Clove oil based co-surfactant free microemulsion of flurbiprofen: Improved solubility with ameliorated drug-induced gastritis

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Abstract: Flurbiprofen, an NSAID, is a water insoluble drug that is also notorious for gastric irritation and inflammation. This study was aimed at using a natural gastroprotective oil as the internal phase to develop flurbiprofen microemulsion (ME) to improve it solubility and ameliorate its gastric side effects. Upon screening of ME components for drug solubility, clove oil, tween 80 and transcutol were identified as the oil, surfactant and cosurfactant, respectively, with higher flurbiprofen solubility. Pseudo-ternary phase diagrams revealed that the ME made with surfactant only and without co-surfactant displayed the similar ME region as made with the mixture of surfactant and co-surfactant. Furthermore, drug loaded oil was also used to draw pseudo-ternary phase diagram and a very little decrease in the ME region was observed. Therefore, co-surfactant free flurbiprofen loaded ME was developed to avoid side effects associated with the use of excessive surfactant quantities. ME were found to possess size in the range of 11-41 nm with PDI <0.5 and a slightly negative charge. Conductivity, pH and refractive indices of the selected MEs were well in the range. Drug release studies indicated maximum drug release from MEs within 5 min. Analysis of the gastric mucosa of rats after oral administration of drug solution and drug loaded ME confirmed that clove oil based ME provided significant protection against the NSAIDs induced gastric damage.

Keywords: Microemulsion, NSAIDs, clove oil, solubility, gastroprotective effect.

INTRODUCTION

Among the greatest challenges in developing formulations is poor water solubility of drugs resulting in low oral bioavailability. Most of the drugs being marketed today are administered orally. The effectiveness of these drugs depends on several factors, among them aqueous solubility of drug is the most important factor (Chaudhary *et al*., 2012). One of the most useful approaches for increasing the solubility of poorly water soluble drugs is oil based formulation. As solubility of drug increases, its bioavailability also increases (Khan & Singh, 2016).

Flurbiprofen, a potent NSAID, is mostly prescribed for musculoskeletal disorders, post-operative pain, gout and rheumatic diseases, inflammation and sore throat (Maroof *et al*., 2015). It is a BCS class II drug thus possesses poor water solubility and low bioavailability. Its oral administration results in severe GI tract side effects. In patients, suffering from upper GI tract diseases, flurbiprofen should be given with close monitoring and observation and should be avoided in patients with active peptic ulcer disease (Simsek *et al*., 2018). The major factors involved in gastric damage by the use of NSAIDs are prostaglandin deficiency and ulcerogenic response followed by hyper motility, neutrophils and free radicals (Takeuchi, 2012). Side effects associated with the use of NSAIDs might decrease patient compliance, thus limiting their overall therapeutic potential (Nagi *et al*., 2015).

Clove oil, extracted from the medicinal plant, *Syzygium aromaticum*is, is commonly used to treat many disorders like toothache, GI disturbances, respiratory disorders, inflammation and also have antibacterial properties (Cui *et al*., 2015). It has been reported to show anti-ulcer and gastroprotective activity in rat models of indomethacin and ethanol induced ulcers. Its active constituent, eugenol, is responsible for the therapeutic activity (Santin *et al*, 2011).

Microemulsions are transparent and thermodynamically stable systems. O/w ME are formed when the oil droplets are covered by surfactants and form internal phase while water forms the external phase. These systems are helpful in incorporating lipophillic drugs, hence resulting in increased solubility in aqueous medium (Kale & Deore, 2017). Microemulsions offer utilization of therapeutic oils as the internal phase to support the encapsulated drug in terms of synergism or limiting the drug induced adverse reactions (Xavier-Junior *et al*., 2017).

We evaluated different oils, surfactants and co-surfactants

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to enhance flurbiprofen solubility. Highest drug solubility was observed in the clove oil. Therefore, clove oil was selected as the oil phase of the microemulsion, which was evaluated for physicochemical properties, drug loading, dissolution and gastric toxicity.

