

**NANOTECHNOLOGY MEDIATED CO-FORMULATION  
OF SIMVASTATIN AND GLIMEPIRIDE**

A  
Thesis

Submitted to



For the award of  
**DOCTOR OF PHILOSOPHY (Ph.D.)**  
in  
**(Pharmaceutics)**

By  
**Narendra Kumar Pandey**  
**(41400121)**

Supervised By  
**Dr. Sachin Kumar Singh**  
**LOVELY FACULTY OF APPLIED MEDICAL SCIENCES**  
**LOVELY PROFESSIONAL UNIVERSITY**  
**PUNJAB**  
**2019**

## **DECLARATION**

I hereby declare that the present dissertation entitled “**Nanotechnology mediated co-formulation of simvastatin and glimepiride**” embodies the original research work carried out by me. It is further stated that no part of this dissertation has been submitted either in part or full for the award of any other degree of Lovely Professional University or any other University/Institution.

**Narendra Kumar Pandey**  
Department of Pharmaceutical Sciences,  
Lovely Professional University,  
Jalandhar-Delhi G.T. Road,  
Phagwara, Punjab 144411

## **CERTIFICATE**

This is to certify that the present dissertation entitled “**Nanotechnology mediated co-formulation of simvastatin and glimepiride**” embodies the original research work carried out by **Narendra Kumar Pandey** under my supervision and guidance. It is further stated that no part of this dissertation has been submitted either fully or in part for any other degree of this or any other university.

**Dr. Sachin Kumar Singh**  
Associate Professor  
Dept. of Pharmaceutical Sciences,  
Lovely Professional University,  
Jalandhar-Delhi G.T. Road,  
Phagwara, Punjab 144411

## ABSTRACT

Simvastatin (SIM) and glimepiride (GLM) are well known antihyperlipidemic and antidiabetic drugs, respectively. Both being lipophilic cause dissolution rate limited oral bioavailability. In the present study two different nanotechnologies *viz.* nanosuspension (NS) and self-nanoemulsifying drug delivery systems (SNEDDS) have been attempted to improve the oral bioavailability of both the drugs as well as their *in-vitro* performance such as dissolution, stability and also evaluated for their antidiabetic potential against high fat diet and streptozotocin induced rat model. Nanosuspension (L-NS) of GLM-SIM mixture was prepared by liquid anti-solvent precipitation method and final formulation was optimized by applying DoE. The liquid SNEDDS (L-SNEDDS) of GLM-SIM mixture was prepared and optimized using ternary phase diagram, thermodynamic, centrifugation and cloud point studies. The optimized L-NS and L-SNEDDS were subjected for solidification using spray drying technique with/without the help of porous carriers. The particle/droplet size of spray dried nanosuspension (SP-NS), L-SNEDDS and solid SNEDDS (S-SNEDDS) were found to be  $127.4 \pm 1.13$  nm,  $55.63 \pm 1.78$  nm, and  $75.26 \pm 2.38$  nm, respectively. The zeta potential for SP-NS, L-SNEDDS and S-SNEDDS were found to be  $-27.32 \pm 2.05$  mV,  $-22.31 \pm 1.66$  mV, and  $-19.54 \pm 1.56$  mV. The drug loading (%) of GLM in SP-NS, L-SNEDDS and S-SNEDDS were found to be  $95.35 \pm 1.39$ ,  $94.5 \pm 1.45$ , and  $95.5 \pm 1.51$ , respectively. Similarly, the drug loading (%) of SIM in SP-NS, L-SNEDDS and S-SNEDDS were found to be  $80.30 \pm 1.33$ ,  $79.2 \pm 1.12$ , and  $92.63 \pm 1.08$ , respectively. The percentage cumulative release of GLM from spray dried nanosuspension (SP-NS), L-SNEDDS and S-SNEDDS were found to be 6.43, 6.44 and 6.44 times higher as compared to unprocessed GLM. Percentage dissolution rate of SIM from SP-NS, L-SNEDDS and S-SNEDDS was observed 4.46, 4.47 and 4.45 times higher than unprocessed SIM. The dissolution rate of both the drugs was found higher from SP-NS as that of SNEDDS. Oral bioavailability of GLM and SIM from SP-NS and S-SNEDDS were found 6.69- and 4.22-folds higher for GLM and 1.76 and 2.68 folds higher for SIM than their unprocessed forms. This study also indicated that AUC of both the drugs were 1.59 and 1.52 higher for SP-NS than SNEDDS. During stability studies, a non-significant difference was observed in

particle/droplet size, zeta potential and dissolution profiles of GLM and SIM from aged and fresh samples of L-SNEDDS, S-SNEDDS and SP-NS. The study on rats indicated that both the drugs showed significant improvement in biochemical parameters such as blood glucose level and lipid profile as well as when they were co-delivered through nanocarriers as compared to their unprocessed form. It was also noted that the combination of both the drugs was found to be more effective than their individual doses. Among nanosuspension and SNEDDS, nanosuspension containing both the drugs (NS-GLM-SIM) was found more efficacious than that of their SNEDDS formulation (SNEDDS-GLM-SIM). As an outcome of study, it was concluded that the dissolution rate, oral bioavailability and pharmacodynamics effect of GLM and SIM was significantly enhanced by both, SP-NS and SNEDDS, however, SP-NS has provided better results as that of SNEDDS.

**Keywords:** Glimepiride; Simvastatin; SP-NS; SNEDDS; Dissolution; Bioavailability; Stability

## **LIST OF ANNEXURES**

1. Publications
2. CPCSEA Approval Certificate
3. Letter of Candidacy
4. Poster presentation Certificates

## ACKNOWLEDGEMENT

First of all, I thank ‘Almighty’, the greatest teacher of all; God for blessing me with the strength to accomplish this work successfully. I feel myself incapable of expressing my innermost into words.

Letters of my vocabulary always find themselves too less and too inadequate when I think of penning them down to express my gratitude for towards my guide **Dr. Sachin Kumar Singh**, Associate professor, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara. I could never have imagined coming of this moment in my life without his unwavering guidance and painstaking efforts. It would not be an exaggeration of words if I render him as a holy spirit who encouraged me always.

My research can't be completed without the support and guidance of **Dr. Monica Gulati**, Professor and Sr. Dean, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara. I wish to thank her for making it all happen for me, by providing excellent facilities and environment to accomplish this endeavour successfully.

I wish to express my heartiest thanks to **Dr. Bimlesh Kumar**, for his continuous support. I wish to express my courteous gratitude and heartiest thanks to all the esteemed faculty members Dr. Deepak N Kapoor, Dr. Saurabh Singh, Dr. Sheetu, Mr. Sudhakar C.K., Dr. Vijay Mishra, Dr. Amit Mittal, Mr. Ankit Kumar Yadav, Dr. Varun Garg, Mr Dileep Singh, Mr. Bhupinder Kapoor and Dr. Navneet Khurana, Mr. Rajesh Kumar, Dr. Pardeep Kumar, Dr. Sheelendra Pratap Singh and Mr. Vijay Ahuja for their unwavering guidance and support.

A very special thanks to Science and Engineering Research Board (SB/YS/LS-102/2013), Department of Science and Technology, New Delhi, India for funding instruments like spray dryer and other facilities for this project. I also take this opportunity to express my thanks to Gattefosse, India, Germany and Grace Materials Technologies, Discovery Sciences, India.

I would also like to thank **Dr. K. Gowthamarajan**, Professor and Head, Department of Pharmaceutics and **Dr. Ashish Wadhvani**, Assistant Professor and head, Department of Biotechnology, JSS College of Pharmacy, Ooty, Tamilnadu, India, for providing facilities like Differential Scanning Calorimetry and Cell line studies.

I wish to express my gratitude to **Mr. Ashok Mittal**, Hon'ble Chancellor, worthy **Mrs. Rashmi Mittal**, Pro Chancellor and **Dr. Rameshwar S Kanwar**, Vice Chancellor Lovely Professional University, Punjab who gave me such kind of research friendly environment in University.

I would like to extend my thanks to my dear students Mr. Parth Sharma, Md. Adil Hussain Malik, Miss Yadav Sarvi Rajesh, Miss Palak Bawa, Mr. Rajan Kumar, Miss Rubiya Khursheed, Mr. Rakesh Kumar and Mr. Dipanjoy Ghosh for their active co-operation during my lab work.

I also take this opportunity to express my heartily thanks to my parents, **Mr. Ram Kundal Pandey** and **Mrs. Ram Kishori Devi** for their blessings, and encouragement all the time. A special thanks to my wife **Mrs. Savita Pandey** for her continuous motivation and support. I am deeply grateful to my son, **Naman Pandey**, for the love. Finally, I would like to express my gratitude to all those who gave me the possibility to complete this thesis. Needless to say, errors and omissions are all mine.

**Narendra Kumar Pandey**



## LIST OF ABBREVIATIONS

Symbol/ Abbreviations	Full form
SNEDDS	Self-nanoemulsifying drug delivery system
GIT	Gastrointestinal tract
L-SNEDDS	Liquid self-nanoemulsifying drug delivery system
S-SNEDDS	Soild self-nanoemulsifying drug delivery system
HLB	Hydrophilic lipophilic balance
PXRD	Powder X-ray diffraction
SEM	Scanning electron microscope
mL	Milliliter
mg	Milligram
%	Percentage
rpm	Rotations per minute
HCL	Hydrochloric acid
°C	Degree Centigrade
DSC	Differential scanning calorimetry
Eg	Example
h	Hour
NaoH	Sodium hydroxide
nm	Nanometer
S.D	Standard deviation
min	Minute
TEM	Transmission electron microscope
PEG	Polyethylene glycol
NIDDM	Non-Insulin Dependent Diabetes Mellitus
ATP	Adenosine triphosphate
AUC	Area under curve
ACN	Acetonitrile
cm	Centimeter
cm <sup>2</sup>	Centimeter square
cm <sup>-1</sup>	Centimeter inverse
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
Na-CMC	Sodium carboxy methyl cellulose
et al.	And co-workers
Fig.	Figure
SEDDS	Self-emulsified drug delivery system
M.R.T	Mean retention time
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl Methylcellulose
IAEC	Institutional Animal Ethics Committee
ICH	International Conference on Harmonization
RT	Retention time
LQC	Lower quantification concentration

---

MQC	Medium quantification concentration
HQC	Higher quantification concentration
NP	Nanoparticle
PDI	Polydispersity Index
RH	Relative Humidity
RP- HPLC	Reserved Phase High Performance Liquid
μL	Microlitre
% RSD	Percent Relative Standard Deviation
SIM	Simvastatin
GLM	Glimepiride
μg	Microgram
LOD	Limits of detection
LQC	Limit of quantification
WS	Working standard
ATV	Atorvastatin
SLS	Sodium lauryl sulphate
PVP	Polyvinylpyrrolidone
BBD	Box–Behnken Design
DoE	Design of experiment
SP-NS	Spray dried nanosuspension
O <sub>mix</sub>	Oil mixture
S <sub>mix</sub>	Surfactant mixture
SMEDDS	Self-microemulsifying drug delivery system
CMCM	Capmul MCM
L MCS	Labrafil M 1944CS
TP	Transcutol P
T80	Tween 80
A-200	Aerosil 200
OAC	Oil adsorption capacity
SXDP	Syloid XDP3150
MS	Magnesium stearate
SFP	Syloid 244 FP
MCC	Micro crystalline cellulose
PVA	Poly vinyl alcohol
NA-CMC	Sodium carboxy methyl cellulose
HPBCD	Hydroxy propyl beta cyclodextrin
ng	Nanogram
Conc.	Concentration
Temp.	Temperature

---

## TABLE OF CONTENTS

1. INTRODUCTION .....	1
2. LITERATURE REVIEW .....	4
2.1. Drug used to treat T2DM .....	4
2.2. Drug used to treat hyperlipidemia .....	4
2.3. Combination therapy to treat T2DM.....	7
2.4. Combination therapy to treat hyperlipidaemia.....	8
2.5. Nanosuspension.....	9
2.5.1. <i>Bottom up technology</i> .....	10
2.5.2. <i>Emulsions as templates</i> .....	10
2.5.3. <i>Microemulsions as templates</i> .....	11
2.5.4. <i>Top down technology</i> .....	12
2.5.5. <i>Media milling</i> .....	14
2.6. Advantages of nanosuspensions.....	15
2.7. Self-Emulsifying Drug Delivery System (SEDDS) .....	16
2.7.1. Composition of SEDDS.....	16
2.7.2. Mechanism of Formation of SEDDS.....	18
2.7.3. Categorization of SEDDS.....	18
2.7.4. Drug Transport Mechanism of SEDDS .....	23
2.7.5. Excipients .....	32
2.8. Characterization of nanosuspensions and SNEDDS.....	39
2.9. Drug Profile.....	40
2.9.1. Simvastatin .....	40
2.9.2. Glimpiride.....	41
3. HYPOTHESIS OF RESEARCH .....	42

4. AIM.....	43
4.1. Objectives.....	43
5. MATERIALS & METHOD .....	44
5.1. Materials.....	44
5.2. Equipment .....	45
6. EXPERIMENTAL WORK.....	46
6.1. Analytical method development .....	46
6.2. Method validation .....	46
6.2.1. Preparation of quality control standards.....	46
6.2.2. Estimation of LOD and LOQ .....	47
6.3. Bioanalytical method development and validation using HPLC .....	48
6.4. Solubility studies of unprocessed simvastatin and glimepiride in water .....	48
6.4.1. Solubility studies of unprocessed simvastatin and glimepiride in various oils, and stabilizers (surfactants and co-surfactants) .....	48
6.5. Preparation of nanosuspensions .....	49
6.5.1. Design of experiments:.....	50
6.6. Solidification of liquid nanosuspensions using spray dryer.....	50
6.7. Preparation and optimization of L-SNEDDS using ternary phase diagram.....	50
6.8. Stability evaluation of optimized L-SNEDDS formulation .....	51
6.9. Oil adsorption capacity (OAC) .....	52
6.10. Preparation of solid SNEDDS (S-SNEDDS) using spray drying .....	52
6.11. Micromeritic evaluation of developed SP-NS and S-SNEDDS formulation..	54
6.12. Calculation of drug loading.....	54
6.13. Droplet size and polydispersity index (PDI).....	55
6.14. Transmission electron microscopy (TEM).....	55
6.15. Powder X-ray Diffraction (PXRD) studies: .....	55

6.16. Differential scanning calorimetry (DSC) analysis .....	56
6.17. Scanning electron microscopy (SEM).....	56
6.18. In vitro dissolution studies .....	56
6.19. Cellular permeability and post-cytotoxicity tests in Caco-2 cell monolayer ..	57
6.20. Pharmacokinetic Study.....	57
6.21. Stability studies .....	58
6.22. Pharmacodynamics study .....	59
6.22.1 Induction of diabetes and obesity .....	59
6.22.2 Treatment design and pharmacological evaluation .....	59
6.22.3 Biochemical studies .....	60
6.22.4 Determination of in vivo antioxidants .....	60
6.22.5 Histopathological studies.....	60
6.23. Statistical analysis .....	60
6.22.5 Histopathological studies.....	61
6.23. Statistical analysis .....	61
7. RESULTS & DISCUSSION.....	62
7.1. Analytical method development and validation.....	62
7.2. Solubility studies .....	68
7.3. Screening of nanosuspension using DoE .....	69
7.4. Optimization of nanosuspension .....	70
7.5. Preparation and optimization of L-SNEDDS using ternary phase diagram.....	82
7.5.1 Construction of ternary phase diagram.....	82
7.5.2. Thermodynamic stability and cloud point determination of selected L-SNEDDS.....	85
7.7. OAC .....	85
7.8. Droplet size and PDI analysis of solid-SNEDDS (S-SNEDDS).....	85

7.9. Micromeritics characteristics of S-SNEDDS and SP-NS .....	87
7.10. In- vitro dissolution studies .....	87
7.11. Cellular permeability and post-cytotoxicity tests in Caco-2 cell monolayer ..	91
7.12. TEM analysis.....	93
7.13. Powder X-ray Diffraction (PXRD) studies .....	94
7.14. Differential Scanning Calorimetry (DSC) studies .....	98
7.15. Scanning Electron Microscopy (SEM) studies .....	102
7.16. Stability study.....	103
7.17. Bioanalytical method development and validation using HPLC .....	107
7.18. Pharmacokinetic Study.....	111
7.19. Pharmacodynamics studies .....	112
7.19.1. Effects on body weight .....	112
7.19.2. Effect on blood glucose level .....	112
7.19.3. Effect on biochemical parametrs .....	113
7.19.4. Effect on GSH level.....	113
7.19.5. Effect on SGOT & SGPT level .....	113
7.19.6. Effect on TBARS, protein and CAT level.....	114
7.19.7. Histopathological studies.....	128
8. CONCLUSION.....	135
9. REFERENCES .....	136

## LIST OF FIGURES

Fig. 1. Various methods used for preparation of nanosuspension. ....	10
Fig. 2. Study procedure used for animal studies.....	59
Fig. 3. Chromatogram of mixture of SIM-GLM in ACN-ortho phosphoric acid.....	62
Fig. 4. Chromatogram of mixture of SIM-GLM in methanol- ortho phosphoric acid	62
Fig. 5. Optimized chromatogram of SIM-GLM in ACN: KH <sub>2</sub> PO <sub>4</sub> (75:25) .....	63
Fig. 6. Calibration curve of (A) GLM and (B) SIM .....	64
Fig. 7. Solubility (mean $\pm$ s.d.) of unprocessed GLM & SIM mixture in different (A) oils and (B) surfactants. ....	69
Fig. 8. Perturbation plot for (A- B) and 2D & 3D-counter plot (C-K) .....	81
Fig. 9. Ternary phase diagram (A) Batch1; (B) Batch 2; (C) Batch 3. ....	84
Fig. 10: Dissolution profiles (mean $\pm$ s.d.) of L-SNEDDS, S-SNEDDS, SP-NS, physical mixture of GLM-SIM and unprocessed GLM and unprocessed SIM (n = 6). ....	89
Fig. 11: Cell permeability (mean $\pm$ s.d.) of A. GLM and B. SIM from nanoformulations and their unprocessed forms (n = 3). ....	92
Fig. 12: Cell permeability (mean $\pm$ s.d.) of A. GLM and B. SIM from nanoformulations and their unprocessed forms (n = 3). ....	93
Fig. 13: TEM image of S-SNEDDS of SIM-GLM mixture. ....	93
Fig. 14: X-ray diffraction patterns of unprocessed drugs, excipients and formulations. ....	98
Fig. 15: DSC patterns of unprocessed drugs, excipients and formulations. ....	102
Fig. 16: SEM images of A. GLM; B. SIM; C. S-SNEDDS containing GLM-SIM; D. SP-NS containing GLM-SIM. ....	103
<b>Fig. 17:</b> Chromatogram of ATV, GLM & SIM.....	107
Fig. 18: Calibration plot of GLM.....	107
Fig. 19: Calibration plot of GLM.....	108

Fig. 20: Mean ( $\pm$ s.d.) Plasma concentration versus time plot of drugs (n = 6).....	112
Fig. 21: Change in total body weight of each group.....	115
Fig. 22: Effect of treatment on Blood glucose level of each group.....	116
Fig. 23: Effect of treatment on GSH level of each group.....	118
Fig. 24: Effect of treatment on SGOT level of each group.....	119
Fig. 25: Effect of treatment on SGPT level of each group.....	120
Fig. 26: Effect of treatment on TBARS level of each group.....	121
Fig. 27: Effect of treatment on total protein level of each group.....	122
Fig. 28: Effect of treatment on CAT level of each group.....	123
<b>Fig. 29:</b> Effect of treatment on TG level of each group.....	124
Fig. 30: Effect of treatment on HDL level of each group.....	125
Fig. 32: Effect of treatment on total cholesterol level of each group.....	127
Fig. 33: Histological sections of kidney.....	131
Fig. 34: Histological sections of liver.....	134



## LIST OF TABLES

Table 1: Drug used to treat T2DM.....	5
Table 2: List of lipid lowering .....	6
Table 3: List of marketed products containing combinations of oral anti-diabetic synthetic drugs .....	7
Table 4: Combination of synthetic anti diabetic drugs with phytochemicals .....	8
Table 5: List of marketed products containing combinations of oral anti-hyperlipidemic synthetic drugs .....	9
Table 6: List of nanosuspension based marketed formulations prepared by top down technique .....	17
Table 7: Patents on nanosuspension formulation.....	17
Table 8: Composition of various S-SEDDS .....	24
Table 9: SNEDDS prepared using Spray drying .....	31
Table 10: Oils/lipids used for SEDDS .....	33
Table 11: Surfactants used for SEDDS (Kang et al., 2012).....	34
Table 12: Co-solvents used for SEDDS.....	34
Table 13: Patents of SEDDS/SMEDDS/SNEDDS (Singh et al., 2009) .....	36
Table 14: Various SNEDDS prepared till date .....	37
Table 15: Techniques to characterize nanoparticles with their advantages and limitations .....	39
Table 16: List of materials used in study .....	44
Table 17: List of equipment used in the study .....	45
Table 18: Solubility of GLM & SIM using different surfactants .....	49
Table 19: Composition of L-SNEDDS .....	53
Table 20: Pharmacodynamic study design (n=6 in each groups) .....	61
Table 21: Results of accuracy studies .....	64

Table 22: Results of precision studies for GLM.....	65
Table 23: Results of precision studies for SIM.....	66
Table 24: Robustness results of various parameters tested for GLM .....	67
Table 25: Robustness results of various parameters tested for SIM.....	67
Table 26: Solubility (Mean $\pm$ s.d.) of GLM and SIM using different surfactants. ....	68
Table 27: DOE of nanosuspension. ....	71
Table 28: Summary of ANOVA of Box–Behnken screening design batches using liquid antisolvent precipitation technique.....	71
Table 29: Composition of selected batches of SIM and GLM loaded L-SNEDDS (% w/w) and evaluation parameters. ....	86
Table 30: Droplet size and PDI of various carriers.....	87
Table 31: Micromeritic characteristics of S-SNEDDS and SP-NS. ....	87
Table 32: In-vitro drug release kinetic models. ....	90
Table 33: Stability studies of nano- formulations.....	105
Table 34: Results of accuracy studies .....	108
Table 35: Results of precision studies for GLM.....	108
Table 36: Results of precision studies for SIM.....	109
Table 37: Robustness results of various parameters tested for GLM .....	110
Table 38: Robustness results of various parameters tested for SIM.....	110
Table 39: Pharmacokinetic parameters of nanoformulations and unprocessed form. ....	111
Table 40: Biochemical Parameters of each group. ....	117



## 1. INTRODUCTION

Diabetes mellitus (DM) is one of the oldest diseases that is recognised by higher blood glucose level either due to insulin resistance (Type 2 diabetes mellitus; T2DM) or the body does not produce insulin (Type 1 diabetes mellitus; T1DM). About 3000 years ago it was first reported in manuscripts of Egyptians (Ahmed, 2002). Treatment of diabetes is known since middle ages and explanation of its pathogenesis was found in 20th century (Piero et al., 2015). It is one of the principle causes of persistent ill health and mortality, moreover, it takes more lives per year as compared to HIV-AIDS with almost 1 death in every 10 sec (Kaul et al., 2013). Globally, due to rise in obesity, diabetes became a global epidemic and continued to increase every year (King et al., 1998). Recent survey from the fact sheet of WHO predicted that about 3.91 folds rise in the incident rate of people suffering from DM between 1980 (108 million) to 2014 (422 million). The number of deaths reported due to DM was 1.5 million in 2012 that raised to 2.2 million by 2017 (WHO, 2016).

First line defence against type 2 diabetes mellitus (T2DM) is to control blood glucose levels and blood pressure as well as changes in living standard. In addition to that diet and weight control should also be followed. If a patient does not respond to the above practices and still there is increased blood glucose then oral antihyperglycemics is prescribed (Khursheed et al., 2019).

Patients having long term history of DM may suffer from complications of vital organs such as heart, eye, kidney, blood vessels and nervous system. DM also causes rise in lipid level of body that is well known as dyslipidemia. Due to this fact there are many cases where people having long history of DM also suffer from hyperlipidemia and take antihyperlipidemic drugs along with drugs used to treat DM. Further, increase in body weight and lipid level is one of the side effects of oral hypoglycemics such as sulphonyl urea (Vyas and Galani, 2010).

Since, DM and hyperlipidemia are closely associated co-morbidities and lives threatening, it becomes important to find novel strategies to treat them simultaneously. In view of this co-administration of drugs could offer a better treatment strategy. Advantage associated with co-administration is ease of

administration in terms of single dosage administration, less amount of excipient to be used per unit of dosage and simultaneous management of DM and hyperlipidemia associated with it.

It is well known fact that the commonly available antihyperlipidemic and antihyperglycemic drugs are associated with poor aqueous solubility. Moreover, statins, that are extensively used antihyperlipidemic, are associated with hepatic first pass metabolism. The poor aqueous solubility and first pass metabolism causes poor oral bioavailability of these drugs. Hence, it is cumbersome to get therapeutic concentration at the required dose itself. Due to this, a known excess dose of drugs is required to be administered to achieve the therapeutic concentration. This strategy is currently being practiced by physicians. In order to reduce their prescribed dose and provide the actual dose that is required, it becomes important to think towards novel strategies to overcome their solubility and bioavailability. This task becomes more challenging, when two lipophilic and gastrointestinal labile drugs are required to be co-administered because every drug has different physicochemical properties such as solubility, partition coefficient, dissociation constant, permeability and oral bioavailability. Hence, single strategy may not work to overcome the challenges of both the drugs.

There are many modifications that are usually being practiced to overcome the dissolution rate limited bioavailability of drugs. This could be done by both physical as well as chemical modifications. Some physical modifications techniques include formation of nanoparticles, complexes, solid dispersions, solid state transformation, co-crystals and vesicular delivery etc. Whereas chemical modifications include supercritical fluid technique, surfactant based systems, hydrotrophy etc. (Sharma et al., 2018). However, they have gained limited success and failed to become universal. This non-universality is related to difference in physicochemical properties of the drugs. Additionally, most of them lack commercialization due to failure during scale-up, instability or toxicity. Hence, there is a need to understand all the aforementioned factors that could significantly affect the formulation so that the product can be commercialized. Although, nanotechnology has also some of its inherent limitations of stability and toxicity, these have been extensively explored by scientist in

developing novel formulations. Hence, better understanding of formulation and process variables through judicious selection of process and excipients could make the process simple, scalable and reproducible (Yadav et al., 2012). In past it has been observed that among all the nanotechnology approaches such as nanosuspensions, self-nanoemulsifying drug delivery systems (SNEDDS), solid lipid nanoparticles, polymeric micelles, nanovesicles, nanostructured lipid carriers and metallic nanoparticles, nanosuspensions and SNEDDS have been reported to be most successful due to their ease of preparation, possibility of scale-up, better drug loading, lesser toxicity and better storage stability.

Among the oral antihyperglycemics, the use of sulfonylureas is being practiced more by physicians and among hyperlipidemics; atorvastatin and simvastatin are being recommended due to their better safety and efficacy profiles. Among sulfonyl ureas, glimepiride is most preferred due to better stimulation of insulin secretion and beneficial extra pancreatic effect (Geinsen, 1988; Kouichi et al., 2005).

Hence, in the present study an attempt has been made to co-administer glimepiride (sulfonylurea) and simvastatin to treat T2DM and hyperlipidaemia associated with it. The administration of simvastatin will reduce the side effects associated with glimepiride. Since both the drugs are poorly soluble, two nanotechnology approaches viz. nanosuspensions and SNEDDS will be attempted to improve their aqueous solubility and reduction in their dose. The efficacy of developed formulations will be evaluated through pre-clinical studies on high fat diet and streptozotocin induced rat model.

## **2. LITERATURE REVIEW**

### ***2.1. Drug used to treat T2DM***

As diabetes is a complex metabolic disorder, possessing a multi-dimensional pathogenesis. Over the past few decades the number of available medications to treat T2DM have increased manifold (Inzucchi, 2018). As a result, management of DM has become significantly complex. In order to control hyperglycemia a large number of choices are available in the market which works either as monotherapy or in combinations. The various classes of anti-diabetic synthetic drugs along with their side effects are listed in Table 1.

### ***2.2. Drug used to treat hyperlipidemia***

Hyperlipidemia is one of the major causes of cardiovascular disease. However, one can reduce the higher lipid levels through proper medication, diet control and exercise. In order to effective management of hyperlipidemia a large number of choices are available in the market which works either as monotherapy or in combinations. The various lipid lowering medication with their dose and side effects are summarized in Table 2 (<https://www.aafp.org/afp/2000/0601/p3371.html>).

**Table 1:** Drug used to treat T2DM

Class	Examples	Marketed Strength	Side Effects	Reference
Thiazolidinedione	Rosiglitazone Pioglitazone	Oral 2 mg; 4 mg; 8 mg Oral 15 mg; 30 mg; 45 mg	↑ chances of heart failure, weight gain, oedema, hepatotoxicity, water retention	(Jerry and Chisholm, 2004)
Lyn Kinase activator	Tolimidone	Phase II Clinical trials	Under investigation	(Ochman et al., 2012)
Sulfonylureas	<i>First generation:</i> Acetohexamide Chlorpropamide Tolazamide Tolbutamide <i>Second generation:</i> Gliclazide Glimepiride Glipizide Glyburide	Discontinued Oral 100 mg; 250 mg Oral 100 mg; 250 mg; 500 mg Oral 500 mg; 1 g Oral 80 mg Oral 1 mg; 2 mg; 4 mg Oral 5 mg; 10 mg Oral 2.5 mg; 5 mg	Hypoglycemia, weight gain, blunt ischaemic preconditioning, hypersensitivity reactions, teratogenic, myocardial infarction	(Sola et al., 2015)
Meglitinide	Repaglinide Nateglinide	Oral 0.5 mg; 1 mg; 2 mg Oral 60 mg; 120 mg	Hypoglycemia	(Guardado-Mendoza et al., 2013)
α-glucosidase inhibitor	Miglitol Acarbose Voglibose	Oral 25 mg; 50 mg; 100 mg Oral 25 mg; 50 mg; 100 mg Oral 0.2 mg; 0.3 mg	Flatulence and diarrhea	(Joshi et al., 2015)
Peptide analogue	Exenatide  Liraglutide  Taspoglutide	Injection 2 mg (Subcutaneous) Injection 1.2 mg; 1.8 mg (Subcutaneous) Phase III Clinical trials halted due to severe hypersensitivity and GIT complications	↑ pulse rate	(Cervera et al., 2008)
Biguanides	Phenformin  Buformin Metformin	Marketed only in combination form with other oral antidiabetics Upto 300 mg daily dose Oral 500 mg; 850 mg; 1000 mg	↑ risk of lactic acidosis ↑ chances of chronic kidney failure ↑ rate of heart failure, Gastrointestinal upset, kidney disorders, ↓ thyroid-stimulating hormone, ↓ luteinizing hormone, and ↓ testosterone	(Rena et al., 2013)
Amylin analogue	Pramlintide	Injection 60 mg (Subcutaneous)	Hypoglycemic conditions	(Femminella et al., 2017; Schmitz et al., 2004)



Sodium-glucose co-transporter-2 inhibitor	Dapagliflozin Canagliflozin Empagliflozin	Oral 5 mg;10 mg Oral 100 mg; 300 mg Oral 10 mg; 25 mg	Risk of dehydration Diabetic ketoacidosis Hypovolemia ↑ LDL-C ↑ rate of heart failure for saxagliptin	(Pecoits-Filho and Perkovic, 2018)
Dipeptidyl Peptidase-4 Inhibitor	Vildagliptin Sitagliptin Saxagliptin Linagliptin Alogliptin Gemigliptin	Oral 50 mg Oral 25 mg; 50 mg; 100 mg Oral 2.5 mg; 5mg Oral 5 mg Oral 25 mg Oral 50 mg		(Barnett, 2006)

**Table 2:** List of lipid lowering drugs

Class	Examples	Dose	Side Effects
HMG-CoA Reductase Inhibitors (Statins)	Atorvastatin	10-80 mg	Headaches, peripheral edema, back pain, dizziness, abdominal pain, insomnia, arthralgias, generalized pain, GI, elevated liver enzyme
	Cerivastatin	0.2-04 mg	
	Fluvastatin	20-80 mg	
	Lovastatin	20-80 mg	
	Pravastatin	10-40 mg	
	Simvastatin	5-40 mg	
Niacin (Nicotinic acid)		50-3000 mg	Flushing, pruritus, nausea, vomiting, glucose intolerance, rare reversible acanthosis nigricans
Fibric Acid Derivatives (Fibrates)	Gemfibrozil	600 mg	Abdominal pain, eczema, headache, muscle or joint pain
	Micronized fenofibrate	67-201 mg	
Bile Acid Sequestrants	Cholestyramine powder	4-20 g 5-30 g	Constipation
	Colestipol granules		

### 2.3. Combination therapy to treat T2DM

The basic concept of combination therapy originated during ancient times and it has seen avid use over the years. In a few decades or so, its importance and necessity came under the limelight. Combination therapies have been treating a plethora of diseases and in most cases seen to alleviate patients suffering from disease quite successfully (Fong et al., 2004).

