EVALUATION AND SYNTHESIS OF NSAID DRUG FOR MUCOADHESIVE MICROEMUSLION DRUG DELIVERY SYSTEM FOR THE TREATMENT OF PARKINSON DISEASE

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Parkinson's disease (PD) is a progressive degenerative disorder of the central nervous system. Worldwide, more than 6.3 million people suffer from PD. The incidence of Parkinsonism varied from 1.12 % to 30.76 % during 2002-2011. Presently, about 360 people among one lakh suffer from this and this figure is likely to increase further with an ageing world. This poses a huge socioeconomic challenge to the world. For the treatment of motor syndrome dopamine agonist and MAO-B inhibitors are the main drugs used. The present study shows the Development of optimal mucoadhesive microemulsion of Zaltoprofen and loxoprofen. Evaluation of efficacy of the developed formulation against PD. Study are done on Pre-formulation study, Characterisation of drug loaded MMEs, Evaluation of MMEs, *In- vitro* drug release study.

KEYWORDS: - Parkinson's disease, evaluation of microemulsion, *In- vitro* study

1. INTRODUCTION

Parkinson's disease (PD) is a progressive degenerative disorder of the central nervous system. Worldwide, more than 6.3 million people suffer from PD. The incidence of Parkinsonism varied from 1.12 % to 30.76 % during 2002-2011. Presently, about 360 people among one lakh suffer from this and this figure is likely to increase further with an ageing world. This poses a huge socioeconomic challenge to the world.[1,2] Direct administration of dopamine by oral or intravenously is not effective as it is not transported to the brain.[3,4,5] Hence its prodrug i.e., Levodopa is used which is transported to brain. long term application over years lead to a typical problem known as "fluctuations in motor activity" i.e., variability in an individual's response to treatments called "dyskinesia".[6,7,8] Protein-rich food is reported in delaying its absorption by the g.i.t. Most frequent observed side effects associated with this therapy are nausea, vomiting, dry mouth, dyskinesia and dizziness. Again, levodopa is susceptible to be degraded by amino acid decarboxylases in GIT as well as systemic circulation.[9,10] To overcome this, it is combined with enzyme inhibitor (Carbidopa). However, the co-administration of enzyme inhibitors suffers from limitations including diarrhoea, flamboyant dreams, visual hallucinations, drowsiness, urine discoloration and dyskinesias. This limits the utility making it unsuitable for long-term treatment as desired for PD. In this scenario, a number of substitutes have been developed over the years. However, none of them have been able to substitute Levodopa. This encouraged us to think for a novel alternative which may be drug or route of administration or formulation as such.[11,12]

2. MATERIALS AND METHODS

2.1 Calibration curve preparation

2.1.1 UV Method

From the stock solution of loxoprofen and Zaltoprofen 100 µg/ml, test solutions of concentration 2, 4, 6, 8, 10, 12, 14 µg/ml were prepared by suitable dilution with hydroalcoholic solvent (Ethanol: PB pH 6.4, 1: 9). Test solution was then taken at the absorption maxima (222 nm) against hydroalcoholic solvent as a blank for loxoprofen and 228 nm for Zaltoprofen. The above procedure was performed in triplicate and average of the absorbance was taken into consideration.

2.1.2 HPLC Method

The solubility study of Loxoprofen was quantified using the developed and reported reversed RP-HPLC method. C18 column is used with a isocratic flow rate of 0.8 ml/min. The elution of drug was done at 230 nm. For Zaltoprofen, the experimental conditions were Acetonitrile and Phosphate buffer pH3 (60:40 v/v), as mobile phase at a flow rate of 1 ml/min and at 254 nm. HPLC column used was 5µm intensil, C18 column (4.6 x 250mm x 5µm).

2.2 Pre-formulation study

2.2.1 Melting Point

Capillary method is used for the determination of melting point. In capillary tube a small amount of drug was inserted and then placed into the melting point apparatus. Finally, the temperature at which drug starts melting, was taken as melting point.

2.2.2 FTIR spectrum of loxoprofen and Zaltoropfen

FTIR spectroscopy is a useful tool for the structure elucidation of drug using KBr disc method. The pure drug sample was triturated and dispersed with micronized IR grade KBr powder followed by application of 8-12 kpa pressure in the hydraulic KBr press to prepare the pellets. The pellets then scanned in the region of 400-4000 nm by FTIR spectrometer. Comparision of obtained FTIR spectrum of test sample was made with reference loxoprofen as well as Zaltoprofen spectrum.

2.2.3 Percentage Purity

Percentage purity was done as per procedure given in IP 2010, the given limits (85% to 115%).

2.2.4 Spectral analysis (Absorption maxima, λmax determination)

50 µg/ml of loxoprofen as well as Zaltoprofen solution was prepared in hydroalcoholic solvent. The absorbance was determined by scanning the sample at entire range of UV range (200-400 nm).

2.2.5 Solubility profile of loxoprofen and Zaltoprofen

The screening criterion to select mucoadhesive microemulsion compositions. The dispersion was shaken in thermostatic orbital shaker for 48 hrs at 37 ± 2˚C (80 rpm). Centrifuged at 8000 rpm for 15 min. To obtain only solubilised loxoprofen as well as Zaltoprofen. Supernatant was filtered through 0.45µm filters and finally analysed using UV-VIS spectrophotometer at appropriate wavelength.

2.2.6 Drug-Excipient compatibility study

Drug-Excipient compatibility study was performed to ensure that the formulation has relatively more biocompatibility. Surfactant, oil and cosurfactant were considered for further development only if physically and chemically compatibility with drug was observed. Adequate quantity of loxoprofen and all formulation components mixed and was kept at room temperature and at 50 C for 7 days FT-IR spectra of the mixture at day 1 (D1) and day 7 (D7) were analysed for compatibility since FT-IR spectrum gives the identification of specific functional groups and so that from this any chemical incompatibility if occurred can be easily identified by change in the peak of functional group.

2.3 Characterization of drug loaded MMEs

2.3.1 Transparency (% Transmittance and Refractive index)

Both developed MMELs and MMEZ were diluted (50 and 100 times) with distilled water. Optical transparency of these formulations was quantified at 560 nm by UV-VIS spectrophotometer against water.

2.3.2 Globule size and Zeta potential analysis.

Both developed MMELs and MMEZs were diluted (50 and 100 times) with distilled water. Malvern zeta sizer is used for determination of zeta potential.

2.3.3 Viscosity analysis.

The viscosity of MMELs and MMEZ was measured to determine rheological properties of formulations. Brookfield HVDV II+ CP viscometer at 30 rpm was used to serve this purpose.

2.3.4 pH of MMELs and MMEZ

pH is very important parameter of microemulsion because it may cause bio-incompatibility and hence the patient inconvenience. The excipients so used to develop formulation. The developed drug loaded MMEs were dispersed in distilled water and the dispersion was finally used for the pH determination using digital pH meter.