MATERIALS AND METHODS

Flurbiprofen was gifted by Axis Pharmaceuticals (Faisalabad, Pakistan). Tween-80, Tween-60, Span-20, Span-80, Polyethylene glycol 400 (PEG 400), Isopropyl Myristate (IPM) and Propylene glycol (PG) were obtained from Daejung Chemicals (Korea); Transcutol-P and Labrafil M 1944 CS (Gattefose®) were given by Morgan Pharmaceuticals LTD, Karachi, Pakistan. Cremophore EL and Cremophore RH 40 were purchased from Lyallpur Scientific Traders, Faisalabad Pakistan. Black seed oil and Citrus lemon oil were purchased from Al-Barkat Tradres, Lahore, Pakistan. Clove oil was purchased from Royal Flavours & Chemical Company, Lahore, Pakistan.

Solubility studies

Screening of suitable oils, surfactants and co-surfactants was performed through solubility studies as mentioned in literature with slight changes (Moghimipour *et al*.,2013). Surplus quantity of drug was added to the each solvent and vortex mixed for 5 minutes. Subsequently, tubes were placed in shaking water bath for 72 hours at 37±0.5°C. Tubes were then centrifuged for 30 minutes at 6000 rpm. Clear supernatant was obtained. The solubility of flurbiprofen was then determined by the analysis of supernatant after required dilution with methanol using UV Spectrophotometer at a wavelength of 246 nm.

Construction of pseudo ternary phase diagram

Phase diagrams were constructed via water titration method using different ratios of oil, surfactant and co-surfactant in order to formulate stable formulation as reported elsewhere with slight modifications (Xiao *et al*., 2013). Components of ME except water were vortexed by a vortex mixture (PCSIR Pakistan) for 2 minutes until uniform stable oily formulation. Water was added in small increments, vortexed for 2 min and left for 60 min to attain the equilibrium. The system was visualized for appearance before another increment of water.

Preparation of flurbiprofen loaded microemulsion

After solubility studies and construction of phase diagram, clove oil and tween 80 were selected as oil and surfactant. Five formulations were selected on the basis of area obtained from phase diagram and ME systems were made. Afterwards, Flurbiprofen was loaded in all five ME systems at a dose of 25mg/g.

Conductivity, pH, refractive index and stability studies

Measurements for conductivity, pH and refractive index were performed in triplicate for both blank ME formulations and flurbiprofen loaded ME formulations at 25 ± 0.5°C by using digital conductivity meter (FP30 Mettler Toledo, Switzerland), pH meter (HI 2210, Hanna, USA) and Refractometer (J457, Rudolph, USA), respectively. In order to test the stability, ME samples were centrifuged at 6000 rpm for 30 minutes and observed for phase separation, flocculation or precipitation of drug.

Drug content

1g from each drug loaded ME formulation was taken in a 15ml falcon tube and volume was made up with methanol to 10ml. The centrifuge tube was then vortexed for 15 minutes followed by sonication for 10-15 minutes in a water bath sonicator. Samples were then taken and diluted accordingly. Measurements were taken by UV Spectrophotometer at a wavelength of 246 nm. The experiment was conducted in triplicate (n=3).

Globule size, polydispersity index (PDI) and zeta potential (ZP) analysis

Blank ME formulations and flurbiprofen loaded ME formulations were evaluated for size, PDI and ZP by zetasizer (Malvern nano ZS90 UK) with three measurements each. The microemulsion formulations were appropriately diluted with distilled water before the measurements.

Dissolution test

Efficacy of dispersion, dissolution and the emulsification of flurbiprofen loaded ME formulations were studied by USP apparatus II at 100 rpm and 37 °C, in 900 mL 0.1N HCl and a pH of 1.2. The samples were taken from at specified time points (2.5, 5, 15, 30, 45, 60, 90 and 120 minutes) and replaced with equal volume of pre-heated dissolution medium. Samples were analyzed by UV Spectrophotometer at a wavelength of 246 nm. The experiments were done in triplicate (n=3).

Evaluation of gastritis

Albino rats (n=5) weighing 250–300g were housed into two groups at room temperature and controlled humidity. One group received FLB dissolved in PEG 400 at a dose of 15mg/kg, while the second group received F3-ME at same dose for 5 days twice a day (Wallace *et al*., 1994). The animals were sacrificed by cervical dislocation. The stomachs were removed and cut along the greater curvature. After rinsing with normal saline, the stomachs were examined with magnifying glass to observe the damage, lesions and scars along the sides of stomach mucosa. Sections of the stomach were also studied for pathological changes by haematoxylin and eosin (HE) staining.