Hence, in order to effectively counter the ever-increasing threat of DM and its multiple accompanying symptoms, focus and priority must be given to all the possible combination therapies between synthetic drugs and phytochemicals, which are being observed as a beacon of hope during these turbulent times of diabetes becoming a worldwide epidemic. Combination of synthetic anti-diabetic drugs available in the market is mentioned in Table 3.

**Table 3:** List of marketed products containing combinations of oral anti-diabetic synthetic drugs

Name	Drug Combinations	Manufacturers
ActoPlus MET	Pioglitazone + Metformin	Takeda Pharmaceutical Company
Avandaryl	Rosiglitazone + Glimepiride	GlaxoSmithKline plc
Benformin	Glibenclamide + Metformin	Orchid Chemicals & Pharmaceuticals Ltd.
Claformin	Gliclazide + Metformin	Orchid Chemicals & Pharmaceuticals Ltd.
Glimitide Forte	Glimepiride + Metformin	Orchid Chemicals & Pharmaceuticals Ltd.
Glimitide Plus	Glimepiride + Metformin	Mano Pharmaceuticals Pvt. Ltd.
Glista M1	Glimepiride + Metformin	Cadila Pharmaceuticals Ltd.
Glista PM2	Glimepiride + Pioglitazone + Metformin	Cadila Pharmaceuticals Ltd.
Glucoavance	Glyburide + Metformin	Merck Serono
Glyloc M	Gliclazide + Metformin	Cadila Pharmaceuticals Ltd.
Metaglip	Glipizide + Metformin	Bristol-Myers Squibb
Metbetic G	Gliclazide + Metformin	Cadila Pharmaceuticals Ltd.
Piocon Forte	Pioglitazone + Metformin	Orchid Chemicals & Pharmaceuticals Ltd.
PioconGM 1	Glimepiride + Pioglitazone + Metformin	Orchid Chemicals & Pharmaceuticals Ltd.
PioconGM 2	Glimepiride + Pioglitazone + Metformin	Orchid Chemicals & Pharmaceuticals Ltd.
Piozulin	Pioglitazone + Metformin	Cadila Pharmaceuticals Ltd.

It has been reported that about 800 different plant species can be utilized as a potent anti-diabetic agent according to reports of the world ethnobotanical information. Among them 450 has been experimentally proven to be anti-diabetic but out of all of them till date. The anti-diabetic mechanism has been clearly understood only for 109

patients (Prabhakar and Doble, 2008). Hence, a synergistic combination therapy of synthetic drugs with phytochemicals should be the ideal target in order to control the blood glucose level as well as to reduce the various additional complications adjoining T2DM. The therapy would be able to resolve various issues (Prabhakar et al., 2014) like:

- a) Quick and prolonged blood glucose level control
- b) Control of other accompanying factors of diabetes mellitus
- c) Treatment of specific sectors which lead to diabetes mellitus

Combination of synthetic drug and phytochemicals with their synergistic effects are highlighted in Table 4.

**Table 4:** Combination of synthetic anti diabetic drugs with phytochemicals

Drug	Phytochemical	Effects
Rosiglitazone	<i>Momordicha charantia</i>	↓BGL, ↑β-cells, ↓ hepatic damage
Metformin (MET)	Arecoline	↑ glucose uptake by cells (IGUC)
MET	Ascorbic acid	↑ IGUC
MET	Caffeic acid	↑ IGUC
MET	Chlorogenic acid	↑ IGUC
MET	Coumaric acid	↑ IGUC
MET	Eugenol	↑ IGUC
MET	Ferulic acid	↑ IGUC, ↓BGL, ↑β-cells
Pioglitazone	Ellagic acid	↑ IGUC
Thiazolidinedione	Arecoline	↑ IGUC
Thiazolidinedione	Caffeic acid	↑ IGUC
Thiazolidinedione	Chlorogenic acid	↑ IGUC
Thiazolidinedione	Coumaric acid	↑ IGUC
Thiazolidinedione	Eugenol	↑ IGUC
Thiazolidinedione	Ferulic acid	↑ IGUC, ↓BGL, ↑β-cells

#### **2.4. Combination therapy to treat hyperlipidaemia**

The management of dyslipidemia, and coronary heart disease is one of the challenging tasks. In that the primary focus is towards proper understanding and utilization of combination therapy to treat the disease rather than monotherapy (<https://www.medscape.com/viewarticle/480603>). Some of the combination therapies explored till date are listed in Table 5.

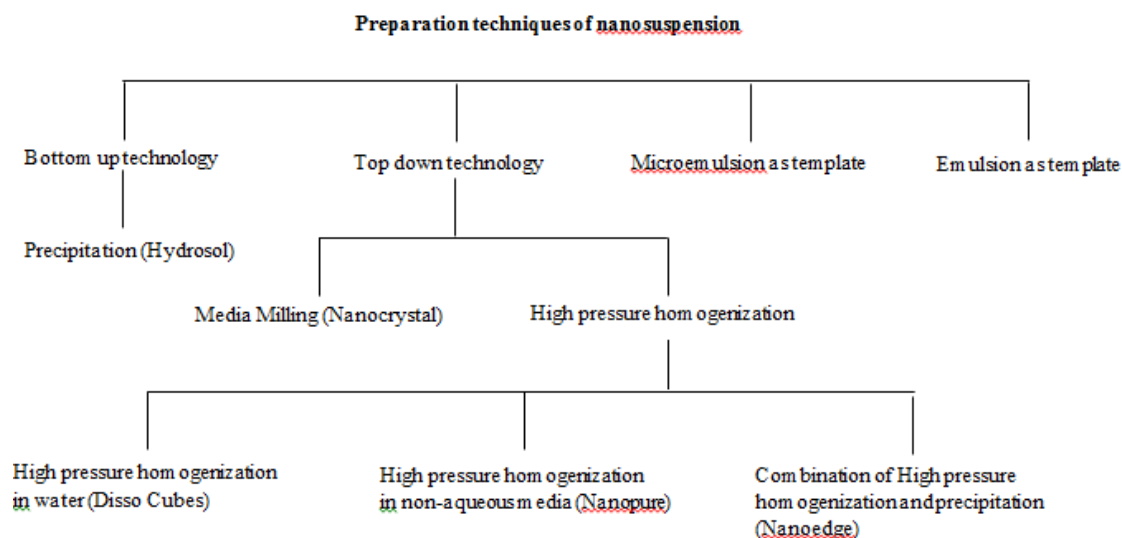
**Table 5:** List of marketed products containing combinations of oral anti-hyperlipidemic synthetic drugs

Name	Drug Combinations	Manufacturers
Vytorin	Simvastatin+ ezetimibe	Merck & Co., Inc
Simcor	Simvastatin+ niacin	AbbVie Inc.
Advicor	Lovastatin + niacin	Kos Pharmaceuticals
Juvisync	Simvastatin + sitagliptin	Merck & Co., Inc
Liptruzet	Atorvastatin + ezetimibe	Merck & Co., Inc.

### **2.5. Nanosuspension**

In nanosuspension, sub-micron drug particles are dispersed in liquid phase containing optimized concentration of stabilizers. These are preferred for drugs exhibiting poor aqueous as well as lipid solubility (Möschwitzer and Müller, 2006). Such compounds have high crystal energy and melting point which reduce their tendency to dissolve, irrespective of solvent used (Rabinow, 2004). Various processes used to prepare nanosuspensions are precipitation (Chen et al., 2015; Yadollahi et al., 2015), pearl milling and high pressure homogenization. The liquid phase can be water or a mixture of water and water miscible liquids or a non-aqueous phase (Debuigne et al., 2001; Liversidge and Conzentino, 1995; Möschwitzer et al., 2004; Trotta et al., 2001; Xia et al., 2014). Main advantage of nanosuspension is increase in saturation solubility, which is specific to the drug molecule as well as temperature dependent. This can be because of increase in dissolution rate of the compound due to increase in surface area. As the particle size reduces below 1  $\mu\text{m}$ , there is increase in saturation solubility (Jacobs et al., 2001). Another advantage of nanosuspension is that there is change in crystal structure. There may be an increase in amorphous fraction and at times, there may be complete conversion of drug particles into amorphous particles (Akkar and Müller, 2003). Based on these general advantages, several compounds have been formulated as nanosuspensions. These include naproxen (I Jethara et al., 2015), clofazamine, bupravaquone (Debuigne et al., 2001), nimesulide (Yadollahi et al., 2015), mitotane (Chen et al., 2015), amphotericin B (Xia et al., 2014), omeprazole (Möschwitzer and Müller, 2006), nifedipine (Möschwitzer et al., 2004) and spironolactone (Kayser et al., 2003). Nanosuspensions are widely prepared by either of the four production methods - (a) Bottom-up technology, (b) Top-down technology, (c) Emulsion as templates and (d) Microemulsion as templates. Fig. 1

shows the schematic representation of the techniques employed for the preparation of nanosuspensions.



**Fig. 1.** Various methods used for preparation of nanosuspension.

### **2.5.1. Bottom up technology**

In case of bottom up technologies, usually the first step is the preparation of drug solution in organic solvent. It is then mixed with an antisolvent, which is miscible with organic solvent. This is a traditional method of preparation of nanoparticles and popularly known as *Via Humid Paratum* (V.H.P). By this method, the nanoparticles are prepared by precipitation. Other approaches used to prepare nanoparticles by bottom up technology are; (i) solvent-anti-solvent method; (ii) supercritical fluid processes; (iii) high gravity controlled precipitation technology, (iv) confined liquid impinging jets, (v) multi-inlet vortex mixer, (vi) sonoprecipitation, (vii) spray drying and (viii) melt emulsification method (Chan and Kwok, 2011; Date and Patravale, 2004; Du et al., 2015; Rabinow, 2004; Sinha et al., 2013).

### **2.5.2. Emulsions as templates**

Emulsions are another form of popular delivery vehicles. They can be used to prepare nanosuspensions by being utilized as templates, especially in case of drugs which are either soluble in volatile solvents or, in solvents that are partially miscible with water. Nanosuspensions can be prepared by two different ways by using this method.

*Method I:* In the first method, the drug is added to an organic solvent or, a mixture of solvents while the aqueous phase is prepared by adding appropriate surfactants. The

organic phase is dispersed in aqueous phase to form an emulsion, which is then subjected to reduced pressure resulting in the evaporation of organic phase. This leads to the instantaneous precipitation of the drug particles, leading to formation of nanosuspensions, which is stabilized by the surfactants present in the formulation (Patravale et al., 2004). In this method, each emulsion droplet gives rise to a particle. Thus, the particle size of the nanosuspension can be controlled by controlling the size of emulsion droplet (Patravale et al., 2004).

Optimizing surfactant concentration could allow increased organic phase ratio and thus increased drug loading in the final emulsion (Patravale et al., 2004). The organic solvents like methylene chloride and chloroform are considered hazardous to environment and human health. Hence there are not used for routine manufacturing processes (Bodmeier and McGinity, 1988). Relatively safer solvents like ethyl acetate and ethyl format are commonly used (Sah, 1997, 2000).

*Method II:* In the second method, hazardous solvents in the dispersed phase are replaced by solvents that are partially miscible in water like butyl, lactate, benzyl alcohol and triacetin (Patravale et al., 2004; Trotta et al., 2001). In this process, first the emulsion is prepared by conventional method. Formation of nanosuspension takes place by subsequent dilution of the prepared emulsion with water. Dilution with aqueous phase results in the diffusion of internal phase into external phase and instant formation of nanosuspension (Patravale et al., 2004). The prepared formulation is subjected to techniques like ultracentrifugation or ultrafiltration to separate the nanosuspension particles from surfactants and internal phase.

A number of reports are there in which emulsions have been used as templates for the formulation of nanosuspensions of some anticancer drugs which have compromised aqueous solubility and bioavailability (Trotta et al., 2001). As an example, Mitotane nanosuspension prepared by this technique exhibited five- fold increase in dissolution rate as compared to its marketed formulations (Trotta et al., 2001).

### ***2.5.3. Microemulsions as templates***

Microemulsions comprise of two immiscible phases i.e. an aqueous and a non-aqueous phase. These are stabilized by surfactants and co-surfactants. The internal phase is a suitable organic solvent or, partially miscible liquid. Drug loading in the

microemulsions can be done by two ways. Either the drug loading is done in the internal phase and microemulsion prepared subsequently or the microemulsion is prepared and then mixed with drug for drug loading. Afterwards, it is processed in the same way as that in the procedure mentioned for preparation of “*emulsions as templates*”.

Griseofulvin nanosuspension has been successfully prepared by using microemulsions as templates (Trotta et al., 2003). About 3 folds increase in dissolution rate of griseofulvin nanosuspensions prepared by this technique has been reported as compared to its marketed formulations.

Two major problems associated with these methods include presence of residual solvents in final formulation and crystal growth after precipitation that may lead to conversion of nanoparticles to micro particles (Möschwitzer and Müller, 2006; Müller et al., 2001). Presence of residual organic solvents is not acceptable as per ICH Q3C (R3) (Guideline) guidelines of residual solvents.

#### ***2.5.4. Top down technology***

Top down process generally includes high pressure homogenization (HPH) and wet ball milling. In both of these technologies, micronized or non-micronized drug particles are suspended in an aqueous or non-aqueous dispersion medium which has surfactants or polymeric stabilizers added to it (Palla and Shah, 2002). In order to reduce the particle size, the prepared suspension is finally passed through ball mill, or subjected to high pressure homogenization (Kreuter, 2001). This results in breakdown of large drug particles into smaller drug nano crystals. The merit of this technique over bottom-up technologies lies in the fact that any drug with poor aqueous and non-aqueous solubility can be processed by its use (Palla and Shah, 2002). Because of its higher industrial relevance, top down process is more popular. The underlying mechanisms involved in the production of nanosuspension by high pressure homogenization and wet ball media milling are discussed below.

##### **2.5.4.1. High Pressure Homogenization (HPH)**

The principle behind HPH is the creation of cavitation forces on passing of drug suspension through high pressure homogenizers. The technique is further classified into two basic types based on the type of medium used for homogenization. If the

medium used is aqueous, the technology is known as “Dissocubes” and in case of non-aqueous medium, it is known as “Nanopure technology”.

#### *2.5.4.1a. High pressure homogenization in water (Disso Cubes)*

In this process the drug is first dispersed in aqueous medium containing surfactants; after which the particle size is reduced by repeatedly passing the suspension through a narrow gap of approximately 25 µm at high pressure i.e. about 1500 bar and a maximum of 20 homogenization cycles (Müller et al., 2001; Patravale et al., 2004). It increases the dynamic pressure and decreases the static pressure below the boiling point of water at room temperature. This results in boiling of water followed by formation of gas bubbles that gets imploded when a gap is left once normal pressure is reached. Due to this, the particle size reduction to nanometer range takes place. Nanosuspension of some drugs like Amphotericin B, Thiomersal, Oridinon, Fenofibrate, Melarsoprol, Buparvaquone, Prednisolone, Carbamazepine and Dexamethasone have been reported to be prepared using this technology (Nagaraju et al., 2010; Patel and Agrawal, 2011).

#### *2.5.4.1b. High pressure homogenization in non-aqueous media (Nanopure)*

In “Nanopure” technology, the particle size reduction of a poorly soluble drug takes place in non-aqueous medium. The drug suspension is homogenized at 0°C and sometimes below the freezing point. In case of high boiling point liquids like water, oils and fatty acids, the drop of static pressure is not enough to begin cavitation, hence, organic solvents are used. Some of the advantages and limitations of “Nanopure” technique are listed below (Paun and Tank, 2012).

#### *2.5.4.1c. Combined precipitation and homogenization (Nanoedge)*

Crystal growth is a common tendency of the precipitated nanocrystals. Hence, it is required to process them at very high pressure so that the size of crystals can be preserved. The factors that need to be controlled include temperature, number of homogenization cycles and pressure of homogenizer.

#### *2.5.4.2. High Pressure Homogenizer*

A high-pressure homogenizer comprises a high-pressure plunger pump and a homogenizing valve which is also called relief valve. Plunger pump provides the energy level required for the relief. The relief valve further comprises a valve seat,



which is fixed and a valve, which is adjustable. Together, these parts result in formation of an adjustable radial precision gap. Depending on the amount of force applied on the valve, the gap conditions and the resistance vary, which, in turn, affect the intensity of homogenizing pressure which is generated. There is an external ring that defines the dimensions of outlet cross-section and prevents the damage to valve casing from the flow of suspension (Müller et al., 1998; Patravale et al., 2004). The HPH is available in two versions: continuous and discontinuous. Continuous HPH is generally employed for the parameter optimization of a homogenization process, whereas discontinuous version is used when either the drug is expensive or is not readily available (Patravale et al., 2004).

#### ***2.5.5. Media milling***

This technology of media milling was first developed by Liversidge et al in 1992 (Liversidge et al., 1992). This technique makes use of milling media which can be yttrium stabilized glass, zirconium oxide or highly cross-linked polystyrene resins. The breakdown of suspended drug particles is caused by the use of this milling media (Juhnke et al., 2010; Liversidge et al., 1992; Patravale et al., 2004). This technique uses high shear media mills or, pearl mills to produce nanosuspensions. A media mill has three main parts: a milling chamber, a milling shaft and a recirculation chamber. Drug along with stabilizers, water and milling media is added to the milling chamber. Milling media or pearls are rotated at a very high shear rate in the chamber. The process of milling is performed under controlled temperatures (Date and Patravale, 2004; Liversidge et al., 1992; Patravale et al., 2004).

The type and quantity of the stabilizers used determines the rate of success in formation of nanosuspensions. Surfactant stabilizers as well as polymeric stabilizers are commonly used (Rabinow, 2004).

A number of formulations produced by wet milling method have reached the market. These include Rapamune<sup>®</sup>, Emend<sup>®</sup>, Tricor<sup>®</sup>, Triglide<sup>®</sup>, Megace<sup>®</sup>, Invega<sup>®</sup> Sustennatm<sup>®</sup> (Liu et al., 2011; Van Eerdenbrugh et al., 2009). Depending upon the product profile, the type of media used may vary e.g. for the media milling technology a highly crossed linked polystyrene (PollyMill<sup>®</sup> milling media) is generally used. This selected media should be able to withstand harsh process

parameters and at the same time should not contribute to the impurities in final product (Merisko-Liversidge and Liversidge, 2011).

The list of marketed nanosuspensions prepared by using top down technologies is given in Table 6 and various patents on nanoparticles are listed in Table 7.

## ***2.6. Advantages of nanosuspensions***

Nanosuspensions of drugs with poor aqueous solubility provide several advantages like:

a) Increase in the dissolution velocity and saturation solubility of the drug

Decrease in the particle size of the drugs causes a significant increase in the surface area which results in enhanced saturation solubility as well as dissolution velocity of the drug (Dressman et al., 1998; Hörter and Dressman, 2001; Patravale et al., 2004). Moreover, as the diffusional distance is reduced, it increases the wettability of the drugs (Mosharraf and Nyström, 1995; Patravale et al., 2004).

b) Improved biological performance

Saturation solubility and dissolution velocity of a drug directly affect its bio-availability (BA). The increase in BA, in turn, increases the therapeutic efficacy of the drug. Thus nanosuspensions of the drugs generally perform better in the in-vivo systems (Patravale et al., 2004).

c) Ease of manufacture and scale-up

Another significant advantage of this technology is that the technique involved can be easily scaled up for commercial application. This fact is reflected in the number of commercially available nanosuspensions.

d) Long-term physical stability

The phenomenon of Ostwald ripening is less prevalent in nanosuspensions as compared to the other nano-delivery systems. These are therefore, physically stable for longer durations (Peters and Muller, 1996). In case of ultrafine dispersed systems, Ostwald ripening results in crystal growth leading to the formation of microparticles. It occurs due to the differences in dissolution pressure or, saturation solubility between small and large particles. As the nanosuspensions have uniform particle size, the differences in the saturation solubilities and concentration gradients (observed in case of particle size variation) are less and Ostwald ripening generally does not occur.

#### e) Versatility

Versatility of application of the nanosuspension is reflected in the fact that they can be incorporated in a variety of dosage forms. These include tablets, pellets, suppositories, hydrogels etc., which can be administered by different routes (Patravale et al., 2004).

### **2.7. Self-Emulsifying Drug Delivery System (SEDDS)**

SEDDS are the isotropic mixtures of oils, surfactants and co-surfactants which upon dilution in a constant volume of water yield transparent emulsion having droplets in the range of nanometer. If the droplet size is less than 100 nm then the obtained emulsion is generally called SNEDDS and if it is above 100 nm but below 250 nm then it is termed as SMEDDS. However, these terms for SEDDS have been found to be varied in different literature.

#### **2.7.1. Composition of SEDDS**

SEDDS are prepared by using three basic formulation components, oil/lipid, surfactant and co-surfactant. These components are screened on the basis of solubility of drugs on them and suitable formulation composition is finalized with the help of ternary phase diagram. Ternary phase diagram is classified into three different zones, SEDDS, SMEDDS and SNEDDS. These zones are assigned on the basis of clarity (size of the globules) and emulsification time after dilution with distilled water (500 mL) (Garg et al., 2016; Rahman et al., 2013).

**Table 6:** List of nanosuspension based marketed formulations prepared by top down technique

S.No.	Product	Active drug	Top down Technique	Dosage form	Therapeutic use	Company	Route of administration
1	Rapamune	Sirolimus	*MM	Tablet	Immunosuppressant	Wyeth Pharmaceuticals – Elan Drug Delivery	Oral
2	Emend	Aprepitant	MM	Capsule	Antiemetic	Merck - Elan Drug Delivery	Oral
3	Megace ES	Megestrol acetate	MM	Nanosuspension	Anorexia, weight loss in AIDS patients	Par Pharmaceuticals - Elan Drug Delivery	Oral
4	Ticor	Fenofibrate	MM	Tablet	Antihyperlipidemic agent	Abbott Laboratories	Oral
5	Triglide	Fenofibrate	**HPH	Tablet	Antihyperlipidemic agent	Skye Pharma - First Horizon	Oral
6	Panzem NCD	2-Methoxyestradiol	-	Nanosuspension	Anti-proliferative and anti-angiogenic effect	Entre Med Inc.	Oral

\* Media milling; \*\*High pressure homogenization

**Table 7:** Patents on nanosuspension formulation

S.No.	Types of nanosuspension	Patent number	Reference
1	Microfluidized nanosuspensions of lipophilic drug	US20110124702 A1	Ming JC et al 2011
2	Process for preparation of crystalline nanoparticle suspensions	WO2011102787 A1	Lindfors L et al 2011
3	Water-insoluble drug particle process	US20020012704 A1	Pace G et al 2002
4	Pharmaceutical formulation of nanonised fenofibrate	US20110311619 A1	Herry C et al 2011
5	Nanoparticles prepared by microprecipitation	US6951656 B2	Kipp JE et al 2005
6	Nanosuspension for dissolution enhancement	US5858410 A	Muller RH et al 1999
7	for producing ultrafine submicronic suspensions	US8034381 B2	Moschwitz J, 2011
8	Process for producing nanometer particles by fluid bed spray drying	WO2001045677 A1	Nicholas JK, 2001
9	Method of producing medicinal nanoparticle suspension	US7597278 88	Asahi T et al 2009
10	Nanosuspension formulation comprising a polydimethyl siloxane hydrophobic phase	WO2011151418 A2	Breitenbach J et al 2011`
11	Nanosuspension of natural materials and preparation method	US10213382B2	(Brand, 2019)
12	Felodipine nano suspension and preparation method thereof	CN103251557A	(Lorry S et al. 2013)
13	Nanosuspension of abiraterone acetate	WO2014009436	(Grahek et al., 2014)
14	Succinpbucol nanosuspension and preparation method thereof	WO2015120799 (A1)	(Li Y et al., 2015)

### **2.7.2. Mechanism of Formation of SEDDS**

SEDDS undergo spontaneous self-emulsification upon dilution in aqueous medium due to increase in entropy to form dispersion than the energy required for increasing emulsion's surface area (Kohli et al., 2010; Singh et al., 2009). Free energy of an emulsion is considered as a direct function of the energy required to create a new surface between any two immiscible phases. The two immiscible phases of an emulsion exhibit a tendency to separate so as to reduce interfacial area to minimum and thus, to minimize free energy of system. These systems are stabilized by use of emulsifying agents that reduce the interfacial tension (Garg et al., 2016; Kohli et al., 2010; Parmar et al., 2011; Singh et al., 2009).

Thus, for SEDDS, such kind of emulsifiers and co-solvents need to be selected that will be able to reduce the interfacial tension. This, in turn, will lower the free energy required by SEDDS so that when they come in contact with aqueous medium in GIT, the self-emulsification process sets in. Fig. 3 depicts the mechanism of SEDDS formation (Garg et al., 2016; Kohli et al., 2010; Singh et al., 2009).

### **2.7.3. Categorization of SEDDS**

#### *2.7.3.1. Liquid SEDDS*

These are self-emulsifying isotropic mixtures of oil, surfactant, and cosolvent in liquid state. These offer the advantages of enhanced solubility of drugs and their increased lymphatic absorption. However, due to their liquid state, they are difficult to be dispensed as dosage form. To make the dosage form more convenient, they need to be incorporated into soft gelatin capsules. This, in turn, adds to the cost of formulation (Garg et al., 2016; Singh et al., 2009).

#### *2.7.3.2. Supersaturable SEDDS*

Concentration of surfactants in the SEDDS formulation is usually in the range of 20–60%. From safety point of view, use of such high concentration of surfactant becomes a concern for the formulator, as their higher concentration is known to lead to some adverse effect (Garg et al., 2016). To overcome this problem, the concept of supersaturable SEDDS was created. In these, the concentration of surfactants is reduced by the inclusion of water soluble polymeric precipitation inhibitor (PPI). These formulations maintain a supersaturable metastable state *in vivo* by reducing

precipitation of drug using PPI. Hydroxypropyl methylcellulose (HPMC) of different grades of viscosity have been widely reported to prevent crystallization as PPI in supersaturable SEDDS (Gao and Morozowich, 2006; Gao et al., 2003; Garg et al., 2016; Raghavan et al., 2000).

#### *2.7.3.3. Solid SEDDS (S-SEDDS)*

Self-emulsifying drug delivery systems were initially developed in liquid form. However these liquid SEDDS faced the difficulty of stability, formation of unit dosage form, high production costs, low stability and portability, low drug loading and few choices of dosage forms. Irreversible drugs/ excipients precipitation may also be problematic (Tang et al., 2008a). S-SEDDS come as a superior alternative to the L-SEDDS. S-SEDDS along with advantages of liquid SEDDS provide better stability, ease of handling, ease of conventional dosage forms like tablets and capsules (Mohsin et al., 2012). Solid self-emulsifying compositions are preferred over liquid ones due to their ability to extend the drug release, higher stability, and ease of handling. Solid SEDDS as name suggests, are solid dosage forms which have the ability to self-emulsify when come in contact with GI media (Cho et al., 2013). S-SEDDS are available in different forms like powders, granules, pellets, tablets and self-emulsifying dispersions (Tarate et al., 2014).

##### *2.7.3.3.1. Techniques Used for Solidification of SEDDS*

###### *2.7.3.3.1.1. Physical adsorption*

Physical adsorption of L-SEDDS on the solid carriers is one of the simplest techniques of solidification. In this process L-SEDDS are added on solid carrier and mixed either via physical blending with hand or motor pestle on lab scale or via use of blenders. Loading factor is calculated as the amount of solid carrier required for adsorption of L-SEDDS so that homogenous powder is obtained. After this, weighed amount of both L-SEDDS and carrier are mixed together until a homogenous solid powder is formed via adsorption of L-SEDDS over solid carriers. This powder should be passed through sieves to break any lumps, if present. The resultant powder can be directly filled into capsules or can be compressed into tablets via addition of some other excipients used for tableting (Chavan et al., 2015; Milović et al., 2014; Tang et

al., 2008a; Zidan et al., 2015). Several carriers like silicon dioxide, syloid have the capacity to adsorb large amount of L SEDDS (Tarate et al., 2014).

Hydrophilic/hydrophobic nature of carrier on which L-SEDDS have to be adsorbed affect the properties of drug e.g. L-SNEDDS of ezetimibe were prepared with Capryol 90, Lauroglycol FCC, ethyl laurate, Cremophor EL and Transcutol P and adsorbed on hydrophobic colloidal silicon dioxide to form self nano emulsifying granules (SNEG). X-ray diffraction (XRD) indicates that drug is in its amorphous form, but when the same SNEDDS were loaded on magnesium stearate a eutectic mixture is resulted (Dixit and Nagarsenker, 2008).

Quantities of ingredients per unit dose can be calculated for S-SEDDS. The following equation was used to calculate the amount of carrier materials (Abdelbary et al., 2013):

$$L = \frac{W}{Qf} \quad (1)$$

L is the liquid loading factor; W is the liquid medication weight; Q is the carrier material weight. The excipient ratio (R) is the ratio between the carrier (Q) and coating material (q) as presented by the following equation (Abdelbary et al., 2013):

$$R = \frac{Q}{q} \quad (2)$$

#### 2.7.3.3.1.2. Melt granulation

Melt granulation is a method in which S-SEDDS are prepared in a single step. In this method there is no need to prepare L-SEDDS and then adsorb on the solid carrier. In this method oil e.g. goat fat, or surfactant which are solid at room temperature are used. In this method all the mixture of oil and surfactant is taken in the desired quantity and heated above the melting point. In this melted mixture drug is added and mixed to form homogenous mixture (Attama and Mpamaugo, 2006).

When this molten mixture is added dropwise with a beaker containing cold water at 4°C at 1000 rpm leads to formation of solid lipid spheres (Attama and Mpamaugo, 2006). The granulation process is controlled by the parameters such as impeller speed, mixing time, binder particle size, and viscosity of the molten binder (Tarate et al.,

2014). These can be filtered out and dried. Attama et al., in 2006, reported the formation of self-emulsifying liposphere using this method using goat fat and Tween 65 (Attama and Mpamaugo, 2006).

#### *2.7.3.3.1.3. Pour moulding method*

Self-emulsifying suppositories and tablets can be prepared via pour moulding method. In this method oil and surfactant are taken and heated together until they homogenize completely. Drug is added into this homogenous mixture and stir thoroughly. This mixture is now poured into moulds and allowed to settle at a temperature of 4 °C. The tablets or suppositories with self-emulsifying ability are taken out from mould and stored at cool place (Attama et al., 2003). Attama et al., in 2003 prepared self-emulsifying tablets using this method (Attama et al., 2003). Though this method is easy to executed, industry friendly and reproducible but chances of degradation at higher temperature are there. Moreover, selection of lipids and surfactant is very important for the stability of these formulations as only those excipient which are solid at room temperature should be selected (Tang et al., 2008a; Tang et al., 2008b).