2.3.5 Drug content

Loxoprofen and Zaltoprofen loaded MMEs was quantified by UV-VIS spectrophotometer. 2 µg/ml of test solution was prepared by diluting MMELs with distilled water. The samples were quantitatively measured as 222 nm for loxoprofen and 228 nm for Zaltoprofen.

2.3.6 Spreadability

A modified apparatus consists of a wooden block to which a pulley is attached to one end and a glass plate is fixed on the same end. 3 gm of MMEL and MMEZ was placed on plate. Both MMEs were than sandwiched. Shorter time interval indicates better spread ability.

2.3.7 Electron Microscope Characterization

Transmission Electron Microscopy (TEM) is the most important technique for the study of microstructures and morphology of microemulsions because it directly produces images at high resolution and it can capture any co-existent structure and micro-structural transitions. The prepared samples of microemulsions were subjected to TEM.

2.4 Evaluation of MMELs.

2.4.1 Measurement of mucoadhesive strength

100 mg of MMELs and MMEZ was kept on agar plates at room temperature. The time is recorded. As a direct evident, the mucoadhesive potential of the developed formulations was determined. A section of nasal tissue with known surface area was mounted to the upper probe using a cyanoacrylate adhesive.

2.4.2 Naso-Cilio Toxicity study.

Excised sheep nasal mucosa was separately treated with same amount of positive control (Iso Propanol), negative control and both MME formulations (separately) for two hours. The effect on the sheep nasal mucosa was observed with help of photomicrographs taken by in terms of cilliary beat and cell destruction.

2.4.3 *In-vitro* drug release study

In-vitro drug release study of microemulsion formulations [MMEI(C) and MMEI(L) as well as MMEZ] were carried out in Franz diffusion cell having volume of 30 ml and an effective permeation area of 7.06 cm2 containing 30 ml of dissolution media as used in the UV determination.

2.4.4 Release kinetics

The following equation is used to study the release kinetics of loxoprofen and Zaltoprofen.

Zero kinetic equation: $Qt = k0t$,

Where, Qt- % drug released at time t

$k0$ – Release rate constant.

First kinetic equation: $\ln (100-Qt) = \ln 100 – k1t$,

Where, $k1$- Release rate constant.

Higuchi's kinetic: $Qt = kHt1/2$,

Where, kH - Higuchi release rate constant

Hixson-Crowell kinetic: $(100-Qt)\ 1/3 = 1001/3 – kHCt$, kHC – Hixson-Crowell rate constant.

Korsmeyer Peppa's model: $Qt/Q\infty = kKPtn$,

Where, $Qt/Q\infty$ - Fraction of drug released at time t, kKP a constant and n, the release exponent indicating the drug release mechanism.

2.4.5 Ex-Vivo Permeation Study

ex vivo drug permeation through nasal mucosa was carried out in Franz diffusion. Freshly excised sheep nasal mucosa was collected from the Slater house, washed several times with PBS pH 6.4. It was loaded with 1 ml of MMELs, MMEZ, IDG, ZDS and IDS. Diffusion was done at 37 ± 0.5 °C and 50 rpm. The receptor medium was analysed by UV-VIS Spectrophotometer. The obtained results taken in triplicate was considered as % drug permeated through.

3. RESULTS AND DISCUSSION

3.1 Pre-formulation Study

3.1.1 Calibration Curve of loxoprofen and Zaltoprofen

3.1.1.1 UV spectroscopy

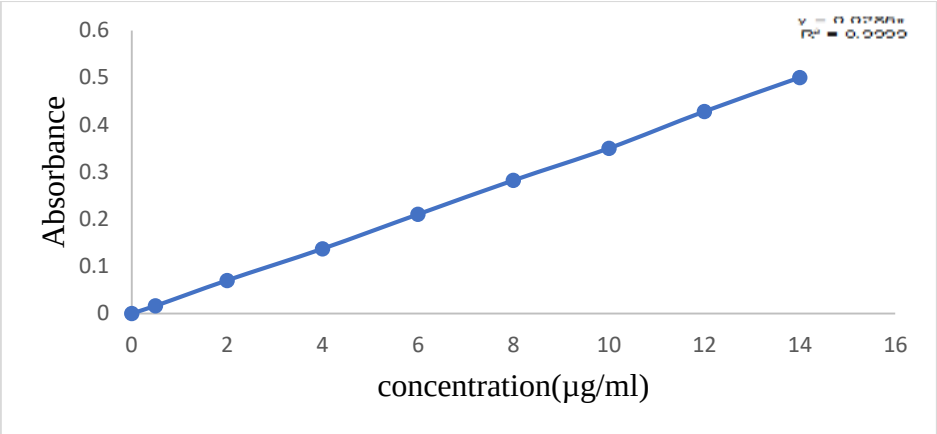


Figure 1. Calibration curve of loxoprofen

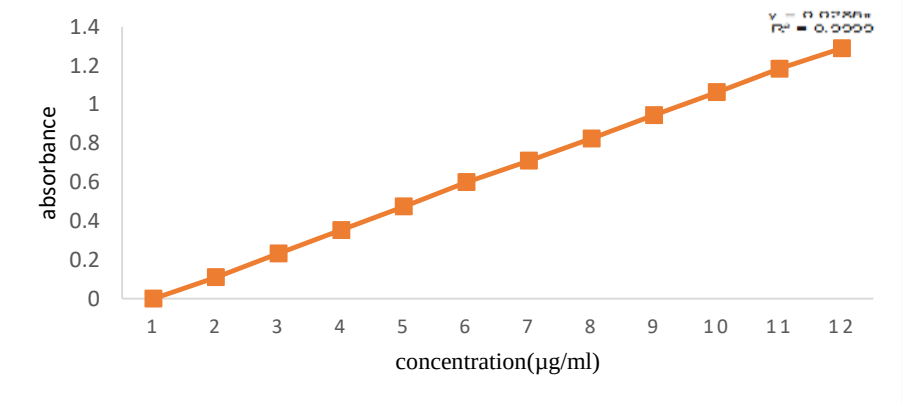


Figure 2. Calibration curve of Zaltoprofen

3.1.1.2 HPLC

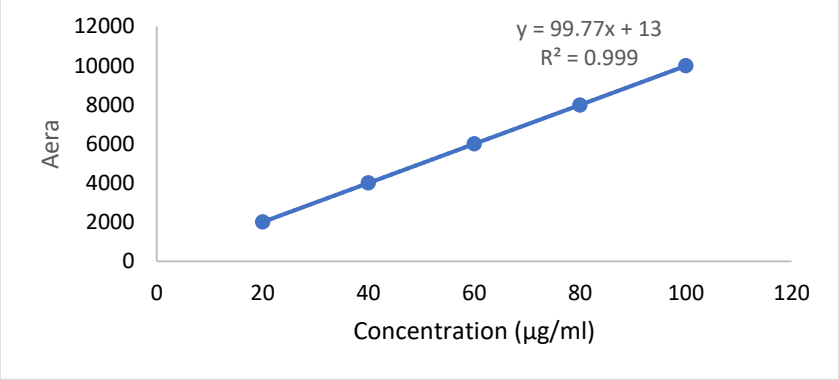


Figure 3. Linear curve of loxoprofen (HPLC)

