Development and Characterization of Colloidal Soft Nano-carriers for Buccoadhesive Delivery of some drug(s)

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SUMMARY

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In recent times, lipid based formulations such as microemulsions have been investigated as an appropriate approach to enhance the oral bioavailability of poorly soluble drugs. Such delivery systems may also enhance oral drug absorption by lymphatic transport via transcellular pathway to avoid the first pass effect, by increasing gastrointestinal membrane permeability or by modifying the metabolism of drugs.

When drug is incorporated into these systems, the active molecule is believed to remain in solution form throughout its transit in the GI tract. Furthermore, the absorption of the drug can be improved by the presence of lipids which stimulate the biliary and pancreatic secretions by the gallbladder, hence augmenting the gastric absorption.

Since microemulsions are capable of incorporating a large range of drug candidates, and also of increasing their solubility and bioavailability, and reducing their toxicity, hence they are promising drug carriers for oral delivery of lipophilic drug candidates.

Oral route is the major route of drug delivery for the chronic treatment of CVDs diseases. However, oral delivery of many drugs is hampered because of the high lipophilicity and first pass effect of the drug(s). Nearly 40% of new drug candidates exhibit low solubility in water, which shows high intra- and inter-subject variability, poor oral bioavailability, and lack of dose proportionality. Thus, for such compounds, the absorption rate from the gastrointestinal (GI) lumen is controlled by dissolution. An improvement of the dissolution rates of aqueous-insoluble and avoidance of the first pass effect of drugs remain the most difficult tasks for the formulation development scientists for their successful formulation, industrial applicability, improved clinical efficacy and marketing.

Hyperlipidemia and hypertension are the major CVD causes of mortality in the world. Conversely, diverse challenges that obstruct the efficient pharmacological therapies aligned with hyperlipidemia and hypertension have been recognized. Low aqueous solubility and pre-systemic metabolism appear to be the major limiting factors which lead to low and erratic oral bioavailability. In the majority of the cases when these drugs are administered orally, due to their poor aqueous solubility, the rate of dissolution in the gastrointestinal (GI) tract is regarded as the rate-limiting step.

Simvastatin (SIM) and Felodipine (FEL) are Biopharmaceutics Classification System class-II drugs. These are poorly water soluble hence their oral absorption is

dissolution rate-limited, and they also have first pass metabolism thereby undergoing erratic and incomplete absorption hence displaying low bioavailability. They are used in management of hyperlipidemia and hypertension.

The purpose of this study was therefore to develop and characterise a successful colloidal soft nano-carrier viz. microemulsion for oral delivery, and microemulsion based buccoadhesive delivery of SIM and FEL. By using this approach we can increase the solubility, avoid the presystemic metabolism and instability in gastric environment and hence, enhance the bioavailability of drug(s). Since hepatic first-pass metabolism of orally administered drugs cannot be bypassed completely hence, an alternative route of drug administration is required to increase the bioavailability of drugs. Alternatively, we also explored design and development of microemulsion based muco/buccoahesive gels for above-mentioned drugs.

The specific objectives were:

(1) To check the solubility of drugs in various components and selection of components.

(2) To construct the phase diagrams with selected components using different co-surfactant mass ratios for the preparation of SIM-loaded and FEL-loaded microemulsion.

(3) Preparation and characterization of SIM-loaded and FEL-loaded microemulsions.

(4) Preparation and evaluation of buccoadhesive gel with optimised SIM-loaded and FEL-loaded microemulsion.

- The drugs in the present study were identified and characterised by FTIR, NMR, MS and UV spectroscopy and found to be pure.
- The solubility of drugs was determined in different oils, surfactants and cosurfactants. Based on the drug solubility studies microemulsion formulation components were selected.
- The optimisation of surfactant: co-surfactant (S/CoS i.e. S_{mix}) mass ratio was performed using pseudoternary phase diagrams by titration method. Among all S_{mix} mass ratio studied for SIM- and FEL-microemulsion formation, S_{mix} ratio (1:1) showed better microemulsion region and more water incorporation to form visually clear, flowable, microemulsions compared to 1:0,

2:1 mass ratio and hence S_{mix} ratio (1:1) was selected for further formulation development.