#### *2.7.3.3.1.4. Spray congealing*

Self-emulsifying microparticles can be produced by spray congealing technology. Fluidized bed equipment is utilized for this purpose. It uses two fluid atomisers with a wide orifice opening i.e. pneumatic nozzle. External mixing of fluid and air or gas occurs outside nozzle orifice, thus atomisation can be varied by changing the air pressure without affecting the liquid flow rate to enable the spraying of high concentration or viscous products. The temperature of feed tank containing molten fluid should be kept higher than melting temperature. Congealing chamber should be cooled using refrigerator system for solidification of droplets. Nozzle sprays the molten fluid in form of fine droplets. These molten drops get hardened because of low temperature of chamber and collected at bottom of congealing chamber (Albertini et al., 2015). Factors that affect the size include the orifice size of pneumatic nozzles, temperature of feed and congealing chamber, rate of atomization and air pressure (Albertini et al., 2015). This method bypasses the use of traditional solvents of spray drying like water and alcohol and relies on the excipients present in typical self-emulsifying formulations (Tarate et al., 2014).



#### 2.7.3.3.1.5. *Spray Drying*

Spray drying is one of the commonly used techniques in formation of S-SEDDS. Spray dryer consists of following components viz. feed delivery system, atomizer, heated air supply, drying chamber, solid-gas separator, and product collection system. In this technique, drug, L-SEDDS and carrier are dissolved or suspended in a solvent to form a homogenous system. This solution is now atomized to produce liquid droplets with the help of spray nozzle in spray dryer. These atomized droplets come in contact with hot air in drying chamber and get converted into fine powder which gets separated and collected in cyclone and collecting container. The product is self-emulsifying in nature. Both hydrophobic and hydrophilic carriers can be used in this process. The atomizer, the temperature, the most suitable air flow pattern and the drying chamber design are important variables affecting product characteristics (Alinaghi et al., 2015; Balakrishnan et al., 2009; Czajkowska-Kośnik et al., 2015; Tarate et al., 2014).

#### 2.7.3.3.1.6. *Extrusion-Spheronization*

S-SEDDS can also be formulated in the form of pellets via extrusion-spheronization. This process includes wet granulation of L-SEDDS with solid excipients, followed by extrusion of wet mass, spheronization of extrudates, drying of the spheroids, sizing, and optionally coating of the pellets. Extruder consists of a die through which material is forced with the help of single or twin screw, and shaped into cylinders of uniform length. This process is used to form uniformly sized pellets. Spheronizer is equipped with a bowl having fixed side walls and rapidly rotating bottom plate. The bottom plate is grooved to provide the equipment-particle interactions for rounding the cylindrical pellets (Abdalla et al., 2008; Tarate et al., 2014).

#### 2.7.3.3.1.7. *Lyophilization*

Lyophilization can also be used for formulating S-SEDDS. In this process, water is evaporated directly via sublimation. It includes several steps i.e. freezing, primary drying, and secondary drying. In this process both carrier and L-SEDDS are dissolved in a common solvent followed by freezing and sublimation process. This method gives a solid product (Tarate et al., 2014). Jain et al., 2014 prepared S-SNEDDS using lyophilization technique. SNEDDS were diluted in minimum quantity of deionized water and thoroughly mixed with Aerosil<sup>®</sup> 200. Lyophilization was done after 15

minutes of equilibration (Jain et al., 2014a). It is a method of choice for thermolabile formulations, proteins, peptides and vaccines, although cost and time is a constrain (Tarate et al., 2014).

#### *2.7.3.3.1.8. Use of Porous Beads*

Porous beads or porous tablets with large surface area may also be used for loading L-SEDDS. Porous polystyrene beads with surface area of 153.12 m<sup>2</sup>/g were used by Patil and Paradkar. Results show good loading efficiency as well drug content (Patel et al., 2012; Patil and Paradkar, 2006). Christiansen et al., in 2014 prepared porous tablet cores for loading of L-SNEDDS using magnesium aluminometasilicate granules with Ac-Di-Sol (disintegrant) and magnesium stearate (anti-adhesive). These cores were then kept in a container along with L-SNEDDS for 2 h to ensure loading of 500 mg of SNEDDS on tablet cores. Excess L-SNEDDS were removed get a dry and shiny tablets loaded with L-SNEDDS (Christiansen et al., 2014).

#### *2.7.3.3.1.9. Self-Emulsifying Solid Dispersion*

Self-emulsifying solid dispersions can also be prepared by melting method. In this method, drug, surfactant and fatty acids are homogenously mixed and slightly heated to get a melted mixture. This melted mixture is then added to a suitable adsorbent like Aerosil<sup>®</sup> 200 and kept at cool temperature. Solid mass obtained is crushed and passed through sieve of suitable size to obtain fine powder (Tran et al., 2013).

### **2.7.4. Drug Transport Mechanism of SEDDS**

SEDDS offer bioavailability of water insoluble drugs even through oral administration. Upon reaching to the GIT these SEDDS undergo absorption in three steps(Charman and Porter, 1996; Garg et al., 2016; Stremmel, 1988)-

*Step-1 (Digestion):* The enzymes present in GIT hydrolyse the emulsion at oil-water interphase and enable SEDDS for absorption. The digestion process stops once these SEDDS form mixed micelles upon interacting with bile salts and fatty acids.

*Step-2 (Absorption):* During absorption these micelles are uptaken through active or passive transport by enterocyte membrane or through lymphatic circulation by chylomicrons.

*Step-3 (Circulatory):* Chylomicrons release the drug into the systemic circulation. The remaining lipids are utilized by body.

**Table 8:** Composition of various S-SEDDS

Drug	Oil	Surfactant	Co-surfactant	Ratio L-SEDDS	Carrier	Technology used	Formulation prepared	Reference
*PLAG	-	Sodium lauryl sulfate (SLS)	-	-	Calcium silicate	** SD	Powder	(Kim et al., 2017)
Sertraline	LBF M 2125 CS + Maisine 35-1; 1:1 ratio	Labrasol	Lauroglycol 90	24.59:50.27: 25.13	Silicon dioxide	*** Ext./Sph.	Pellets	(A Rahman et al., 2016)
Dabigatran etexilate	Maisine 35-1: MCT = 1:1	Gelucire 44/14	Transcutol P	45:37:18	MCC 102, colloidal silica, Mg stearate	**** DC	Dispersible tablets	(Chai et al., 2016)
Vinpocetine	Maisine 35-1	Cremophor EL	Transcutol P	-	Aeroperl	DC	Osmotic tablets	(El-Zahaby et al., 2018)
Amisulpride	Capryol 90	Cremophor RH40	Transcutol P	-	Magnesium Aluminium silicate Aeroperl	***** PA	Powder	(Gamal et al., 2017)
Lopinavir	Maisine	Tween 80	Transcutol P	-	Aeroperl	PA	Powder	(Garg et al., 2016)
Glipizide	Captex 355	Solutol HS15	Imwitor 988	30:45:25	Calcium carbonate	PA	Powder	(Dash et al., 2015)
Cilostazol	Peceol	Tween 20	Labrasol	15:55:30	Calcium silicate	SD	Powder	(Mustapha et al., 2017)
Olmesartan	Capryol 90	Cremophor RH40	Transcutol P	-	Aerosil 200	SD	Powder	(Nasr et al., 2016b)
Meloxicam	Labrafil / SA 5	Cremophor RH 40 / Tween 80, 1:1, w/w	Transcutol P and PEG 400, 1:2, w/w	-	Aeroperl 300	PA	Granules	(Parekh et al., 2017)
Lopinavir	Capmul MCM C8	Cremophor RH40	Propylene glycol	-	Neusilin US2	PA	Powder	(Patel et al., 2016)
Ezetimibe	Capryol 90	Cremophor EL	Tween 80	-	Silicon dioxide	SD	Powder	(Rashid et al., 2015)
Alpha- mangostin	Captex 200P	Tween 80	Capryol 90	20/70/10	Aeroperl 300	PA	Powder	(Sodalee et al., 2016)
Artemether	Suppocire	Gelucire	Transcutol P	110 mg: 800 mg: 50 mg	-	***** MM	Suppositories	(Gugulothu et al., 2010)
Atorvastatin	Oleic Acid	Tween 80	-	1:9	Lactose	** SD	Powder	(Czajkowska-Kośnik et al., 2015)
Atorvastatin	Capryol 90	Tween 80/1,2- Propylene Glycol (1:1)	-	1.5:8.5	Lactose	** SD	Powder	(Czajkowska-Kośnik et al., 2015)

( Table 8 continued.....)

Drug	Oil	Surfactant	Co-surfactant	Ratio L- SEDDS	Carrier	Technology used	Formulation prepared	Reference
Brucea javanica oil	Caprylic/capric triglyceride (GTCC)	Cremophor RH-40	PEG400	11.1:66.7:11.1	PVPP	#### AB	Granules	(Shao et al., 2013)
Candesarn Cilexetil	Miglyol 812	Tween 80 / Cremophor EL	Labrasol	12:37:75	colloidal silicon dioxide and MCC	PA	Powder	(Nekkanti et al., 2010)
Carbamazepine	Mygliol 812	Labrasol (caprylocaproyl macrogol-8 glycerides)	Phosal 50 PG/propylene glycol	-	diatom	PA	Suspension	(Milović et al., 2014)
Carvedilol	Capmul MCM	Nikkol HCO 50 (Solid)	-	262.0 mg : 225.0 mg)	Nikkol HCO 50	Congealing	Powder	(Singh et al., 2013)
Carvedilol Celastrol	Capmul MCM Ethyl oleate	HCO 50 OP-10	Lutrol F 68 Transcutol P	- 25:60:15	- MCC KG 802	- # WGCM	- Dispersible tablets	(Singh et al., 2008) (Qi et al., 2014)
Ciclosporin A	Miglyol 810N (solid)	Cremophor EL	Transcutol P	6:3:2	Gelatin,D-Sorbitol, SDS and Ethylcelluse and pectin (98:2%)	Extrusion	Coated minisphere for colon targeting	(Keohane et al., 2016)
Cilnidipine	Capryol 90	Tween 80	Transcutol P	3:6:1	Neusilin US2	PA	Powder	(Bakhle and Avari, 2015)
Cinnarizine	Sesame oil	Cremophor RH 40, Oleic acid	Brij 97, Ethanol	20.6:45:15.4 :9:10	Magnesium aluminametasilicate granules	Adsorption	Porous tablet cores	(Christiansen et al., 2014)
clopidogrel napadisilate	Peceol	Cremophor RH60	Transcutol P	2:3:5	Aerosil 200	SD	Powder	(Kim et al., 2014)
Coenzyme Q10	Lemon oil	Cremophor EL	Capmul MCM-C8	-	Kollidon VA 64, Glucidex IT 12, and Avicel PH-112	Blending	Tablet	(Nazzal and Khan, 2006)
Coenzyme Q10	medium-chain triglyceride (MCT)	sucrose ester of fatty acid	-	5 g: 40 g	Hydroxypropyl cellulose (HPC-SSL)	** SD	Powder	(Onoue et al., 2012)

( Table 8 continued.....)

Drug	Oil	Surfactant	Co-surfactant	Ratio L- SEDDS	Carrier	Technology used	Formulation prepared	Reference
CRM	Capryol 90, Labrafac PG	Cremophor EL	Labrasol, PEG 400	8.1 g: 8.1 g: 18.9 g: 18.9 g	Silicon dioxide and glyceryl behenate,	Ext./Sph.	Pellets	(Setthacheewakul et al., 2010)
CRM	Capryol 90	HPMCAS-HF	-	-	Aerosil-200	## MQESDM	Nano capsules	(Wadhwa et al., 2014)
Cyclosporine	Sweet orange oil	Emulphor EL- 620	Capmul MCM- C8	20: 60.2: 8.5	amorphous silica (Rxcipients GL200)	Lyophilization	Tablets	(Zidan et al., 2015)
Cyclosporin A	Labrafil M 1944 CS	Cremophor EL	Transcutol P	9:7:14	10 % PVP- K30 (w/v) as coating material and non- pareil cores	### FBC	Pellets	(Lei et al., 2011)
Danazol	Capmul MCM	Tween 80	Transcutol P	1:2:1	Aerosil 380	PA	Powder	(Alinaghi et al., 2015)
Danazol	Capmul MCM	Tween 80	Transcutol P	1:2:1	Aerosil 380 (5 % w/w)	SD	Powder	(Alinaghi et al., 2015)
Danazol	Capmul MCM	soya lecithin	-	100:0.6	Aerosil 380 (5 % w/w)	SD	Microparticl es	(Alinaghi et al., 2015)
Danazol	Captex 355 (36% w/w), Capmul MCM (18% w/w)	Cremophor EL	Ethanol	54: 36:10	Neusilin US2	PA	Powder	(Speybroeck et al., 2012)
Danazol	soybean oil (30% w/w), Maisine 35-1 (30% w/w)	Cremophor EL	ethanol	60:30:10	Neusilin US2	PA	Powder	(Van Speybroeck et al., 2012)
Darunavir	Capmul MCM C8	Tween 80	Transcutol P	16.6:41.7:41.7	Neusilin US2	PA	Powder	(Inugala et al., 2015)
Dexibuprofen	Labrafil M 1944 CS	Labrasol	Capryol 90	1.5:8:0.5	Aerosil 200	SD	Powder	(Balakrishnan et al., 2009)
Diclofenac	Goat fat	Tween 65	-	20:10 to 8:2	-	Pour moulding	Tablets	(Attama and Mpamaugo, 2006)
Diazepam	Cithrol MCM	Solutol HS 15	-	-	Avicel PH 101	Ext./ Sph.	Pellets	(Abdalla and Mäder, 2007)
Zedoary turmeric oil	Ethyl oleate	Tween 85	-	-	HPMCAS-LG), Talc and Aerosil 200	MQESDM	Microsphere	(You et al., 2005)

( Table 8 continued.....)

Drug	Oil	Surfactant	Co-surfactant	Ratio L- SEDDS	Carrier	Technology used	Formulation prepared	Reference
Docetaxel	Capryol 90	Labrasol	Transcutol P	10:75:15	Colloidal Silicon dioxide	SD	Powder	(Seo et al., 2013)
Embelin	Capryol-90	Acrysol EL 135	PEG 400	49.50 mg: 115.50 mg and 24.75 mg	Neusilin US2 (Carrier) and Aerosil 200 (Coating agent)	#### PAB	Granules and Tablet	(Parmar et al., 2015)
Erlotinib	Labrafil M 2125 CS	Labrasol	Transcutol P	5:65:30	Aerosil 200	SD	Powder	(Truong et al., 2016)
Ezetimibe	Capryol 90	Cremophor- EL	Cremophor- EL	-	Aerosil 200	Mixing	Powder	(Dixit and Nagarsenker, 2008)
Fenofibrate	Labrafac WL1349	Cremophor- EL and PEG 6000	Gelucire 44/14	20:30::30:20	PEG 6000	Congealing	Capsules	(Kanaujia et al., 2014)
Fenofibrate	Labrafac WL1349	TPGS 1000 (solid surfactant)	Gelucire 44/14	25:50:25	TPGS 1000	Congealing	Capsules	(Kanaujia et al., 2014)
Fenofibrate	M812 and I988 (7:3)				Neusilin US2	PAB	Powder	(Shazly and Mohsin, 2015)
Fenofibrate and Probuco	monoesters of fatty acids with glycerol or propylene glycol	Poloxamer 188	-	-	Poloxamer 188	Congealing	Powder	(Shah and Serajuddin, 2012)
Flurbiprofen	Labrafil M 1944 CS	Labrasol	Transcutol P	12.5:80:7.5	Silicon dioxide	SD	Powder	(Kang et al., 2012)
Flurbiprofen	Labrafil M 1944 CS	Labrasol	Transcutol P	12.5:80:7.5	HP-β-CD	SD	solid dispersions	(Kang et al., 2012)
Furosemide and propranolol	medium-chain triglyceride	Cremophors ELP, RH40, and RH60	-	1.5:1	Avicel PH-101	Ext. / Sph.	Pellets	(Nikolakakis and Malamataris, 2014)
Glipizide	Phosal 53MCT	Tween 80	Transcutol P		Syloid 244 FP	PA	Powder	(Agrawal et al., 2015)
Ibuprofen	Capryol 90	Cremophor EL	Labrasol	3:4:3	Fujicalin®	PAB	Tablets	(Kang et al., 2011)
Ibuprofen	-	Labrasol	-	-	Neusilin SG2	PAB	Powder	(Krupa et al., 2014)
Lovastatin	Capmul MCM	Nikkol HCO-50	Lutrol F127	-	-	MM	Powder	(Beg et al., 2015)

( Table 8 continued.....)

Drug	Oil	Surfactant	Co-surfactant	Ratio L- SEDDS	Carrier	Technology used	Formulation prepared	Reference
Glibenclamide	-	Myverol 18-04 Myvatex mighty soft Gelucire - 50/13 Gelucire 44/14 Cremophor EL 188 (solid at room temp.)	PEG 4000 (solid at room temperature)	-	-	Congealing	Microparticles	(Albertini et al., 2015)
Ibuprofen	-	Labrasol	PEG 200	1:1	Neusilin SG2 (70%) and MCC (30 %)	Ext./sph. and FBC	Pellets	(Krupa et al., 2015)
Isradipine	Labrafil M 2125 CS	Capmul MCM L8	Cremophor EL	-	Neusilin US2	PA	Powders	(Ramasahayam et al., 2015)
Isradipine	-	-	-	-	Poloxamer 407	MM	Solid dispersion tablet	(Tran et al., 2013)
Ketoprofen	Captex 200	Tween 80	Capmul MCM	-	Aerosil 200	-	Gelled SEDDS	(Patil et al., 2004)
Loratidine	Liquid paraffin	Span 20	Capriole, Transcutol	73.8:24.5:6.1 5:0.5	Aerosil 200 Crosscarmellose (10) lactose (20 - 30), Avicel (40)	Ext./ Sph.	Pellet	(Abbaspour et al., 2014)
Lutein	Phosal 53 MCT	Labrasol	Transcutol P	25:60:15	Aerosil 200	SD	Powder	(Shanmugam et al., 2011b)
Loratadine	Captex 200 & Capmul MCM	Cremophor- EL	Cremophor- EL	-	Porous polystyrene	*#BFBE	Powder	(Khan et al., 2004)
Lovastatin	Capmul MCM	HCO 50	Lutrol F 68	-	-	-	Powder	(Singh et al., 2008)
Mixture of mono- and diglyceridesP olsorbate 80, and water.	-	-	-	-	Avicel PH101 and Lactose	Ext./Sph.	Pellets	(Newton et al., 2001)
Sirolimus	Capryol PGMC:	Vitamin E TPGS	glycofurol	3:4:3	Sucroester 15 and mannitol	WG	Powder	(Cho et al., 2013)

( Table 8 continued.....)

Drug	Oil	Surfactant	Co-surfactant	Ratio L- SEDDS	Carrier	Technology used	Formulation prepared	Reference
Methyl Paraben & propyl Paraben Nifedipine	Imwitor 742	Tween 80	-	-	Avicel PH101	Ext./ Sph.	Powder	(Serratoni et al., 2007)
Nile red	-	-	-	Microemulsior (Tween-80 (27.2% wt/wt), Span-80 (0.8% and IPM (5.4%),)	Alginate	Gelling followed by Lyophilizati on	alginate sponges	(Josef and Bianco- Peled, 2013)
Nimesulide	Cithrol GMO	Tween 80	-	-	Microcel 101	SD	Powder	Franceschinis et al., 2004
Nimodipine Nitrendipine	Ethyl oleate Miglyol 812	Labrasol Cromophor RH 40, Tween 80	Cremophor RH40 - Transcutol P	-	Dextran 40 Syloid 244 FP Kollidon CL-SF Flowlac 100 Avicel PH	SD Ext./ Sph.	Powder -	(Yi et al., 2008) (Wang et al., 2010)
Oleanolic acid Piroxicam	Ethyl oleate Capra hircus	Labrasol Tween 65	Transcutol P -	15:71:14 4:11	Mannitol -	**# WG Congealing and precipitation method	Powder Liposphere	(Ma et al., 2014) (Attama and Mpamaugo, 2006)
Probuocol	Capmul MCM; Captex 355, Cremophor EL	Capmul MCM; Captex 355, Cremophor EL	Capmul MCM; Captex 355, Cremophor EL	-	Neusilin® US2	PA	Tablet	(Gumaste et al., 2013)
Progesterone	Captex 355:Capmul MCM (2:1)	Solutol HS15	-	6:4	Avicel PH 101microcrystalline cellulose	Ext./Sph.	Pellet	(Abdalla et al., 2008)
Telmisartan	Castor oil	Tween 20	Propylene glycol	30:55:15	MCC	PAB	Powder	(Jaiswal et al., 2014)



( Table 8 continued.....)

Drug	Oil	Surfactant	Co-surfactant	Ratio L- SEDDS	Carrier	Technology used	Formulation prepared	Reference
Scutellarin	Maisine 35-1 and Labrafac Lipophile WL 1349 (1:1, w/w	Labrasol and Cremophor EL (1:2, w/w)	Transcutol P	-	lactose, HPMC, MCC	SD	Powder	(Li et al., 2013)
Silymarin	Akoline MCM, Miglyol	Tween 80, soy lecithin	Propylene glycol	-	MCC and lactose	Ext./Sph.	Pellets	(Iosio et al., 2011)
Simvastatin	Labrafil,	Tween 80	Transcutol P	1:6:3	Avicel or Starch 1500 (5 or 10) and Aeroperl (1) as coating	liquisolid powders via blending	Tablet	(Abdelbary et al., 2013)
Simvastatin	Lauroglycol™ 90	Cremophor EL	Transcutol P	Water(60):2 0:15:5	mixture composed of 70% (w/w) of MCC, 27% (w/w) of Lac and 3% (w/w) of PVP	high shear mixer	Granules	(Franceschinis et al., 2015)
Sirolimus	Labrafil M1944CS	Cremophor EL,	Transcutol P	22.4, 38.4, 19.2 mg	MCC, Lactose and CMS-Na	Ext./Sph.	Pellets	(Hu et al., 2012)
Tacrolimus	Lauroglycol FCC	Cremophor RH	PEG 400	1:6:3	Florite RE	PAB	Powder	(Patel et al., 2013)
Tacrolimus	Labrafac	Labrasol	Lauroglycol	15:70:15	Aerosil200	SD	Powder	(Seo et al., 2015)
Tamoxifen and Quercetin	Capmul MCM)	Cremophor RH 40	Labrafil 1944CS	4:3:3	Aerosil 200	Lyophilization	Powder	(Jain et al., 2014a, b)
Valsartan	Capmul MCM (117.50 µL),	Labrasol (171.00 µL)	Tween 20 (171.00 µL)	-	Aerosil 200, Sylsilia (350, 550, and 730) and Neusilin US2	WG	Granules and Tablets	(Beg et al., 2012)
Vitamin A acetate	Soyabean oil, Capmul MCM- C8	Cremophore EL	-	-	Avicel	Mixing and compression	Tablets	(Taha et al., 2009)
Vinpocetine	Akoline MCM, Peanut oil	Polysorbate 80	-	-	Microcel 101 Ac- DI-Sol	Ext./Sph.	Bi- layered pellets	(Iosio et al., 2008)

\* 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol; \*\* Spray drying; \*\*\* Extrusion/Spheronization; \*\*\*\* Direct compression; \*\*\*\*\* Physical adsorption; \*\*\*\*\* Melting method; # Wet granulation compression method; ## Modified quasi- emulsion solvent diffusion method; ### Fluidised bed coating; #### Physical adsorption and blending; \*# Beads formation by evaporation; \*\*# WG

**Table 9:** SNEDDS prepared using Spray drying

Drug	Excipient	Solvent	Volume of L-SEDS	Excipient solvent ratio	Inlet temp. (°C)	Outlet temp. (°C)	Aspiration	Feed rate	Reference
Cilostazol	Calcium silicate	Water	-	-	100	50-55	100 %	4 kg/cm <sup>2</sup>	(Mustapha et al., 2017)
Olmесartan	Aerosil 200	Ethanol		1g in 200 mL	60	35	85	5 mL/min	(Nasr et al., 2016a)
Ezetimib	HPC	Water	-	-	115	75-85	100 %	5mL/min	(Rashid et al., 2015)
Atorvastatin	Lactose	Water	10 g	10 g in 100 mL	60	40	100 %	4 mL/min	(Czajkowska-Kośnik et al., 2015)
clopidogrel napadisilate	Aerosil 200	Ethanol	1 g	0,75 g in 100 mL	70	40	100 %	6 mL/min	(Kim et al., 2014)
Coenzyme Q10	HPC-SSL	25 % Ethanol	45 g	50 g in 2000 mL	160	75	-	3.9 kg/h	(Onoue et al., 2012)
Dexibuprofen	Aerosil 200	Ethanol	1 mL	500 mg in 100 mL	60	35	85 %	5 mL/min	(Balakrishnan et al., 2009)
Docetaxel	Lactose	Water	600 mg and 1000 mg	800 mg in 100 mL and 1300 mg in 160 mL water	120	65	500 N l/h;	5 mL/min	(Chen et al., 2011)
Docetaxel	Aerosil 200	Ethanol	3 mL	3 g for 500 mL	62	32	-25 mbar	-	(Seo et al., 2013)
Erlotinib	Aerosil 200	Ethanol	5 g	5 g for 150 mL	70	58	80	-	(Truong et al., 2016)
Erlotinib	Dextran 40	Water	5 g	5 g for 150 mL	130	100	80	-	(Truong et al., 2016)
Flurbiprofen	silicon dioxide and magnesium stearate	Ethanol	1 mL	1g in 100 mL	60	40	-25 mbar	5 mL/min	(Kang et al., 2012)
Flurbiprofen	PVA, Na- CMC and HP- β-CD	Water	1 mL	1g in 100 mL	100	80	-25 mbar	5 mL/min	(Kang et al., 2012)
Scutellarin	Lactose, HPMC and MCC	Water	4 g	4 g in 200 mL	140	66	90 %	5 mL/min	(Li et al., 2013)
Tacrolimus	Aerosil 200	Ethanol	4 g	1 g for 400 mL	62	35	85 %	5 mL/min	(Seo et al., 2015)
Lutein	Aerosil 200	Ethanol	1 mL	500 for 100 mL	60	35	85 %	5 mL/min	(Shanmugam et al., 2011b)

### **2.7.5. Excipients**

Formation of SEDDS utilizes oils, surfactants and co-surfactants. Oils used to prepare SEDDS are biodegradable, ready to hydrolyse and with low HLB value. These oils help in improving solubility as well as transport of the drug through lymphatic routes and thereby helps in enhancing their bioavailability. Various oils used to prepare SEDDS are listed in Table 10 (Dash et al., 2015; Garg et al., 2016; Mandawgade et al., 2008);(Garg et al., 2016; Porter et al., 2008; Pouton and Porter, 2008; Singh et al., 2009).

The surfactant having high HLB value and have very good safety profile are used to prepare SEDDS. Their amphiphilic property helps in solubilizing the drug in the mixture of both, oil and water. These helps in improving the oral bioavailability of lipophilic drugs by enhancing their dissolution rate. Various surfactants use to prepare SEDDS are listed in Table 11. (Eaimtrakarn et al., 2002; Garg et al., 2016; Koga et al., 2006) (Balakumar et al., 2013; Chistyakov, 2001; Devraj et al., 2013; Garg et al., 2016; Porter et al., 2008; Pouton and Porter, 2008; Tarate et al., 2014).

In order to reduce the amount of surfactant, decrease the droplet size, increase drug loading. The use of co-surfactants is recommended. These co-surfactants also help in improving solubility of hydrophilic surfactants in the isotropic mixture. The list of co-surfactants use to prepare SEDDS are given in Table 12 (Shahba et al., 2012). (Garg et al., 2016; Pouton, 2000; Pouton and Porter, 2008; Singh et al., 2009).

**Table 10:** Oils/lipids used for SEDDS

Lipid/Oil	Chemical name	Reference
Bean phospholipids	-	(Lv et al., 2012)
Capmul MCM EP	GlycerylCaprylate/Caprinate	(Jain et al., 2014a, b)
Caprylic/capric triglyceride	Caprylic/capric triglyceride	(Shao et al., 2013)
Capryol 90	Propylene glycol monocaprylate (type II) NF	(Inugala et al., 2015)
Captex 355	GlycerylTricaprylate/Tricaprate	(Inugala et al., 2015)
Capmum MCM C8	GlycerylMonocaprylate	(Inugala et al., 2015)
Castor oil	Castor oil	(Tran et al., 2014)
Chuanxiong oil		(Cai et al., 2007; Cai et al., 2008)
Cinnamon oil	Cinnamon oil	(Zhang et al., 2008b)
Cotton seed oil	Cotton seed oil	(Kang et al., 2012)
Corn oil	Corn oil	(Kang et al., 2012)
Cradamol GTCC	Caprylic/Capric Triglyceride	(Yao et al., 2008)
Cremophor EL Castor oil	Macroglycerol Ricinoleate , Polyoxyl 35 Castor Oil USP	(Inugala et al., 2015)
Ethyl oleate	Ethyl oleate	(Cui et al., 2005)
Gelcire 44/14	Lauroyl macrogol-32 glycerides EP Lauroyl polyoxyl-32 glycerides NF	(Mandawgade et al., 2008)
Isopropyl myristate	Myristic acid isopropyl ester	(Wang et al., 2009)
Labrafac PG	Propylene glycol dicaprylocaprinate EP Propylene glycol dicaprylate/dicaprate NF	(Setthacheewakul et al., 2010)
Lauroglycol FCC	Propylene glycol mono laurate	(Rao and Shao, 2008; Rao et al., 2008)
Labrafac CC	Caprylic/Capric Triglyceride	(Inugala et al., 2015; Kang et al., 2012)
Mineral oil	Higher alkanes from mineral source	(Kang et al., 2012)
Maisine oil	Glycerylmonolinoleate	(Parmar et al., 2011; Zhang et al., 2008a)
Miglyol 812	Liquid lipids/C8/C10 triglycerides	(Ma et al., 2012)
Myvacet 9-45	Myvacet 9-45K NF	(Kommuru et al., 2001)
Methyl decanoate	Decanoic acid methyl ester	(Wang et al., 2009)
Methyl oleate	Oleic acid methyl ester	(Wang et al., 2009)
Oleic acid	Octadecenoic acid	(Qi et al., 2011; Rao and Shao, 2008; Rao et al., 2008)
Olive oil	Olive oil	(Qi et al., 2011)
Peanut oil	Peanut oil	(Kang et al., 2012)
Peceol	Glycerol monooleate	Rao et al., 2008
Phosal 53 MCT	Lecithin in caprylic/capric triglycerides, alcohol, glyceryl stearate, oleic acid and ascorbylpalmitate	(Shanmugam et al., 2011a; Shanmugam et al., 2011b)
Polyoxyethylene castor oil	Polyoxyethylene castor oil	(Mekjaruskul et al., 2013)
Sesame oil	Sesame oil	(Kang et al., 2012)
Sunflower oil	Sunflower oil	(Kang et al., 2012)
Soybean oil	Soybean oil	(Qi et al., 2011)
Trilaurin	Glycerol trilaurate	(Elgart et al., 2013)

**Table 11:** Surfactants used for SEDDS (Kang et al., 2012)

Surfactant	Chemical name	Reference
Capmul	mono-diglyceride of medium chain fatty acids	(Basalious et al., 2010)
Cremophor RH40	PEG-40 hydrogenated castor oil	(Rao and Shao, 2008)
Cremophor-EL	PEG-35 castor oil	(Parmar et al., 2011)
Labrafil M 2125	PEG-6 corn oil	(Inugala et al., 2015; Kang et al., 2012)
CS		
Labrafil M1944CS	PEG-6 apricot kernel oil	(Inugala et al., 2015; Kang et al., 2012)
Labrasol	Caprylocaprylmacrogol glycerides	(Inugala et al., 2015; Parmar et al., 2011; Rao and Shao, 2008; Rao et al., 2008)
Polysorbate 80	Polyoxy ethylene 20 sorbitan mono oleate	(Rao and Shao, 2008)
Polysorbate 20	Polyoxy ethylene 20 sorbitan mono laurate	(Rao and Shao, 2008)
Polyoxamer 407	Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)	(Date and Nagarsenker, 2007)
Polyoxamer 188	Pluronic F-68 solution	(Date and Nagarsenker, 2007)
Solutol HS 15	Macrogol (15)-hydroxystearate	(Date and Nagarsenker, 2007)
Span 20	Sorbitanmonolaurate	(Kang et al., 2012; Qi et al., 2011)
Span 80	Sorbitanmonooleate	
Span 85	Sorbitantrioleate	(Qi et al., 2011)
Tween20	PEG-20 sorbitanmonolaurate	(Date and Nagarsenker, 2007)
Tween-80	PEG-20 sorbitanmonooleate	(Date and Nagarsenker, 2007; Qi et al., 2011; Singh et al., 2009)
Tween-85	PEG-20 sorbitantrioleate	(Singh et al., 2009)

**Table 12:** Co-solvents used for SEDDS

Cosurfactant	Chemical name	HLB	Reference
1,2 octane diol	1,2 octane diol		(Wang et al., 2009)
Akoline MCM	Caprylic/ Capric glycerides	5-6	(Date and Nagarsenker, 2007)
Akomed	Oil containing triacylglycerols of caprylic and capric acid		(Date and Nagarsenker, 2007)
Capmul MCM-C8	Glycerylcaprylate	5-6	(Singh et al., 2009)
Caproyl 90	Propylene glycol mono caprylate	6	(Kang et al., 2012; Parmar et al., 2011)
HCO-60	PEG-60 hydrogenated castor oil	14	(Singh et al., 2009)
Imwitor 742	Caprylic/Capric Glycerides	4	(Date and Nagarsenker, 2007)
Labrafil 1944 CS	PEG-6 apricot kernel oil	4	(Date and Nagarsenker, 2007)
Lauroglycol 90	Propylene glycol monolaurate	5	(Inugala et al., 2015)
Lauroglycol FCC	Propylene glycol monolaurate	4	(Rao et al., 2008)
Lutrol F127	PolyoxamersPh Eur., Polyoxamers		(Beg et al., 2015)
PEG 400	Polyethylene glycol 400	11.6	(Rao and Shao, 2008)
PG	Propylene glycol		(Date and Nagarsenker, 2007; Shahba et al., 2012)
PlurolDioleique CC 497	Polyglyceryl-3 dioleate NF Polyglyceryl-3 oleate (USA FDA IIG)	3	(Date and Nagarsenker, 2007)
Transcutol P	Diethylene glycol mono ethyl ether	-	(Date and Nagarsenker, 2007; Parmar et al., 2011)

#### *2.7.5.1. Excipients used for S-SEDDS*

Apart from oils and surfactants which are used in L-SEDDS different excipients are used in formulation of S-SEDDS. Nature of excipient depends upon the type of S-SEDDS formulated, the method by which SEDDS are prepared, compatibility with the drug, nature of release pattern of dosage form prepared (Abbaspour et al., 2014; Nazzal and Khan, 2006; Singh et al., 2013). Mainly for those methods in which L-SEDDS are converted to S-SEDDS (powder, granules or tablets) carrier with high loading capacity are used. These carriers are porous in nature and have the capacity to load higher amount of oil or L-SEDDS on their porous molecules. Different grades of Silicon dioxide, Magnesium aluminium silicate are used as carrier for L-SEDDS. Different Industries have come up with several grades of these carriers with higher carrying capacity due to their porous nature (Chavan et al., 2015; Kang et al., 2011; Krupa et al., 2015). But there are some methods in which S-SEDDS can be prepared directly without preparing L-SEDDS by use of solid oil or surfactants.