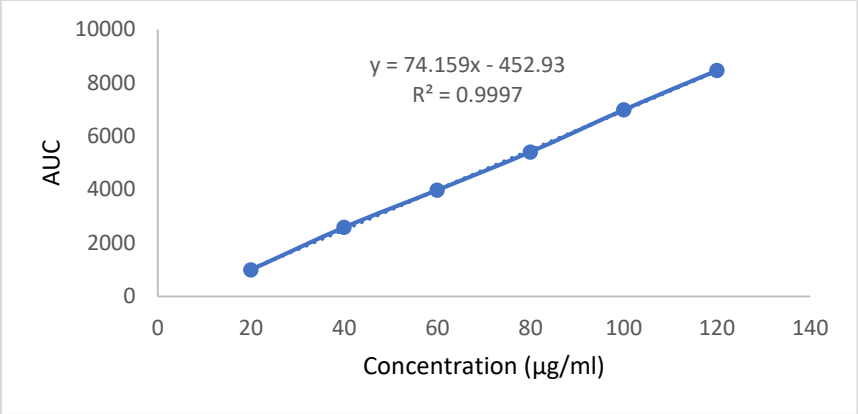


Figure 4. Linear curve of Zaltoprofen (HPLC)

3..1.1.3 Solubility

Solubility of drug in different solvents was measured by UV-VIS Spectrophotometer. The data showed that loxoprofen is water insoluble and maximum solubility was found in ethanol which helps to develop the solvent system for UV-VIS method of estimation. Data for Zaltoprofen was showed the water insolubility.

Drug	Solvents	Solubility	Inferences
Loxoprofen	Water	0.545±0.13	Water insoluble
	Ethanol	79.58±0.78	Highly soluble
	Acetone	50.65±1.78	Highly soluble
Zaltoprofen	Water	0.489±0.38	Water insoluble
	Ethanol	76.12±1.29	Highly soluble
	Acetone	55.58±1.89	Highly soluble

Table 1. Solubility of loxoprofen and Zaltoprofen in different solvents

3.1.1.4 Drug and Excipient compatibility study

Physical compatibility data are shown in Table 8. Data are suggesting that there is no physical incompatibility between loxoprofen and screened formulation compositions like Capmul MCM, Labrafil M 1944CS, mixture Tween 80 and Transcutol P as stabilizing part and Polycarbophil as mucoadhesive polymer. Similarly, no physical incompatibility between Zaltoprofen, Labrafil M 1944CS and Transcutol P was observed. So, all the above screened formulation compositions separately. were used for the development of an effective MMEI and MMEZ for nasal delivery of loxoprofen and Zaltoprofen respectively.

Formulation	Precipitation		Phase separation		Colour change	
	D3	D7	D3	D7	D3	D7
Loxo+C+A+Tr+P	×	×	×	×	×	×
Loxo+L+A+Tr+P	×	×	×	×	×	×
Zal+L+A+Tr+P	×	×	×	×	×	×

loxo- loxoprofen, Zal- Zaltoprofen, C- Capmul MCM, L- Labrafil, Tr-Transcutol P, P- Polycarbophil, T-Tween-80

Table 2. Drug-excipients compatibility study

Further drug and excipients chemical compatibility were carried out by means of FTIR studies. The interpretation of FTIR spectra indicates that no physicochemical interaction in between loxoprofen and other formulation compositions.

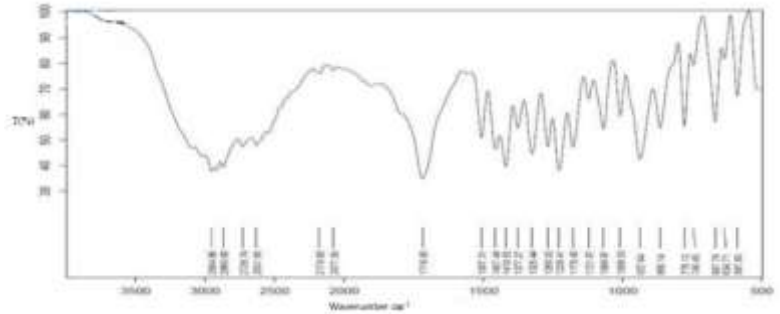


Figure 5. MMEL including Loxoprofen (Day 1)

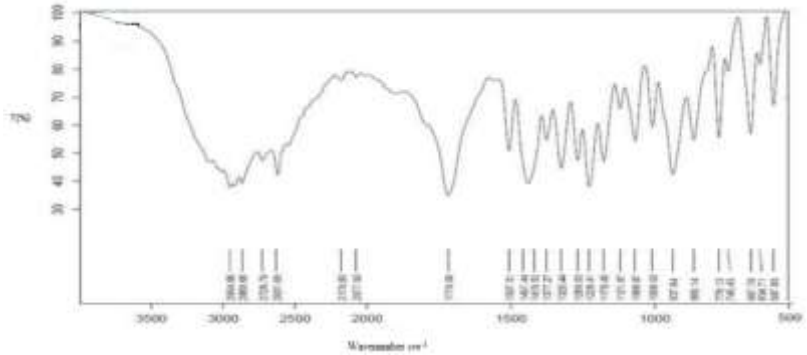


Figure 6. MMEL(C) including loxoprofen (Day 7)

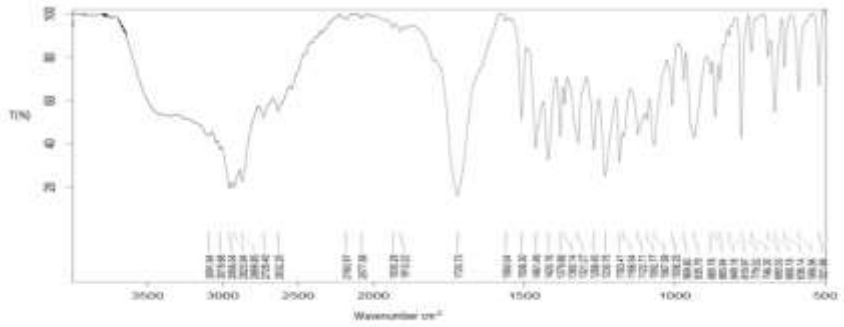


Figure 7. MMEL(L) including Loxoprofen (Day 7)

Functional group	Peak composition day 1	Peak composition day 7	
-C=O	1720	1720	1721
-CH	2629	2634	2635
CH-CH Stretching	2870	2875	2870
Aromatic	2961	2955	2957

Table 3. interpretation of FTIR spectra of MME(C) and MMEL(L)