STATISTICAL ANALYSIS

Mean, standard deviation and statistical analysis of the data were obtained by using GraphPad Prism. One way

analysis of variance (ANOVA) was employed for statistical comparison and the p value < 0.05 was considered as significant.

RESULTS

Solubility screening

Successful incorporation of a drug in a ME depends upon its solubility in that ME. As ME is a mixture of oil, surfactant and co-surfactant, so when we add surfactant in a formulation, it gets adsorbed in the layer present between the oil and surfactant and causes decrease in interfacial tension which results in stable formulation as ME. fig. 1 presents the solubility data of flurbiprofen in various oils, surfactants and co-surfactants. Clove oil, tween 80 and transcutol P showed highest flurbiprofen solubility among oils, surfactants and co-surfactants, respectively.

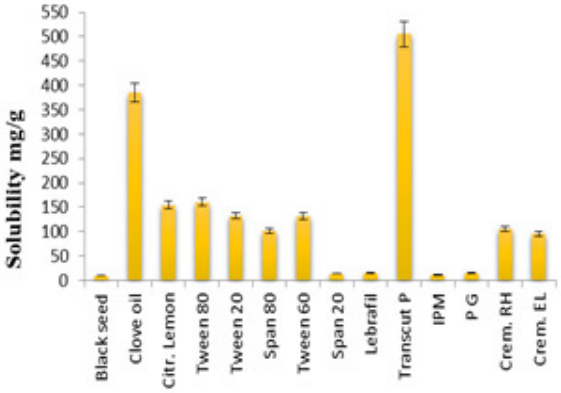

Fig. 1: Solubility of FLB in different oils, surfactants and co-surfactants

Pseudo-ternary phase diagram

ME regions were identified by constructing pseudo-ternary phase diagrams to facilitate the selections of levels of components of ME. Clove oil was found to offer a wide range of ME region in the pseudo-ternary phase diagram as shown in fig. 2 (a). Even in the absence of co-surfactant (fig. 2b), the decrease in the ME region was minimal. Furthermore, drug loaded oil (25mg/g) was also utilized to identify the regions of ME in co-surfactant free ME system (fig. 2c), to indicate the ability of the ME to remain stable upon drug loading.

The area of ME region in co-surfactant free ME system without incorporation of drug was 26.1 cm², whereas that of formulated with incorporation of drug was 22.14 cm². However, the overall decrease was not significant. Based on the findings of pseudo-ternary phase diagrams (fig. 2c), 5 ME formulations were selected as blank and drug loaded for further testing as shown in table 1.

Conductivity, pH and refractive index

Conductivity measurement is used to detect the type of emulsion. From fig. 3, it can be clearly seen that the conductivity among ME formulations (58 - 140 µs/cm), both blank and drug loaded, was significantly affected by the amount of oil and surfactant incorporated in them. As the amount of oil increased from 10% to 25%, along with a decrease in water content from 50% to 40%, there was a significant decrease in conductivity. These results are in good agreement with a previous report for o/w ME systems (Cilek *et al*., 2005). Moreover, presence of drug increases the hydrophobicity of the ME system, thus leading to decrease in ability of the system to conduct electricity. This could explain the decrea of drug loaded MEs in our experiments.