**Table 13:** Patents of SEDDS/SMEDDS/SNEDDS (Singh et al., 2009)

Patent number	Year	Inventor	Company
US2009124670 (A1)	2009	Sakai Kenichi	
WO2009040776 (A1)	2009	Nakhat Premchand and Mandaogade Prashant	Wockhardt Research Centre, India
US/2009/0186926	2009	Agam R. Sheth, Bhagwant Rege, Soumojeet Ghosh, Laman L. Alani, Maria T. Cruanes and Craig A. Mckelvey	Merck and Co., US
WO2008128960 (A1)	2008	Schwarz, Franz, Xaver	Sandoz, Switzerland
US20070104741	2008	Ram B. Murty, K.Y. Lexington and Santos B. Murty	Murty Pharmaceuticals, Inc., US
KR20020071037 (A)	2008	Baek Kwang Seok and Choi Young Wook	
US 2007/0104740 A1	2007	Jody Firmin Marceline Voorspoels	
US2007012895 (A1)	2007	Sandner Bernhard, Stanica Cristina and Jiang Longying	
US 2006/0014788 A1	2006	Michael J. Gumkowski, Lombardo Franco, Sharad B. Murdande and Michael E. Perlman	Pfizer Inc., US
US 7022337	2006	Likan Liang, Amir H. Shojaei, Scott A. Ibrahim and Beth A. Burnside	Shire Laboratories Inc., US
US 2002/0131945 A1	2006	Robert Wayne Glenn, James Charles Dunbar and Tharwat Tadros	
US/2006/0292186	2006	Jean-Sebastien Garrigue, Gregory Lambert, Alain Razafindratsita, Simon Benita, Shicheng Yang and Neslihan Gursoy	
CA 2578130	2006,	Zhentao Liu, Liying Yang, Hanyu Yang, Yuqing Gao, Dongmin Shen, Wenmin Guo,	Shijiazhuang Pharma, China
MX2007002335 (A)	2007	Xiaolong Feng and Jia Zheng	
CA 2579449	2006	Jean Pachot	Aventis Pharma, France
7022337B2	2006	Likan Liang, Amir H. Shojaei, Scott A. Ibrahim and Beth A. Burnside	Shire Laboratories Inc., US
WO/2005/037251	2005	John Ong, Gregg Stetsko, Odile Esther Levy, S.S. Ghosh	Amylin Pharmaceuticals Inc., US
US2005232952(A1)	2005	Alain Razafindratsita, Gregory Lambert, Jean-Sebastien Garrigue, Neslihan Gursoy, Shicheng Yang and Simon Benita,	Novagali Pharma, SA
AU2003214538			
CA2003 2478424			
KR20050011323 (A)	2005	Cho Sun Hang and Jeong Sang Young	Korea Research Institute of Chemical Technology
US 2005/0025792 A1		Peracchia Maria-Teresa, Cote Sophie and Gaudel Gilbert	Aventis Pharma, France
US6555558	2003	Shirlynn Chen and Jocelyn A. Gunn	Boehringer Ingelheim Pharmaceuticals Inc.
EP1340497(A1)	2003	Lambert Gregory and Razafindratsita Alain	Novagali SAS, France
CA 2003/2478424			
US 6221391	2001	Mark T. Rouffer	Accucaps Industries Limited, Cannada
WO0066140 (A1)	2000	Mulye Nirmal	Pharmasolutions Inc, US

US20060034797	2000				Johnson & Johnson Pharmasolutions, Inc., US
US2000/6057289	2000	Nirmal Mulye			
US2002103139 (A1)	2000	M. Weisspapir and J. Schwarz			
US 2003/0147927 A1	2002	Mansoor A. Khan and Sami Nazzal			
US7276113		Mark G. Le Page, William Zavadoski, Shigeru Kishida and Yoshiaki Kawasaki,			U.S.Cosmetics Corporation, US
US 8,835,509 B2	2014	Kanchan Kohli, Sunny Chopra, Saurabh Arora, Roop K. Khar, Kolappa K. Pillai			Arbro Pharmaceuticals Ltd., New Delhi

**Table 14:** Various SNEDDS prepared till date

Drug	Composition of L-SNEDDS	Techniques of solidification	Formulation prepared	Carrier Used	Stage of development	References
Loratidin	Liquid paraffin, Capriole, Span 20 and Transcutol	Extrusion Spheronization	S-SNEDDS	Aerosil	Formulation and development	(Abbaspour et al., 2014)
Carvedilol	Capmul MCM, Nikkol HCO 50	Congeaing	S-SNEDDS	Nikkol HCO 50	Preclinical phase	(Singh et al., 2013)
Lovastatin	Capmul MCXM, Nikkol HCO-50, Lutrol F127	Melting method	S-SNEDDS	-	Preclinical phase	(Beg et al., 2015)
Loratadine	Captex 200, Capmul MCM, Cremophor - EL, Cremophor EL	Bead formation by evaporation	S-SNEDDS	Porous polystyrene	Clinical phase	(Han et al., 2004)
Nifedipine	Imwitor 742	Physical adsorption by triturate	S-SNEDDS	Aerosil 200	Formulation and development	(Weerapol et al., 2014)
Vitamin A acetate	Soyabean oil, Capmul MCM-C8, Cremophore EL	Mixing and compression into tablets	SNEDDS tablets	Avicel	Formulation and development	(Taha et al., 2009)
Darunavir	Capmul MCM, Tween 80, Transcutol P,	Physical adsorption	S-SNEDDS	Neusilin US2	Preclinical phase	(Inugala et al., 2015)
Cilostazol	Peceol, Tween 20, Labrasol	Spray dried	S-SNEDDS	Calcium silicate	Preclinical phase	(Mustapha et al., 2017)
Embelin	Capryol 90, Acrysol EL 135, PEG 400	Physical adsorption	S-SNEDDS	Aerosil, Neusilin US2	Formulation and development	(Parmar et al., 2015)
Rosuvastatin calcium,	Garlic oil, olive oil, Tween-80, PEG 400	Physical mixing	Solid supersaturable SNEDDS	Maltodextrin and MCC 102	Preclinical phase	(Abo Enin and Abdel-Bar, 2016)
Tacrolimus	Capryol PGMC, Transcutol HP, Labrasol	Absorption method	S-SNEDDS	Colloidal silica	Preclinical phase	(Seo et al., 2015)



Valsartan	Capmul MCM, Labrasol, Tween 20	Adsorption method	S-SNEDDS	Aerosil 200, Sylsya (350, 550, 730), Neusilin US2	Preclinical phase	(Beg et al., 2012)
Loratidine	Solutol HS 15, Capmul MCM C8	Adsorption method	S-SNEDDS	Aerosil (A200), Aerosil (AR972)	Preclinical phase	(Verma et al., 2016)
Celecoxib	Capryol 90, Cremophor RH 40, Propylene glycol	-	SNEDDS	-		(Kaur et al., 2013)
Rosuvastatin	Capryol 90, poloxamer 407, Transcutol P	Spray dried	S-SNEDDS	Mannitol	Formulation and development	(Kamel and Mahmoud, 2013)
Flurbiprofen	Labrafil M 1944, Labrasol, Transcutol HP	Spray dried	S-SNEDDS	Hydrophobic and hydrophilic carriers	Formulation and development	(Kang et al., 2012)
Glimepiride	Tween 80, PEG and Mygliol 812	Physical adsorption	S-SNEDDS	Aerosol@ 200	Preclinical phase	(Mohd et al., 2015)
Olmesartan medoxomil	Oelic acid, Tween 80 and Transcutol HP	Surface adsorption method	S-SNEDDS	Aerosil 200, Aeroperl GT, Sylsya 550, Neusilin US2 and Fujicalin SG	Preclinical phase	(Beg et al., 2016)
Repaglinide	Olive oil, Miglyol Cremophore RH 40, Capryol 90 and Labrasol	Adsorption technique	S-SNEDDS	Neusilin US2	Formulation and development	(Reddy et al., 2014)
Erlotinib	Labrafil M2125CS, Labrasol, and Transcutol HP	Spray dried	S-SEDDS	Dextran or Aerosil	Preclinical phase	(Truong et al., 2016)
Docetaxel	Capryol 90, Cremophore EL and Transcutol HP	Absorption method	S-SNEDDS	Colloidal silica	Preclinical phase	(Quan et al., 2012)
Simvastatin	Capryol 90, Cremophore RH 40, Transcutol HP	Adsorption technique	S-SNEDDS	Crospovidone	Formulation and development	(Sunitha Reddy and Sowjanya, 2015)
Irbesartan	Capryol 90, Cremophor RH40 and Transcutol HP	Spray dried	S-SNEDDS	Aerosil 200	Research	(Nasr et al., 2016)
Glipizide	Captex 355, Solutol HS15 and Imwitor 988	Physical mixing	S-SNEDDS	Calcium carbonate	Formulation and development	(Dash et al., 2015)

## 2.8. Characterization of nanosuspensions and SNEDDS

Characterization of developed nanoformulations is one of important steps in formulation development and optimization. Different evaluation methods used to characterise the nanoformulations along with their advantages and limitation were summarized in Table 15.

**Table 15:** Techniques to characterize nanoparticles with their advantages and limitations

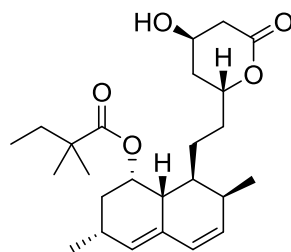
Parameters	Technique used	Advantages	Limitations
Particle size and size distribution	Laser diffraction	a. Wide range of measurement b. Rapid c. Non-invasive d. Apply to both liquid suspension and dry powder samples	Particles are assumed to be spherical
	Coulter counter	More precised	Apply only to spherical particles
	PCS/DLS	rapid, non-invasive	a. Limited measurement range b. Apply only to liquid suspension
Particle surface charge/zeta potential	Laser Doppler electrophoresis	Precised and rapid	-
Particle size and morphology	SEM/TEM	a. Evaluate both particle morphology and size b. Very small quantity of sample required	a. Challenging to acquire statistical size distribution b. Time-consuming c. Usually invasive,
	AFM	a. Non-invasive b. Evaluate both particle morphology and size c. Very small quantity of sample required.	a. time-consuming b. Challenging to acquire statistical size distribution
Crystallinity state	XRD/DSC	Provides information of drug crystallinity, polymorphism as well as crystal stability	-
Chemical interactions	HPLC/FTIR/ATR-IR/NMR/MS/LCMS	Sensitive and selective	-

## 2.9. Drug Profile

### 2.9.1. Simvastatin

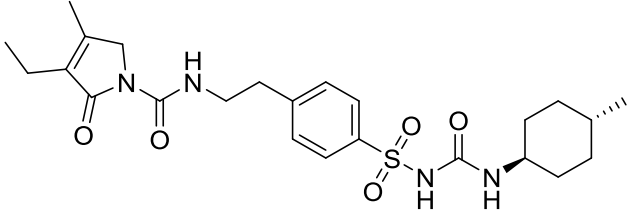
Characteristic	Description (www.drugbank.com)
Drug name	Simvastatin
Category	Antihyperlipidemic
Formula	C <sub>25</sub> H <sub>38</sub> O <sub>5</sub>
Molecular weight	418.566 g/mol
Synonyms	Simvastatin, Simvastatina, Zocor
IUPAC name	(1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate

Chemical structure



Water Solubility	Insoluble
Melting point	135-138 °C
Log P	4.68
Absorption	100%
Protein binding	Approximately 95%
Half life	Approx. 3 hours

## 2.9.2. Glimepiride

Characteristic	Description (www.drugbank.com)
Drug name	Glimepiride
Category	Antidiabetic
Formula	C <sub>24</sub> H <sub>34</sub> N <sub>4</sub> O <sub>5</sub> S
Molecular weight	490.619 g/mol
Synonyms	Amaryl, glimepiride, glimepiride, glimepiridum
IUPAC name	3-ethyl-4-methyl-N-{{2-[4-{{(4-methylcyclohexyl)carbonyl}amino)sulfonyl}phenyl]ethyl}-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide.
Chemical structure	 <p>The chemical structure of Glimepiride is shown. It consists of a 3-ethyl-4-methyl-1H-pyrrole-2(1H)-one ring system. The nitrogen atom of the pyrrole ring is substituted with a carbonyl group, which is further substituted with a 2-ethyl-4-((4-methylcyclohexyl)carbonylamino)sulfonylphenyl group. The cyclohexane ring is shown with a dashed bond to indicate stereochemistry.</p>
Water Solubility	Insoluble
Melting point	207°C
Log P	3.5
Absorption	100 %
Protein binding	More than 99.5%
Half life	Approx 5 hours

### **3. HYPOTHESIS OF RESEARCH**

The reports of WHO state that among diabetics about 40-60% are obese and suffer from diabetic dyslipidaemia. From 1980 to till date, the global prevalence of people suffering from DM has been doubled, rising from 4.7% to 8.5% in the adults. The data also reflects towards rise in associated risk factors due to obesity or overweight. Oral sulphonyl ureas and statins are widely used together for the treatment of hyperlipidemia and hyperglycaemia, two disorders which are known to be closely associated with each other. Among oral sulphonyl ureas, glimepiride is the one which is frequently prescribed by physicians for treatment type – II diabetes mellitus. Simvastatin is one of the commonly used statins to treat hyperlipidemia in diabetic patients. Long term use of oral sulphonyl ureas is reported to result in fat depositions in the vital organs of body. Hence, formulation of binary mixture of these two drugs is expected to provide a rational combination therapy for the treatment of patients suffering from the commonly prevalent co-morbidities i.e. atherosclerosis and type II diabetes mellitus.

The major challenge associated with glimepiride and atorvastatin is that they belong to BCS (biopharmaceutical classification system) class II and exhibit poor solubility and thereby dissolution rate limited bioavailability. As discussed earlier, preparation of nanosuspension could be a promising approach to overcome their dissolution limited bioavailability issues. In present study these two drugs will be formulated in a single unit dosage form by the preparation of nanosuspension.

#### **4. AIM**

Formulation and evaluation of nanosuspensions and solid-SNEDDS containing simvastatin and glimepiride to treat T2DM and hyperlipidaemia associated with it.

##### ***4.1. Objectives***

Formulation development of nanosuspension containing glimepiride and simvastatin.

- Formulation development of SNEDDS containing glimepiride and simvastatin.
- In vitro evaluation of nanosuspension and SNEDDS containing glimepiride and simvastatin
- In vivo evaluation of nanosuspension and SNEDDS containing glimepiride and simvastatin

## 5. MATERIALS & METHOD

### 5.1. Materials

**Table 16:** List of materials used in study

Chemicals	Manufacturers
Simvastatin	Yarrow Chem, Pvt. Ltd, Mumbai, India
Glimepiride	Yarrow Chem, Pvt. Ltd, Mumbai, India
Acetonitrile HPLC Grade	Lobachemie Pvt. Ltd., Mumbai, India
Sodium Hydroxide pellets	Central drug house Pvt. Ltd, New Delhi, India
Orthophosphoric acid	Lobachemie Pvt. Ltd., Mumbai, India
Triethylamine	Lobachemie Pvt. Ltd., Mumbai, India
Ethanol	Central drug house Pvt. Ltd, New Delhi, India
Aerosil 200	Central drug house Pvt. Ltd, New Delhi, India
Potassium Dihydrogen Orthophosphate (KH <sub>2</sub> PO <sub>4</sub> )	Central drug house Pvt. Ltd, New Delhi, India
Hydrochloric acid	Lobachemie Pvt. Ltd., Mumbai, India
Ammonium acetate	Lobachemie Pvt. Ltd., Mumbai, India
Millipore water	Bio-Age Equipment Ltd., Mohali, India
Hydrochloric acid	Lobachemie Pvt. Ltd., Mumbai, India
Lauroglycol FCC	Gattefosse Pvt. Ltd, Mumbai, India
Tween (80,20 and 60)	Central drug house Pvt. Ltd, New Delhi, India
Span(20,40,60 and 80)	Central drug house Pvt. Ltd, New Delhi, India
PEG (200,400,600 and 800)	Central drug house Pvt. Ltd, New Delhi, India
Pluronic F-68	Central drug house Pvt. Ltd, New Delhi, India
Sesame oil	Central drug house Pvt. Ltd, New Delhi, India
Peanut oil	Central drug house Pvt. Ltd, New Delhi, India
Sunflower oil	Central drug house Pvt. Ltd, New Delhi, India
Cotton seed oil	Central drug house Pvt. Ltd, New Delhi, India
Soyabean oil	Central drug house Pvt. Ltd, New Delhi, India
Mustard oil	Central drug house Pvt. Ltd, New Delhi, India
Oleic acid	Central drug house Pvt. Ltd, New Delhi, India
Olive oil	Central drug house Pvt. Ltd, New Delhi, India
Eucalyptus oil	Central drug house Pvt. Ltd, New Delhi, India
Castor oil	Central drug house Pvt. Ltd, New Delhi, India
Hydroxy propyl beta cyclodextrin (HPBCD)	Central drug house Pvt. Ltd, New Delhi, India
Polyvinyl alcohol (PVA)	Central drug house Pvt. Ltd, New Delhi, India
Sodium carboxy methyl cellulose (NA-CMC)	Central drug house Pvt. Ltd, New Delhi, India
Formic acid	Lobachemie Pvt. Ltd., Mumbai, India
Trehalose	Lobachemie Pvt. Ltd., Mumbai, India
Mannitol	Lobachemie Pvt. Ltd., Mumbai, India
Sorbitol	Lobachemie Pvt. Ltd., Mumbai, India
Labrafac CC	Gattefosse Pvt. Ltd., Mumbai, India
Labrafil MI944CS	Gattefosse Pvt. Ltd., Mumbai, India
Labrafil M2125	Gattefosse Pvt. Ltd., Mumbai, India
Labrasol	Gattefosse Pvt. Ltd., Mumbai, India
Maisine 35-1	Gattefosse Pvt. Ltd., Mumbai, India
Capryol 90	Gattefosse Pvt. Ltd., Mumbai, India
Miglyol 812N	Cremer Ole GmbH& Co.KG, Germany
Syloid XDP3150	Grace Material Technologies, Discovery Sciences, Pune ,India
Capmul MCM	M/S Abitec Corp., Ohio
Transcutol P	Gattefosse Pvt. Ltd., Mumbai, India
Syloid 244 FP	Grace Material Technologies, Discovery Sciences, Pune ,India
Cithrol GMS	Croda Chemicals Pvt. Ltd, Navi Mumbai, India

Triacetin	Sigma Aldrich, St. Louis, USA
Egg phosphatidyl Choline	Lipoid GmbH, Ludwigshafen, Germany
Soya phosphatidyl Choline	Sigma Aldrich, St. Louis, USA
Lactose	Lobachemie Pvt. Ltd., Mumbai, India

## 5.2. Equipment

**Table 17:** List of equipment used in the study

Equipments	Model/Manufacturer
Electronic weighing balance	CY360, Shimadzu Co. Ltd., Kyoto, Japan
Dissolution apparatus	DS 8000 (Manual) Lab India, Mumbai, India
pH meter	Phan, Lab India, Mumbai, India
High performance liquid chromatography	HPLC LC-20AD, Shimadzu Co. Ltd., Kyoto, Japan
UV spectrophotometer	UV-1800, Shimadzu Co. Ltd., Kyoto, Japan
Spray dryer	JISL Spray Mate, Jay Instruments, Navi Mumbai, India
Ultrasonication bath	Loba Life, Lobachemie, Mumbai, India
Hot air oven	Cadmach Drying Oven, Cadmach Machinery Ltd., Ahmadabad, India
Sieves	Sieve No. 44, Bhushan Engineering & Scientific Traders, Ambala, India
Magnetic stirrer	Remi 5MLH, Vasai, Mumbai, India
FTIR spectrophotometer	Shimadzu Co. Ltd., Kyoto, Japan
Stability chamber	Remi CHM 10S, Remi Sales & Engineering Ltd., Mumbai, India
Differential scanning calorimeter	DSC Q200 V24.4 Build 116
Scanning electron microscope	Hitachi S-3400N
Transmission electron microscope	FEI Tecnai G 2 F20 model, Netherlands
XRD analyzer	PAN analytical X'pert 3 Pro, Netherlands
Partilce size	Zetasizer, Malvern Instruments Ltd., Malvern, U. K.



## **6. EXPERIMENTAL WORK**

### ***6.1. Analytical method development***

The HPLC system consisted of a mobile phase delivery pump (LC-20 AD; Shimadzu, Japan), a photodiode array detector (SPDM20A; Shimadzu, Japan), a 20 $\mu$ L loop (Rheodyne) and LC Solution software. A C-18 reverse-phase column (Nucleodur C18, 250 mm  $\times$  4.6 mm i.d., 5 $\mu$ ) was utilized for estimation and separation of simvastatin (SIM) and glimepiride (GLM) in SIM-GLM mixture, using acetonitrile and potassium dihydrogen phosphate buffer pH 5 (75:25, v/v) as mobile phase. The flow rate was 1 mL min<sup>-1</sup> and detection wavelength was 232 nm. Standard solutions (2, 4, 6, 8 and 10  $\mu$ g/mL) were prepared in mobile phase and analysed. The developed method was validated as per ICH Q2 (R1) guidelines.

### ***6.2. Method validation***

#### **6.2.1. Preparation of quality control standards**

The quality control standards were prepared at three different levels i.e., lower quality control standards (LQC), Medium quality control standards (MQC) and Higher quality control standards (HQC) of calibration curve. Hence, 6 $\mu$ g/mL was kept as 100% (MQC) level and 80% of 6 $\mu$ g/mL (i.e., 4.8  $\mu$ g/mL) as LQC and 120% of 6 $\mu$ g/mL (i.e. 7.2  $\mu$ g/mL) was kept as HQC levels. All the three concentrations were prepared in plasma as well as in mobile phase.

##### ***6.2.1.1. Linearity and range:***

The calibration curve was developed by plotting the graph between mean peak area of five replicates versus corresponding concentrations of SIM and GLM, and the regression equation was recorded.

##### ***6.2.1.2. Accuracy:***

The accuracy of method was developed through calculation of recovery of the drug from the quality control standard solutions prepared in mobile phase and plasma. The LQC, MQC and HQC standard solutions were injected 6 times to HPLC and its mean of response was recorded. Percentage recovery was calculated by dividing the actual recovery of drug to their theoretical concentration and multiplying them by hundred.

The mean of response was recorded and percentage relative standard deviation was calculated as per equation -1

$$\text{Percent recovery} = \frac{\text{Actual concentration recovered}}{\text{Theoretical concentration}} \times 100 \quad \text{Eq. (1)}$$

#### 6.2.1.3. Precision:

Precision of the method was evaluated in terms of repeatability and intermediate precision. Repeatability was tested by injecting six times the samples of LQC, MQC and HQC on the same day and under same experimental conditions. The intermediate precision was evaluated by determining LQC, MQC and HQC samples six times on each of three different days (inter-day) as well as by the three different analysts (inter-analyst) under the same experimental conditions. The mean of response was recorded and percentage relative standard deviation was calculated.

#### 6.2.1.4. Robustness:

In order to check the effect of small changes on robustness of the developed method, the study was carried out by varying pH of the mobile phase (3.8, 4.0 and 4.2), flow rate (0.8, 1 and 1.2 mL/min) and ratio of mobile phase phosphate buffer: methanol as [73:27; 75:25, and 77:23 v/v], respectively. Six replicates of medium concentration (6µg/mL) were injected and their effect on area of the peak, recovery and retention time was observed and mean of response was recorded.

### 6.2.2. Estimation of LOD and LOQ

LOD and LOQ were determined by standard deviation of response (sigma) and slope of calibration curve (S). Standard deviation of Y intercepts of regression line was used as standard deviation.

$$LOD = \frac{3.3 \sigma}{S} \quad \text{Eq. (2)}$$

$$LOQ = \frac{10 \sigma}{S} \quad \text{Eq. (3)}$$

### ***6.3. Bioanalytical method development and validation using HPLC***

For quantification of drugs in rat plasma, a bioanalytical method was developed. Method specificity was evaluated by spiking blank plasma and standard drug solution (150 ng/mL) that was prepared in plasma. Suitable dilutions in the range of 50-250 ng/mL were prepared using stock solution of 1000 µg/mL containing GLM and SIM. To all these dilutions 0.5 mL plasma and 1 mL of ATV (1 µg/mL) was spiked as internal standard. To this mixture 1 mL acetone was added and the contents were centrifuged at 35000 g for 15 min. The supernatant was collected and evaporated. Reconstitution was done by using mobile phase and injected to HPLC for analysis at 232 nm.

### ***6.4. Solubility studies of unprocessed simvastatin and glimepiride in water***

Solubility study of unprocessed SIM and GLM in water was performed by shake-flask method at room temperature. In this method, excess amount of pure drug was added into 10 mL of distilled water to get saturated solution. Solution was placed on mechanical shaker that was agitated for 48 h at  $37 \pm 0.2^\circ\text{C}$  in a shaking water bath at 50 rpm. Further solution was kept for 24 h to get equilibrium between dissolved and undissolved drug at room temperature and finally excess drug was removed by filtration using Whatman No.1 filter paper. Sample was evaluated by using RP-HPLC method and solubility of both unprocessed drugs was calculated using calibration curve.

#### **6.4.1. Solubility studies of unprocessed simvastatin and glimepiride in various oils, and stabilizers (surfactants and co-surfactants)**

For preparation of nanosuspensions solubility study of unprocessed GLM (100 mg) and SIM (100 mg) was performed in 50 mL of water using SLS, PEG 4000, and PVP K-30 wherein the drug to solubilizer ratio was varied from 0.25 to 2.0 using mechanical shaker. The speed of shaker was maintained at 50 rpm for 48 h at temperature of  $37 \pm 0.2^\circ\text{C}$  (Mahesh et al., 2014).

To formulate SNEDDS, solubility of both the drugs was done in various oils, surfactants and co-surfactants. Both the drugs (100 mg each) were added in 1 mL of various oils (olive oil, castor oil, coconut oil, sesame oil, sunflower oil, peanut oil, eucalyptus oil, oleic acid, mustard oil, cotton seed oil, Labrafac, Triacetin, LMCS,

CMCM, LM2125, soyabean oil, C 90, LFCC, M 35-1, M812N, and CGMS 40), surfactants (PEG 200, PEG 400, PEG 600, PEG 800, PG, T20, T60, T80, S20, S40, S60, S80, egg phosphatidyl choline (EPC), soya phosphatidyl choline (SPC) and Labrasol) and co-surfactants (TP, ethanol). Solutions (1% w/v) of EPC and SPC were prepared using ethanol and water mixture in the ratio of 1:1. The prepared samples were transferred in glass vials (5 mL capacity). The liquid samples were mixed using cyclone mixer (CM 101, REMI, India) for 2 min and closed using rubber cap. Mechanical shaking was carried out for all vials for 48 h. The speed of shaker was maintained at 50 rpm at temperature of bath was  $37 \pm 0.2^\circ\text{C}$ . Upon completion of shaking, all the samples were centrifuged at 11200 g for 15 min and supernatant was collected. The samples were diluted using ethanol and injected to HPLC for estimation of drug (Garg et al., 2017; Inugala et al., 2015; Rajesh et al., 2018). The experiments were carried out in triplicate and mean data was recorded.

**Table 18:** Solubility of GLM & SIM using different surfactants

Name of surfactant	Drug to surfactant ratio
Sodium lauryl sulphate (SLS)	1/0.25
	1/0.50
	1/0.75
	1/1
	1/1.5
	1/2
Poly ethylene glycol 4000 (PEG 4000)	1/0.25
	1/0.50
	1/0.75
	1/1
	1/1.5
	1/2
Poly vinyl pyrrolidone (PVP K-30)	1/0.25
	1/0.50
	1/0.75
	1/1
	1/1.5
	1/2

### ***6.5. Preparation of nanosuspensions***

Nanosuspension was prepared using bottom-up technique i.e. anti- solvent addition and drug precipitation method. The prepared liquid nanosuspension was solidified into free flowing powder by spray drying technique. The various process variables

affecting size and charge of nanosuspensions were optimized using design of experiments. Different variables that were screened during initial studies were drug to surfactant ratio, polymer to drug ratio, solvent to anti-solvent ratio, solvent addition rate, time of mixing and speed of mixing. It was observed that surfactant to drug ratio, polymer to drug ratio, solvent to antisolvent ratio and speed of mixing significantly affected the responses. Hence in order to find out their optimum ratio, Box–Behnken Design (BBD) was explored.

#### **6.5.1. Design of experiments:**

Surfactant to drug ratio, polymer to drug ratio, solvent to antisolvent ratio and speed of mixing were varied at three levels (+1, 0, -1) keeping their type constant. Design expert 10. Stat Ease. USA software was used to perform the above study. Table 26 showed the factors with design level and different composition of nanosuspensions respectively. SIM (1 g) and GLM (0.4 g) were accurately weighed and dissolved in 40 mL of acetone as solvent. These were added to a glass beaker of 1000 mL capacity containing 500 mL of water (antisolvent) containing SLS and PVP K-30 using a glass burette (50 mL). The liquid inside beaker was stirred using Silverson's homogenizer (REMI, India) at a particular speed for 4 h. The amount of excipients, solvent to antisolvent ratio and stirring speed were kept as per Table 1.

