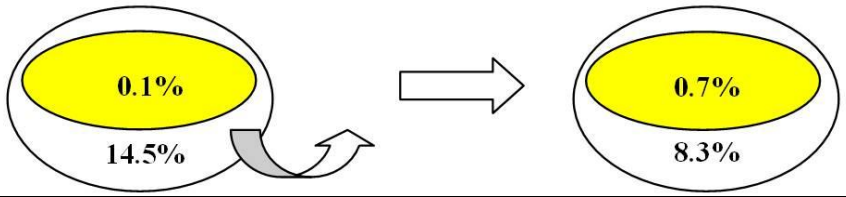
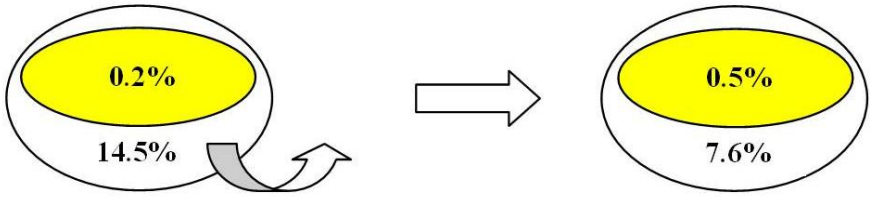
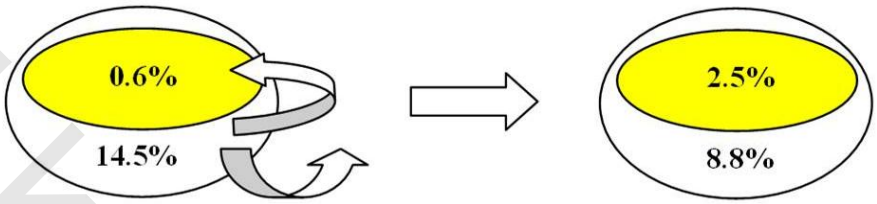
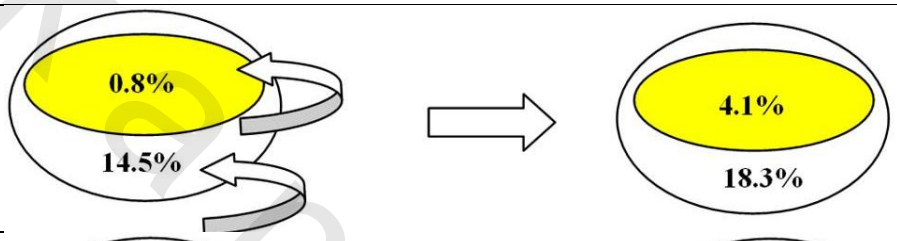
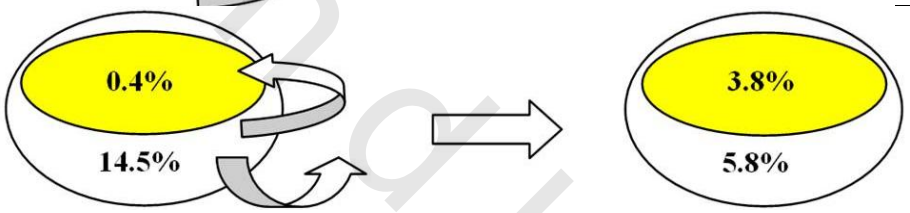
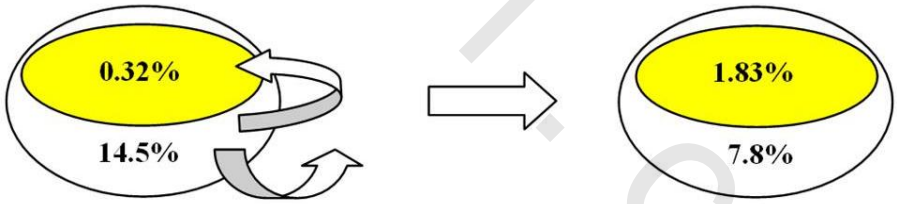
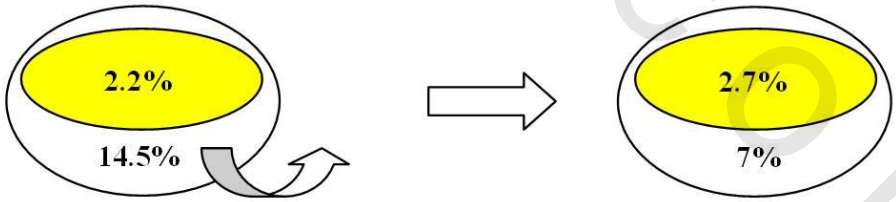
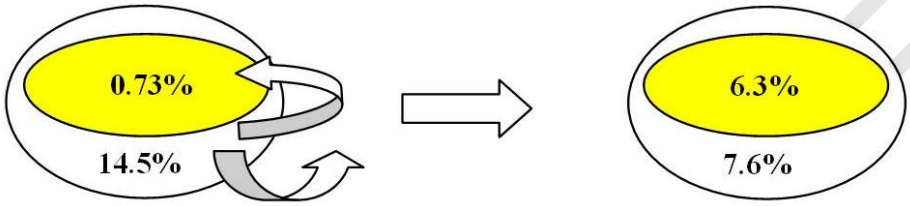
Formula	A Results of water migration study B
F 1	
F 2	
F 4	
F 6	
F 7	
F 8	
F 9	
F 10	

Figure 18: Water migration behavior of ETO softgels; (A) at zero time, (B) after 7 days, %RH= 20-30%

In case of F6, the results showed a sharp decrease in the mechanical properties of the shell. This was referred to the migration of propylene glycol from the fill to the shell and act as a plasticizer which in turn lead to be the softgel shell more elastic and increase the possibility for deformation[21].

In case of F8 and F10, the results revealed an increase in the mechanical properties especially for F10 leading to reduce its elasticity and increased its brittleness where the fill content composed of low molecular weight of polyethylene glycol (PEG 400) which have a higher affinity for water and glycerin used in the shell formulations leading to the migration of these shell components into the PEG fill[21]. Migration of plasticizer from the shell into the fill in a softgel could result in the reduced elasticity (flexibility) and increased brittleness of the shell shortly after production or on storage, especially when exposed to cold temperature[21].