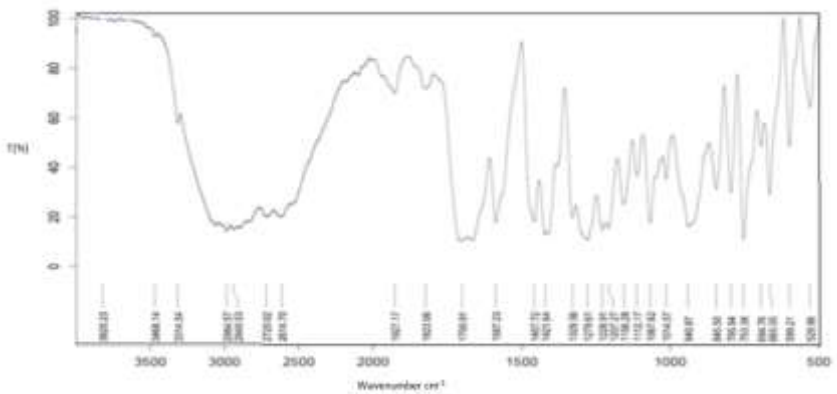


Figure 8. MMEZ (Day 1)

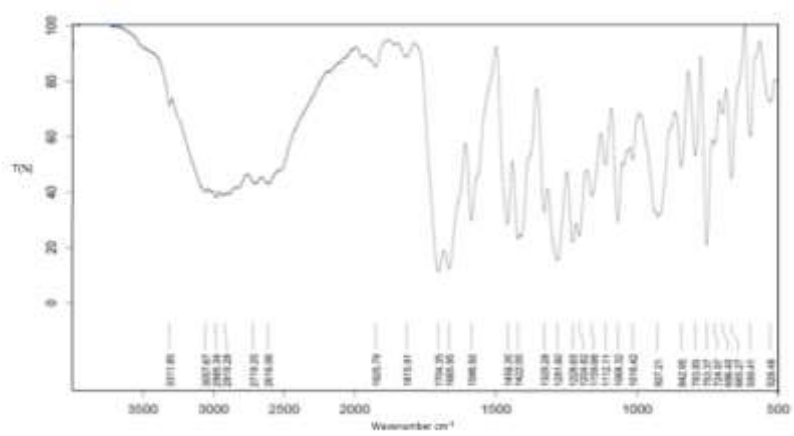


Figure 9. MMEZ (Day 7)

Functional group	Peak Composition Day 1	Peak composition day 7
OH stretching	3052	3049
Aliphatic CH stret	2983	2989
-C=O (ketone)	1670	1669
-C=O(Acid)	1708	1706
-C=C(Aromatic)	1658	1674

Table 4. Interpretation of FTIR spectra for MMETZ

The observed peak of the spectrum was not significantly differed from the functional group on Day 1 and Day 7 for both formulation composition and hence it was confirmed that all formulation compositions of mucoadhesive microemulsion was compatible with loxoprofen as well as Zaltoprofen respectively. From the above data it is clear that the selected drug is physically and chemically stable with the excipients and therefore they can be further considered for formulation.

3.1.1.5 Melting point.

Melting point of loxoprofen was found to be 196 ± 1^0 C and was found to be near reference data (198^0 C) so drug was identified as loxoprofen. Similarly, the melting point of procured Zaltoprofen (133-138^0 C) was matching with the reference (135- 139^0 C) and hence the drug was complied with the melting point.

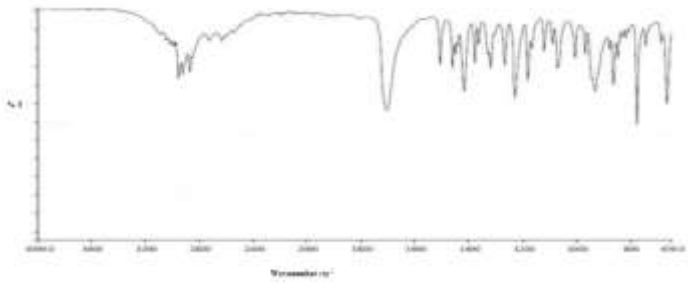


Figure 10. FTIR spectrum of loxoprofen

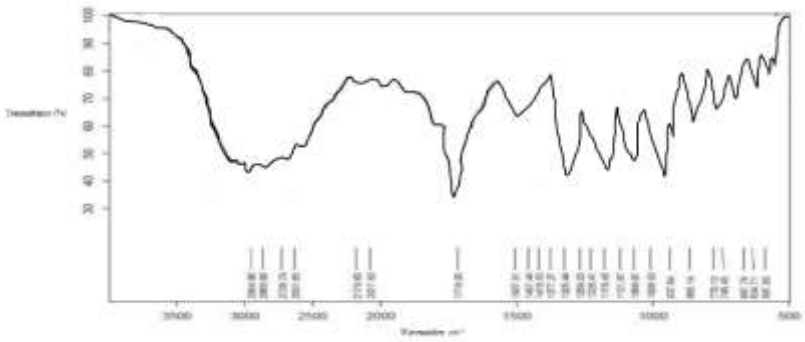


Figure 11. FTIR spectrum of procured loxoprofen

Functional group	Peak in graph	Peak of drug
-C=O	1719	1723
-CH	2625	2632
CH-CH stretching	2880	2869
Aromatic	2960	2955

Table 5. Interpretation of FTIR spectra

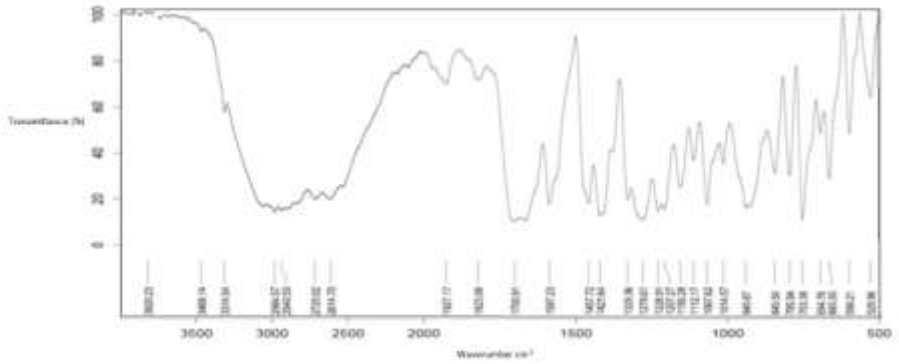


Figure 12. FTIR spectrum of Zaltoprofen

Function group	Peak in graph	Peak of Drug
OH stretching	3100-3500	3050-3314
Aliphatic CH stretch	2950-3000	2985-2945
-CH	2625	2645
CH-CH stretching	28890	2875
Aromatic	2961	2967

Table 6. Interpretation of FTIR spectra of Zaltoprofen

3.1.1.6 Percentage Purity

Percentage purity was done as per procedure given in IP 2010 and was found to be 99.53 ± 0.61% for both the drugs that complies with the given limits (85% to 115%).

3.1.1.7 Spectral analysis of loxoprofen and Zaltoprofen

Wavelength of Detection: 222 nm in ethanolic phosphate buffer (pH 6.4) (1:9). A representative spectrum of loxoprofen showing wavelength maxima at 222 nm in ethanolic buffer (PB, pH 6.4) at 9:1 ratio for different concentration was absorption maxima for Zaltoprofen.