#### **6.6. Solidification of liquid nanosuspensions using spray dryer**

Optimized batch of liquid nanosuspensions was dried using spray dryer. The suspension was sprayed to the nozzle of 0.7 mm diameter with atomization air pressure of 4 kg/cm<sup>2</sup>. Suspension was fed using a peristaltic pump at a flow rate of 20 mL/min. The inlet was kept 120°C and a recorded outlet temperature was 55°C. The spray dried nanosuspension powder (SP-NS) was stored in a desiccator till further use.

#### **6.7. Preparation and optimization of L-SNEDDS using ternary phase diagram**

Based on the outcomes of solubility studies, CMCM and LMCS were chosen as oils, T80 and TP were chosen as surfactant and co-surfactant respectively. The individual oils and surfactant and co-surfactant were mixed in the ratio of 1:9 to 9:1 and diluted to triple distilled water (500 mL) to form SNEDDS. However, the formed emulsion

was turbid and shows phase separation within 2 h of storage. Hence, these were discontinued from further evaluation. In the similar way, total 81 SNEDDS prototypes have been developed by combination of CMCM and LMCS as  $O_{mix}$  and combination of T80 and TP as ( $S_{mix}$ ) in the ratio of 1:9 to 9:1, wherein, internal ratios of both,  $O_{mix}$  and  $S_{mix}$  were varied from 1:1, 2:1 to 1:2. In small increments, GLM and SIM (5 mg & 2 mg respectively) were added in combination to all prepared formulations and blended using vortex mixer to form a monophasic system. The prepared isotropic mixtures were stored in clean glass vials (screw capped) at room temperature until their further assessment (Garg et al., 2019a; Garg et al., 2017; Inugala et al., 2015). Different compositions of isotropic mixtures are shown in Table 19. The prepared isotropic mixtures were diluted in triple distilled water (500 mL) to analyse their quality based on parameters such as level of transparency upon dilution, drug precipitation, rapidity of formation of emulsion and phase separation. To understand the formulation of SNEDDS, glass beaker (500 mL capacity) was filled with water (500 mL) and kept rotated on a magnetic stirrer (REMI, India) at 100 rpm using glass bead at temperature of  $37 \pm 0.2^\circ\text{C}$ . The L- SNEDDS sample (1 mL) was dropped in the beaker and emulsion formation was noted. Ternary phase diagram was constructed using Triplot software (version 4.1.2 by Todd-Thompson), wherein, the obtained emulsions were categorised as transparent (SNEDDS), translucent (SMEDDS), opaque (emulsion) and phase separation. The formulations falling under SNEDDS region were selected and subjected to further evaluation.

#### ***6.8. Stability evaluation of optimized L-SNEDDS formulation***

Stability of the optimized L-SNEDDS formulation was evaluated using three parameters; a. temperature variation; b. centrifugation; c. cloud point. The formulation was subjected to thermal stress by heating cooling cycles ( $4^\circ\text{C}$  and  $40^\circ\text{C}$ ), freeze thaw cycles ( $-21^\circ\text{C}$  and  $+25^\circ\text{C}$ ), and storage stability at  $40^\circ\text{C}$  for 48 h. Centrifugation stress was provided by centrifuging the diluted SNEDDS sample at 11200 g for 15 min. The diluted SNEDDS were prepared by addition of 1 mL of the formulation to 500 mL of distilled water. After centrifugation, the SNEDDS were visually observed for instability (phase separation and drug precipitation) (Inugala et al., 2015; Kallakunta et al., 2012). Cloud point was determined by heating 100 mL of diluted L-SNEDDS

on a water bath at temperature that was gradually increased from 25 to 100°C. Heating was stopped upon appearance of cloudiness and temperature was recorded as its cloud point (Inugala et al., 2015; Zhang et al., 2008b).

#### **6.9. Oil adsorption capacity (OAC)**

L-SNEDDS were converted into free-flowing powder using series of porous carriers. Hydrophobic carriers used were A-200, SXDP, MS, SFP, lactose and MCC PH102. Hydrophilic carriers used were PVA, Na-CMC and HPBCD, were used. OAC was determined using gravimetric method. OAC was calculated as the amount of porous carrier required to transform the unit dose of oily liquid formulation into the free-flowing powder (Malaysia, 2012; Modasiya et al., 2009). OAC will be considered high if the amount of carrier required will be less (Kumar et al., 2018).

#### **6.10. Preparation of solid SNEDDS (S-SNEDDS) using spray drying**

A series of S-SNEDDS were prepared by dissolving 1g of each of the carriers (A-200, SFP, SXDP, MCC PH 102, MS, PVA, Na-CMC and HPBCD) by dissolving them in 100 mL of solvent. Hydrophilic and hydrophobic carriers were dissolved in water and ethanol, respectively. To each of these dispersions, 1 mL of L-SNEDDS was added and stirred at 100 rpm using magnetic stirrer to achieve homogenous dispersion (Kumar et al., 2018). The formed dispersions were spray dried under following conditions:

Nozzle diameter: 0.7 mm

Peristaltic pump flow rate: 16 mL/min

Atomization air pressure: 4 Kg/cm<sup>2</sup>

Aspirator filter pressure: -25 mbar

Inlet air temperatures: 70°C (for dispersions made in ethanol) and 120°C (for dispersions prepared in water).

Recorded outlet temperatures: 36°C (for dispersions made in ethanol) and 57°C (for dispersions prepared in water).

**Table 19:** Composition of L-SNEDDS

Formulation	*O <sub>mix</sub> (1:1)	**S <sub>mix</sub> (1:1)	Formulation	*O <sub>mix</sub> (1:2)	**S <sub>mix</sub> (1:1)	Formulation	*O <sub>mix</sub> (2:1)	**S <sub>mix</sub> (1:1)
F <sub>1</sub>	0.5:0.5	4.5:4.5	F <sub>28</sub>	0.3:0.7	4.5:4.5	F <sub>55</sub>	0.7:0.3	4.5:4.5
F <sub>2</sub>	1:1	4:4	F <sub>29</sub>	0.7:1.3	4:4	F <sub>56</sub>	1.3:0.7	4:4
F <sub>3</sub>	1.5:1.5	3.5:3.5	F <sub>30</sub>	1:2	3.5:3.5	F <sub>57</sub>	2:1	3.5:3.5
F <sub>4</sub>	2:2	3:3	F <sub>31</sub>	13:2.7	3:3	F <sub>58</sub>	2.7:1.3	3:3
F <sub>5</sub>	2.5:2.5	2.5:2.5	F <sub>32</sub>	1.7:3.3	2.5:2.5	F <sub>59</sub>	3.3:1.7	2.5:2.5
F <sub>6</sub>	3:3	2:2	F <sub>33</sub>	2:4	2:2	F <sub>60</sub>	4:2	2:2
F <sub>7</sub>	3.5:3.5	1.5:1.5	F <sub>34</sub>	2.3:4.7	1.5:1.5	F <sub>61</sub>	4.7:2.3	1.5:1.5
F <sub>8</sub>	4:4	1:1	F <sub>35</sub>	2.7:5.3	1:1	F <sub>62</sub>	5.3:2.7	1:1
F <sub>9</sub>	4.5:4.5	0.5:0.5	F <sub>36</sub>	3:6	0.5:0.5	F <sub>63</sub>	6:3	0.5:0.5
		**S <sub>mix</sub> (1:2)			**S <sub>mix</sub> (1:2)			**S <sub>mix</sub> (1:2)
F <sub>10</sub>	0.5:0.5	3:6	F <sub>37</sub>	0.3:0.7	3:6	F <sub>64</sub>	0.7:0.3	3:6
F <sub>11</sub>	1:1	2.7:5.3	F <sub>38</sub>	0.7:1.3	2.7:5.3	F <sub>65</sub>	1.3:0.7	2.7:5.3
F <sub>12</sub>	1.5:1.5	2.3:4.7	F <sub>39</sub>	1:2	2.3:4.7	F <sub>66</sub>	2:1	2.3:4.7
F <sub>13</sub>	2:2	2:4	F <sub>40</sub>	13:2.7	2:4	F <sub>67</sub>	2.7:1.3	2:4
F <sub>14</sub>	2.5:2.5	1.7:3.3	F <sub>41</sub>	1.7:3.3	1.7:3.3	F <sub>68</sub>	3.3:1.7	1.7:3.3
F <sub>15</sub>	3:3	1.3:2.7	F <sub>42</sub>	2:4	1.3:2.7	F <sub>69</sub>	4:2	1.3:2.7
F <sub>16</sub>	3.5:3.5	1:2	F <sub>43</sub>	2.3:4.7	1:2	F <sub>70</sub>	4.7:2.3	1:2
F <sub>17</sub>	4:4	0.7:1.3	F <sub>44</sub>	2.7:5.3	0.7:1.3	F <sub>71</sub>	5.3:2.7	0.7:1.3
F <sub>18</sub>	4.5:4.5	0.3:0.7	F <sub>45</sub>	3:6	0.3:0.7	F <sub>72</sub>	6:3	0.3:0.7
		**S <sub>mix</sub> (2:1)			**S <sub>mix</sub> (2:1)			**S <sub>mix</sub> (2:1)
F <sub>19</sub>	0.5:0.5	6:3	F <sub>46</sub>	0.3:0.7	6:3	F <sub>73</sub>	0.7:0.3	6:3
F <sub>20</sub>	1:1	5.3:2.7	F <sub>47</sub>	0.7:1.3	5.3:2.7	F <sub>74</sub>	1.3:0.7	5.3:2.7
F <sub>21</sub>	1.5:1.5	4.7:2.3	F <sub>48</sub>	1:2	4.7:2.3	F <sub>75</sub>	2:1	4.7:2.3
F <sub>22</sub>	2:2	4:2	F <sub>49</sub>	13:2.7	4:2	F <sub>76</sub>	2.7:1.3	4:2
F <sub>23</sub>	2.5:2.5	3.3:1.7	F <sub>50</sub>	1.7:3.3	3.3:1.7	F <sub>77</sub>	3.3:1.7	3.3:1.7
F <sub>24</sub>	3:3	2.7:1.3	F <sub>51</sub>	2:4	2.7:1.3	F <sub>78</sub>	4:2	2.7:1.3
F <sub>25</sub>	3.5:3.5	2:1	F <sub>52</sub>	2.3:4.7	2:1	F <sub>79</sub>	4.7:2.3	2:1
F <sub>26</sub>	4:4	1.3:0.7	F <sub>53</sub>	2.7:5.3	1.3:0.7	F <sub>80</sub>	5.3:2.7	1.3:0.7
F <sub>27</sub>	4.5:4.5	0.7:0.3	F <sub>54</sub>	3:6	0.7:0.3	F <sub>81</sub>	6:3	0.7:0.3

\*O<sub>mix</sub> - CMCM; L MCS; \*\*S<sub>mix</sub>: T80: TP



### **6.11. Micromeritic evaluation of developed SP-NS and S-SNEDDS formulation**

The micromeritic behaviour of SP-NS powder and S-SNEDDS powder were determined by calculating true, bulk and tapped density, flow rate, angle of repose and Carr's compressibility index. The experiments were carried out as reported by Kaur et al., (2015) (Kaur et al., 2015). Angle of repose (AOR) was done by pouring accurately weighed powders over the funnel (clamped above a graph paper) until the formation of conical pile on a graph paper. The gap between paper and funnel was kept 7 mm. The AOR was calculated using Eq. 4. The untapped volume occupied by powder was measured as bulk volume and bulk density was calculated by the formula given in Eq. 5. Tapped volume was calculated by tapping 100 times the cylinder filled with powder and Tapped density ( $\rho_t$ ) as well as Carr's compressibility index (CI) were calculated using the Eqs. 6. and 7.

$$\tan\theta = \frac{2h}{D} \quad \text{Eq. (4)}$$

Here, h = Height of the heap of powder; D = Diameter of the base of the heap of powder

$$\rho_b = \frac{M}{V_b} \quad \text{Eq. (5)}$$

Here,  $\rho_b$  = Bulk density;  $V_b$  = Bulk volume; M = Weight of powder

$$\rho_t = \frac{M}{V_t} \quad \text{Eq. (6)}$$

Where,  $V_t$  = Minimum volume occupied by the blend in the cylinder; M = Weight of the blend.

$$CI = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \quad \text{Eq. (7)}$$

### **6.12. Calculation of drug loading**

The L-SNEDDS and S-SNEDDS containing SIM (equivalent to 5 mg) and SIM (equivalent to 2.5 mg) were added to 1 mL of optimized batch of L-SNEDDS and S-SNEDDS and mixture was vortexed for 15 min. This mixture was diluted with double distilled water (500 mL) and stirred at 500 rpm. The temperature of water was

maintained at 37°C. Sample (5 mL) was withdrawn and centrifuged at 11200 g for 15 min for removal of the undissolved SIM and GLM. Suitable dilutions were made using distilled water and area of diluted samples was recorded by injecting samples to HPLC at 232 nm. The percentage drug loading was calculated as per the formula given in Eq. 8. The process was repeated for SP-NS. An amount of powder containing SIM (equivalent to 5 mg) and GLM (equivalent to 2.5 mg) was taken and dilute to 100 mL of double distilled water. Suitable dilutions were made and sample analysis was carried out as reported for SNEDDS samples.

$$\% \text{ Drug loading} = \frac{\text{Area of test drug present in SNEDDS}}{\text{Area of known standard}} \times 100 \quad \text{Eq. (8)}$$

### **6.13. Droplet size and polydispersity index (PDI)**

Droplet size, PDI and zeta potential of SP-NS, L-SNEDDS and S-SNEDDS were determined using zeta sizer (nano ZS90, Malvern Instruments Ltd., UK). The readings were noted (in triplicate) using a laser beam (50 mV) at an angle of 90° in a disposable polystyrene cells maintained at 25°C. After suitable dilution, samples were subjected for 12 sub-runs within 2 min. to record the results (Sood et al., 2014).

### **6.14. Transmission electron microscopy (TEM)**

The morphology of optimized S-SNEDDS droplets was measured using TEM (H-7500, Hitachi, Japan). The sample (100 µL) was diluted to 10 mL using double distilled water. One drop of emulsion was taken and spread over carbon-coated copper grid for formation of film and kept for drying. Afterwards it was negatively stained using one drop of 2%w/v phosphotungstic acid (PTA) solution and it was air dried. The sample was analyzed through TEM (Inugala et al., 2015).

### **6.15. Powder X-ray Diffraction (PXRD) studies:**

The PXRD patterns of unprocessed SIM and GLM, SLS, PVPK-30, A-200, physical mixture of SIM and GLM, SP-NS of SIM-GLM and optimized S- SNEDDS powder were recorded using an X-ray diffractometer. Samples were scanned using copper line as radiation source at scanning rate of 0.010°min<sup>-1</sup> over a 2θ range between 3-80° angle at 40-kV voltage and 40-mA current (Renuka et al., 2014). For the experiment Bruker axs (D8 Advance, Coventry, U.K.) instrument was used.

### **6.16. Differential scanning calorimetry (DSC) analysis**

The thermograms for unprocessed SIM and GLM, SLS, PVPK-30, A-200, physical mixture of SIM and GLM, SP-NS of SIM and GLM and optimized S-SNEDDS powder. The instrument used was DSC, Q200 TA (Bangalore, India). The analysis was carried out as per Renuka et al., 2014. Two aluminium pans were taken and to one of the pans samples (5 mg) were crimped and one was kept as blank. The pans were heated from 0 to 300°C with a rise in temperature of 10°C/min with continuous supply of nitrogen (flow rate -50 mL/min). TA-Universal Analysis 2000 software (version 4.7A) was used as data station to record the melting points ( $T_m$ ) (Renuka et al., 2014).

### **6.17. Scanning electron microscopy (SEM)**

Unprocessed SIM and GLM, A-200, physical mixture, SP-NS powder and S-SNEDDS were scanned for surface analysis using scanning electron microscopy (SEM). Prior to analysis the samples were fixed on a metallic stub using a conductive tape (12 mm diameter). The data station used was - Supra 35VP (Oberkochen, Zeiss, Germany). The voltage used to accelerate electrons was 1.00 kV (Renuka et al., 2014).

### **6.18. In vitro dissolution studies**

The dissolution study was carried for unprocessed SIM and GLM, L-SNEDDS, S-SNEDDS powder, SP-NS containing an amount equivalent of 5 mg SIM and 2.5 mg GLM. USP type I dissolution apparatus (DS8000, Lab India, Mumbai, India) containing 900 mL of simulated gastric fluid (SGF) (pH 1.2) was used. The temperature of medium was kept at  $37 \pm 0.5^\circ\text{C}$  and speed at  $50 \pm 4$  rpm. Unprocessed SIM and GLM, S-SNEDDS powder, L-SNEDDS and SP-NS were weighed and filled individually into size "0" hard gelatin capsules and kept in basket. The study was carried out for 60 min. and samples (5 mL) were withdrawn at predetermined intervals and filtered using membrane filter (0.2  $\mu\text{m}$ , Millipore, Germany). Centrifugation of filtered samples at 10000 g was done for 15 min and supernatant was collected. The collected samples were analyzed at 232 nm using HPLC. The study was carried out in hexaplicate and mean data ( $\pm$  s.d.) was recorded.

### **6.19. Cellular permeability and post-cytotoxicity tests in Caco-2 cell monolayer**

For better correlation of *in-vitro* dissolution and *ex-vivo* permeability of drugs, the permeability of formed S-SNEDDS and SP-NS and PM carried out using Caco-2 cell monolayer. Experiment was performed by following the steps reported by Rajesh et al. (2018) (Rajesh et al., 2018). In brief, “Caco-2 cells” were seeded at a density of  $1.0 \times 10^5$  cells/well on 12 mm Transwell polycarbonate membrane inserts with 0.4 mm pores and cultured for 21 days. Inserts with a higher transepithelial electrical resistance value than  $300 \Omega/\text{cm}^2$  were washed three times with Hank’s Balanced Salt Solution (pH 6.5) before the transcellular transport assessment. Transport buffer was put into both the apical (A, 0.5 mL) and basolateral sides (B, 1.5 mL). The GLM (2.5 mg) and SIM (5 mg) solutions were obtained by dissolving the drugs with 0.1% dimethyl sulfoxide. SP-NS containing GLM (equivalent to 2.5 mg) and SIM (equivalent to 5 mg) were diluted with water (10 mL). The drug solutions and SNEDDS were added into the apical and basolateral sides of the cell inserts, respectively, to reach a content of 20  $\mu\text{M}$ . Then, 0.1 mL of the basolateral or apical side solutions were sampled at predetermined times intervals of 1, 2, 3 and 4 h. The withdrawn volume was replaced with the equivalent volume of fresh transport media. Samples were filtered and analyzed by HPLC. Apparent permeability coefficient ( $P_{app}$ ) was calculated as given in Eq. 9.

$$P_{app} = \frac{\Delta Q}{(\Delta t. A. C_0)} \quad \text{Eq. (9)}$$

Where,  $\Delta Q$  is the transfer amount (nmol),  $A$  is the filter surface area ( $\text{cm}^2$ ),  $t$  is the time of incubation(s), and  $C_0$  is the initial concentration ( $\mu\text{M}$ ).

After incubation, the Caco-2 cells were treated with 80  $\mu\text{L}$  of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS)-based CellTiter 96 Aqueous One Solution Cell Proliferation Assay Reagent in 5%  $\text{CO}_2$  atmosphere condition at  $37^\circ\text{C}$  for 4 h. The absorbance was detected at 232 nm wavelength by EMax precision microplate reader” (Ke et al., 2016; Rajesh et al., 2018).

### **6.20. Pharmacokinetic Study**

The rats weighing in the range of 250-300 g having age in range of 7-8 weeks were purchased from National Institute of Pharmaceutical Education and Research

(NIPER), Mohali, India. The polypropylene cages lined with husk were used to store the rats. The rats were stored at  $25 \pm 2^\circ\text{C}$  and  $55 \pm 10\%$  relative humidity in a 12 h each of light and dark cycle. Standard pellet diet and water ad libitum were used to feed rats. Prior to conduct of experiment the protocol was scrutinized and got approval for ethical conduct of experiment by Institutional Animal Ethics Committee of School of Pharmaceutical Sciences, Lovely Professional University. The protocol number for study was LPU/IAEC/2018/24.

The crossover study was carried out using 18 rats that were divided into three groups, each group containing 6 rats. The oral dose used for GLM was 2.5 mg and SIM was 5 mg. Rats of group 1 received unprocessed GLM and SIM, group 2 received SNEDDS of GLM and SIM and group 3 received nanosuspension of GLM and SIM. The rats received the formulations in an empty stomach. After a washout period of 7 days, group 1 rats received SNEDDS of GLM and SIM and group 2 rats received GLM and SIM. Further, after washout period of 7 days, group 1 rats received nanosuspension of GLM and SIM and group 3 rats received GLM and SIM. In all the cases, blood samples (0.2 mL) were collected at 0, 0.5, 1 and 2 h from first 4 rats of all the groups and at 5, 10, 18 and 24 h from next 4 rats of all the groups in vials containing potassium oxalate as anticoagulant. The blood samples were mixed well, centrifuged at 35000 g for 15 min. and the plasma was transferred to 5 mL vials, capped tightly and processed. The area under the curve ( $\text{AUC}_{0-t}$  and  $\text{AUC}_{0-\infty}$ ) was carried out by using PK solver 2.0 software. The relative oral bioavailability was calculated by the formula given in Eq. 10.

$$\text{Relative bioavailability (Fr)} = \frac{(\text{AUC})_{\text{test}} \times \text{D}_{\text{std}}}{(\text{AUC})_{\text{std}} \times \text{D}_{\text{test}}} \quad \text{Eq. 10}$$

Where, AUC – Area under the curve; D – Dose administered

### **6.21. Stability studies**

The formulations, L-SNEDDS, S-SNEDDS and SP-NS were kept for stability studies for six months at  $25 \pm 0.2^\circ\text{C}/65 \pm 5\%$  relative humidity and  $40 \pm 0.2^\circ\text{C}/75 \pm 5\%$  relative humidity in a stability chamber (Remi Electrotechnik, Mumbai, India). The results of aged samples such as droplet/particle size, zeta potential, % drug loading,

dissolution and angle of repose were calculated and compared with their freshly prepared samples. The dissolution profiles of aged and fresh samples were compared using student 't' test and model independent analysis (f2 value). The values were found to be significant if 'p' value was less than 0.05 and profiles were found similar if 'f2' value was more than 50 (Shah et al., 1998).

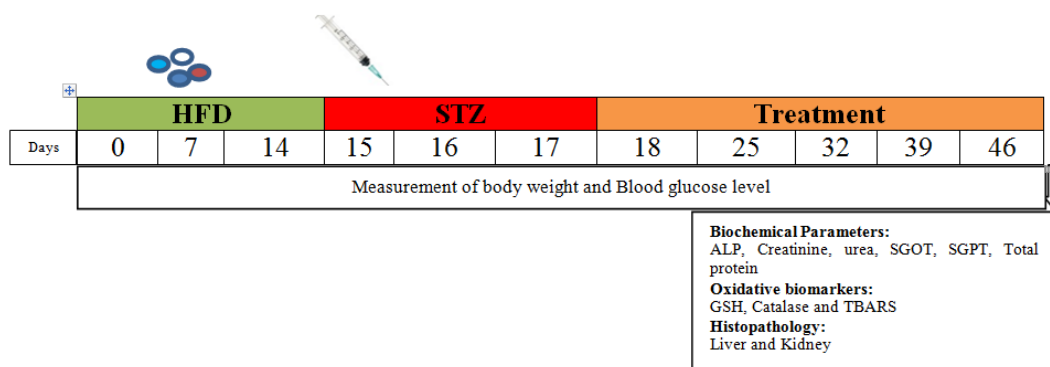
## 6.22. Pharmacodynamics study

### 6.22.1 Induction of diabetes and obesity

All the animals were fed with high fat diet (HFD) for 2 weeks after that induction of diabetes was performed using a single intraperitoneal injection of 45 mg/kg streptozotocin (STZ) in ice cold 0.1 M sodium-citrate buffer (pH 4.5). Age-matched control rats have received an equivalent amount of sodium-citrate buffer. The rats with blood glucose higher than 200 mg/dL were considered as diabetic and considered for further study (Garg et al., 2019b).

### 6.22.2 Treatment design and pharmacological evaluation

All the male Wistar rats were randomly divided into 17 groups after administration of HFD and STZ. All the animals were received their respective treatment from day 18<sup>th</sup> as indicated in Table 20 and continued daily for period of 4 weeks. The biochemical parameters, oxidative biomarkers and histopathology were performed 24 h after administration of last dose at 46<sup>th</sup> day (Fig. 2).



**Fig. 2.** Study procedure used for animal studies

### **6.22.3 Biochemical studies**

For determination of biochemical parameters blood was withdrawn from retro-orbital plexus and collected in plain Eppendorf tubes. Blood samples were processed to separate serum and it was stored in deep freezer before biochemical analysis. Kits of Erba Diagnostics, India (Arunachalam and Parimelazhagan, 2013; Garg et al., 2017) were used to estimate the biochemical parameters. Serum levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT) were estimated to check the liver function of rats of each group. Serum lipid profiles were determined by measuring triglycerides (TG), total cholesterol (TC) and high density lipoprotein (HDL).

### **6.22.4 Determination of in vivo antioxidants**

The kidneys have been separated, weighed and washed for blood removal in ice-cold saline. Isolated kidneys were cut into parts and homogenized (Glass-Teflon pot-ter homogenizer, Thomas Scientific, USA) using pH 7.5 buffer (0.025 M Tris-HCl). Homogenate tissue was centrifuged for 10 min at 4° C at 10,000 rpm. The supernatant was isolated and used for different estimates of antioxidant enzymes. Protein concentrations were estimated by the method of Lowry et al. (1951) (Lowry et al., 1951). Liver homogenates were prepared in ice-cold 10% (w/v) potassium chloride solution, levels and activities of various markers were measured. These include: catalase (CAT) (Sinha et al., 1972), lipid peroxidation (LPO) (Ohkawa et al., 1979), and reduced glutathione (GSH) (Ellman et al., 1959).

### **6.22.5 Histopathological studies**

Paraffin sections of kidney and liver tissues were made and stained with haematoxylin and eosin to observe histopathological changes (Garg et al., 2017b) on 450X under microscope.

### **6.23. Statistical analysis**

All the experimental data are expressed as mean  $\pm$  standard deviation (SD). Statistical assessment of the acquired information was performed using analysis of variance or Tukey's multiple comparison test using GraphPad Prism version 7.0 (GraphPad Software Inc., CA, USA). A  $P < 0.05$  value showed a substantial difference in the outcomes achieved.

**Table 20:** Pharmacodynamic study design (n=6 in each groups)

Groups	Treatment	Dose (Route of Administration)
I	Normal control (NC) 45 mg/kg Streptozotocin (STZ) in ice cold 0.1 M sodium-citrate buffer (pH 4.5)+ HFD treatment, Intraperitoneal injection for STZ and HFD orally	Ice cold 0.1 M sodium-citrate buffer (pH 4.5), p.o
II	Experimental control (EC)	
III	Placebo of SNEDDS formulation (P-SNEDDS)	Placebo SNEDDS (without drugs), p.o
IV	Placebo of nanosuspension formulation (P-NS)	Placebo nanosuspension (without drugs), p.o
V	Unprocessed GLM (U-GLM)	2mg (GLM), p.o
VI	Unprocessed SIM (U-SIM)	5mg (SIM), p.o
VII	Unprocessed GLM-SIM (U-GLM-SIM)	2mg (GLM)+5 mg (SIM), p.o
VIII	Nanosuspension GLM formulation at high dose (NS-GLM-H)	2mg (GLM), p.o
IX	Nanosuspension GLM formulation at low dose (NS-GLM-L)	1mg (GLM), p.o
X	Nanosuspension SIM formulation at high dose (NS-SIM-H)	5 mg (SIM), p.o
XI	Nanosuspension SIM formulation at low dose (NS-SIM-L)	2.5 mg (SIM) , p.o
XII	Nanosuspension GLM-SIM formulation (NS-GLM-SIM)	1mg (GLM)+2.5 mg (SIM), p.o
XIII	SNEDDS GLM formulation at high dose (SNEDDS-GLM-H)	2mg (GLM), p.o
XIV	SNEDDS GLM formulation at low dose (SNEDDS-GLM-L)	1mg (GLM), p.o
XV	SNEDDS SIM formulation at high dose (SNEDDS-SIM-H)	5 mg (SIM), p.o
XVI	SNEDDS SIM formulation at low dose (SNEDDS-SIM-L)	2.5 mg (SIM), p.o
XVII	SNEDDS GLM-SIM formulation (SNEDDS-GLM-SIM)	1mg (GLM)+2.5 mg (SIM), p.o

### 6.22.5 Histopathological studies

Paraffin sections of kidney and liver tissues were made and stained with haematoxylin and eosin to observe histopathological changes (Garg et al., 2017b) on 450X under microscope.

### 6.23. Statistical analysis

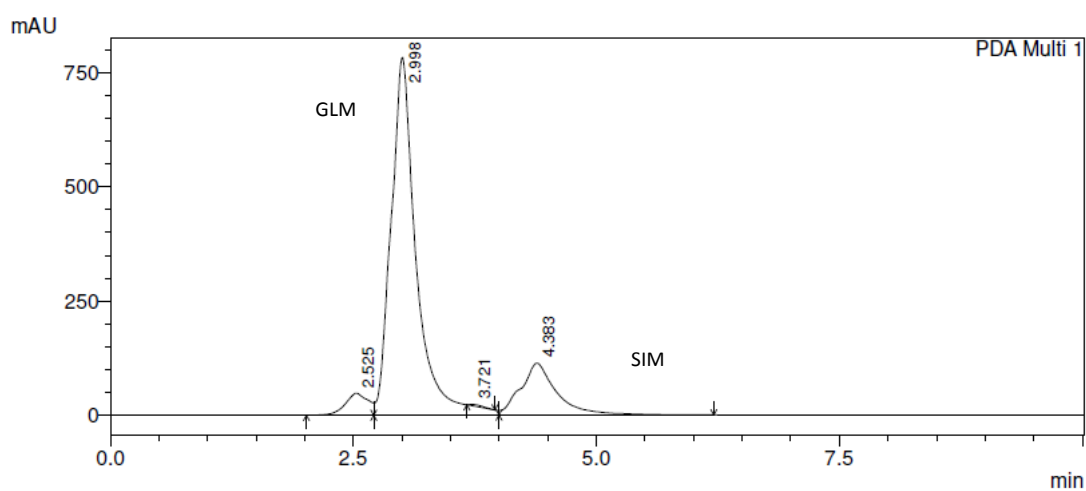
All the experimental data are expressed as mean  $\pm$  standard deviation (SD). Statistical assessment of the acquired information was performed using analysis of variance or Tukey's multiple comparison test using GraphPad Prism version 7.0 (GraphPad Software Inc., CA, USA). The  $P < 0.05$  (Wherever applicable) value showed a substantial difference in the outcomes achieved.



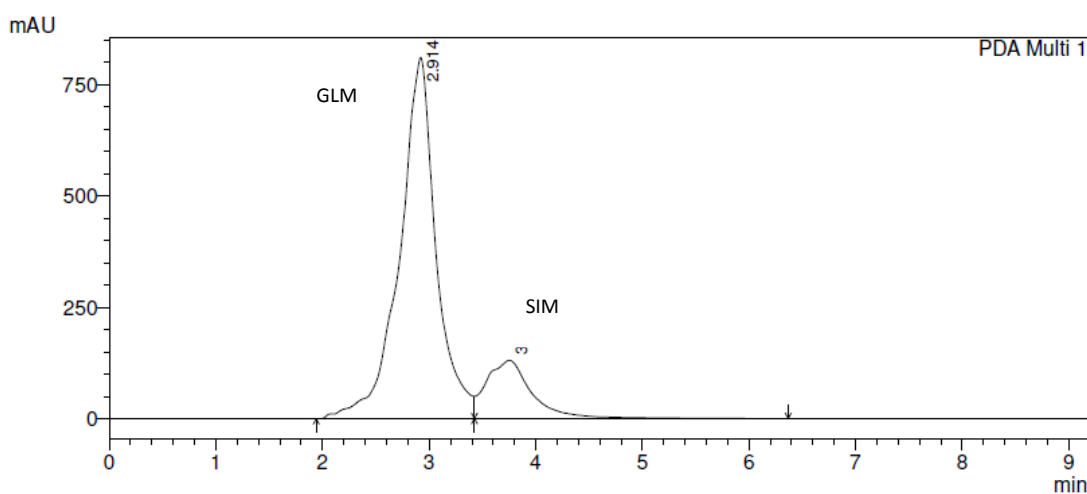
## 7. RESULTS & DISCUSSION

### 7.1. Analytical method development and validation

The retention time for GLM and SIM was 4.725 min and 9.940 min respectively. Linearity was observed in the range of 2-10  $\mu\text{g/mL}$  with coefficient of regression 0.999. The RSD was found to be less than 2% and percentage recovery was between 95 to 105%.