Part I Preparation of SIM-loaded microemulsion for oral delivery: *In vitro* and *in vivo* characterization

- For SIM, microemulsion formulations (MES1-MES6) were prepared using α-linolenic acid as the oil phase, Kolliphor EL 40 as surfactant and Transcutol HP as co-surfactant. In all the formulations, the level of SIM was kept constant (10 mg/ml).
- SIM based microemulsion formulations were characterised for globules size, PDI, pH, drug content, and *in vitro* release. Based on the results of percent cumulative release, particle size and flowability, formulation batches MES1, MES3 and MES5 were rejected whereas remaining formulations i.e. MES2, MES4 and MES6 were further subjected to *ex vivo* intestinal permeation study and *ex vivo* permeation study which also compared the results with conventional formulation i.e. marketed tablet suspension, oily solution and emulsion of the SIM.
- The droplet size analysis of the SIM microemulsion formulations (MES1-MES6) showed the mean droplet size to be in the range of 143.1 to 297.1 nm, and PDI values varied from 0.261 to 0.315, respectively. Formulations demonstrated low PDI values, which indicate uniform droplet size distribution. The smaller the droplet size larger is the surface area available for partitioning of the drug, which may enhance the rate of intestinal absorption of SIM. The dilution test was performed on microemulsion confirming the type of microemulsion to be o/w.
- The amount of the drug content in all formulations of microemulsion was found to be in the range of 9.62-10.02 mg/ml of the added amount. The results of drug assay showed the appropriateness of the system for high entrapment of drug in the internal phase.
- All the formulations were showed pH values in the range of 5.8 to 6.6 of SIM microemulsion. Thus it can be supposed that drug is not diffusing in the external phase and remains in the oil phase.
- *In vitro* release of SIM microemulsion formulations were studied in phosphate buffer (pH 6.8). Microemulsion formulations MES1 to MES4 released >94%,

MES5, and MES6 released >80% and SIM marketed tablet showed 20.46±4.54%, SIM suspension (pure drug) 33.67±3.98%, oily solution 35.92±6.02, emulsion 46.79±4.85% drug release at the end of 1 h in the dissolution medium.

- This *in vitro* study concludes that release of SIM was very fast by microemulsion as more than 80% of the drug release was released in 1 hour compared to conventional formulations.
- *Ex vivo* rat everted gut sac permeation study was performed using Kreb's Ringer Phosphate buffer (KRPb, pH 6.8) with optimised microemulsion formulations and conventional formulation. SIM-loaded microemulsions showed P_{app} 4.20×10^{-5} cm/sec, 4.18×10^{-5} cm/sec and 3.62×10^{-5} cm/sec for MES2, MES4 and MES6 respectively after 1hr, while conventional formulations showed a maximum Papp of 2.36×10^{-5} cm/sec, 2.88×10^{-5} cm/sec and 2.69×10^{-5} cm/sec from drug suspension, emulsion, and oily solution, respectively. It can be concluded that nanosized globules have better interaction with the biological membrane and are able to permeate through intestinal membranes. Based on the apparent permeability, (Paap) MES4 formulation was selected for surface morphology analysis by TEM, rheological behaviour, cell cytotoxicity, cellular uptake, *in vivo* pharmacodynamic and pharmacokinetics studies, and histopathology.
- Surface morphology analysis of SIM-loaded optimised microemulsion (MES4) formulation was performed by using TEM analysis. MES4 revealed spherical globule formation. The inter-phase of oils and Kolliphor EL40 and Transcutol HP displayed a denser region which indicates film formation by S_{mix}, which prevented the globules from coalescence.
- The rheological analysis of MES4 showed that the low viscosity. It appeared to decrease at low shear rates and remained almost constant at higher shear rates while the flow curves confirmed that the prepared MES4 system revealed a linear relationship between the shear stress and shear rate, which is a characteristic of Newtonian flow.
- SIM-loaded MES4 was found to be non-toxic, where 94.52±2.36 % of the cells was found to be viable as compared to placebo MES4 with 99.14±1.42% at concentration 100 µg/ml. Results indicated non-toxicity and good

biocompatibility of the MES4 microemulsion towards cells and this was confirmed by the cell viabilities after 24 h of incubation.