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Table 9: Shows the mechanical properties of ETO softgels

Formula	Mechanical properties			
	Puncture force (PF) (N)	**Tensile strength (TS) (Mpa)**	**Young's modulus (Mpa)**	**Elongation at break (%)**
Empty capsule	9.53 ± 2.4	0.43 ±0.2	0.14 ± 0.07	306 ± 0.21
F1	8.6 ± 3.97	0.27 ± 0.185	0.14 ± 0.078	171.5 ± 0.2
F2	15.89 ±2.128	0.748 ± 0.125	0.22 ± 0.038	344 ± 0.6
F4	17.15 ± 4.41	0.786 ± 0.091	0.292 ± 0.12	295 ± 0.93
F6	3.4 ± 0.577	0.165 ± 0.041	0.06 ± 0.0095	272 ± 0.4
F7	16.276 ±1.12	0.85 ± 0.166	0.3 ± 0.054	286 ± 0.6
F8	11.368 ±2.03	0.637 ± 0.13	0.216 ±0.0583	302.6 ± 0.53
F9	16.66 ± 2.07	0.915 ± 0.22	0.32 ± 0.1574	311 ± 0.77
F10	31.135 ±1.67	1.745 ± 0.3	0.68 ± 0.192	270 ± 0.74

4.9. Stability studies

4.9.1. Accelerated stability study

4.9.1.1. High performance liquid chromatography (HPLC) chemical stability test

Due to possible interaction between hydroxyl compounds used in softgels (eg. Polyethylene glycol, sorbitol and glycerin) and ETO being has a carboxylic acid group in its structure leading to ETO hydrolysis[105]. F 1 and F 10 were subjected to chemical stability analysis test by using HPLC system. Results revealed that no degradation for ETO in F 1 and F 10 as shown in Figures 20 and 21 in comparison with standard ETO sample shown in Figure 19.

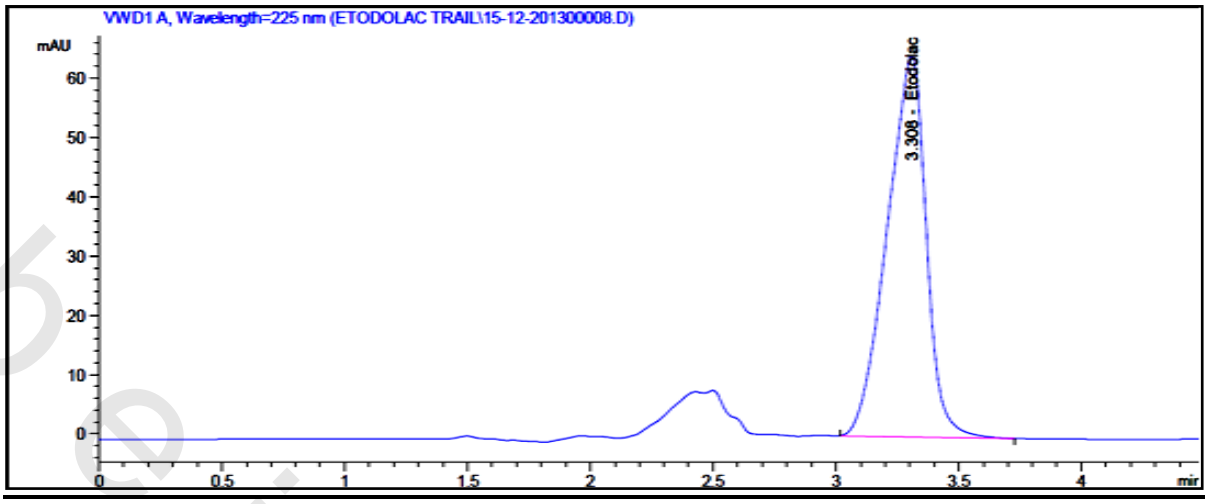

Figure 19: HPLC chart of standard ETO solubilized in methanol at retention time 3.308 min

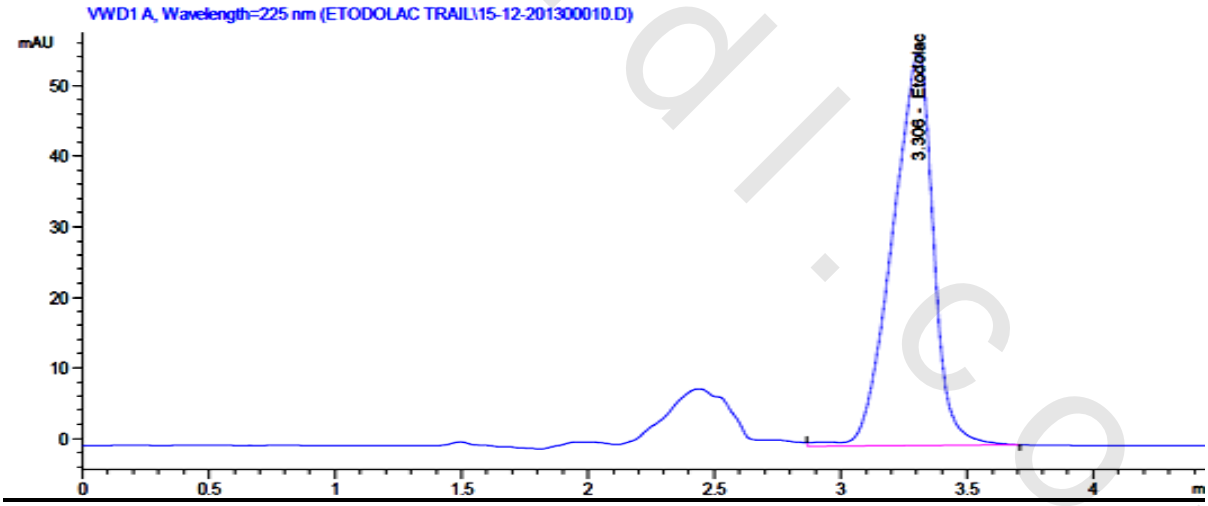

Figure 20: HPLC chart of ETO in F1 solubilized in methanol at retention time 3.306 min.