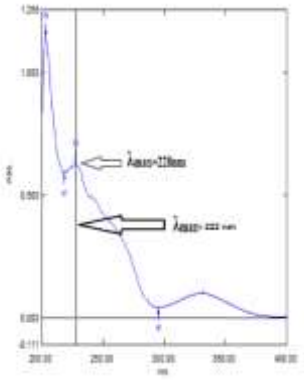


Figure 13. λmax determination of loxoprofen and Zaltoprofen

3.2 Characterization of drug loaded MMEs

Formulation	Transmittance (%)	Refractive index	Globule size (nm)	Zeta potential(mV)	PdI
MMEL(C)	98.6±0.38	3.78	66.30±4.19	-20.1±3.58	0.189±0.20
MMEL (L)	99.8±0.29	3.65	48.49±3.49	-24.5±3.58	0.210±0.18
MMEZ	98.2±0.19	3.49	90.84±4.65	-20.2±1.29	0.198±0.29

MMEL(L)- Labrafil based MMEI; MMEL(C)- Capmum MCM based MMEI; MMEZ Labrafil M1944 CS based MMEZ

Table 7. Characterization data of optimized MMEs

3.2.1 Transparency

% Transmittance was found to be near to 100 % (98.6 ± 0.38, 99.8 ± 0.29 and 98.2 ± 0.19 %) which indicated that both MMEIs are optically transparent. The developed MMEIs were having smaller globule size with narrow size distribution that enabled light to diffract less. Isotropic property of both MMEIs was indicated by refractive index. Obtained result of refractive index not significantly differed to that of distilled water.

3.2.2 Globule size and PdI

Optimal MMEL with 3 % Capmul MCM, 36 % Transcutol P (Smix-3:1), 61% of 0.5% Polycarbophil and 30 mg of loxoprofen for 10ml of batch size, are transparent with globule size 66.29 ± 5.44 nm. 3% v/v Labrafil M 1944CS, mixture of Tween-80 and Transcutol P (26% - 9%v/v) with 0.5% aq. Polycarbophil dispersion in distilled water showed globule size 46.73 ± 3.11 nm. Globule size and PdIs of both MMEIs (0.183 ± 0.19 and 0.201 ± 0.19) indicating the nanosize range of the formulation. It also depicts the monodisperse property as well as the narrow size distribution of the optimized MMEI which help in dissolution and permeation in turn effective brain targeting through nasal route. For MMEZ, average globule size and size distribution.

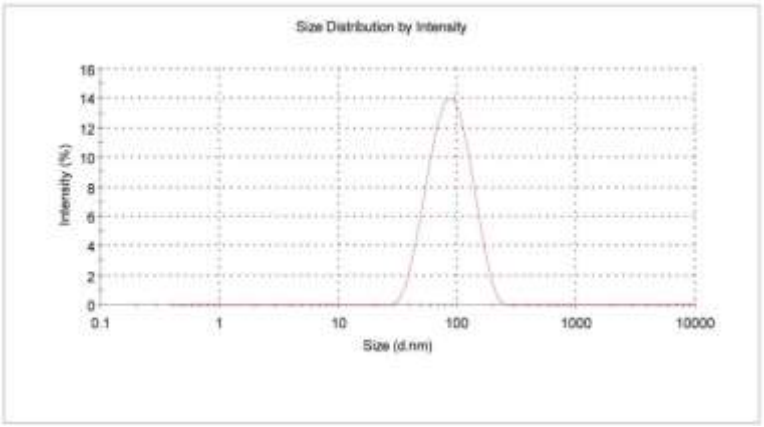


Figure 14. Result of Globule size with PdI of optimized MMEL(C)

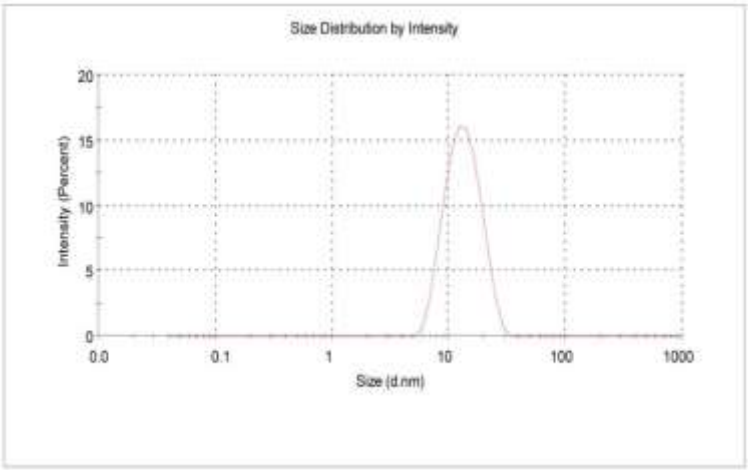


Figure 15. Result of Globule size with PdI of optimized MMEL(L) size with PdI

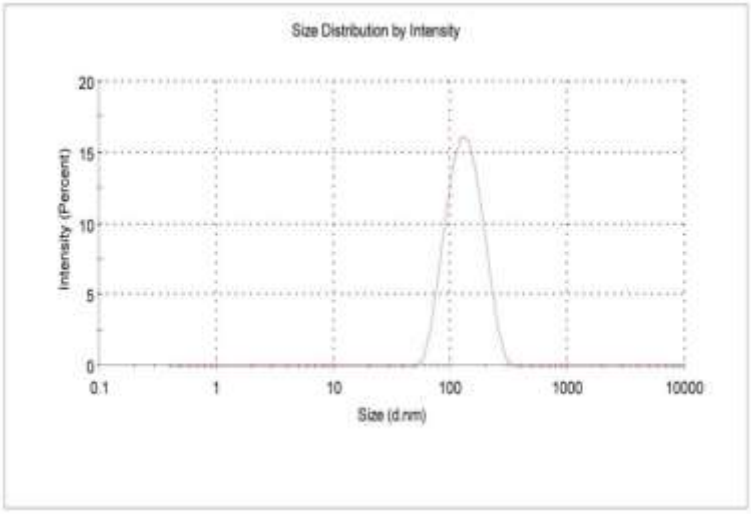


Figure 16. Result of Globule size with PdI of optimized MMEZ

3.2.3 Zeta potential

Zeta potential of optimized MMEL(C) was found to be -24.5 ± 3.27 mV. Zeta potential gives stability of microemulsion. According to DLVO theory, repulsion due to electric double layer stabilizes microemulsion and hence, aggregation is not expected to take place. Result of zeta potential and increased surface area also affect the stability of the formulation, since the sedimentation rate was minimal due to the smaller size. Labrafil based MMEL showed zeta potential -20.9 ± 3.98 mV.