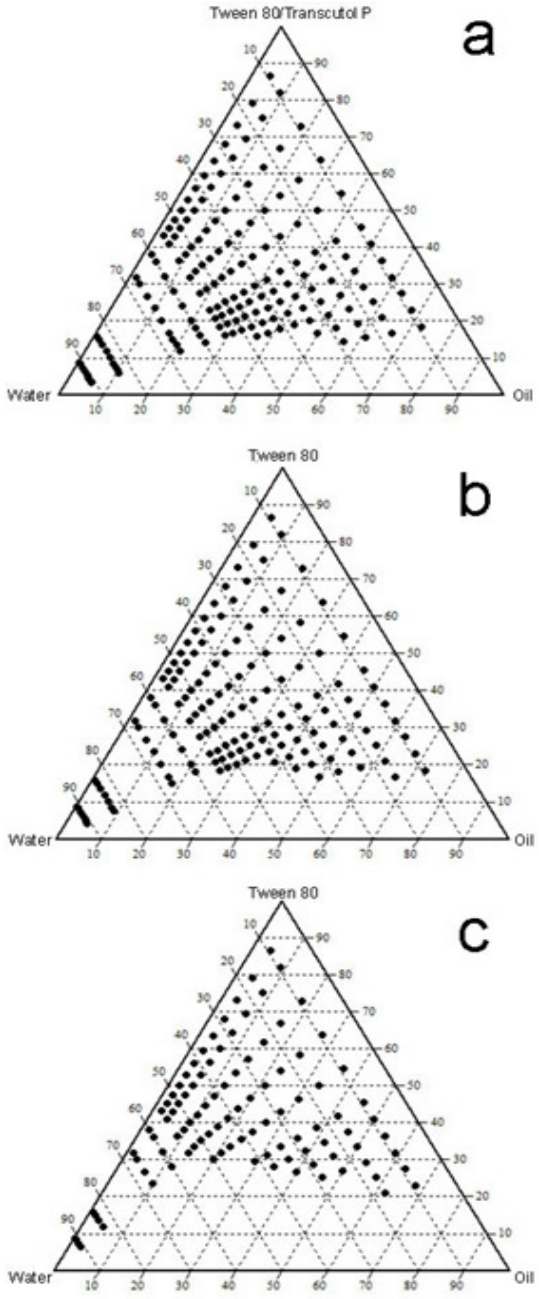

Fig. 2: Pseudo ternary phase diagrams based on a) tween 80 and transcutol P, clove oil and water; b) tween 80,

clove oil and water; c) tween 80, drug loaded clove oil and water.

The pH values of blank ME formulations ranged from 6.18-6.49 and that of drug loaded ME formulations ranged from 5.39-5.61. Among the formulations, the difference in pH was not significant. The slight decrease in pH of the drug loaded ME formulations could be attributed to the acidic nature of drug as flurbiprofen is a propionic acid derivative. Overall, the pH of the ME formulations are suitable for oral administration.

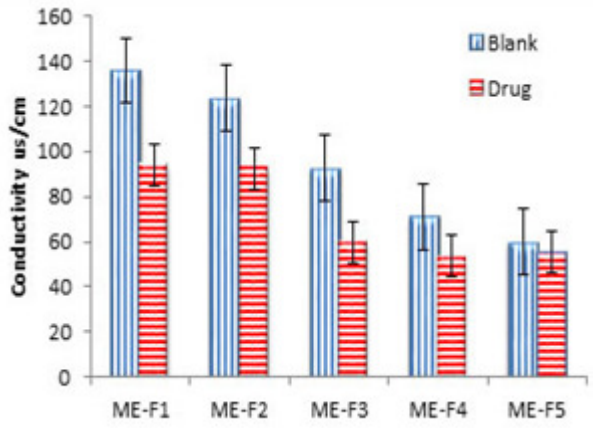

Fig. 3: Conductivity of different ME formulations

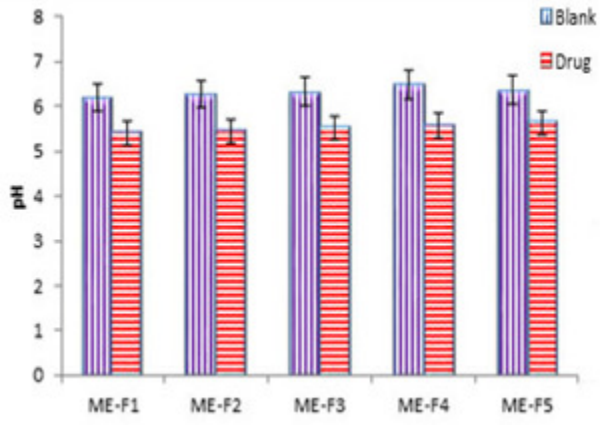

Fig. 4: pH of different ME formulations

Fig. 5 displays the refractive index values for both blank and drug loaded MEs. Drug loaded ME and blank ME range from1.40 – 1.42 in refractive index. Difference in refractive index values may be due to decrease in water content among the ME. As the water content decreases, the refractive index values increase (Olariu *et al*., 2014; Vlaia *et al*., 2016). However, drug loaded MEs showed marginally higher refractive indices as compared to their blank formulations.

Drug content

Fig. 6 shows the findings of the percentage drug content determination of drug loaded MEs. Drug content was found to be more than 95 % for ME-F1, ME-F3 and ME-F4. Whereas, ME-F4 showed drug loading of almost 80 %. The least loading efficiency was found in the ME-F2, which was around 75%.