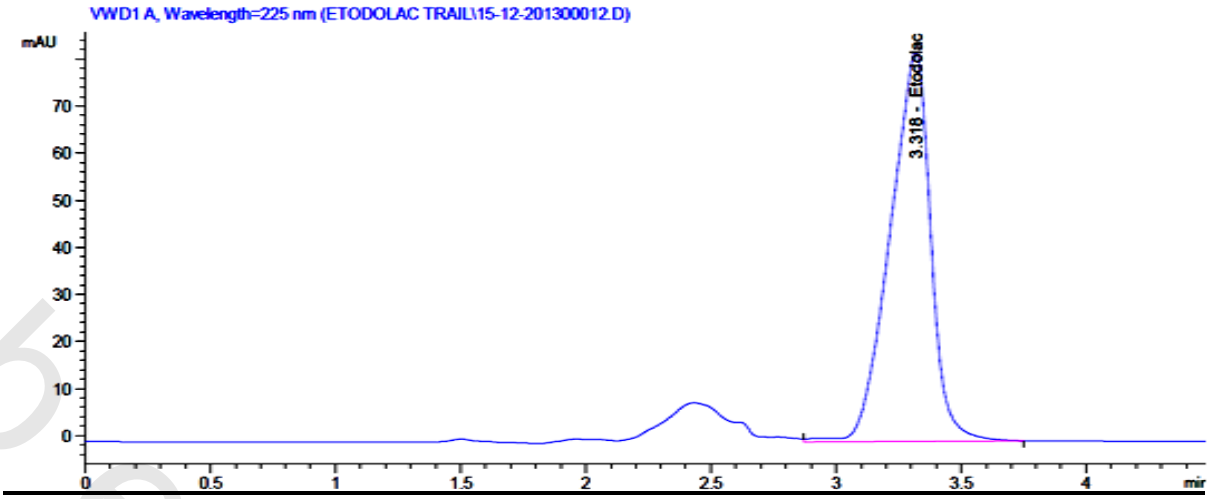

Figure 21: HPLC chart of ETO in F 10 solubilized in methanol at retention time 3.318 min

4.9.1.2. *In vitro* dissolution study

Because of the previous explained results of in-vitro dissolution study at zero time; F1, F2 and F3 formulations were excluded from this study due to the failure of ETO dissolution results according to USP requirements for ETO dissolution test.

Although F4 and F5 dissolution results line with the pharmacopeial specification, they were excluded from this study because their results were at border line with USP requirements. On other hand, F 6 was excluded from this study because of the leakage of the fill contents from softgel capsules upon storage in the accelerated stability cabinet for 3 months.

F7, F8, F9 and F10 were subjected to this study in comparable with their dissolution results at zero time. Results revealed in Figure 22 in a sharp decrease of the dissolution results of F8 to reach to 31% after 30 mins. This because of presence of CRM EL in the fill content where CRM EL is encountered to be a material containing polyoxyethylene moiety in its structure which is susceptible to autoxidation in the presence of oxygen and produce reactive organic peroxide[106-108]. Also it had been postulated that polyoxyethylene moieties undergo oxidative decomposition at high temperature in the presence of water to ethylene glycol which may then be oxidized further to form formaldehyde[109]. Although F7 fill content composed of 20% PLX 407, results revealed in Figure 23 that the dissolution result of ETO after 30 min is the same as the result at zero time which shows that PLX 407 doesn't undergo autoxidation process because of its high molecular weight leading to decrease in the mobility of the polymer chains resulting in decrease in the autoxidation reactions.[107]

Although F9 results shown in Figure 24 revealed a decrease in ETO dissolution to 81% after 30 mins, it lined with pharmacopeial requirements of ETO. This decrease may be due to autoxidation reaction for CRM RH 40 being one of the materials containing polyoxyethylene moiety in its structure but lesser extent if compared to F 8 formulation[107, 110].
In case of F 10, results revealed that there was no change in the dissolution behavior of ETO if compared to the same formula at zero time as shown in Figure 25.

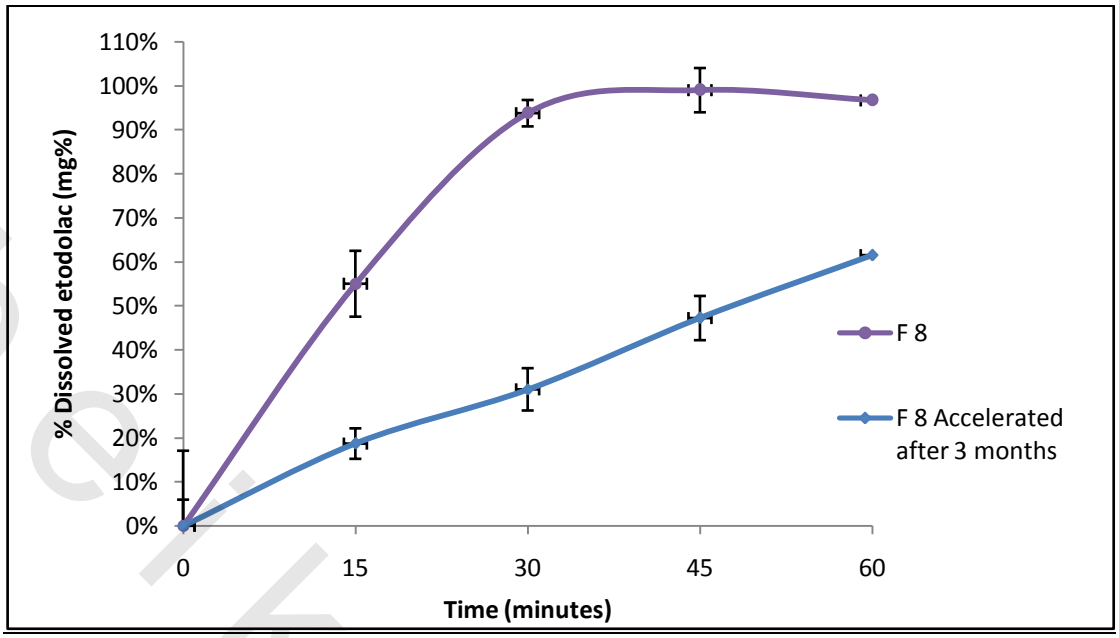

Figure 22: Comparative dissolution behavior of F 8 after accelerated stability and at zero time.