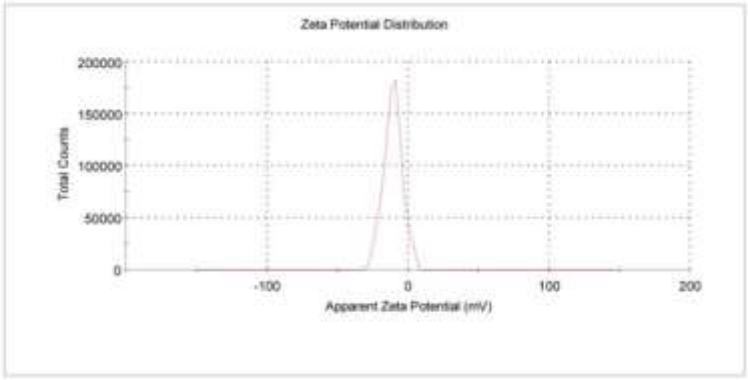


Figure 17. Zeta potential of optimized Capmul MCM based MMEL

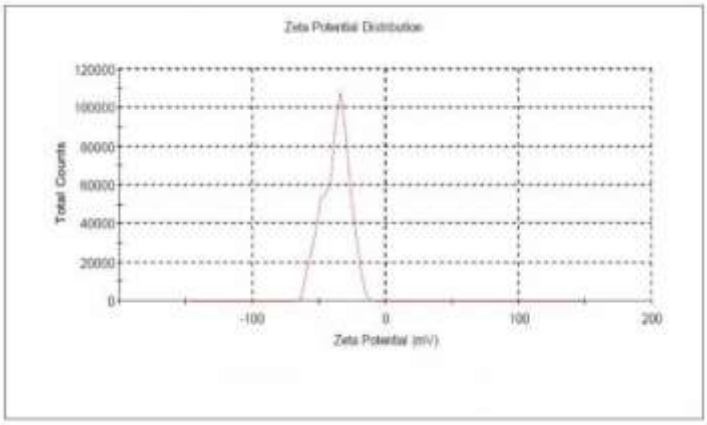


Figure18. Zeta potential of optimized Labrafil based MMEL

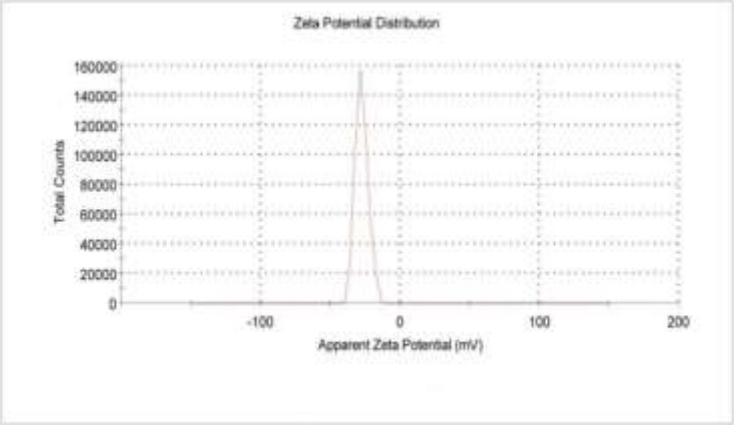


Figure 19. Zeta potential of optimized Labrafil based MMEZ

3.2.4 Viscosity and Spreadability

Result showed that viscosity of final MMEs (37.74×103cPs to 41.80×103cPs at 25°C) were enough to adhere to the nasal mucosa, hence increased residence time. Rapid penetration across nasal mucosa was facilitated. Spreadability was found to be 7- 13 sec range, hence good spreadability and hence developed MMEs suitable for the trans nasal application from the patient compliance point of view.

3.2.5 pH

Optimized MMEs showed pH i.e., 6.4 ± 0.31, suggesting the compatibility to the nasal mucosal route of administration because no local irritation will be produced in the treated area of nasal mucosa, hence totally suitable for nasal application.

3.2.6 % Drug content and content uniformity

Drug content of MMEs was found to be 98.4 ± 0.51 % and 98.77 ± 0.89 % which is very near to 100 % indicating the chemical stability of the formulation. Drug content of MMEs at three test points were in between 98.1 to 97.5 (n = 3) suggesting uniform drug distribution, hence the developed MMEs was found complying the content uniform.

3.2.7 TEM study

TEM images of optimized MMEs were indicating the spherical shape as well as narrow size distribution, which also confirmed through the globule size and PdI results. No aggregation of droplets was also observed from these taken photographs.

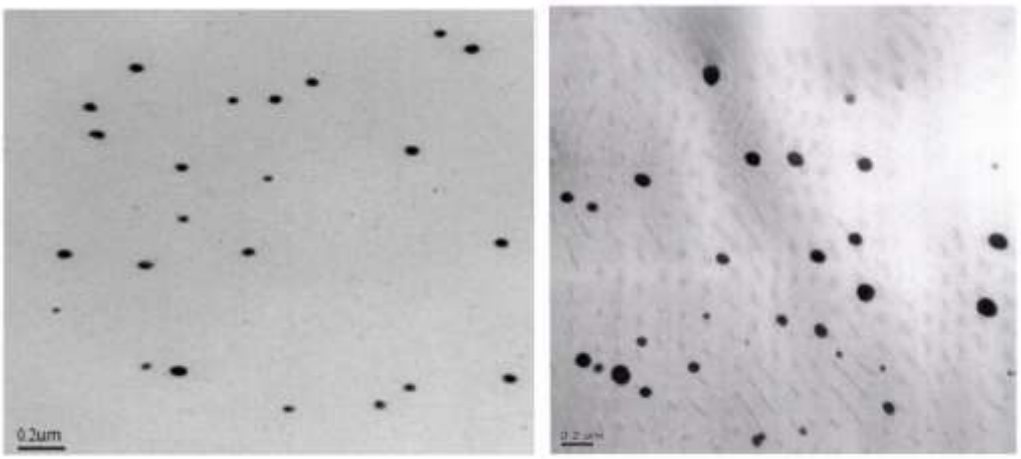


Figure 20. TEM image of optimized MMEL and MMEZ

3.3 Evaluation of MMEIs

3.3.1 Mucoadhesive strength of MMEs

The retention time as the reflect of mucoadhesive strength of MMEL(C), MMEL(L), MMEZ, IDG and ZDG were found be 24.5 ± 1.2 min, 22.4 ± 1.3, 25.3 ± 1.3, min 22.8 ± 1.2 and 24.3± 0.2. The mucoadhesive strength was 4285.86 dynes/cm and 4311.28 dynes/cm2 for developed MMEL(C), MMEL(L) and 4311.27 dynes/cm2 for MMEZ respectively while IDS and IDS found to have well below i.e., 2784 dynes/cm2 and 226.49 dynes/cm2 respectively.

3.3.2 Naso-Cilio Toxicity study

The retention time as the reflect of mucoadhesive strength of MMEL(C), MMEL(L), MMEZ, IDG and ZDG were found be 24.5 ± 1.2 min, 22.4 ± 1.3, 25.3 ± 1.3, min 22.8 ± 1.2 and 24.3± 0.2. The mucoadhesive strength was 4285.86 dynes/cm^2. Results of nasal ciliotoxicity studies reveal the non-toxicity effect of the developed MMEs and hence it was found suitable for application on the

nasal mucosa. The data of pH, globule size, spreadability, mucoadhesive strength and nasal non-ciliotoxicity of the developed MMEs confirming the suitability of nasal use for the brain targeting to treat Parkinson's disease.