**Fig. 3.** Chromatogram of mixture of SIM-GLM in ACN-ortho phosphoric acid



**Fig. 4.** Chromatogram of mixture of SIM-GLM in methanol- ortho phosphoric acid

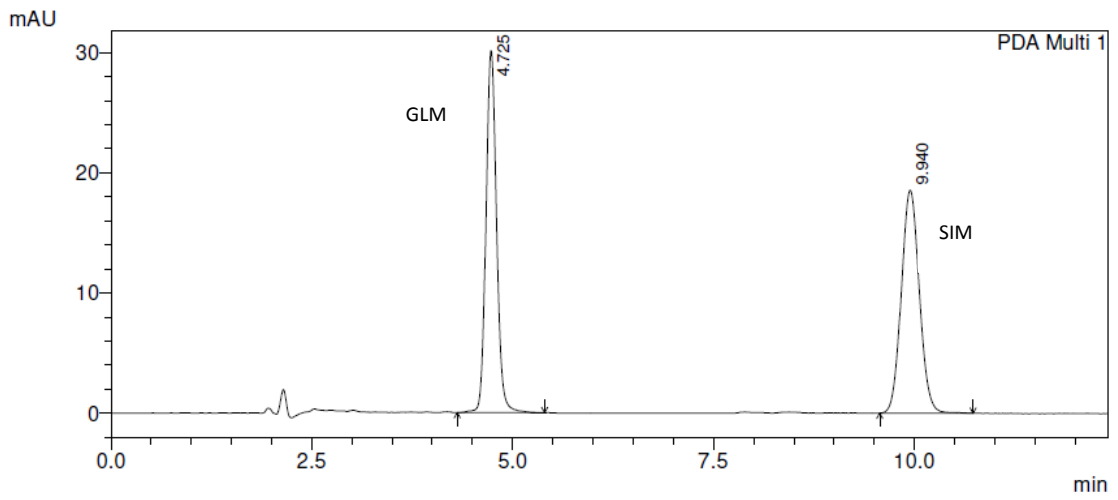
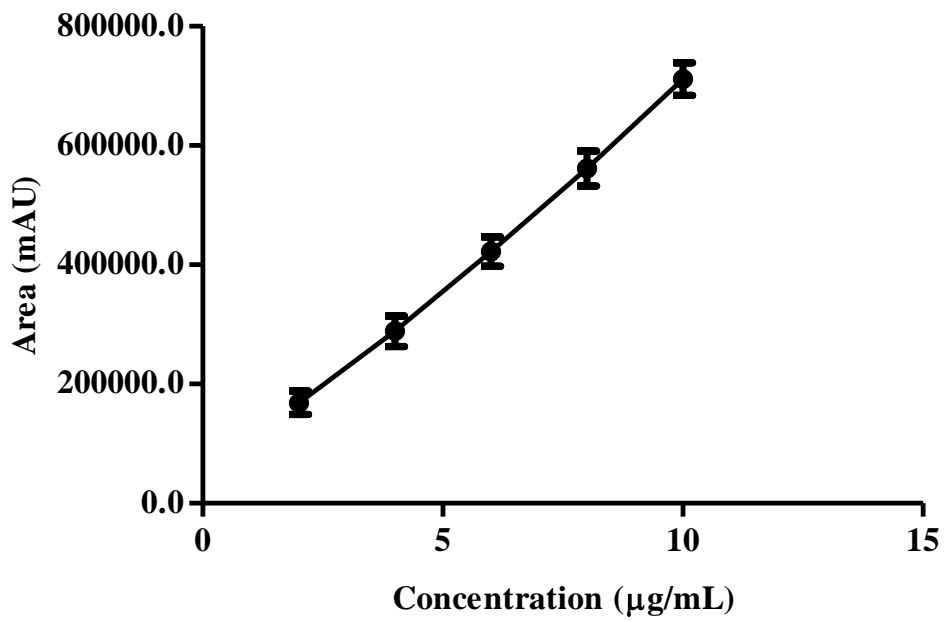
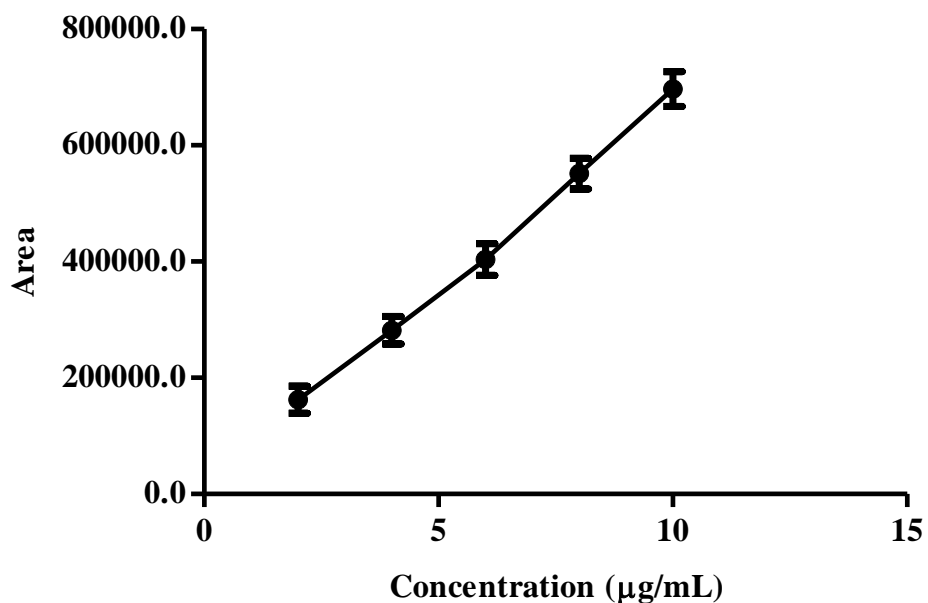


Fig. 5. Optimized chromatogram of SIM-GLM in ACN:  $\text{KH}_2\text{PO}_4$  (75:25)



(6A) Calibration curve of GLM



(6B) Calibration curve of SIM

Fig. 6. Calibration curve of (A) GLM and (B) SIM

Table 21: Results of accuracy studies

Levels	Concentration of standard solution (µg/mL)	Concentration of sample Solution (µg/mL)	Total concentration of solution (actual) (µg/mL)	Concentration of drug recovery from mobile phase (µg/mL) *(N=5)	Recovery (%)	Mean Recovery (%)
GLM						
LQC	4.80	6.00	10.80	10.40 ± 1.24	96.30	98.70
MQC	6.00	6.00	12.00	11.80 ± 1.68	98.30	
HQC	7.20	6.00	13.20	13.40 ± 1.20	101.50	
SIM						
LQC	4.80	6.00	10.80	10.50 ± 1.31	97.20	97.93
MQC	6.00	6.00	12.00	11.70 ± 1.53	97.50	
HQC	7.20	6.00	13.20	13.10 ± 1.50	99.10	

**Table 22:** Results of precision studies for GLM

Parameters	Level	Concentration (µg/mL)	Analytical responses (area), injections						Mean (*N=6)	SD	%RSD
			1	2	3	4	5	6			
Repeatability (intraday precision)	LQC	4.80	234538	239931	239490	240068	237295	240992	238719.00	2389.91	1.00
	MQC	6.00	333709	334581	339807	330986	329153	325986	332370.30	4796.05	1.44
	HQC	7.20	761009	757841	752328	754292	763383	762742	758599.20	4567.48	0.60
Intermediate precision (interday)											
Day 1	LQC	4.80	235633	240931	239360	238068	242305	243952	240041.50	3000.02	1.25
	MQC	6.00	332519	335661	342107	331725	327738	326916	332777.70	5590.75	1.68
	HQC	7.20	753849	753141	762810	751213	775361	763500	759979.00	9145.44	1.20
Day 2	LQC	4.80	239177	240064	234717	239214	242260	248500	240655.30	4560.48	1.89
	MQC	6.00	328849	329007	330295	329371	327389	321194	327684.20	3315.96	1.01
	HQC	7.20	744824	753977	750359	765157	747446	750869	752105.30	7115.54	0.94
Day 3	LQC	4.80	291262	288824	291628	295900	291569	300090	293212.20	4069.34	1.38
	MQC	6.00	353207	357967	349249	345520	357674	357445	353510.30	5191.84	1.46
	HQC	7.20	770311	770493	771935	778614	777642	775851	774141.00	3688.68	0.47
Intermediate precision (inter analyst)											
Analyst 1	LQC	4.80	234538	229931	229490	230168	233273	232718	231686.30	2094.00	0.90
	MQC	6.00	343709	334581	330837	340676	339053	328985	336306.80	5795.34	1.72
	HQC	7.20	764319	759337	760028	754567	759889	762139	760046.50	3261.48	0.43
Analyst 2	LQC	4.80	239639	241176	239984	236853	243264	248516	241572.00	3994.14	1.65
	MQC	6.00	338857	341377	338486	330375	333578	341174	337307.80	4410.65	1.31
	HQC	7.20	754824	750367	749359	761467	754346	761735	755349.70	5295.14	0.70
Analyst 3	LQC	4.80	250132	245360	239672	242425	238793	241163	242924.20	4216.16	1.73
	MQC	6.00	351432	356134	347892	352881	351984	347644	351327.80	3204.01	0.91
	HQC	7.20	749356	761189	760043	752346	756289	755436	755776.50	4491.50	0.59

**Table 23:** Results of precision studies for SIM

Parameters	Level	Concentration (µg/mL)	Analytical responses (area), injections						Mean (*N=6)	SD	%RSD	
			1	2	3	4	5	6				
Repeatability (intraday precision)	LQC	4.80	245674	250324	245692	246231	251089	243567	247096.20	2951.59	1.19	
	MQC	6.00	363892	358799	359933	362236	360899	361234	361165.50	1776.22	0.49	
	HQC	7.20	439872	442234	441976	442108	440034	437994	440703.00	1697.96	0.38	
Intermediate precision (interday)	Day 1	LQC	4.80	257476	264007	260530	258136	255188	263039	259729.30	3000.02	1.25
		MQC	6.00	361727	357938	365216	352269	353268	352254	357112.00	5590.75	1.68
		HQC	7.20	446674	447270	457717	450060	462948	447006	451945.80	9145.44	1.20
	Day 2	LQC	4.80	253669	265136	260238	253503	258037	253191	257295.70	4560.48	1.89
		MQC	6.00	348054	341393	356953	352294	353382	349078	350192.30	3315.96	1.01
		HQC	7.20	444108	442285	458229	460266	452165	460327	452896.70	7115.54	0.94
	Day 3	LQC	4.80	323501	323849	323755	326302	321871	333086	325394.00	4069.34	1.38
		MQC	6.00	382686	383741	377071	373265	387233	384927	381487.20	5191.84	1.46
		HQC	7.20	470126	463550	471085	474865	473349	469357	470388.70	3688.68	0.47
Intermediate precision (inter analyst)	Analyst 1	LQC	4.80	248964	250453	241456	249777	248788	251345	248463.80	3562.22	1.43
		MQC	6.00	362887	353349	359902	363312	357998	360785	359705.50	3678.40	1.02
		HQC	7.20	451290	449936	447755	452031	451132	447451	449932.50	1927.93	0.42
	Analyst 2	LQC	4.80	239978	248779	249933	250155	248873	251332	248175.00	4123.60	1.66
		MQC	6.00	356334	357835	348977	360021	348886	351443	353916.00	4780.93	1.35
		HQC	7.20	471133	462759	458871	453347	458873	458599	460597.00	5969.71	1.29
	Analyst 3	LQC	4.80	251764	247789	249977	246733	250342	251138	249623.80	1961.48	0.78
		MQC	6.00	348872	357745	352246	353351	349983	348892	351848.20	3413.64	0.97
		HQC	7.20	380031	374433	368897	367745	364338	367339	370463.80	5734.50	1.54

**Table 24:** Robustness results of various parameters tested for GLM

Variables	Value	Concentration (µg/mL)	Peak area (mean±SD) (*N=5)	Mean of peak areas of three values (*N=3)	Retention time (mean±SD) (*N=5)	Mean of retention times of three values (*N=3)	% Recovery (mean±SD) (*N=5)	Mean of % recoveries of three values (*N=3)
pH	4.30	6.00	327684.20 ± 3315.96	327316.40	4.76 ± 0.001	4.77	96.40 ± 1.12	97.40
	4.50	6.00	322777.70 ± 5590.75	SD = 4366.40	4.77 ± 0.01	SD = 0.01	97.30 ± 1.09	SD = 1.05
	4.70	6.00	331487.20 ± 5262.52	%RSD = 1.33	4.77 ± 0.01	%RSD = 0.21	98.50 ± 1.15	%RSD = 1.08
Flow rate (ml/min)	0.80	6.00	398412.20 ± 7167.23	396236.00	4.77 ± 0.01	4.74	98.30 ± 1.33	99.60
	1.00	6.00	402562.30 ± 7418.56	SD = 7650.20	4.73 ± 0.01	SD = 0.00	101.20 ± 1.14	SD = 1.47
	1.20	6.00	387733.40 ± 6987.27	%RSD = 1.93	4.73 ± 0.00	%RSD = 0.06	97.30 ± 1.57	%RSD = 1.48
Mobile phase ratio (A: B) v/v	73:27	6.00	389842.00 ± 7261.32	395121.30	4.73 ± 0.02	4.77	98.56 ± 1.12	99.10
	75:25	6.00	393338.00 ± 7337.13	SD = 6361.30	4.76 ± 0.02	SD = 0.04	98.88 ± 1.03	SD = 1.16
	77:23	6.00	402184.00 ± 7194.45	%RSD = 1.61	4.81 ± 0.01	%RSD = 0.93	99.87 ± 1.32	%RSD = 1.35

**Table 25:** Robustness results of various parameters tested for SIM

Variables	Value	Concentration (µg/mL)	Peak area (mean±SD) (*N=5)	Mean of peak areas of three values (*N=3)	Retention time (mean±SD) (*N=5)	Mean of retention times of three values (*N=3)	% Recovery (mean±SD) (*N=5)	Mean of % recoveries of three values (*N=3)
pH	4.30	6.00	359189.70 ± 3006.20	359481.80	9.63 ± 0.01	9.72	97.90 ± 1.03	98.60
	4.50	6.00	359067.40 ± 3678.40	SD = 3333.50	9.77 ± 0.01	SD = 0.01	98.40 ± 1.11	SD = 1.04
	4.70	6.00	360188.30 ± 3315.90	%RSD = 0.93	9.77 ± 0.01	%RSD = 0.08	99.50 ± 0.98	%RSD = 1.05
Flow rate (ml/min)	0.80	6.00	359253.60 ± 5590.80	355885.60	9.83 ± 0.01	9.76	99.70 ± 1.23	99.70
	1.00	6.00	359223.50 ± 4796.10	SD = 5192.90	9.72 ± 0.01	SD = 0.01	97.90 ± 1.15	SD = 1.20
	1.20	6.00	349179.80 ± 5191.80	%RSD = 1.46	9.73 ± 0.00	%RSD = 0.06	101.40 ± 1.21	%RSD = 1.20
Mobile phase ratio (A: B) v/v	73:27	6.00	359198.50 ± 3678.40	359176.40	9.83 ± 0.02	9.80	98.70 ± 1.12	98.90
	75:25	6.00	357145.40 ± 3688.70	SD = 3622.80	9.76 ± 0.02	SD = 0.01	99.50 ± 1.03	SD = 1.17
	77:23	6.00	361185.30 ± 3501.30	%RSD = 1.01	9.81 ± 0.01	%RSD = 0.17	98.40 ± 1.32	%RSD = 1.17

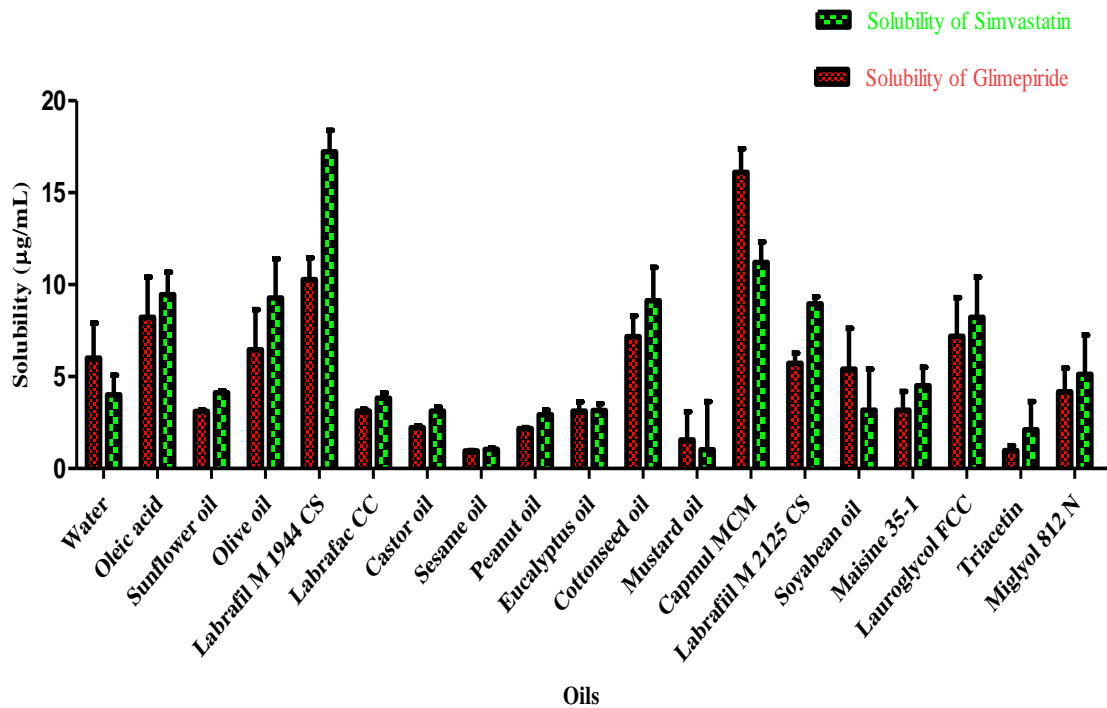
## 7.2. Solubility studies

The results of solubility studies for preparation of nanosuspensions showed (Table 26) that both the drugs have maximum solubility in SLS followed by PVPK-30. The least solubility was observed in all the batches prepared using PEG 6000. Hence, SLS and PVPK-30 were taken as electrostatic and steric stabilizer respectively for the preparation of nanosuspensions. It was also observed that for both the drugs, maximum solubility was observed between drug to SLS ratio 0.25 to 0.75 afterwards the solubility was found to be decrease with increase in SLS to drug ratio. Similarly, higher solubility for both the drugs was observed when the drugs to PVPK-30 ratio were varying from 0.25 to 0.50. Hence, the drug to SLS ratio and drug to PVPK-30 ratio were taken between 0.20 to 0.60 and 0.15 to 0.45, respectively for the preparation of nanosuspensions using DoE.

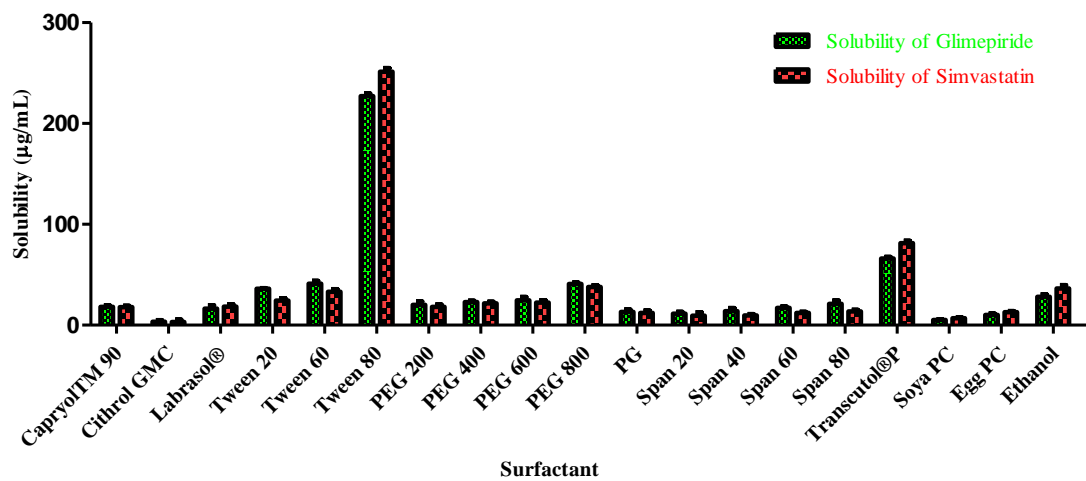
Solubility of SIM and GLM in different oils and stabilizers are shown in Fig.7A. and Fig.7B. Maximum solubility of SIM and GLM were observed in LMCS oil and CMCM respectively. Both drugs were showing maximum solubility in TP and T80. On the basis of above results, CMCM and LMCS were selected as an oil and TP, T80 as stabilizer for further studies.

**Table 26:** Solubility (Mean  $\pm$  s.d.) of GLM and SIM using different surfactants.

Surfactant	Drug to surfactant ratio	Solubility of GLM (%) (Mean $\pm$ S.D.)	Solubility of SIM (%) (Mean $\pm$ S.D.)
Sodium lauryl sulphate (SLS)	1/0.25	31.37 $\pm$ 0.048	17.11 $\pm$ 0.08
	1/0.50	72.21 $\pm$ 1.16	54.23 $\pm$ 0.22
	1/0.75	58.45 $\pm$ 1.09	55.78 $\pm$ 0.53
	1/1	54.04 $\pm$ 2.12	34.89 $\pm$ 1.12
	1/1.5	54.25 $\pm$ 0.78	41.62 $\pm$ 0.66
	1/2	57.54 $\pm$ 0.92	43.77 $\pm$ 0.76
Poly ethylene glycol 4000 (PEG 4000)	1/0.25	14.46 $\pm$ 1.18	9.32 $\pm$ 0.33
	1/0.50	12.22 $\pm$ 2.18	15.63 $\pm$ 0.97
	1/0.75	20.21 $\pm$ 0.98	16.87 $\pm$ 0.54
	1/1	18.11 $\pm$ 1.26	19.32 $\pm$ 1.02
	1/1.5	20.98 $\pm$ 1.45	21.46 $\pm$ 0.42
	1/2	16.84 $\pm$ 0.86	22.11 $\pm$ 0.87
Poly vinyl pyrrolidone (PVP K-30)	1/0.25	37.22 $\pm$ 3.55	9.52 $\pm$ 0.53
	1/0.50	43.14 $\pm$ 2.99	10.56 $\pm$ 0.44
	1/0.75	56.22 $\pm$ 4.16	11.03 $\pm$ 0.78
	1/1	25.68 $\pm$ 0.65	11.08 $\pm$ 1.13
	1/1.5	33.54 $\pm$ 1.12	27.33 $\pm$ 0.98
	1/2	48.56 $\pm$ 1.47	36.23 $\pm$ 1.05



(7A)



(7B)

**Fig. 7.** Solubility (mean  $\pm$ s.d.) of unprocessed GLM & SIM mixture in different (A) oils and (B) surfactants.

### 7.3. Screening of nanosuspension using DoE

Twenty-six experiments were run to investigate the effect of formulation (SLS to GS & PVP K-30 to GS) and processing factors (Mixing speed) affecting the responses.



The results of experiment are shown in in Table 27. The significance and magnitude of the effect of independent variables as well as adequacy of model was determined using analysis of variance (ANOVA). The P value less than 0.05 confirmed that the model was adequate. Using BBD two different polynomial equations were generated, one for particle size (Eq. 7) and other for zeta potential (Eq. 11). These equations helped in generating different counter plots for different independent factors.

$$\text{Particle size} = +245.83 - 46.52 \times A - 53.62 \times B + 39.02 \times C - 86.28 \times D \quad \text{Eq. (7)}$$

$$\begin{aligned} \text{Zeta potential} = & -36.45 - 8.09 \times A + 1.29 \times B + 0.34 \times C - 1.93 \times D + 3.58 \times AB \\ & + 2.42 \times AC + 0.29 \times AD + 4.45 \times BC - 1.09 \times BD - 1.06 \times CD + 4.71 \times A^2 + 2.44 \times B^2 \\ & - 3.65 \times C^2 - 0.44 \times D^2 \end{aligned} \quad \text{Eq. (11)}$$

The perturbation and the counter plots (Fig. 8A-8J) showed that response Y1 (Particle size) was more influenced by C (ratio of solvent/anti solvent) and D (speed of mixer) whereas A (ratio of surfactant SLS to drug) and B (ratio of polymer PVP K-30 to drug) has similar effect. Zeta potential (Response Y2) was highly influenced by factor A and equally affected by B and C and little effect of factor D was observed on zeta potential as per perturbation plot.

Polynomial equation showed that particle size got decreased with increase in factor A, B & D and particle size increased with increase in factor C. Polynomial equation also showed that zeta potential of nanosuspensions got increased with decreased in factor A, C & D and inversely relation observed with factor B. Same effects were observed in contour plot and 3-D response surface observed.

#### **7.4. Optimization of nanosuspension**

According to the BBD, the optimized values for various parameters was: the drug to SLS ratio was 0.6, the drug to PVP K-30 ratio was 0.45, solvent to antisolvent ratio 0.08 and speed of mixer 4000 rpm. Using the predicted values for particle size of SP-NS should be  $47.31 \pm 0.93$  nm and zeta potential within the limit of -25.75 to -50.17 mV. The obtained particle size of optimized batch of L-NS was found to be  $45.30 \pm 1.05$  nm with polydispersibility index of  $0.25 \pm 0.06$ . The zeta potential of optimized batch was found to be  $-28.33 \pm 1.34$  mV. All these values are found within the predicted value of DoE. This optimized L-NS was spray dried and the particle size of

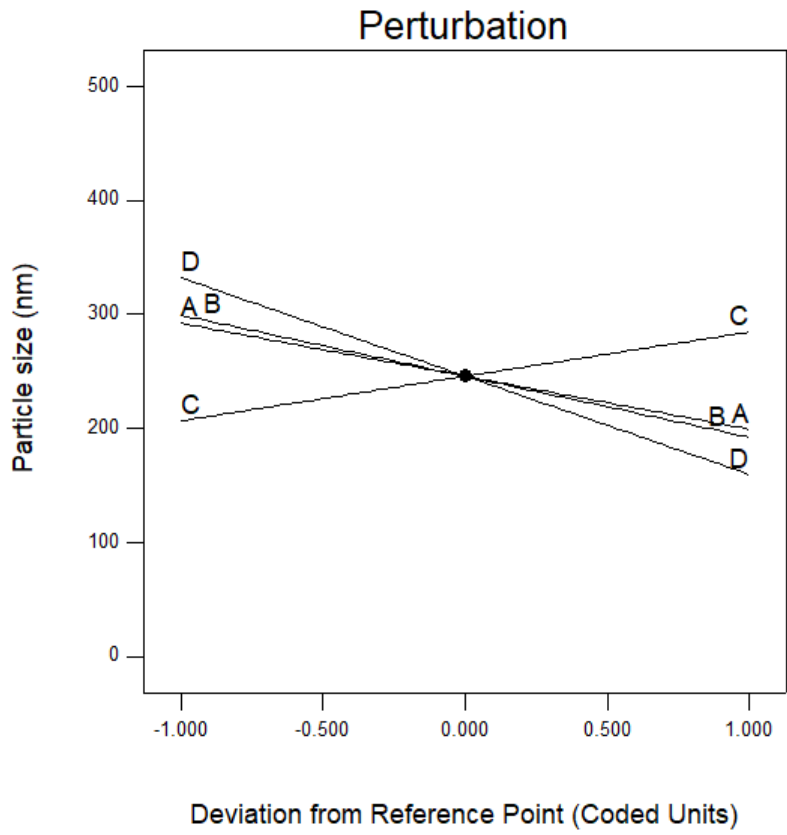
SP-NS was found to be  $133.30 \pm 1.13$  nm with PDI  $0.34 \pm 0.05$ . Zeta potential was found to be  $-27.32 \pm 2.05$  mV.