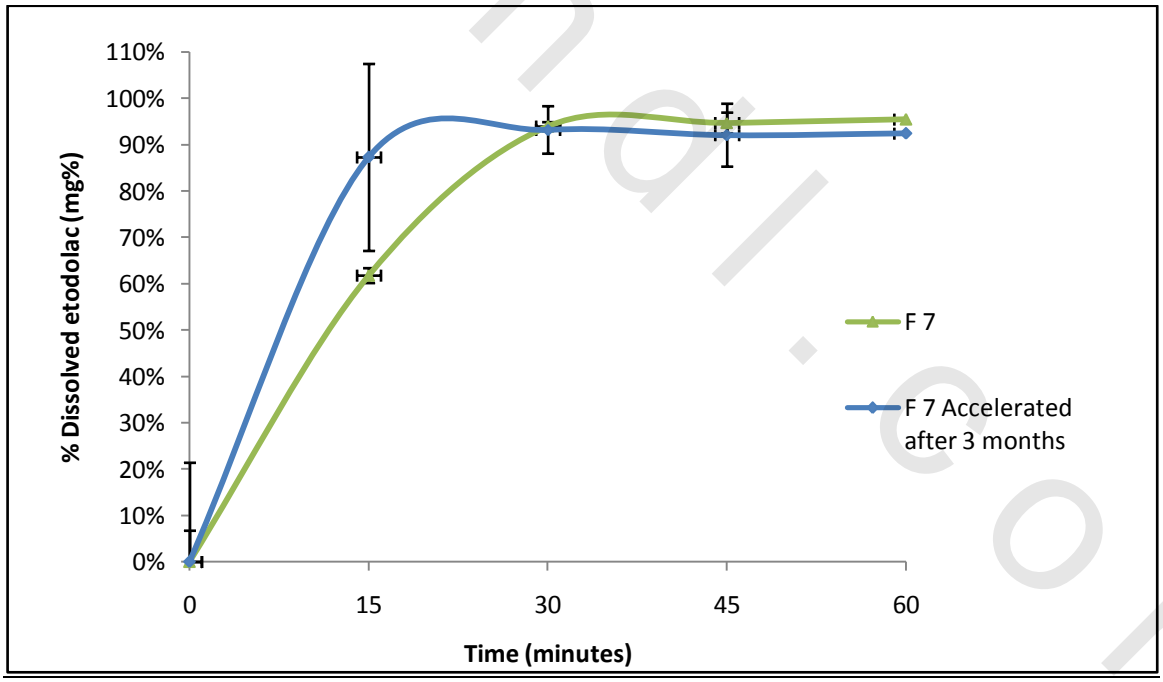

Figure 23: Comparative dissolution behavior of F 7 after accelerated stability and at zero time.

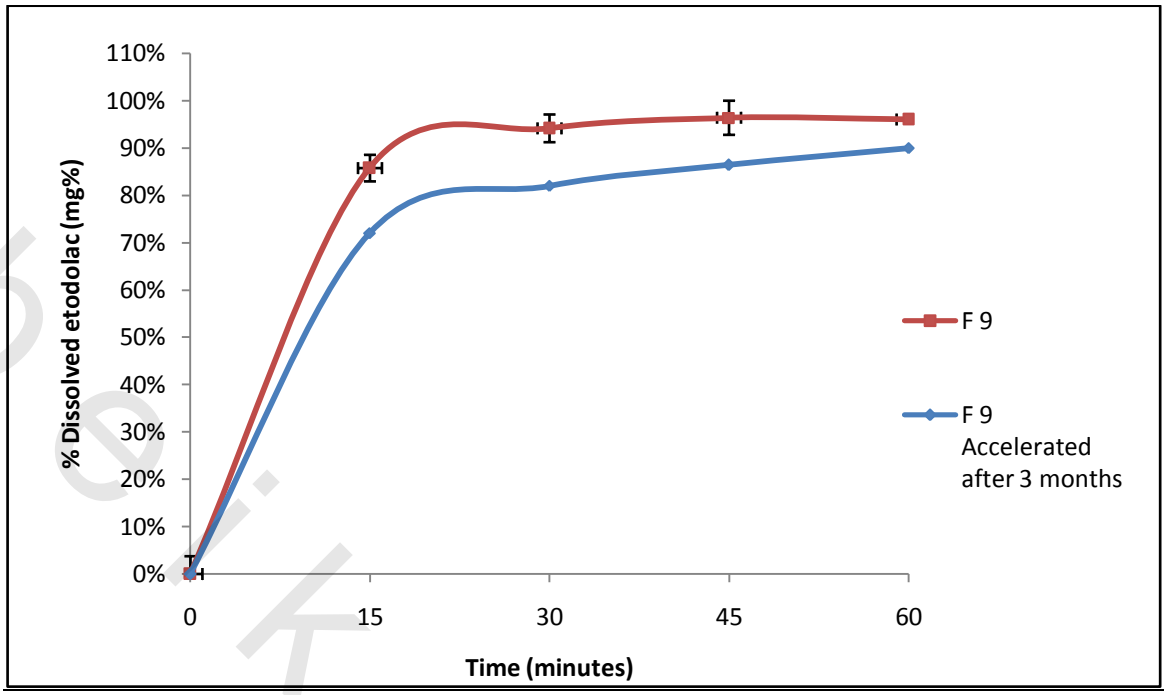

Figure 24: Comparative dissolution behavior of F 9 after accelerated stability and at zero time.

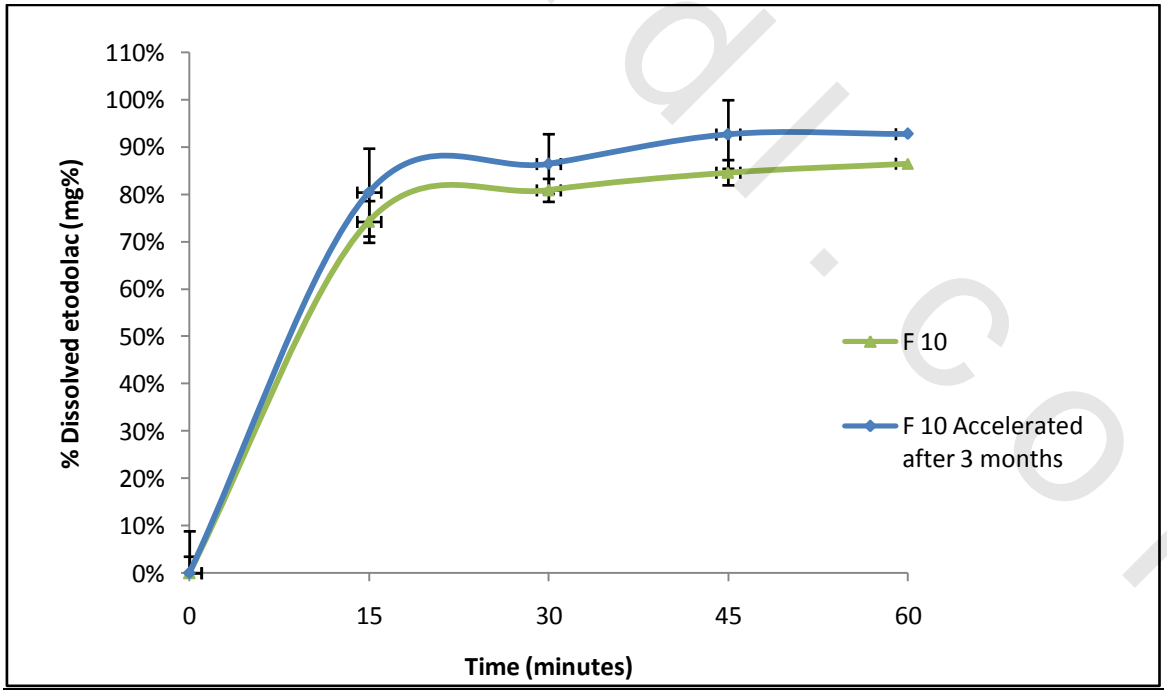

Figure 25: Comparative dissolution behavior of F10 after accelerated stability and at zero time.