- Confocal microscopy revealed intracellular uptake of the optimised MES4 microemulsion in J774 cells. Results suggested that the microemulsion efficiently transported the payload to the cellular sites in the cell and not in the nucleus of the cell.
- *In vivo* anti-hyperlipidemic of MES4 was performed and compared with the marketed tablet suspension. For induction of hyperlipidemia in rats, triton was used. Different lipid parameters such as serum cholesterol (SC), serum triglycerides (ST), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), atherogenic index (AI), LDL/HDL ratio, H/M ratio was evaluated.
- *In vivo* anti-hyperlipidemic results revealed significant activity ($P<0.001$) towards lipid profiles. An increase in HDL level was found in the SIM-loaded optimised MES4 (48.933±5.07mg/dl) when compared with marketed tablet (29.77±5.351947 mg/dl). Significant reduction ($P < 0.001$) in the SC, ST and LDL levels was observed with optimized MES4 with values of (37.23±5.65 mg/dl), (33.60±4.88 mg/dl) and (10.07±3.52 mg/dl) respectively as compared to marketed tablet having values of (54.67±6.03mg/dl), (59.33±13.05 mg/dl) and (27.13±4.16 mg/dl) respectively and VLDL significantly reduced ($P <0.01$) for optimized MES4 (3.97±2.35 mg/dl) as compared to marketed tablet suspension (11.87±2.61 mg/dl). Optimized MES4 significantly increased the level of HDL while significantly reducing the levels of SC, ST, LDL and VLDL when compared with SIM marketed tablet. That could be attributed to the solubilization of drug leading to increased absorption of the drug and therefore, improved lipid levels. These results indicate that the prepared microemulsion was efficient in controlling lipid levels as compared to marketed tablet suspension.
- *In vivo* anti-hyperlipidemic studies also revealed a significant ($P < 0.01$) difference in AI ratios, when the AI of optimised MES4 (0.76±0.112) was compared with marketed tablet (1.88±0.406) and significant difference ($P < 0.05$) in LDL/HDL ratios were observed, when optimized MES4 (0.206±0.0703) was compared with marketed (0.91±0.0318) tablet. It was

therefore concluded that optimised MES4 significantly reduces AI and LDL/HDL ratios when compared with a marketed tablet. A significant reduction in AI and LDL/HDL ratio with prepared formulation could give a better protection from cardiovascular risks.

- The H/M ratio was found to be (1.81±0.32) for control, (1.25±0.14) for toxic, (2.95±0.20) for marketed tablet, (4.52±0.19) for SIM loaded optimised MES4 formulation, and (1.41±0.59) for dummy MES4 respectively. In MES4 formulation treated groups, cholesterol synthesis in liver was significantly lower (high H/M ratio) as compared to marketed tablet treated groups ($P < 0.001$).
- *In vivo* pharmacokinetic study after oral administration of the marketed tablet suspension and SIM-loaded optimised microemulsion (MES4) formulation revealed significantly higher plasma concentration (C_{max}, 107.84±8.95ng/ml) ($P<0.05$) compared to marketed formulation (C_{max} 57.65± 4.48 ng/ml). The AUC_{last} for MES4 was found to be 409.46±22.54ng h/ml, which was significantly higher ($P<0.01$) than that of marketed formulations (155.40±12.78 ng h/ml). $AUMC_{last}$ for MES4 was found to be 1611.56±32.68 ng h^2/ml, which was significantly higher ($P < 0.01$) than that of marketed formulations (461.44±30.58ng h^2/ml). The area under the curve (AUC) for optimised MES4 showed a 2.635-fold improvement from AUC generated after administering marketed tablet formulation, indicating a significant improvement of SIM bioavailability when given orally as microemulsion which could be due to increased surface area because of nanosizing and improved dissolution rate. The relative bioavailability of the MES4 increased 263.5% with respect to marketed tablet suspension. When T_{max} of the optimised MES4 formulation was compared with a marketed formulation, T_{max} of the optimised formulation was observed to be lesser than marketed formulation which could be attributed to faster dissolution rate.
- Histopathology of the liver sections of rats treated with SIM loaded optimised MES4 showed more recovery of hepatic architecture with preserved parenchymal structures than marketed tablet treated rats.

Part II Preparation of buccoadhesive gel of SIM: *ex vivo* and *in vivo* evaluation

- SIM-loaded optimized MES4 was further utilised for the formulation of buccal gel using carbopol 71G (CP71G) gelling agent. MES4 microemulsion based

buccal gels were named MEBG4, MEBG5, and MEBG6 containing 4%, 5%, and 6% w/v CP 71G respectively, prepared and evaluated for pH, drug content, buccoadhesive strength, and viscosity. Consequently, prepared gels were compared with optimised microemulsion (MES4) and pure drug solution in water for buccal permeation study. Based on these evaluations, optimised gel was selected and texture analysis, rheology and pharmacokinetic studies were performed.