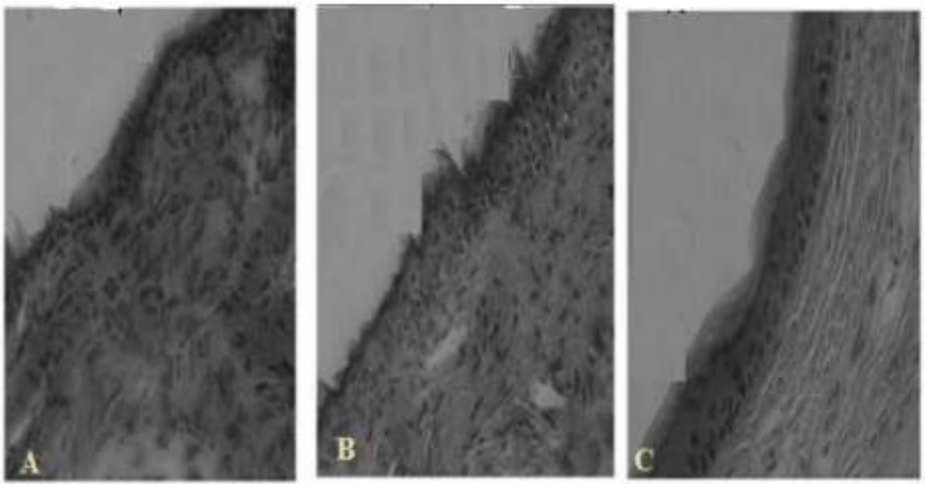


Figure 21. Naso-ciliotoxicity study result showing non-toxicity of developed MMEL(C).

A- unaffected nasocilliary part, B- Damaged Nasocilliary part, C- Unaffected Nasocilliary part

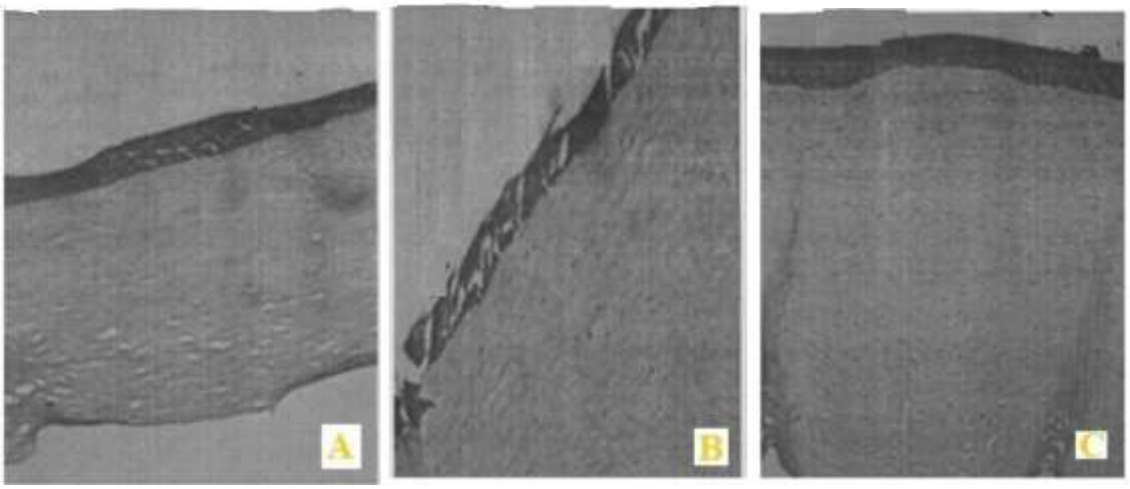


Figure 22. Result of naso-ciliotoxicity study showing non-toxicity of developed MMEZ

A- unaffected nasocilliary part, B- Damaged Nasocilliary part, C- Unaffected Nasocilliary part

3.4 *In-vitro* release study

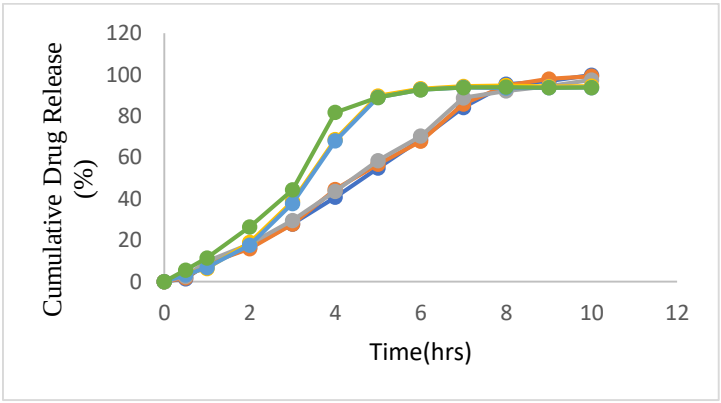
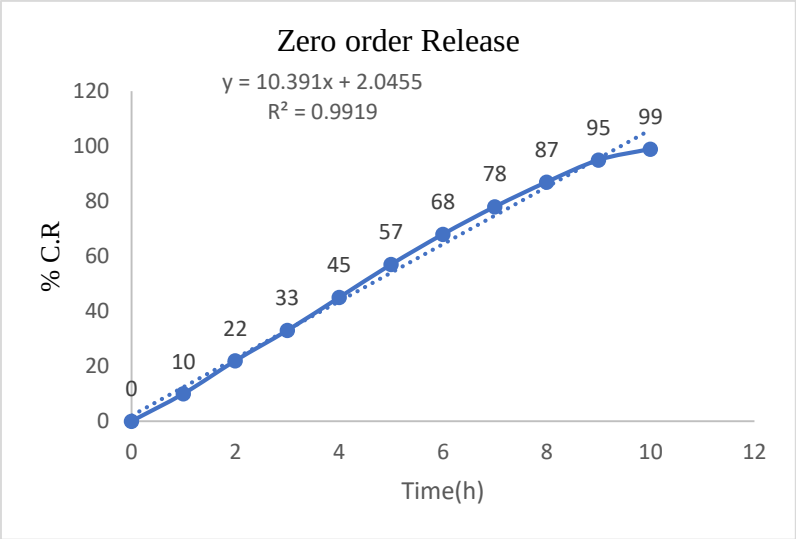
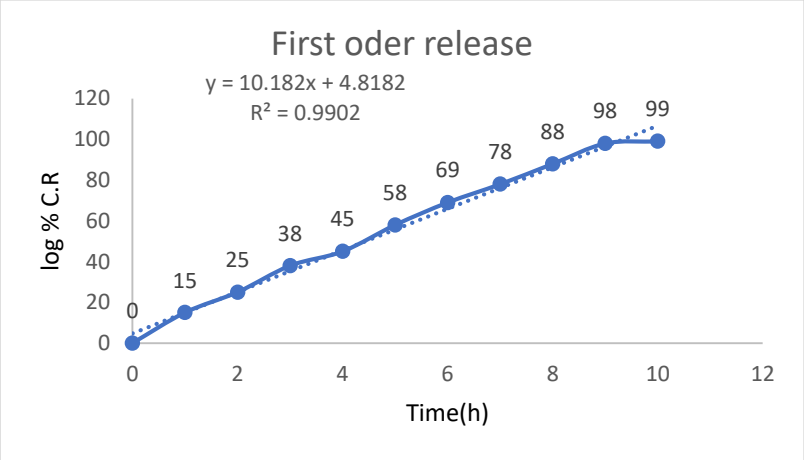
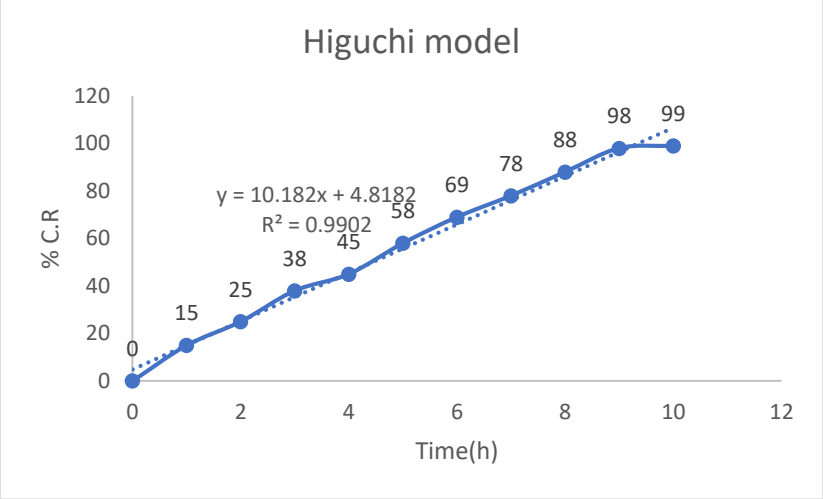