4.9.1.3. Rupture test for ETO softgels

F1, F2, F4, F7, F8, F9 and F10 softgels were subjected for this test. The results demonstrated at table 10 revealed that in case of F1, F2, F7 and F10; the fill contents cause no deterioration to the softgel shell as shown in Figure 26.

In case of F4, F8 and F9; the results showed that the fill contents for these formulation affect on the rupture of the shell of the softgels as shown in Figure 27. This was because of containing CRM EL and CRM RH 40 as a materials with polyoxyethylene moiety in the fill contents leading to its autoxidation producing reactive organic peroxides which further degraded to produce short chain carboxylic acids and aldehydes.

Chemically, aldehydes were known to form methylene bonds between two amino groups on adjacent gelatin chains or within the same chain as illustrated in Figure 28. The aldehydes induced cross-linking of gelatin was thought to involve the ε- amino functional groups present in the lysine moieties and the guanidino functional groups present in the arginine moieties of the gelatin chain[111, 112].

Although the result of gelatin cross-linking in F9, it may be due to CRM RH 40 autoxidation reaction, it possessed good dissolution results. The reason that only 20% of CRM RH 40 was required to promote gelatin cross-linking as shown in F 4 and F 8 and the rest of CRM RH 40 was sufficient to promote emulsification of ETO in aqueous medium.

Table 10: Rupture test results of ETO softgels after accelerated stability

Formula	Results	
	After 15 mins	After 30 mins
F1	Ruptured	……..
F2	Ruptured	……..
F4	Failed to rupture	Failed to rupture
F7	Failed to rupture	Ruptured
F8	Failed to rupture	Failed to rupture
F9	Failed to rupture	Failed to rupture
F10	Failed to rupture	Ruptured

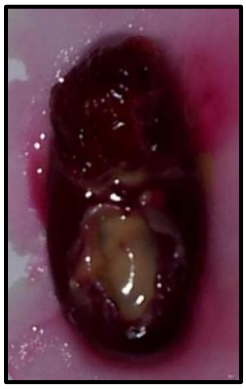

Figure 26: Ruptured ETO softgels.

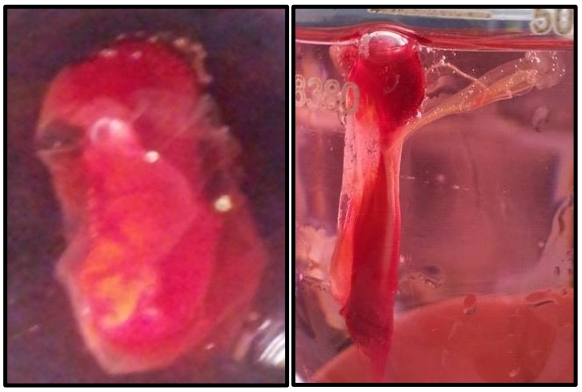

Figure 27: Unruptured ETO softgels

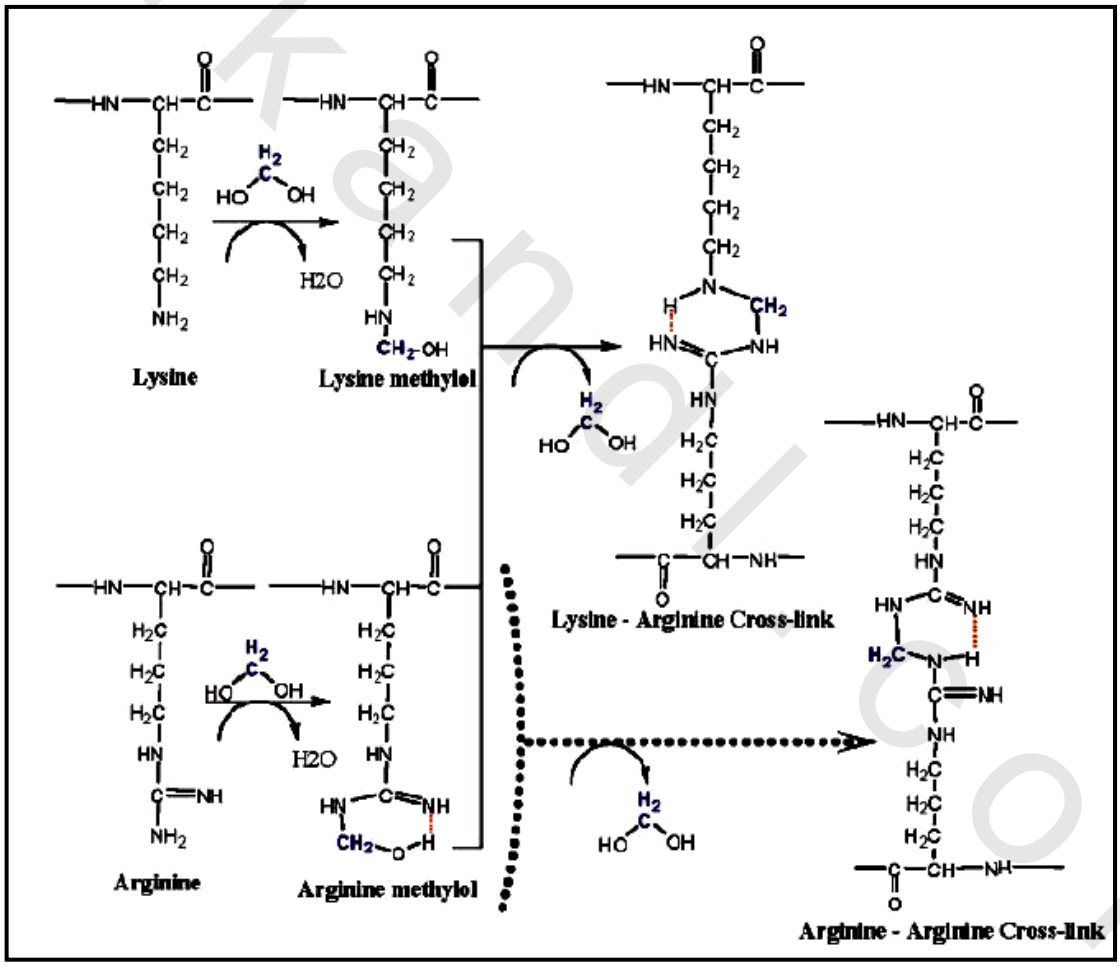

Figure 28: Possible mechanism of formation of methylols of lysine and arginine and subsequent cross-links formation in gelatin (adopted from Taylor *et al,* Albert *et al,* Gold *et al*,[(111)])[(21)].