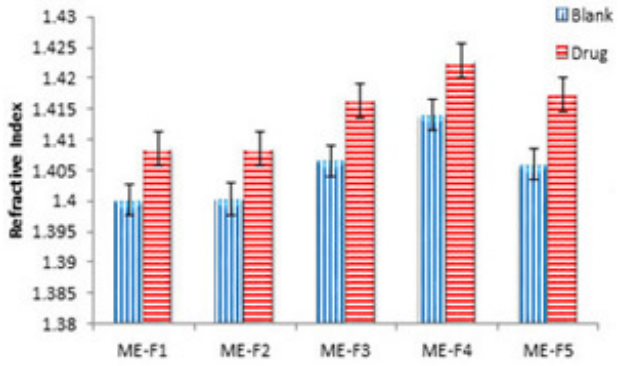

Fig. 5: Refractive index of different ME formulations

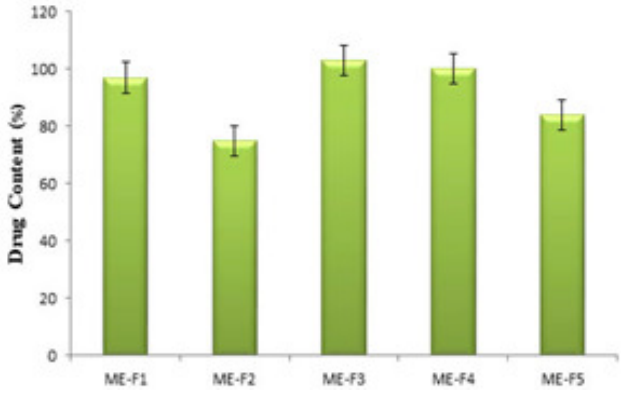

Fig. 6: Drug content (%) of different ME formulations

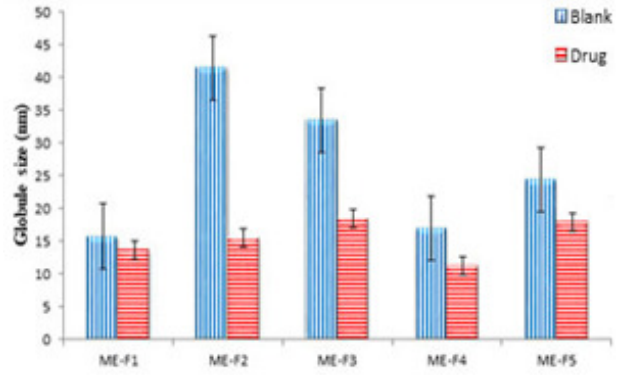

Fig. 7: Globule size of different ME formulations

Stability studies for the drug loaded MEs were performed by checking phase separation when subjected to centrifugation at 6000 rpm for 30 minutes. No phase separation or flocculation was observed and all the ME systems were found to be clear and transparent.

Globule size, PDI and ZP

Fig. 7 shows the globule size of both blank and drug loaded MEs. The size ranged from 15.82 - 41.46 nm in

blank MEs. Formulations loaded with drug were smaller in size than their blank counterparts (11.25 – 18.53 nm). PDI defines that whether the globule or droplet size of formulation is uniform or not and how much the formulation is homogeneous with respect to droplet size. fig. 8 presents the PDI of blank and drug loaded MEs. The values of droplet size close to zero show greater uniformity. PDI less than 0.5 has been reported to be within range (Golmohammadzadeh *et al*., 2017).