- All the gel formulations (MEBG4, MEBG5, and MEBG6) were homogenous, smooth with acceptable consistency with a pH range of 6.91±0.015 to 7.18±0.024, which was within pH range (5.6-7.0) of the oral mucosa. The optimised MEBG4 gel, having a pH of 7.18, could be administered easily through buccal mucosa, for systemic delivery of the drug and considered as a suitable delivery system, for avoiding pain, damage or irritation to the oral mucosa tissues.
- Drug content of all prepared formulations (MEBG4, MEBG5, and MEBG6) was found to be in the range of 9.88±0.12 to 9.93±0.20 mg/g of gel and indicated the uniformity of drug distribution in the developed formulations.
- The viscosity of all the prepared gel formulations (MEBG4, MEBG5, and MEBG6) was found to be in the range of 30,716.83± 125.1 to 50,613.78±218.3cP. The viscosity was found to increase with increase in the polymer concentration from 4 to 6% w/v. This could be attributed to the augmentation in intermolecular bonds or cross-linking between the polymer chains, hence increasing the gel network complexity and may lead to adhesive interactions and can enhance polymer retention time over mucosal surfaces where the gel is applied.
- Buccoadhesive strength values for the buccal delivery system were between the ranges of 185-374 mN at CP71G concentrations of 4% to 6%w/v for all the gel formulations (MEBG4, MEBG5, and MEBG6). Therefore, it can be concluded that CP71G gels of 4-6% w/v have the good mucoadhesive strength and can be employed to prolong the residence time of the drug at the application site in the oral buccal mucosa.
- Buccal permeation study of the aqueous suspensions, MEBG4 MEBG5, MEBG6 and MES4 exhibited 24.75±7.80%, 81.44±4.38%, 63.50±8.25%,

46.84±5.45, and 92.20±6.05% drug permeation respectively, at the end of 6h. Formulations MEBG4 and MEBG5 based gel showed significantly higher ($p<0.001$) drug permeation compared to the aqueous suspension of the SIM. The steady state permeation flux (Jss) of the formulations MES4 (0.482 mg/cm^2/h) and MEBG4 (0.443 mg/cm^2/h) were showed significantly higher flux ($p<0.001$) than the aqueous suspension (0.132 mg/cm^2/h) while MEBG5 (0.332 mg/cm^2/h) showed significant higher flux ($p<0.01$) than aqueous suspension. Results demonstrate that MES4 showed significant enhancement in permeation of SIM. This could be due to the presence of microemulsion in the gel formulations that enhanced the permeation of simvastatin through the buccal mucosa.

- Release kinetic modeling of the release profile of MEBG4, PDS, MES4 formulations were followed Korsmeyer-Peppas (KP) model while MEBG5 and MEBG6 followed zero order release kinetics. MEBG4, PDS, MES4 formulation followed Korsmeyer-Peppas (KP) model which usually illustrates a drug's release when the release mechanism is unknown or more than one type of release mechanism is involved. This model considers mechanisms, the drug diffusion as well as polymer relaxation. All the prepared gels (MEBG4 to MEBG6) formulation and MES4 showed 'n' values between 0.50 and 1.00 except the drug suspension which indicated anomalous transport or a combination of both diffusion mechanisms and Case II transport.
- Based on the permeation studies, formulation MEBG4 was selected as the optimised buccal gel for *in vivo* pharmacokinetics studies, histopathological, texture analysis and stability studies.
- *In vivo* pharmacokinetics studies showed that the Cmax value (131.208±21.563 ng/ml) of buccoadhesive gel (MEBG4) was found significantly higher ($P<0.001$) when compared with the same dose of oral route Cmax (68.513±9.821 ng/ml). The mean value of AUC_{last} via buccal route (1076.319±97.648 ng h/ml) was found to be 3.853 folds higher as compared to oral suspension. The relative bioavailability of SIM (the MEBG4 buccal gel) increased 385.3% with respect to marketed tablet suspension. This could be due to the fact that the permeability of the drug increased through capillary vessels present in the buccal cavity thereby enabling direct entry of drug into the

systemic circulation and also due to the prevention of drug degradation by intestine and liver.