4.9.2. Shelf stability study

4.9.2.1. High performance liquid chromatography (HPLC) chemical stability test

F1 and F10 were selected for chemical shelf stability analysis test by using HPLC system. Results revealed that there is no degradation for ETO after 3 months shelf stability as shown in Figures 29 and 30 in comparison with HPLC chart of standard ETO solubilized in methanol as mentioned before (Figure 19).

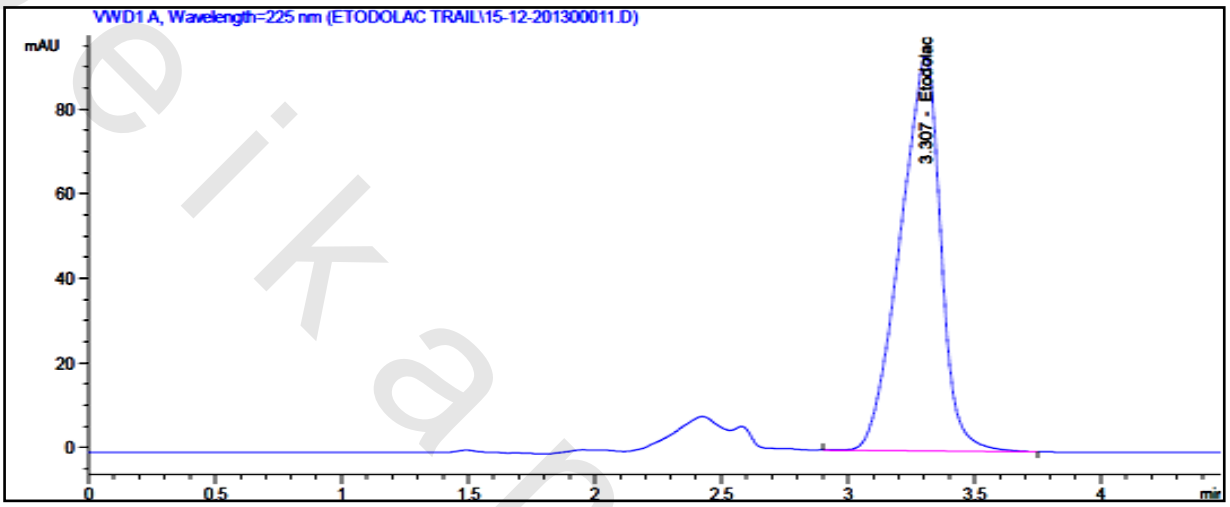

Figure 29: HPLC chart of ETO in F 1 solubilized in methanol at retention time 3.307 min.

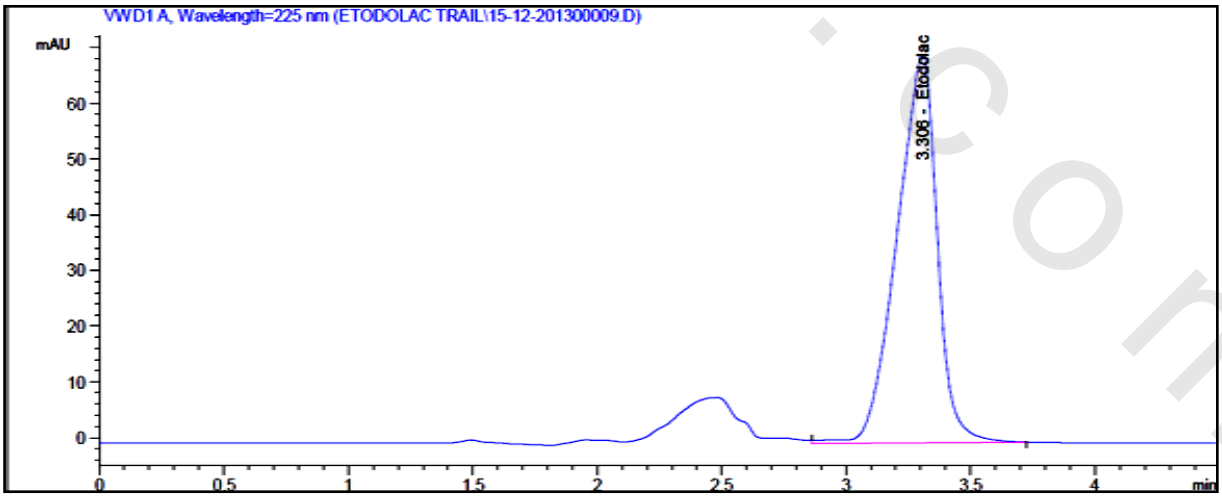

Figure 30: HPLC chart of ETO in F10 solubilized in methanol at retention time 3.306 min.

4.9.2.2. *In vitro* dissolution study

In addition to F7, F8, F9 and F10; F6 softgel were subjected to this study in comparable with their dissolution results at zero time. In case of F6; results revealed in Figure 31, a decrease in the dissolution result of ETO after 30 mins to reach 85.43%, this is due to migration of a portion of propylene glycol which is encountered to be a co-solvent to shell of the capsule leading to decrease the concentration of propylene glycol in the fill leading to decrease in the dissolution result if compared to the dissolution result at zero time.

Although, results revealed in F8 in Figure 33, a slight decrease in the dissolution result of ETO after 30 min to reach 76.43% due to the previously discussed reason, it shows better results if compared to the dissolution results of ETO after the accelerated stability study indicating incomplete autoxidation reaction of CRM EL. In case of F7, F9 and F10, there is no change in the dissolution results of ETO if compared to the results at zero time as shown in Figures 32, 34 and 35.

4.9.2.3. Rupture test for ETO softgels.

F1, F2, F4, F6, F7, F8, F9 and F10 softgels were subjected for this test. On comparing the results of rupture test of ETO softgels after accelerated stability, it was revealed that the fill content of all tested formula has no effect on the dissolution of the shell of the softgels after shelf stability study.