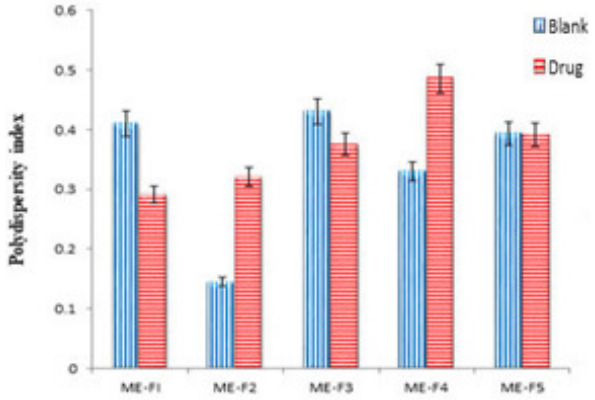

Fig. 8: PDI of different ME formulations

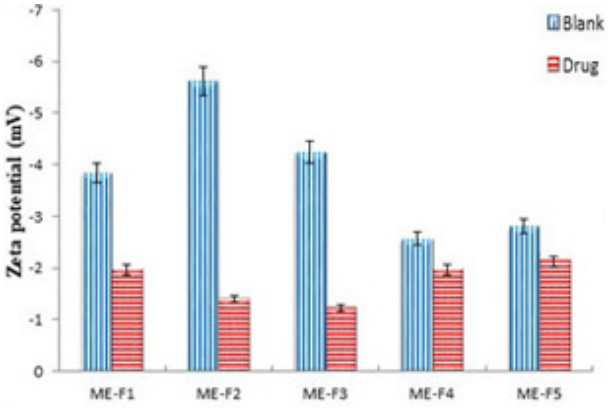

Fig. 9: ZP of different ME formulations

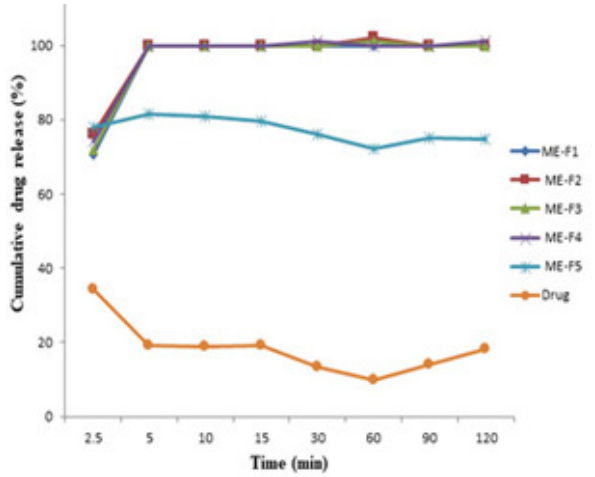

Fig. 10: Cumulative % drug release of flurbiprofen loaded microemulsions.

ZP for blank MEs ranged from -2.57 to -5.62 and that for drug incorporated MEs were between -1.23 to -2.12, as shown in fig. 9. The negative charge could originate from POE (polyethoxylate) group found in surfactant (tween 80) which results in formation of H-bonding at the border of o/w microemulsion (Ibrahim *et al*., 2015).

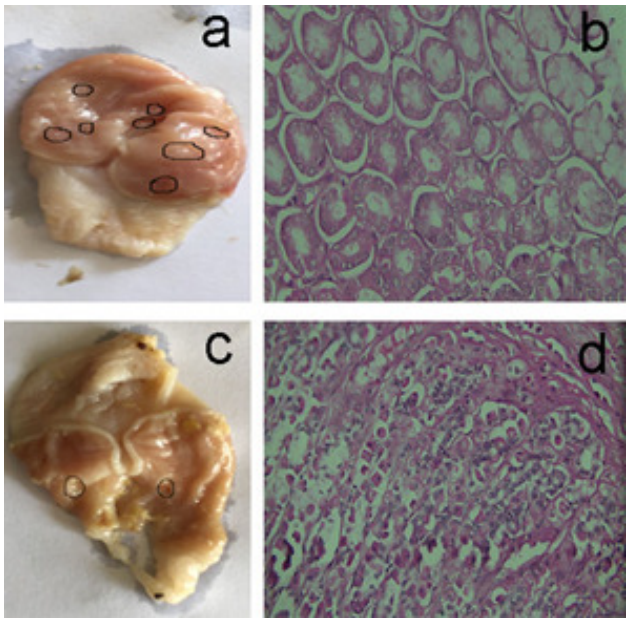

Fig. 11: Stomach mucosa and HE stained stomach mucosa sections of rats treated with drug dissolved in PEG 400 (a & b) and ME-F3 (c & d).