- The texture analysis unveiled that the MEBG4 gel showed good gel strength, adequate adhesiveness, and ease of spreading, which is necessary for application and increased time of contact of the formulation in the oral buccal cavity.
- Rheology of MEBG4 gel showed pseudoplastic behaviour, in which the increased shear rate resulted in decreased viscosity. As the shear stress increases the viscosity decreases, assisting in the spreading of formulations over tissue.
- The histological analysis of buccal mucosa showed that all the epithelial sections had been successfully detached from the connective tissues with intact morphology and integrity and no histological changes were observed in formulation treated buccal mucosa when compared with control.

Part III Preparation and characterization of FEL-loaded microemulsion for oral delivery

- For FEL, formulations (MEF1-MEF15) prepared using α-linolenic acid as the oil phase, Tween 80 as the surfactant and Isopropyl alcohol as co-surfactant by using Box-Behnken design.
- The average globule size of all the FEL loaded microemulsion (MEF1-MEF15) was found in the range of 162.5–629.8 nm. After analysis of response surface plots, it was concluded that surfactant and oil concentration showed a significant effect on particle size of the microemulsions containing FEL.
- The *in vitro* dissolution behaviour of the FEL was studied in phosphate buffer (pH 6.8). The dissolutions of FEL microemulsions (MEF1-MEF15) released more than 70% drug in 1h. On the basis of the response surface plot, it was concluded that release of FEL was mainly dependent on the oil as well as surfactant concentration in the formulation.
- On the basis of all the characterization results, i.e. particle size and *in vitro* release for FEL microemulsion, further optimisation of microemulsion was done using overlay plot analysis. On the basis of overlay plot, one FEL optimised (OPT-MEF) microemulsion was predicted. Predicted OPT-MEF FEL microemulsion was evaluated for particle size, PDI, surface morphology, *in*

vitro release, *ex vivo* intestine permeation, rheological analysis, cytotoxicity, cellular uptake study, pharmacokinetic study and stability studies.

- Particle size and PDI for the OPT-MEF was found 221.5 nm and PDI 0.152, particle size was found slightly lesser than the predicted value (232.2 nm) while *in vitro* release found 96.23% which was slightly more than the predicted value (93.04%). Results are indicating that the software showed good simulation ability.
- Surface morphology analysis by TEM of the optimised OPT-MEF microemulsion indicated the spherical nature of o/w microemulsion.
- The rheological investigation showed that the viscosity was low for prepared optimised microemulsion (OPT-MEF). The flow curve was confirmed that the OPT-MEF microemulsion revealed a linear relationship between the shear stress and shear rate, which is a characteristic of Newtonian flow. It can be concluded that low viscosity and Newtonian flow are suitable for oral drug delivery and the formulation has the capability of being filled in soft or hard gelatin capsules.
- The intestinal permeability of FEL from OPT-MEF microemulsion formulation was found to be significantly higher ($P < 0.01$) than the marketed tablet, oily solution and drug emulsion, indicating that the nanosized globules of microemulsion absorbed faster than the conventional formulations. The OPT-MEF microemulsion showed Papp of 7.918×10^{-5} cm/sec after 1hr, while conventional formulations showed a maximum Papp of 3.013×10^{-5} cm/sec, 4.428×10^{-5} cm/sec and 5.335×10^{-5} cm/sec after 1hr from marketed tablet, drug oily solution, and drug emulsion, respectively.
- MTT assay of OPT-MEF microemulsion showed no toxicity where 96.28±2.06 % of the cells were found to be viable as compared to placebo OPT-MEF with 99.09±2.75% at concentration 100 µg/ml. Results indicated the non-toxicity and good biocompatibility of the OPT-MEF FEL microemulsion towards cells and this was confirmed by the cell viabilities.
- Confocal microscopy revealed intracellular uptake of the OPT-MEF microemulsion in incubated cells, majorly co-localized in the cell cytoplasm. Results put forward that the microemulsion formulation proficiently transported the payload at the cellular sites in the cell and not in the nucleus of the cell.