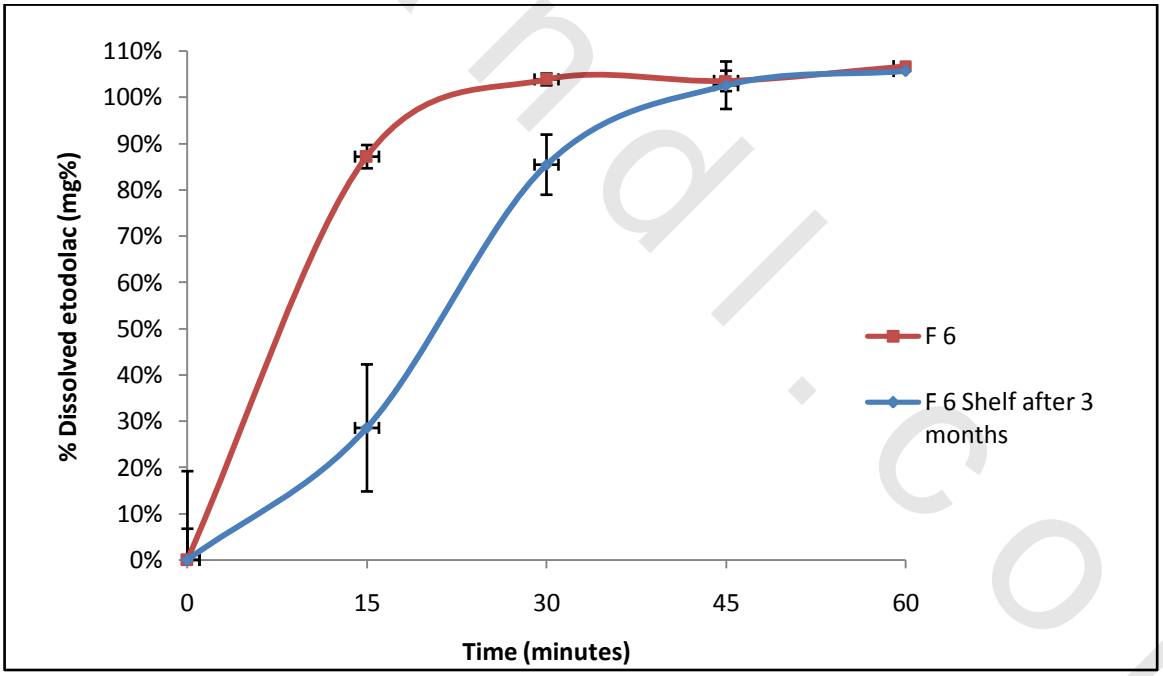

Figure 31: Comparative dissolution behavior of F6 after shelf stability and at zero time.

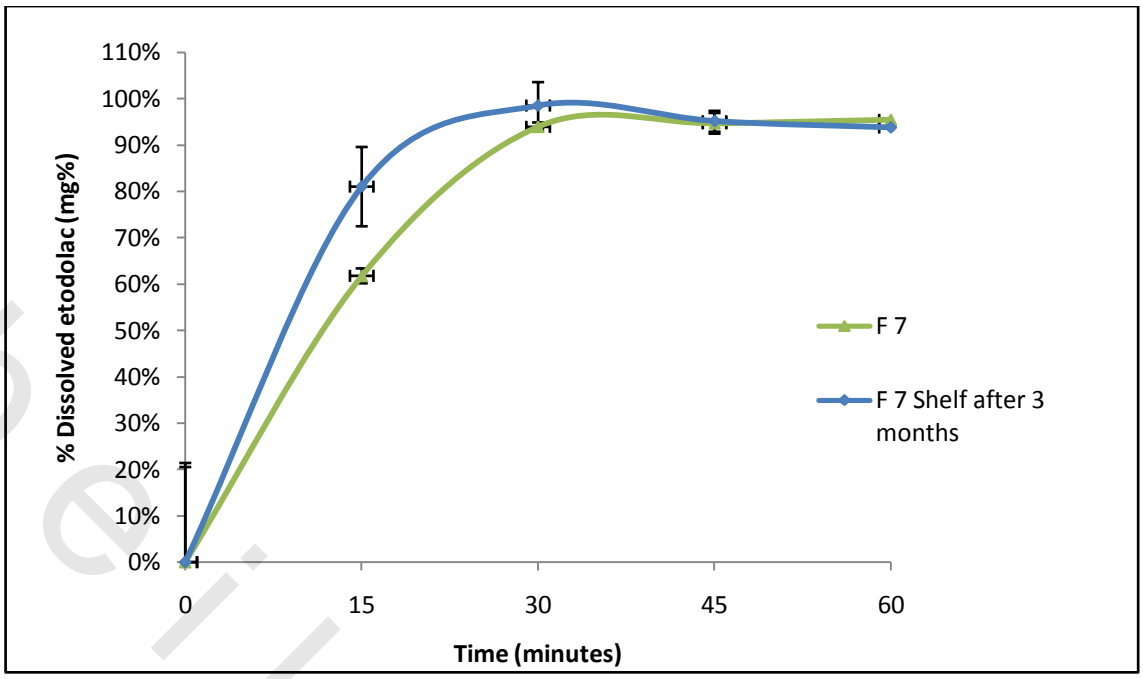

Figure 32: Comparative dissolution behavior of F 7 after shelf stability and at zero time.