Figure 23. *In-vitro* drug release study of optimized MMEL (L), MMEL (C), MMEZ, IDG and IDS

3.5 Release Kinetic modelling







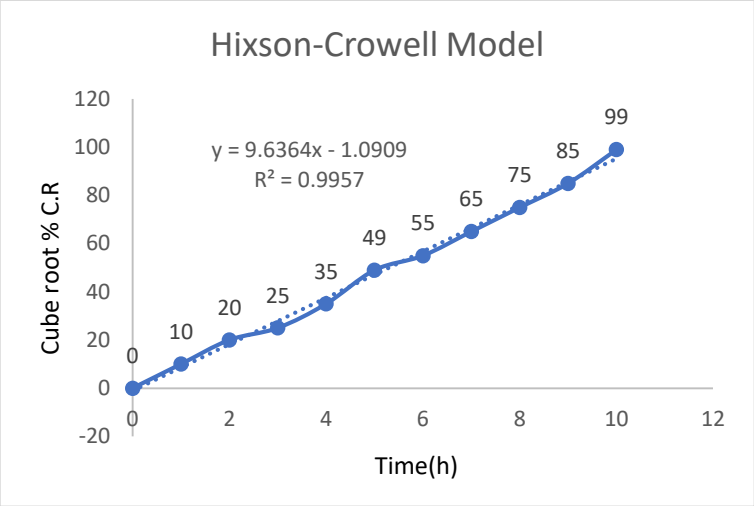


Figure 24. Kinetic models

3.6 *Ex vivo* permeation study

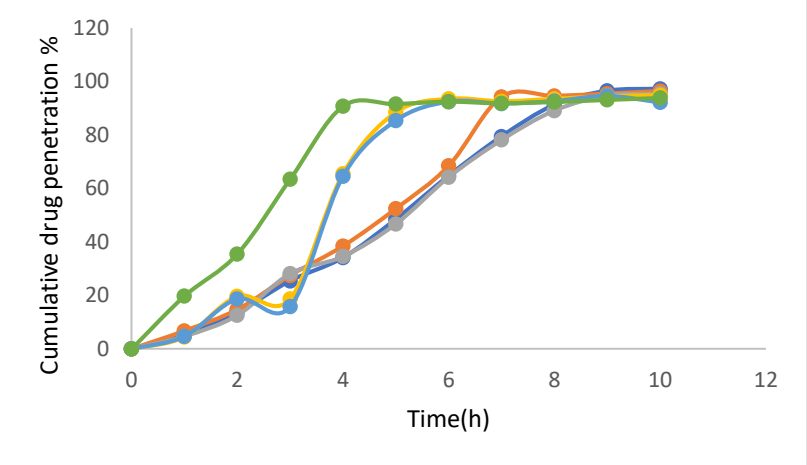


Figure 25. Comparative ex-vitro drug permeation study of optimized MMEs, IDG, ZDG and IDS

4. SUMMARY & CONCLUSION

Parkinson's disease is a neurodegenerative disorder of the central nervous system. PD poses a huge socioeconomic challenge to the world. Neuroinflammation is one of the major contributing factor for PD. Solubility of practically insoluble loxoprofen was found to be highest in biocompatible for nasal route, Capmul MCM (39.3 ± 3.13 mg/ml) and Labrafil M 1944 CS (46.59 ± 2.66 mg/ml) as compared to other screened oils. For Zaltoprofen, the highest solubility was observed in Labrafil M 1944 CS 45.19 ± 2.59 mg/ml. Thus, Capmul MCM and Labrafil M 1944 CS were selected for loxoprofen and Labrafil M 1944 CS alone for Zaltoprofen and used as internal or dispersed phase for the development of mucoadhesive microemulsion formulation. Microemulsion formulations were developed by water titration method and optimised using factorial design. From the formulation optimization study, MMEL containing 3% oil, 37.0 % (38.0 % for Labrafil M 1944 CS based) Smix and 0.5% Polycarbophil for MMEL(C) and MMEL(L) were optimised formulation. Similarly, optimised formulation composition of MMEZ was 3% Labrafil M 1944 CS, 37.0 % of Smix and 0.5% polyvcarbophil. Globule size of MMEL(C), MMEI(L) and MMEZ was 66.29 nm ± 4.15, 46.73 nm ± 3.11 and 89.98 nm ± 4.66 with PdI value 0.183 ± 0.19, 0.201 ± 0.19 and 0.184 ± 2.23 respectively, indicating the monodisperse nature of system. PdI value and TEM results of both developed formulations indicated the mono-dispersity property and narrow size distribution. Zeta potential of MMEL(C), MMEI(L) and MMEZ was -24.4 mV ± 3.27, -21.4 mV ± 2.99 and -20.9 mV ± 1.28. *In-vitro* drug release data revealed the control release and release kinetic studies showed the formulations follows Fickian diffusion and Higuchi model. Ex-vivo drug permeation studies and flux result showed the significance of developed MMELs and MMEZ for the rapid permeation through nasal mucosa to target loxoprofen to brain through olfactory route.

5. REFERENCES

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