Table 1: Composition of ME formulations

Formulation	Clove oil (%)	Tween 80 (%)	Water (%)
ME-F1	10	40	50
ME-F2	15	35	50
ME-F3	20	35	45
ME-F4	20	40	40
ME-F5	25	35	40

Dissolution test

The dissolution test was performed with 25mg/g flurbiprofen loaded ME formulations as presented in. fig. 10. It can be clearly seen that drug concentration increases with time. However, the decrease in concentration of flurbiprofen for ME-F5 reveals that the drug could precipitate due to recrystallization as mentioned elsewhere (Patel *et al*., 2012).

Evaluation of gastritis

From fig. 11 (a) and (b), it can be clearly seen that the stomach mucosa of rats treated with flurbiprofen dissolved in PEG showed significantly higher numbers lesions and inflammation as compared to the ME group. Flurbiprofen loaded ME-F3 was selected for the gastroprotective effect, based on the findings of

physicochemical evaluation of the drug loaded MEs. Fig.11 (c) and (d) shows that gastric mucosa of drug loaded ME treated animals was least affected by the drug toxicity, in terms of lesions and inflammation.

DISCUSSIONS

ME is a mixture of oil, surfactant and co-surfactant, so when surfactants are added in a formulation, they get adsorbed in the layer present between the oil and water, which causes decrease in interfacial tension. Pseudo-ternary phase diagram helps to identify the best region for stable formulations with least possible ratio of oil, surfactant and co-surfactant. Here the ME was prepared without using co-surfactant to make the system more simple and conspicuous. Single surfactant based ME of clove oil has been reported in the literature with tween 20 (Gupta *et al*., 2005).

Fig. 2(b) and (c) presents the phase diagram without use of co-surfactant. There was a marginal decrease in the ME region when shifted to the single surfactant system. Due to the higher surfactant levels, ME systems have been reported for the toxicity (Vadlamudi *et al*., 2014). Therefore, we opted for the co-surfactant free ME to make our formulation more biocompatible. Hence, phase diagram with drug loaded oil was made to establish the stability of the co-surfactant free ME. We noted a decrease in the region of the ME. This indicates that the drug's hydrophobicity leads to increase in the hydrophobicity of the system, which requires slightly greater surfactant quantities to make a stable ME. Nonetheless, the long chain mono unsaturated fatty acids present in tween 80 and high HLB value might affect the hydrophobic nature of drug to improve solubility and effectively stabilize the ME (Roohinejad *et al*., 2015).

The decrease in conductivity and pH for the drug loaded ME were attributed to the poor water solubility and acidic nature of drug, respectively. As concentration of oil increased from ME-F1 to ME-F5, the droplet size also increased. Similar phenomenon has also been reported previously (Mortazavi & Pishrochi, 2013). Interestingly, the drug loaded MEs showed lower size range than the blank formulations. The hydrophobic nature of the flurbiprofen could have resulted in the compressing of the globule of ME. As per literature, 10 – 140 nm ME globule size falls in the satisfactory limit of formulations (Golmohammadzadeh *et al*., 2017). ZP of ME was slightly negative to neutral, due to the presence of nonionic tween 80. Centrifugation of the ME confirmed that the co-surfactant free MEs were stable and showed no signs of phase separation or flocculation. These observations confirmed the credibility of the phase diagram for the selection of the levels of ME components. Dissolution testing reiterated the ability of the designed ME system to improve the solubility and dissolution behavior of poorly soluble drugs.

ME-F3 was selected for assessment of the formulation to ameliorate the NSAIDs induced gastritis, on the basis of drug loading, oil content and other physicochemical attributes. Evaluation of gastritis revealed that clove oil based ME has gastroprotective properties. Rat mucosa treated with ME-F3 showed limited damage versus flurbiprofen dissolved in PEG 400. Gastric mucus is viscid, clear gel in nature that forms by the blend of water and glycoproteins that cover entire mucosa thus preventing it from damage. The possible mechanism involved in gastroprotection by the use of clove oil against the use of NSAIDs, could the enhanced mucus production by mucosal cells (Santin *et al*., 2011).

CONCLUSION

ME is effective in enhancing the solubility of poorly soluble lipophillic drugs belonging to BCS class II drugs. Flurbiprofen ME was found to be stable and showed good release profile. Encapsulation in gastroprotective oil also limited NSAIDs induced damage against GI tract of rats. This ME system could be helpful in extending the therapeutic applications of NSAIDs with fewer chances of gastrointestinal side effects. However, ME developed in this research needs further evaluation for pharmacokinetic and pharmacodynamics performance.

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