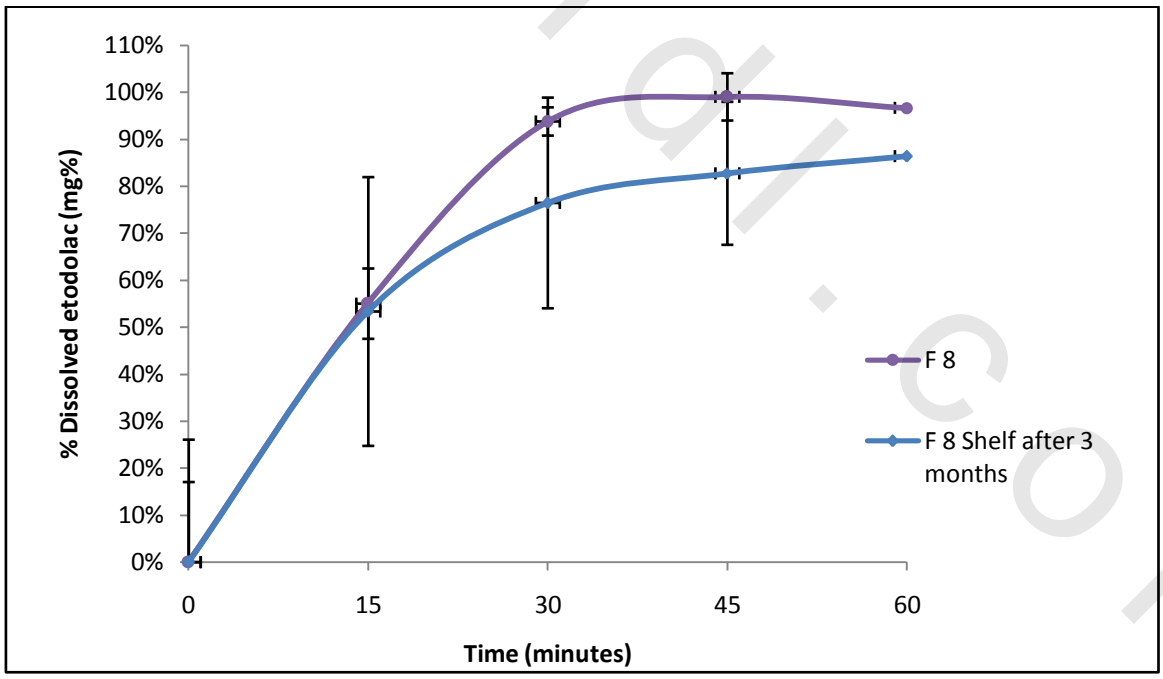

Figure 33: Comparative dissolution behavior of F 8 after shelf stability and at zero time.

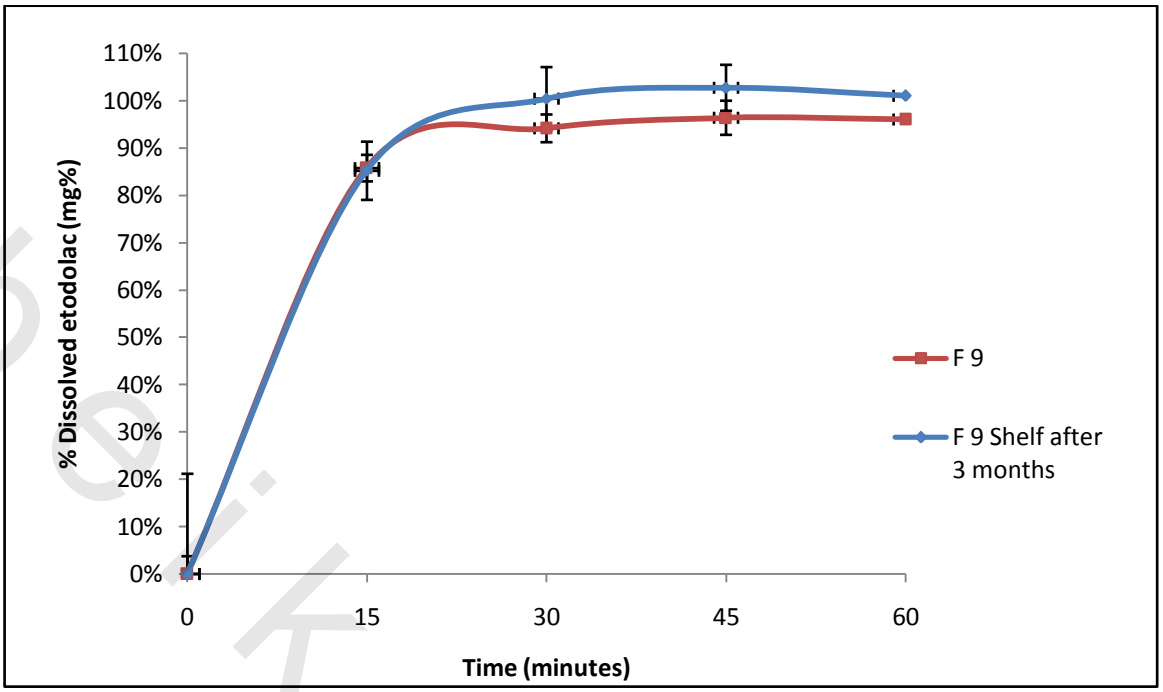

Figure 34: Comparative dissolution behavior of F 9 after shelf stability and at zero time.

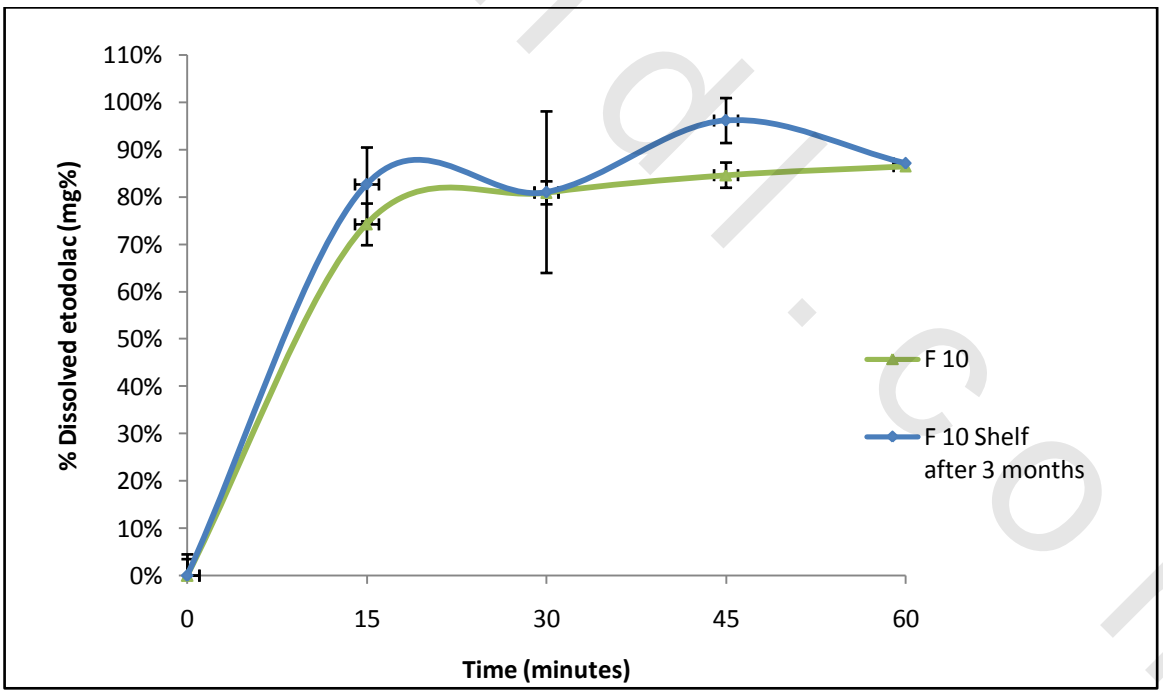

Figure 35: Comparative dissolution behavior of F 10 after shelf stability and at zero time.