- The OPT-MEF formulation showed significantly higher plasma concentration (C_{max}, 7.12±1.04 µg/ml) ($P < 0.01$) compared to marketed formulation (C_{max} 2.44± 1.03 µg/ml). The AUC_{last} for OPT-MEF was found to be 84.53±10.73 µg h/ml, which was significantly higher ($P<0.01$, unpaired t-test with Welch correction) than that of marketed formulations (27.41± 5.54 µg h/ml). $AUMC_{last}$ for OPT-MEF was found to be 1068.37±73.46 µg h^2/ml, which was significantly higher ($P < 0.01$) than that of marketed formulations (326.25±32.68 µg h^2/ml). The enhancement in C_{max} and AUC_{last} may possibly be due to the fact that drug molecules were absorbed faster from the gastrointestinal tract, due to the reduction in globules size, increased surface area for absorption and also augmented dissolution rate.
- T_{max} of the optimised formulation was observed to be lesser than marketed formulation which could be attributed to faster dissolution rate and higher T_{max} could be observed for marketed formulation due to crystalline nature of the pure (FEL) drug.
- The area under the curve (AUC) for optimised OPT-MEF showed a 3.083-fold improvement from AUC generated after administering marketed tablet formulation, indicating a significant improvement of FEL bioavailability when given orally as a microemulsion. Hence, the relative bioavailability of FEL increased 308.3% when given as microemulsion formulation (OPT-MEF) with respect to marketed tablet suspension.

Part IV Preparation and evaluation of buccoadhesive gel containing optimized FEL-loaded microemulsion

- FEL loaded optimised microemulsion (OPT-MEF) was used for the buccoadhesive gel utilising HPMC E4M and PCP as gelling agents. Microemulsion based buccal gels containing 1% w/w (MEF-E4M1), 2% w/w (MEF-E4M2) and 3%w/w (MEF-E4M3) HPMC K4M and 1% w/w (MEF-PCP1), 2% w/w (MEF-PCP2), 3%w/w (MEF-PCP3) PCP were prepared respectively. Prepared gels were assessed for homogeneity, colour, texture, pH, drug content, buccoadhesive strength, viscosity. Consequently, prepared gels were compared with optimised FEL microemulsion (OPT-MEF) and pure drug solution in water for buccal permeation study. Based on these evaluations, the

optimized buccoadhesive gel was selected for texture analysis, rheology and pharmacokinetic studies.

- All the prepared gel formulations were homogenous, smooth with acceptable consistency with a pH range of 6.73±0.015 to 7.08±0.012, which was within pH range (5.6-7.0) of the oral mucosa. The pH of all the developed formulations was found near to neutral and hence the prepared gels can be considered as a suitable delivery system, for avoiding pain, damage or irritation to the oral mucosa tissues. Therefore, the optimised MEF-PCP1 gel, have a pH of 7.08, can be administered easily to the oral buccal mucosa.
- Drug content of all prepared buccoadhesive gel formulations (MEF-PCP1 to MEF-E4M3) was found to be in the range of 9.57±1.18 to 9.79±1.53 mg/g of gel. This indicated uniform drug distribution in the prepared gel formulations.
- The viscosity of all the buccal gels was found to be in the range of 3788.34± 210.5 to 61,953.59±132.8cP. The viscosity was found to increase by increasing the polymer concentration from 1 to 3% w/w. This may possibly be due to the cross-linking between the polymer chains, or intensification in intermolecular bonds thus increasing the gel network complexity. This behaviour is important for buccoadhesive drug delivery because it can lead to adhesive interactions and be able to improve polymer retention time over mucosal surfaces where the gel is applied.
- The buccoadhesive strength values for the buccal delivery system were found between the ranges of 111.12±5.49-467.63±16.32 mN at concentrations of 1% to 3%w/v HPMC E4M and PCP for all the prepared gel formulations. HPMC E4M based buccal gels were showed less mucoadhesive strength compared to PCP gel formulation, this could be due to may have less interaction with mucoadhesive membrane of HPMC E4M. Therefore, it can be concluded that PCP buccal gels of 1-3% w/w have good mucoadhesive strength compared to HPMC E4M gels and PCP based gels can be employed to extend the residence time of the drug at the relevance site in the oral buccal mucosa.
- The MEF-E4M1, MEF-E4M2, MEF-E4M3, HPMC K4M, MEF-PCP1, MEF-PCP2, MEF-PCP3, OPT-MEF and aqueous dispersion showed 81.165±3.97, 76.797±5.56, 65.071±5.64, 82.161±6.04, 64.222±3.79, 53.961±5.77, 91.178±5.67 and 31.411±2.78% drug permeation respectively at the end of 6h.

The gel formulations MEF-E4M1, MEF-E4M2, MEF-E4M3, HPMC K4M, MEF-PCP1, MEF-PCP2, and MEF-PCP3 showed significantly higher ($p<0.05$) drug permeation compared to aqueous suspension.