**Table 27:** DOE of nanosuspension.

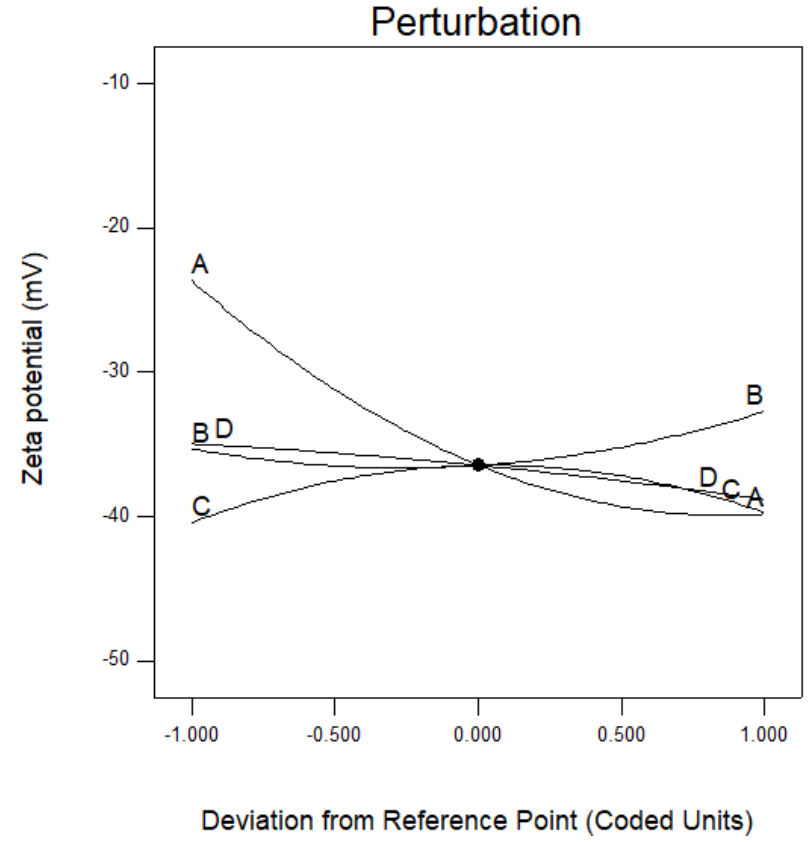
Formulation	Ratio of surfactant SLS to drug (g)	Ratio of polymer PVP K-30 to drug (g)	Ratio of solvent/anti solvent (ml) Acetone/water	Speed of mixer (rpm)	Particle Size (nm)	Zeta Potential (mV)
F1	0.40 (0)	0.30 (0)	0.06 (-1)	2000 (-1)	216.00	-40.60
F2	0.20 (-1)	0.30 (0)	0.06 (-1)	3000 (0)	403.12	-26.00
F3	0.40 (0)	0.30 (0)	0.12 (+1)	2000 (-1)	353.57	-35.90
F4	0.20 (-1)	0.30 (0)	0.09 (0)	2000 (-1)	362.22	-16.44
F5	0.60 (+1)	0.30 (0)	0.09 (0)	2000 (-1)	256.22	-40.17
F6	0.40 (0)	0.45 (+1)	0.09 (0)	2000 (-1)	323.45	-34.22
F7	0.40 (0)	0.15 (-1)	0.09 (0)	2000 (-1)	278.22	-35.41
F8	0.60 (+1)	0.30 (0)	0.06 (-1)	3000 (0)	216.72	-43.67
F9	0.40 (0)	0.45 (+1)	0.06 (-1)	3000 (0)	45.59	-45.40
F10	0.40 (0)	0.15 (-1)	0.06 (-1)	3000 (0)	310.22	-33.33
F11	0.20 (-1)	0.15 (-1)	0.09 (0)	3000 (0)	438.30	-24.00
F12	0.40 (0)	0.30 (0)	0.09 (0)	3000 (0)	265.34	-36.18
F13	0.20 (-1)	0.45 (+1)	0.09 (0)	3000 (0)	191.63	-19.22
F14	0.60 (+1)	0.45 (+1)	0.09 (0)	3000 (0)	90.00	-24.60
F15	0.40 (0)	0.30 (0)	0.09 (0)	3000 (0)	266.78	-36.22
F16	0.60 (+1)	0.15 (-1)	0.09 (0)	3000 (0)	288.20	-43.70
F17	0.60 (+1)	0.30 (0)	0.12 (+1)	3000 (0)	413.26	-42.40
F18	0.40 (0)	0.15 (-1)	0.12 (+1)	3000 (0)	188.32	-39.18
F19	0.40 (0)	0.45 (+1)	0.12 (+1)	3000 (0)	212.14	-35.67
F20	0.20 (-1)	0.30 (0)	0.12 (+1)	3000 (0)	403.81	-34.40
F21	0.40 (0)	0.30 (0)	0.06 (-1)	4000 (+1)	98.15	-40.22
F22	0.40 (0)	0.45 (+1)	0.09 (0)	4000 (+1)	133.12	-38.16
F23	0.60 (+1)	0.30 (0)	0.09 (0)	4000 (+1)	88.22	-47.71
F24	0.20 (-1)	0.30 (0)	0.09 (0)	4000 (+1)	111.76	-25.13
F25	0.40 (0)	0.15 (-1)	0.09 (0)	4000 (+1)	216.54	-34.97
F26	0.40 (0)	0.30 (0)	0.12 (+1)	4000 (+1)	106.54	-39.76

**Table 28:** Summary of ANOVA of Box–Behnken screening design batches using liquid antisolvent precipitation technique.

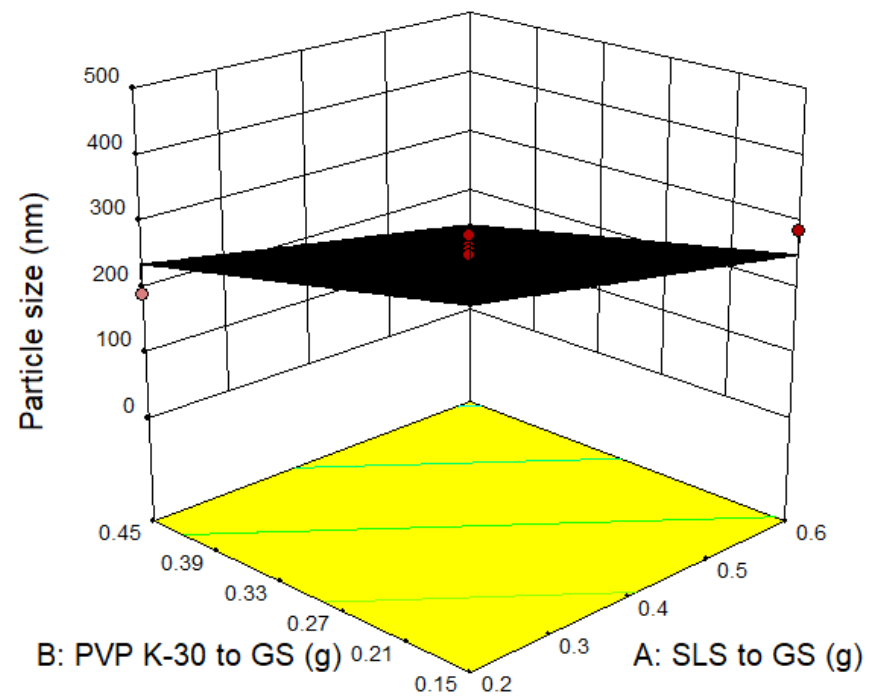
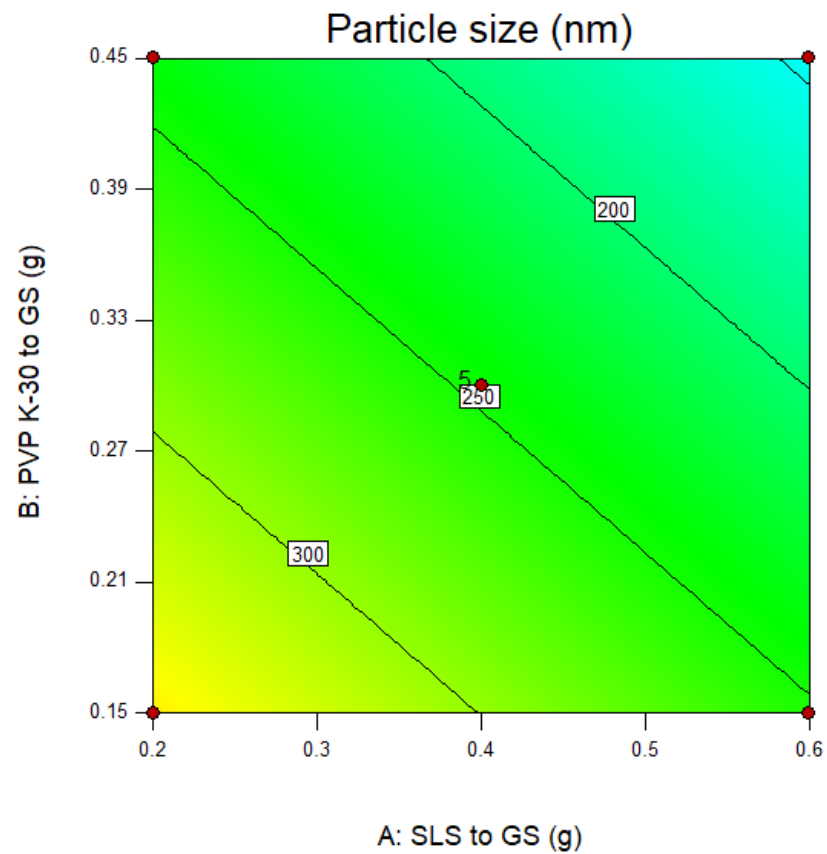
Response variables	Regression parameters		P value	
	R <sup>2</sup>	F <sub>cal</sub>		
Particle Size	0.5149	247.91	< 0.0001	
Zeta Potential	0.8218	133.80	0.0001	



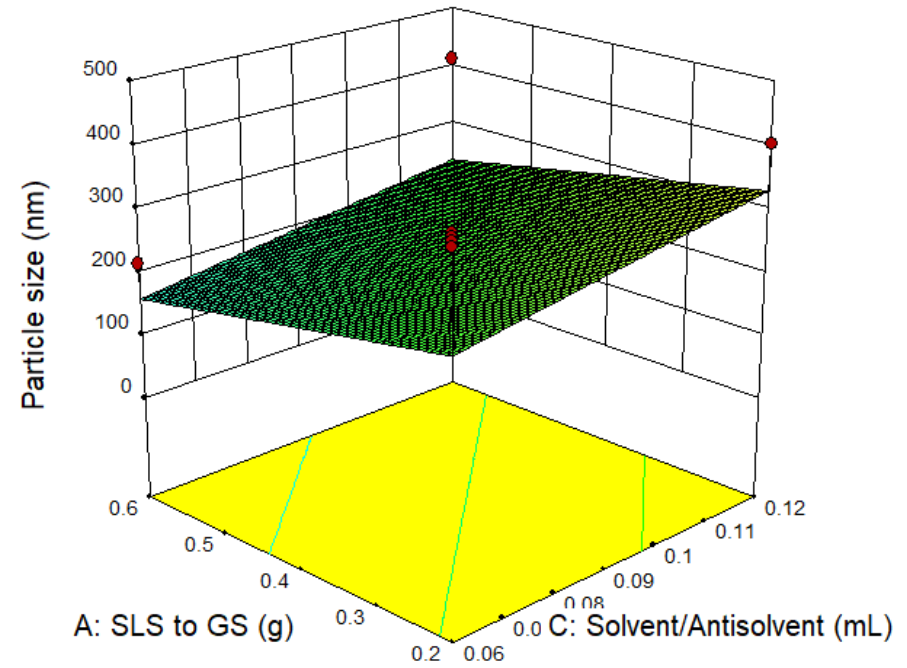
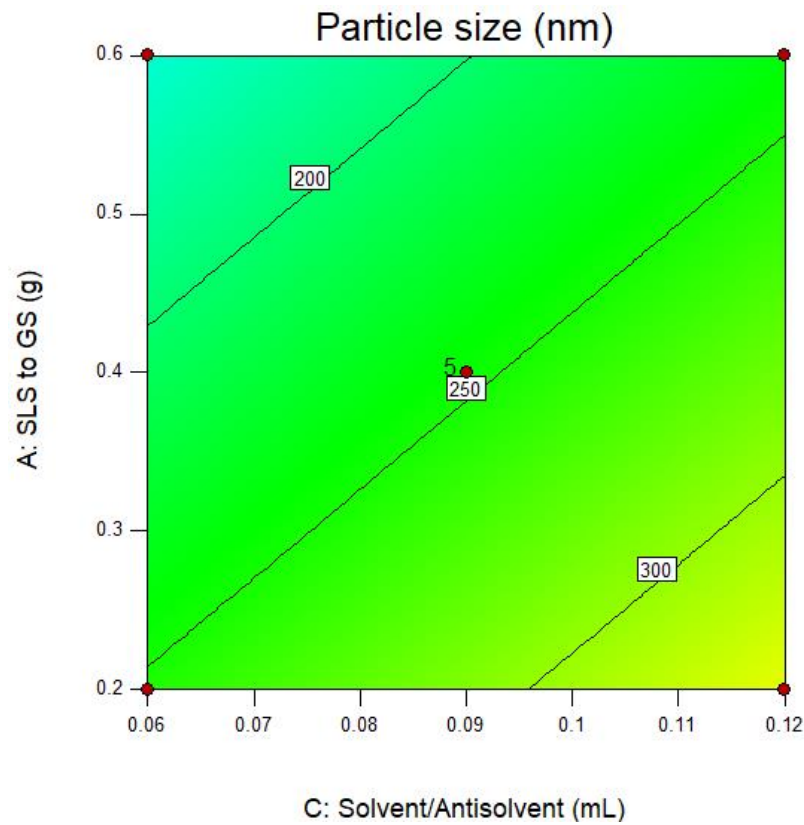
**(8A)** Perturbation plot for particle size (B) Zeta potential



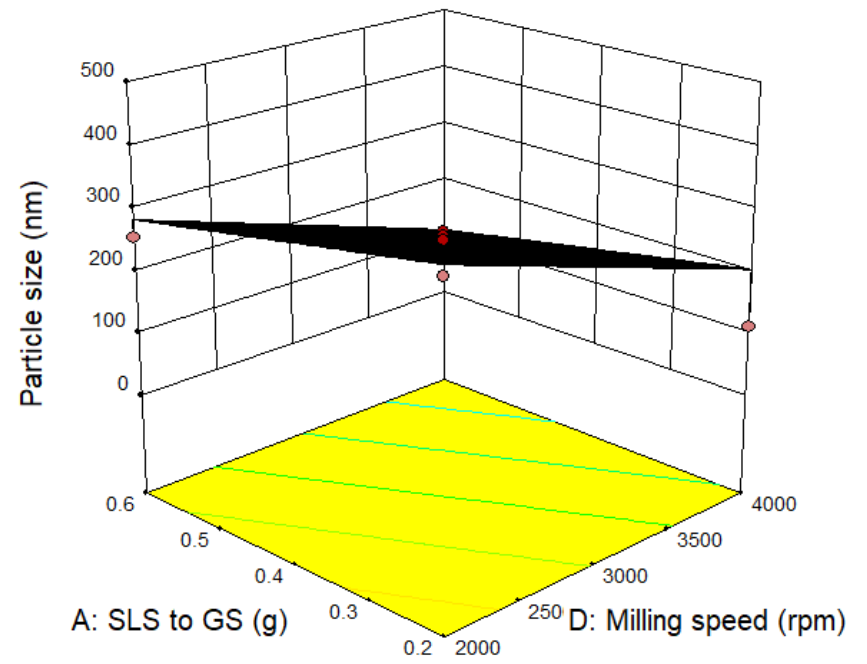
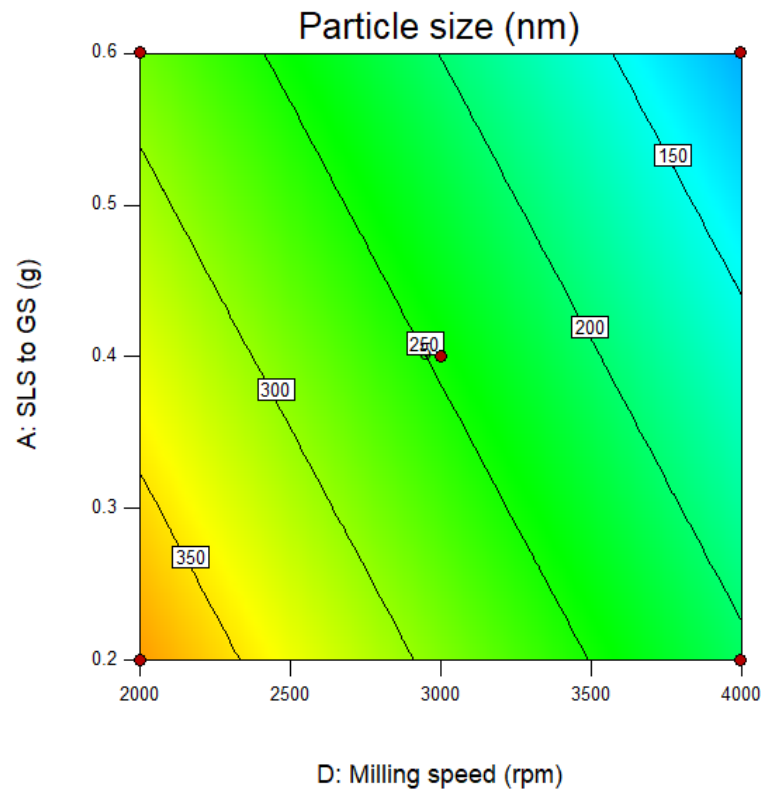
**(8B)** Perturbation plot for Zeta potential



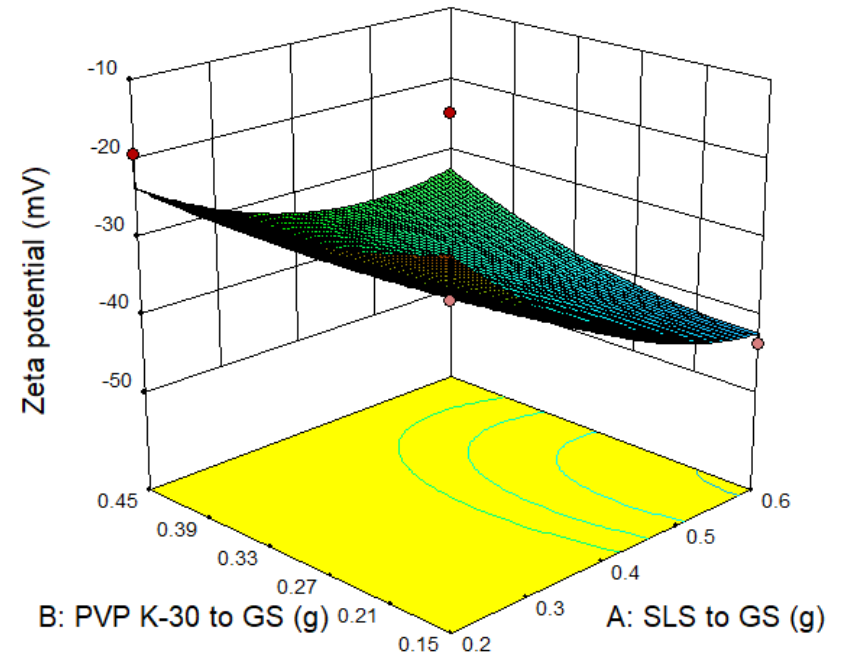
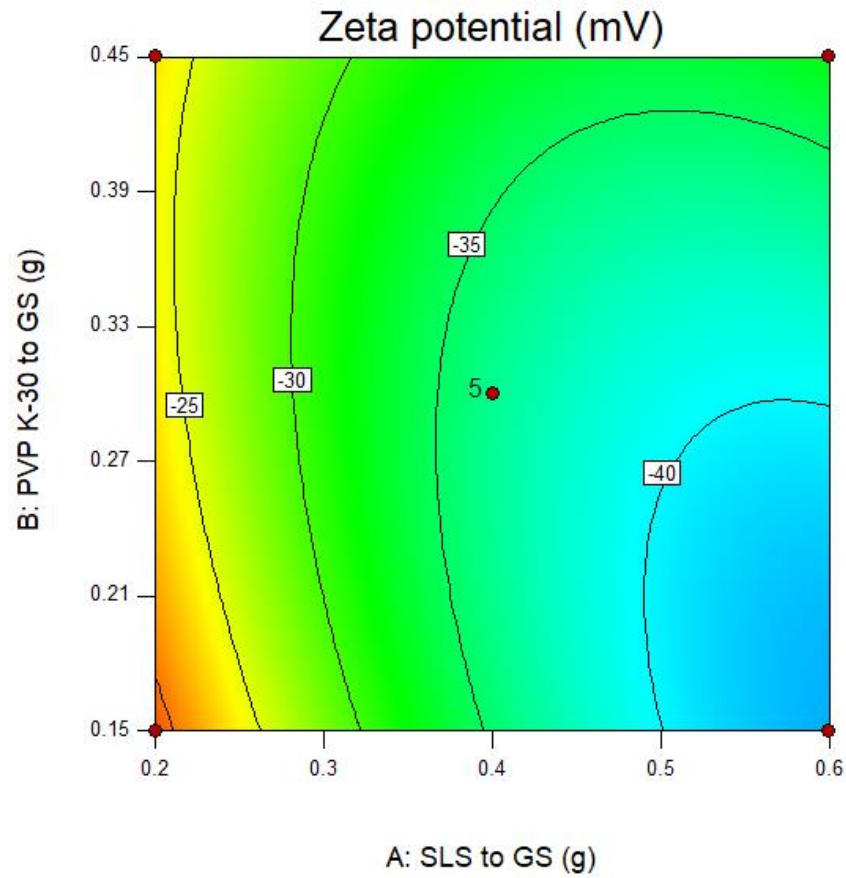
(8C) 2D & 3D-counter plot for effects of PVPK-30 to GS and SLS to GS on particle size of nanosuspension



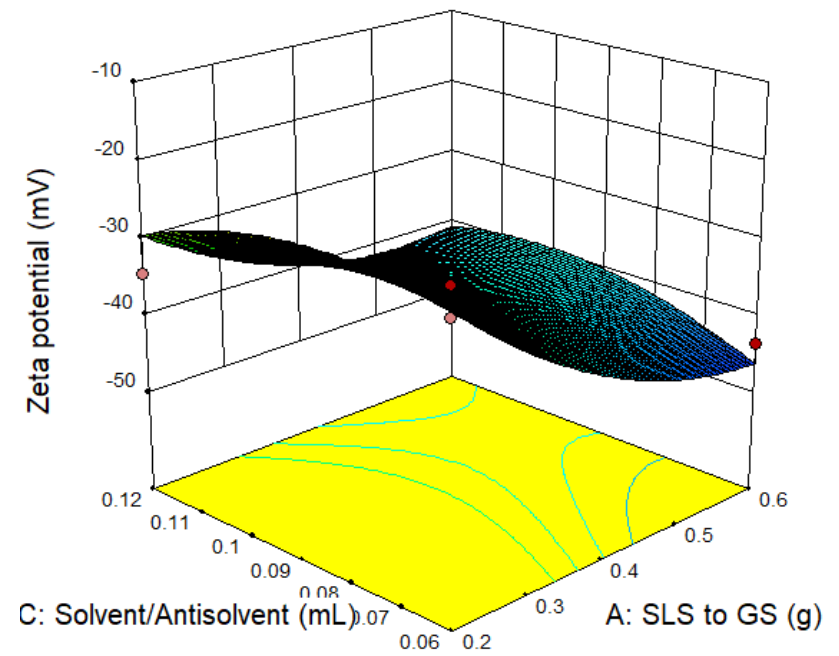
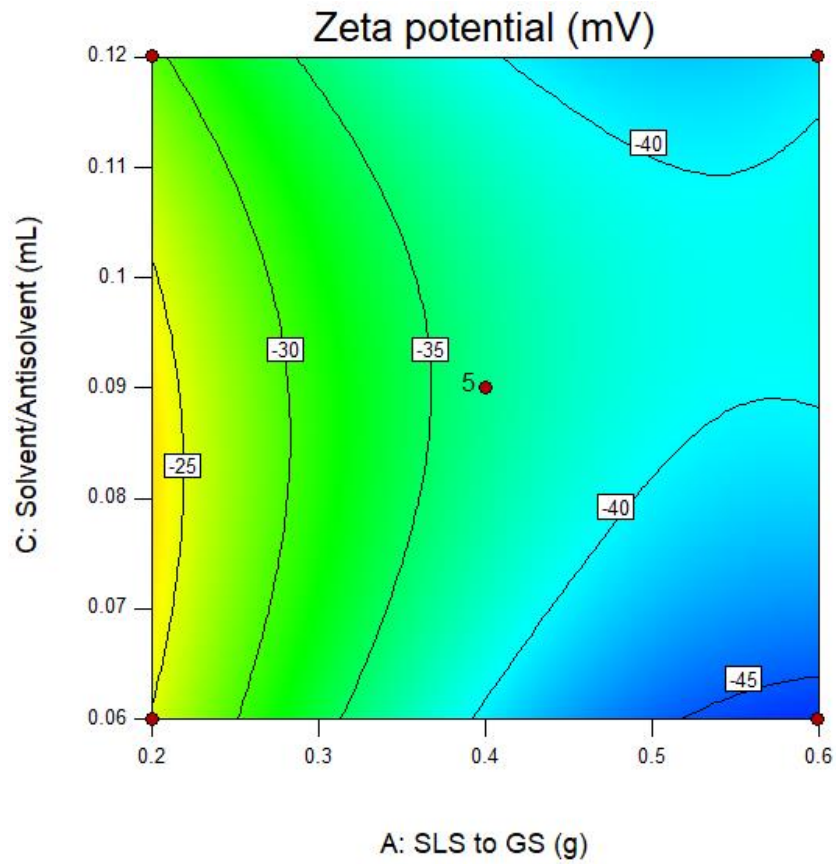
(8D) 2D & 3D-counter plot for effects of SLS to GS and solvent/antisolvent on particle size of nanosuspension



(8E) 2D & 3D-counter plot for effects of Milling speed and SLS to GS on particle size of nanosuspension

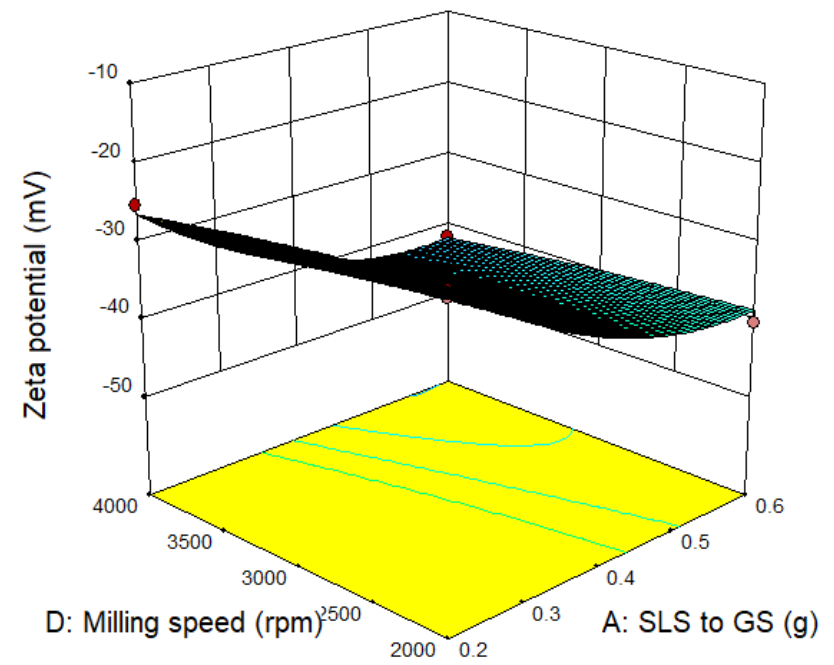
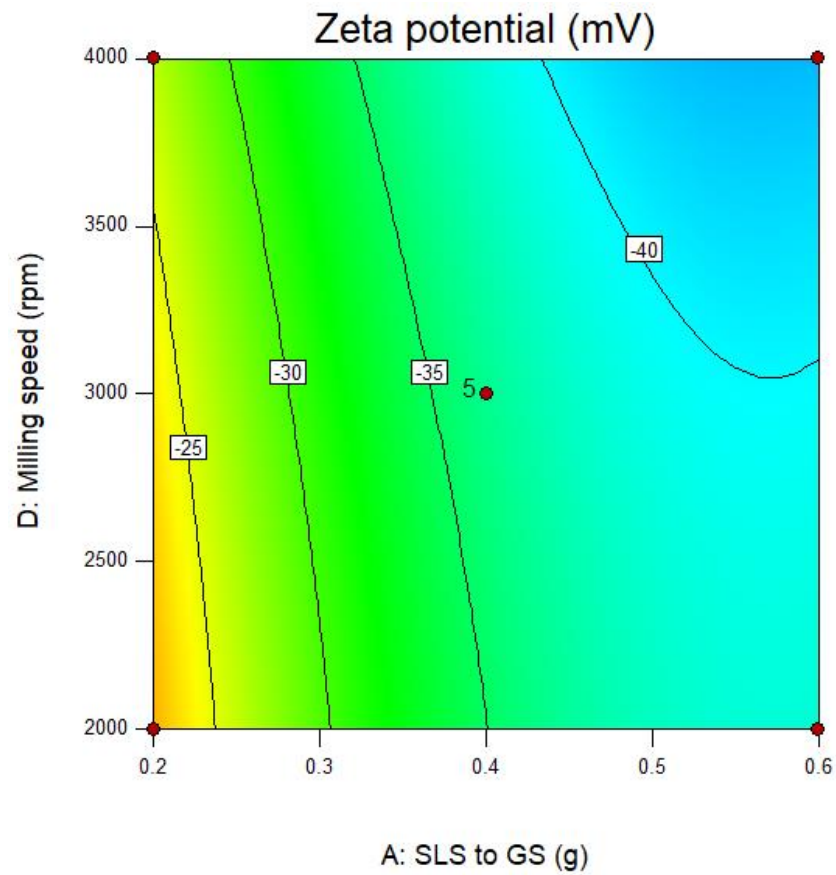


(8F). 2D & 3D-counter plot for effects of PVPK-30 to GS and SLS to GS on particle size of nanosuspension

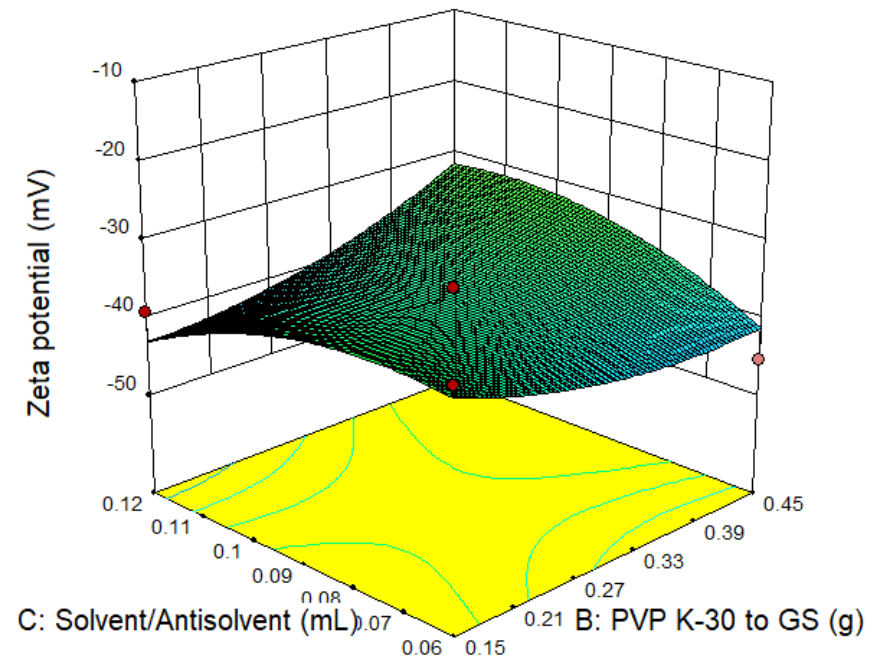
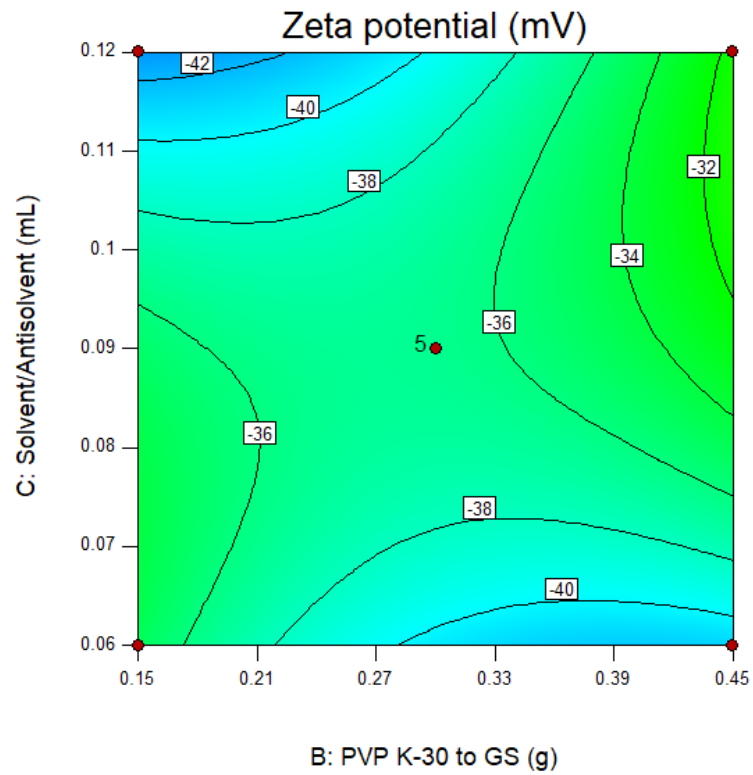


(8G) 2D & 3D-counter plot for effects of Solvent/antisolvent and SLS to GS on zeta potential of nanosuspension