- The OPT-MEF (0.526 mg/cm^2/h) and MEF-PCP1 (0.436 mg/cm^2/h) showed significantly higher flux ($p<0.01$) than the aqueous suspension (0.168 mg/cm^2/h). The permeation augmentation ratio of MEF-PCP1 buccal gel was 3.13 times higher comparing to the aqueous suspension of the FEL through oral buccal mucosa. Results reveal that OPT-MEF showed significant improvement in permeation of FEL compared to aqueous suspension. Hence, it can be concluded that the polymer concentration and presence of microemulsion in the gel formulations plays a significant role in permeation of drug via buccal oral mucosa and gives an improved permeation of drug when compared with an aqueous suspension of pure drug.
- The release kinetic of FEL from MEF-PCP1, MEF-E4M3, OPT-MEF showed first-order (FO) release which indicates the concentration of drug in the release media is directly proportional to time, whereas MEF-PCP2, MEF-MCP3, MEF-E4M2 followed FO as well as korsemeyer-peppas (KP) type release, and MEF-E4M1 showed KP type release mechanism. KP model generally demonstrates a drug release when the release mechanism is unidentified or more than one type of release mechanism is involved. All the prepared microemulsion based buccal gels showed 'n' values between 0.50 and 1.00 except drug suspension (n=1.218) which indicates anomalous transport or a combination of both diffusion mechanisms and Case II transport.
- Based on the *ex vivo* permeation study of the prepared microemulsion based buccal gels, MEF-PCP1 was selected for further evaluations like rheological behaviour, texture analysis, stability studies, histopathology of the buccal membrane and *in vivo* pharmacokinetics study.
- Rheology of MEF-PCP1 gel showed pseudoplastic behaviour, in which the increased shear rate resulted in decreased viscosity. A pseudoplastic behaviour is suitable for buccal delivery purpose, since as the shear stress increases the viscosity decreases, assisting in the spreading of formulations over a tissue.
- The texture analysis revealed that the FEL-loaded MEF-PCP1 gel showed admirable gel strength, ample adhesiveness, and easiness of spreading, which

are essential for application and keep hold of the gel formulation in the buccal cavity.

- *In vivo* pharmacokinetics studies were illustrated that the C_{max} value (3.513±1.74 µg/ml) of MEF-PCP1 (buccoadhesive gel) were found significantly higher ($P<0.01$) when compared with the same dose of oral route C_{max} (9.208±2.88 µg/ml). The AUC_{last} through buccal route was found to be 3.973 folds higher when compared with oral marketed tablet suspension. The relative bioavailability of FEL (MEF-PCP1 buccal gel) was enhanced 397.30% with respect to orally administered marketed tablet suspension. This may possibly be due to permeability of the drug increasing via the capillary vessels present in the oral buccal cavity hence the drug directly entered into the systemic circulation and in addition, because of avoidance of drug degradation through intestine and liver.

Conclusion

- According to physicochemical, *in vitro* and *in vivo* characterization, it may be concluded that SIM and FEL were incorporated into the microemulsion successfully. Results indicated that developed microemulsions are a promising delivery system for the oral drug delivery of SIM and FEL to enhance the bioavailability.
- Finally, it was concluded that the experiments could be prudently extrapolated to develop a novel colloidal soft-nanocarriers containing α-linolenic acid as the oil phase, providing appropriate platform technology(ies) for enhancing the oral bioavailability of other BCS class-II drugs, especially those undergoing extensive hepatic first-pass metabolism. Further, there is a need to develop an *in vitro* and *in vivo* correlation between the developed and marketed formulation.
- The microemulsion based buccal gels were effectively developed and evaluated for systemic delivery of SIM and FEL through buccal route. Buccoadhesive gel containing SIM- and FEL- loaded microemulsion were evaluated for physicochemical parameters, *ex vivo* and *in vivo* performances which demonstrate the formation of a consistent, stable and effective delivery system. The results of the permeation study demonstrated the role of the microemulsion for effective buccal permeation of SIM and FEL via the buccal route. The pharmacokinetic studies revealed adeptness of prepared gels toward

proficient absorption of SIM and FEL through the buccal mucosa. It was finally concluded that the buccal gel containing drug loaded microemulsion could be a good choice for circumventing the first pass metabolism, intestinal degradation and also for enhancement of the bioavailability of poorly water-soluble drugs.