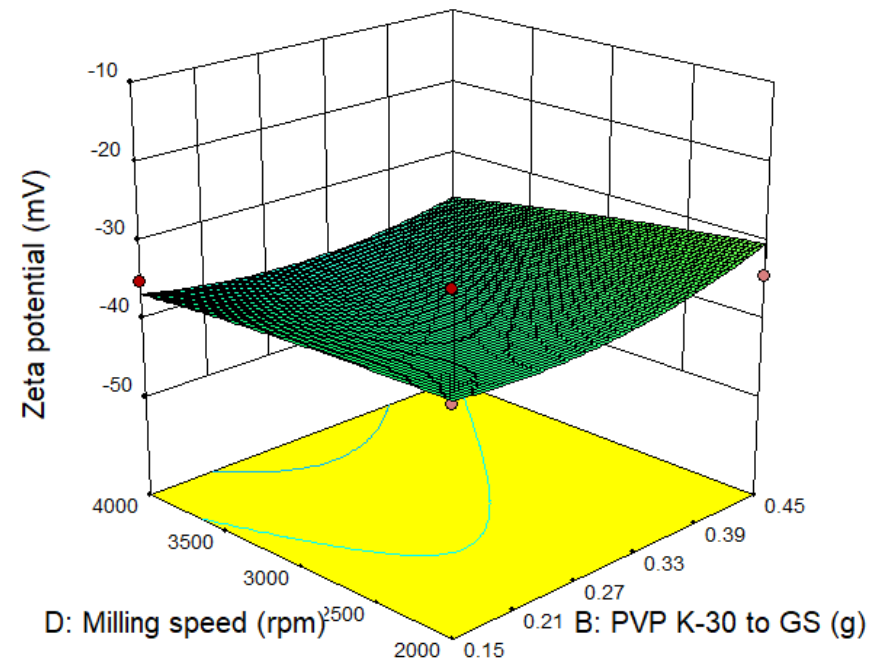
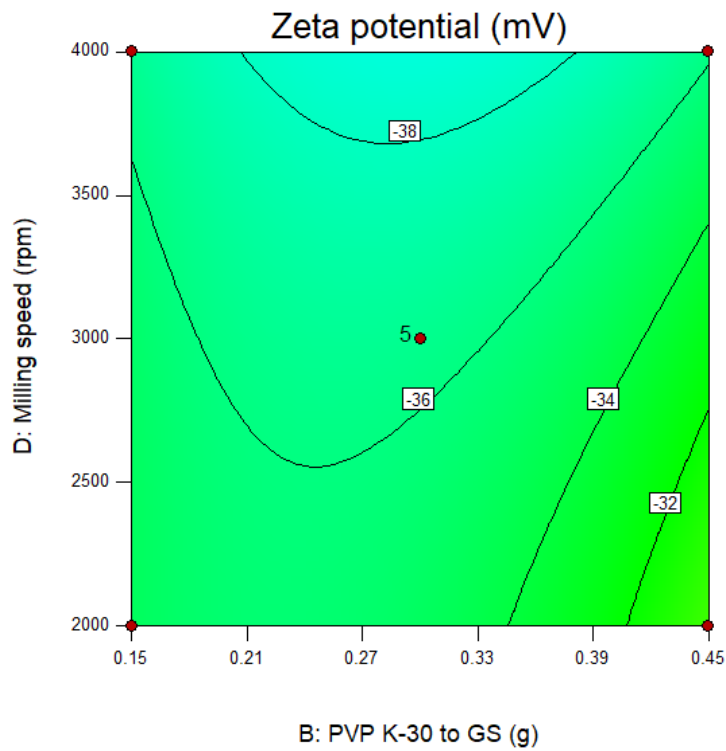




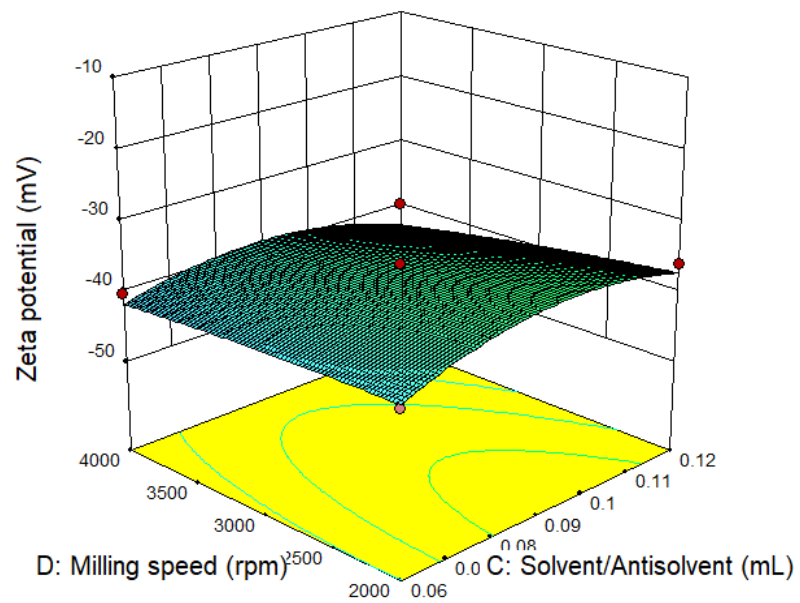
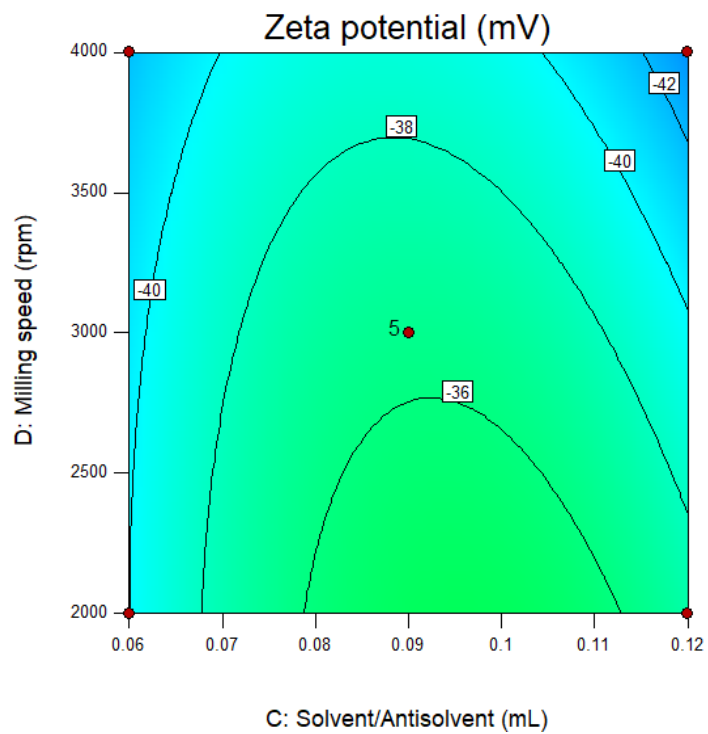
(8H) 2D & 3D-counter plot for effects of Milling speed and SLS to GS on zeta potential of nanosuspension.



(8I) 2D & 3D-counter plot for effects of Solvent/antisolvent and PVP K-30 to GS on zeta potential of nanosuspension



**(8J)** 2D & 3D-counter plot for effects of Milling speed & PVP K-30 to GS on zeta potential of nanosuspension.



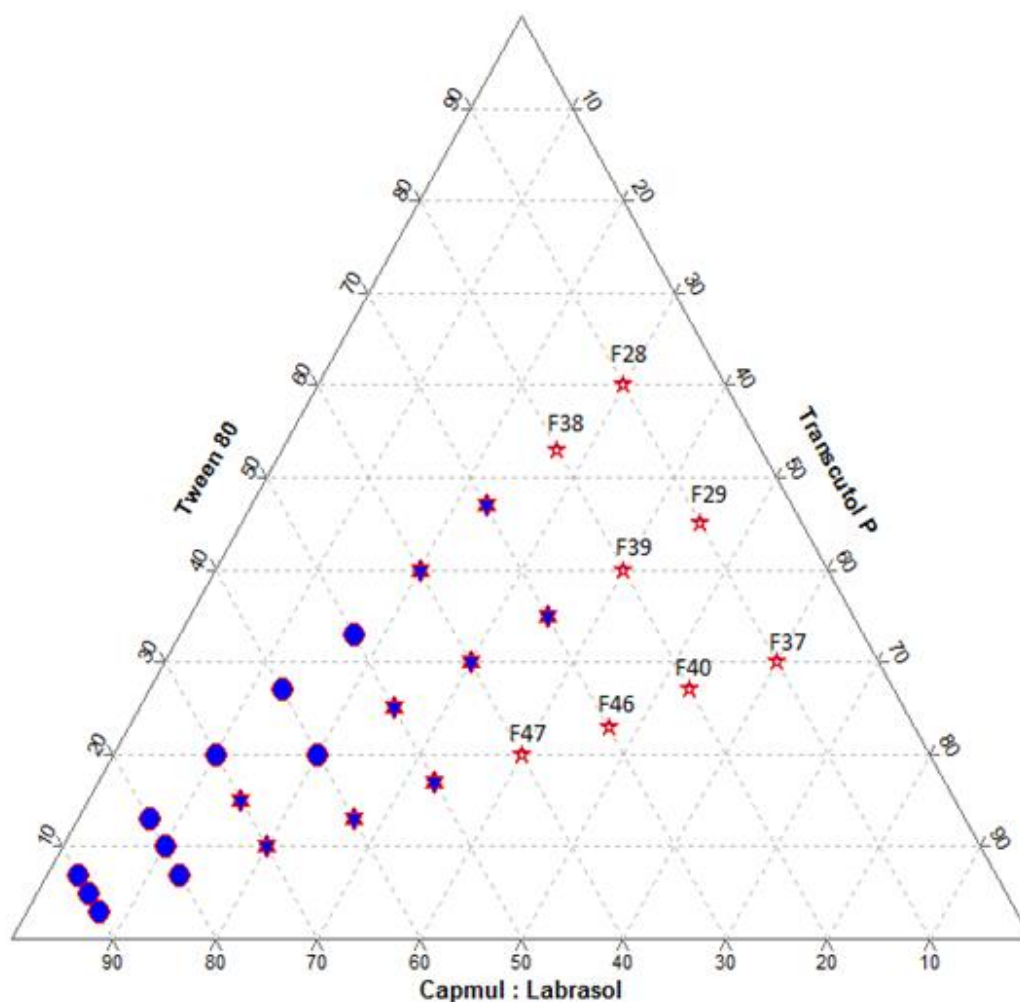
(8K) 2D & 3D-counter plot for effects of Milling speed and solvent/antisolvent on zeta potential of nanosuspensions.

Fig. 8. Perturbation plot for (A- B) and 2D & 3D-counter plot (C-K)

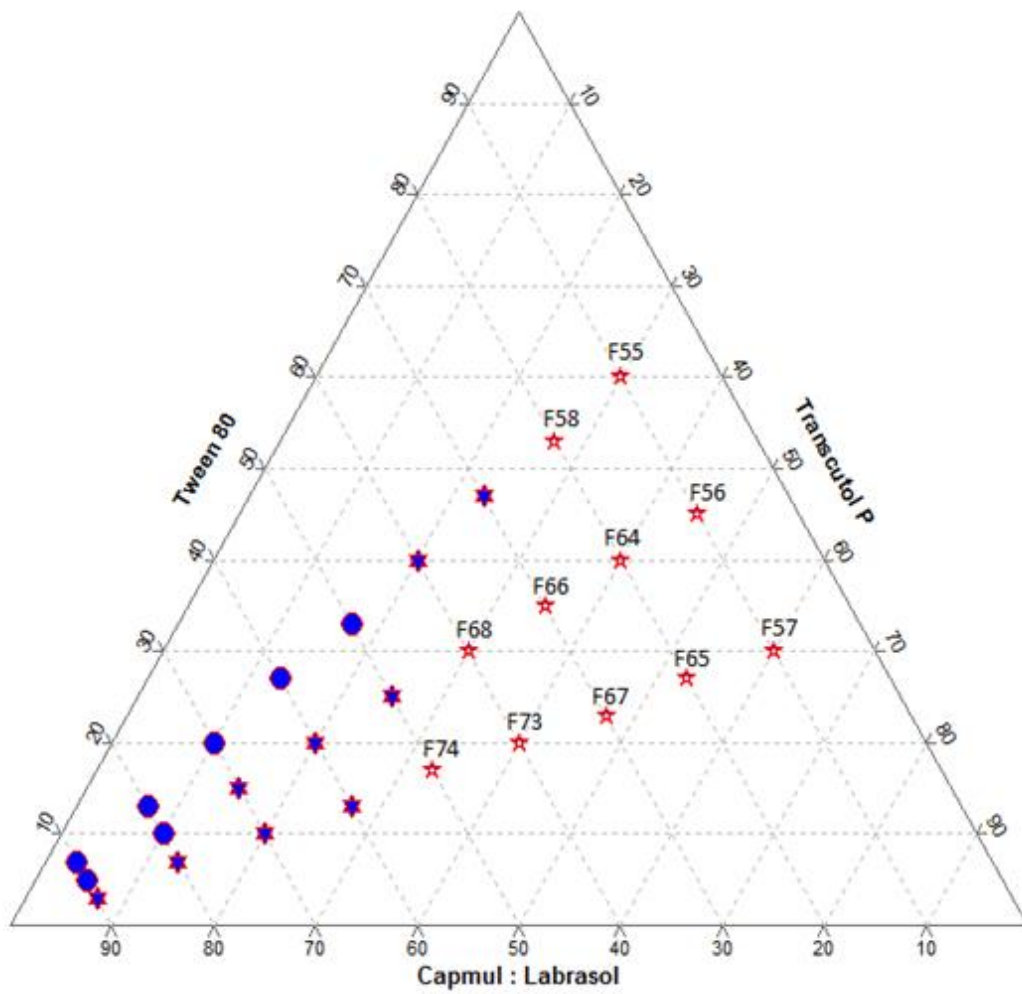
## 7.5. Preparation and optimization of L-SNEDDS using ternary phase diagram

### 7.5.1 Construction of ternary phase diagram

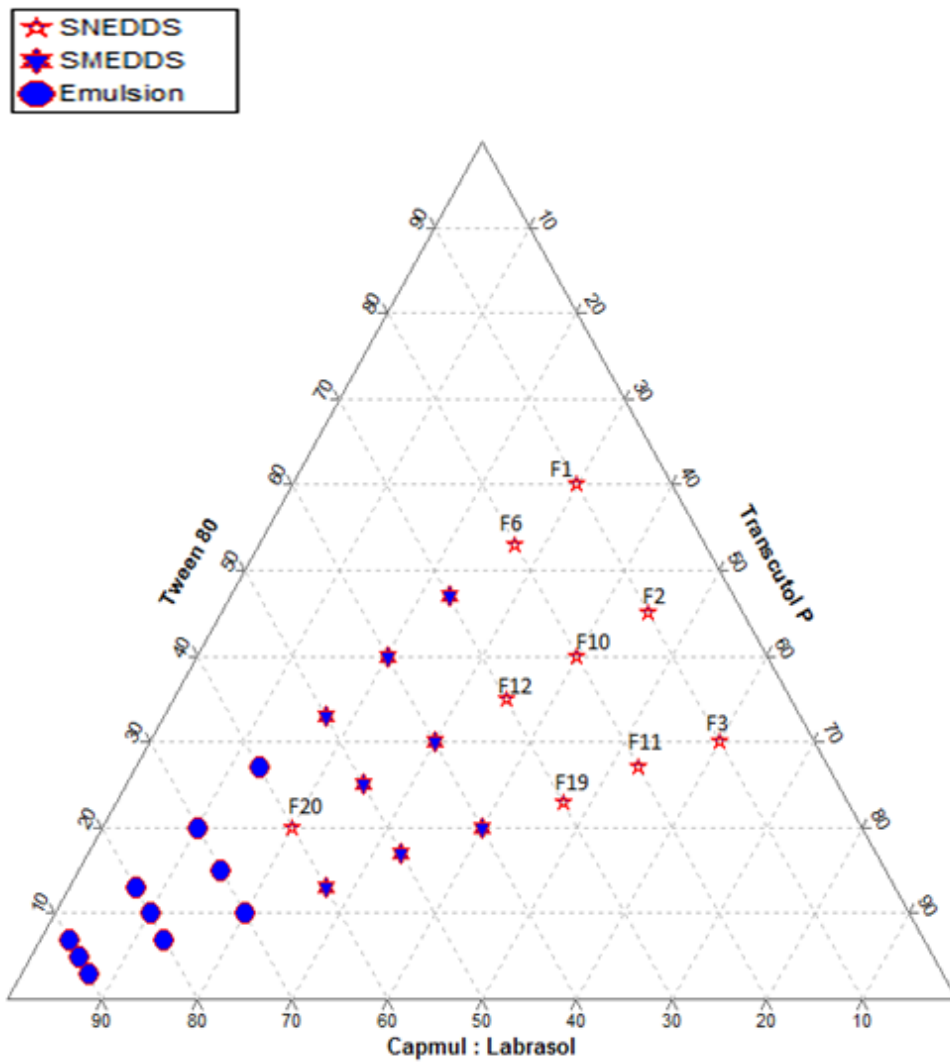
On the basis of level of transparency after dilution with distilled water, ternary phase diagram of batch 1 (Fig. 9A), batch 2 (Fig. 9B) and for batch 3 (Fig. 9C) were constructed and nano- region of ternary phase diagram was labelled. Nanoemulsions F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>6</sub>, F<sub>10</sub>, F<sub>11</sub>, F<sub>12</sub>, F<sub>19</sub>, F<sub>20</sub>, F<sub>28</sub>, F<sub>29</sub>, F<sub>37</sub>, F<sub>38</sub>, F<sub>39</sub>, F<sub>40</sub>, F<sub>46</sub> and F<sub>47</sub>, F<sub>55</sub>, F<sub>56</sub>, F<sub>57</sub>, F<sub>58</sub>, F<sub>64</sub>, F<sub>65</sub>, F<sub>66</sub>, F<sub>67</sub>, F<sub>68</sub>, F<sub>73</sub> and F<sub>74</sub> were selected from nanoregion and subjected for further studies.



(9A)



(9B)



(9C)

Fig. 9. Ternary phase diagram (A) Batch1; (B) Batch 2; (C) Batch 3.

### **7.5.2. Thermodynamic stability and cloud point determination of selected L-SNEDDS**

The selected 29 formulations using ternary phase diagram were subjected for thermodynamic stability studies. These L-SNEDDS were screened for droplet size, drug loading (%), cloud point temperature, appearance, phase separation after storage at 40°C for 48 h, centrifugation, heating /cooling cycles and after freeze/thaw cycles, respectively (Table 28). It was observed that among the selected 28 SNEDDS prototypes, formulation F<sub>68</sub> has shown least droplet size, highest drug loading and better stability against thermodynamic stress and kinetic stress (centrifugation).

### **7.6. Drug loading of S-SNEDDS and SP-NS**

Drug loading of S-SNEDDS (F<sub>68</sub>) was found to be 95.5% and 92.63% for GLM and SIM respectively. Drug loading of SP-NS was found to be 95.25% and 80.30% for GLM and SIM respectively.

### **7.7. OAC**

A-200 showed the highest OAC than any other carriers used to prepare S-SNEDDS.

The OAC of carriers was observed in the following decreasing order:

A-200 (185 mg) > SFP (230 mg) > SXDP (405 mg) > MCC PH 102 (415 mg) > HP-β-CD (490 mg) > Na-CMC (610 mg) > Lactose (1580 mg) > MS (1720 mg)

### **7.8. Droplet size and PDI analysis of solid-SNEDDS (S-SNEDDS)**

The average droplet diameter and PDI of the formulation S-SNEDDS and L-SNEDDS is shown in Table 30. The optimized L-SNEDDS average droplet size was  $55.63 \pm 1.78$  nm with very low  $0.24 \pm 0.06$  PDI. The results revealed strong influence of solidification process and strong carriers on responses. During their dilution in water, S-SNEDDS powders showed fast dispersion (within 30 sec). It is also essential to note that better outcomes have been achieved by hydrophobic carriers. Lactose, zinc stearate, Na-CMC and HPβ-CD has shown greater droplet size. Only A-200 showed the droplet diameter value closer to the L-SNEDDS value. The increasing order of droplet size using various carriers is shown below:

A-200 < SXDP < SFP < MCC PH102 < HPβ-CD < Na-CMC < MS < Lactose



**Table 29:** Composition of selected batches of SIM and GLM loaded L-SNEDDS (% w/w) and evaluation parameters.

Formulation code	Mean droplet size (nm)	% Drug Loading (GLM)	% Drug Loading (SIM)	Cloud point (°C)	Appearance	Phase separation after 48h at 40°C	Phase separation after centrifugation	Phase separation after heating /cooling cycles	Phase separation after freeze / thaw cycle
F <sub>1</sub>	127.30 ± 2.11	67.30 ± 1.65	43.50 ± 0.43	94.16	TP*				
F <sub>2</sub>	171.22 ± 1.57	71.50 ± 1.32	51.40 ± 1.23	92.54	TP				
F <sub>3</sub>	272.06 ± 3.21	59.70 ± 2.11	37.80 ± 0.77	93.18	TL**				
F <sub>6</sub>	232.73 ± 2.78	54.20 ± 1.78	57.90 ± 1.47	97.54	TL				
F <sub>10</sub>	111.54 ± 1.08	59.50 ± 0.87	45.10 ± 1.11	92.48	TP				
F <sub>11</sub>	98.67 ± 1.23	67.20 ± 0.78	39.80 ± 0.52	91.18	TP				
F <sub>12</sub>	256.44 ± 3.04	77.30 ± 1.03	60.10 ± 0.89	94.42	TL				
F <sub>19</sub>	156.93 ± 1.89	69.40 ± 0.59	55.30 ± 1.63	91.17	TP	No	No	No	No
F <sub>20</sub>	112.67 ± 2.63	66.30 ± 1.24	48.10 ± 2.11	88.16	TP				
F <sub>28</sub>	267.20 ± 3.67	52.80 ± 0.88	33.80 ± 0.96	85.31	TL				
F <sub>29</sub>	282.04 ± 4.11	62.20 ± 2.06	51.70 ± 1.37	91.67	TL				
F <sub>37</sub>	139.98 ± 1.45	73.60 ± 1.43	55.30 ± 2.33	88.24	TP				
F <sub>39</sub>	189.56 ± 2.22	80.30 ± 2.33	50.80 ± 2.08	97.13	TP				
F <sub>40</sub>	210.34 ± 3.08	55.70 ± 1.39	57.40 ± 1.89	92.17	TL				
F <sub>46</sub>	157.11 ± 0.98	64.10 ± 1.65	47.40 ± 1.54	92.50	TP				
F <sub>47</sub>	166.09 ± 1.07	72.80 ± 0.98	59.30 ± 1.67	86.72	TP				
F <sub>55</sub>	145.10 ± 0.88	58.30 ± 0.75	57.20 ± 1.35	89.44	TP				
F <sub>56</sub>	222.42 ± 2.33	77.90 ± 1.11	55.60 ± 1.48	93.78	TL				
F <sub>57</sub>	236.13 ± 1.55	61.40 ± 1.36	60.40 ± 2.22	94.21	TL				
F <sub>58</sub>	267.77 ± 3.11	56.40 ± 2.11	49.60 ± 1.67	95.33	TL				
F <sub>64</sub>	189.34 ± 1.45	81.30 ± 1.71	52.80 ± 1.82	94.26	TP				
F <sub>65</sub>	174.33 ± 1.55	68.20 ± 1.05	47.90 ± 0.77	93.54	TP				
F <sub>66</sub>	123.67 ± 1.09	70.70 ± 1.77	49.60 ± 1.44	89.90	TP				
F <sub>67</sub>	118.22 ± 1.22	66.90 ± 2.45	53.70 ± 1.11	92.15	TP				
F <sub>68</sub>	55.63 ± 1.78	94.50 ± 2.74	79.20 ± 1.94	93.48	TP				
F <sub>73</sub>	108.11 ± 1.41	73.90 ± 1.67	60.40 ± 0.65	90.54	TP				
F <sub>74</sub>	140.76 ± 1.52	67.40 ± 1.49	58.80 ± 0.56	91.67	TP				

TP\* - Transparent; TL\*\* - Translucent

**Table 30:** Droplet size and PDI of various carriers

Formulations of S-SNEDDS prepared using different carriers	Droplet size (nm)	Polydispersity Indices (PDI)
L-SNEDDS	55.63 ± 1.78	0.24 ± 0.06
A-200	75.26 ± 2.38	0.27 ± 0.09
SXDP	89.19 ± 2.31	0.32 ± 0.01
SFP	96.38 ± 1.16	0.56 ± 0.02
MCC PH102	266.67 ± 1.46	0.66 ± 0.03
HPβ-CD	387.26 ± 3.23	0.42 ± 0.00
Na-CMC	418.16 ± 1.34	0.51 ± 0.02
MS	486.18 ± 6.69	0.43 ± 0.02
Lactose	566.18 ± 7.69	0.65 ± 0.03

### 7.9. Micromeritics characteristics of S-SNEDDS and SP-NS

The findings about micromeritics characteristics studies of SP-NS and S-SNEDDS are shown in Table 31. It was found that the bulk density of S-SNEDDS prepared by using different carriers was ranging from  $0.21 \pm 0.22$  and  $0.33 \pm 0.09$  g/cm<sup>3</sup>, and tapped density from  $0.24 \pm 0.22$  and  $0.45 \pm 0.22$  g/cm<sup>3</sup>. The angle of repose was ranging from  $18.33 \pm 1.16$  to  $43.53 \pm 1.18$  (°), and Carr's index from  $12.50 \pm 0.04$  to  $34.37 \pm 1.54$ , respectively. The difference in physiochemical properties and OAC of the materials resulted in variable micromeritic properties of porous carriers. A-200 resulted promising results in development of S-SNEDDS, hence, other carriers were discontinued from further studies. It is important to know that all the micromeritic properties of SP-NS were found within the pharmacopoeial limits.

**Table 31:** Micromeritic characteristics of S-SNEDDS and SP-NS.

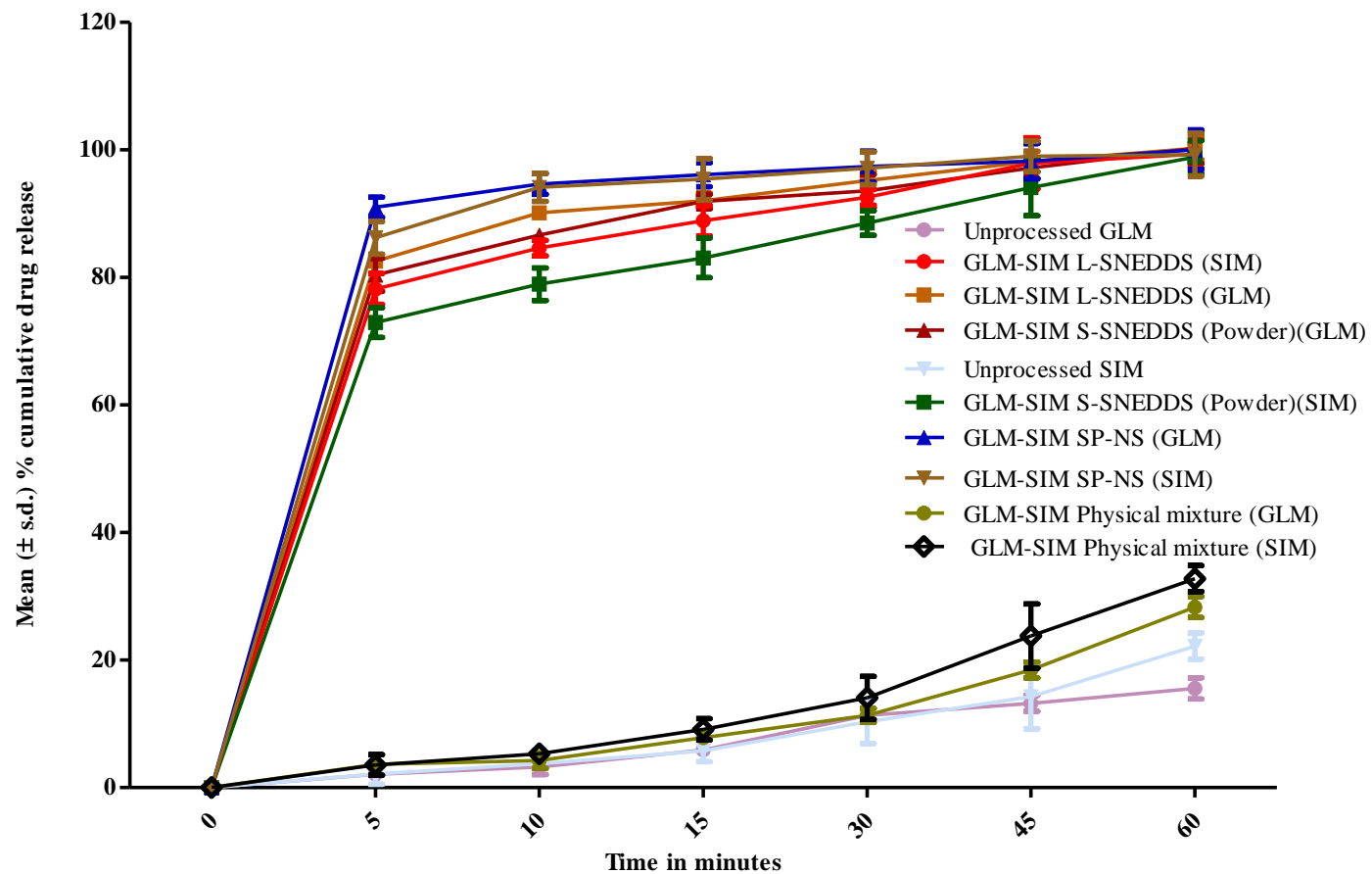
Component	Angle of repose (°)	Bulk Density (g/cm <sup>3</sup> )	Tap Density (g/cm <sup>3</sup> )	Carr's index
A-200	18.33 ± 1.16	0.21 ± 0.22	0.24 ± 0.22	12.50 ± 0.04
SFP	33.42 ± 1.22	0.27 ± 0.09	0.36 ± 0.01	25.00 ± 1.38
SXDP	24.22 ± 1.18	0.26 ± 0.09	0.32 ± 0.04	18.75 ± 1.84
MS	34.79 ± 1.46	0.33 ± 0.09	0.43 ± 0.03	23.26 ± 0.81
MCC PH102	43.53 ± 1.18	0.22 ± 0.14	0.33 ± 0.22	33.33 ± 0.90
Na-CMC	24.14 ± 1.87	0.29 ± 0.07	0.41 ± 0.09	29.27 ± 1.30
HPβ-CD	37.50 ± 1.44	0.21 ± 0.03	0.32 ± 0.08	34.37 ± 1.54
Lactose	35.22 ± 1.23	0.31 ± 0.18	0.45 ± 0.22	31.11 ± 2.10
SP-NS	29.68 ± 1.07	0.23 ± 0.07	0.32 ± 0.04	28.13 ± 1.67

### 7.10. In- vitro dissolution studies

Dissolution study of L-SNEDDS, S-SNEDDS, SP-NS, physical mixture of GLM-SIM and unprocessed GLM and unprocessed SIM in phosphate buffer pH 6.8 is shown in

Fig. 10. *In-vitro* drug release studies showed that L-SNEDDS, S-SNEDDS and SP-NS exhibited significant ( $P < 0.05$ ) faster drug release than PM and their unprocessed forms. The % cumulative release of GLM from SP-NS, L-SNEDDS and S-SNEDDS were shown  $100.04 \pm 3.10$ ,  $100.24 \pm 1.80$  and  $100.24 \pm 2.40$  respectively. Similarly, *in-vitro* release of SIM from SP-NS, L-SNEDDS and S-SNEDDS were observed  $99.23 \pm 3.35$ ,  $99.33 \pm 2.20$  and  $98.88 \pm 2.56$  respectively within 60 min. The percentage cumulative release of GLM from PM and unprocessed form were found to be  $28.32 \pm 1.64$  and  $15.56 \pm 1.64$  respectively. Percentage release rate of SIM from PM and unprocessed form were observed  $32.77 \pm 2.14$  and  $22.20 \pm 2.04$  respectively within 60 min of dissolution studies. These results indicated that there is no significant ( $P > 0.05$ ) difference were observed in dissolution profiles of both the drugs within the nano formulations. The dissolution profiles were subjected to release kinetic studies to understand the release mechanism drugs from each formulation as well as unprocessed drugs.

From the largest values of the acquired correlation coefficient ( $r$ ), the best kinetic order for *in-vitro* release of drugs from SP-NS, L-SNEDDS and S-SNEDDS formulation was calculated. Table 32 showed that the *in-vitro* release of GLM from SNEDDS (L & S) follows first order kinetics whereas Peppas model was observed in SP-NS. Release of SIM from all nano formulations obeys first order release model only.



**Fig. 10:** Dissolution profiles (mean  $\pm$  s.d.) of L-SNEDDS, S-SNEDDS, SP-NS, physical mixture of GLM-SIM and unprocessed GLM and unprocessed SIM (n = 6).

**Table 32:** *In-vitro* drug release kinetic models.

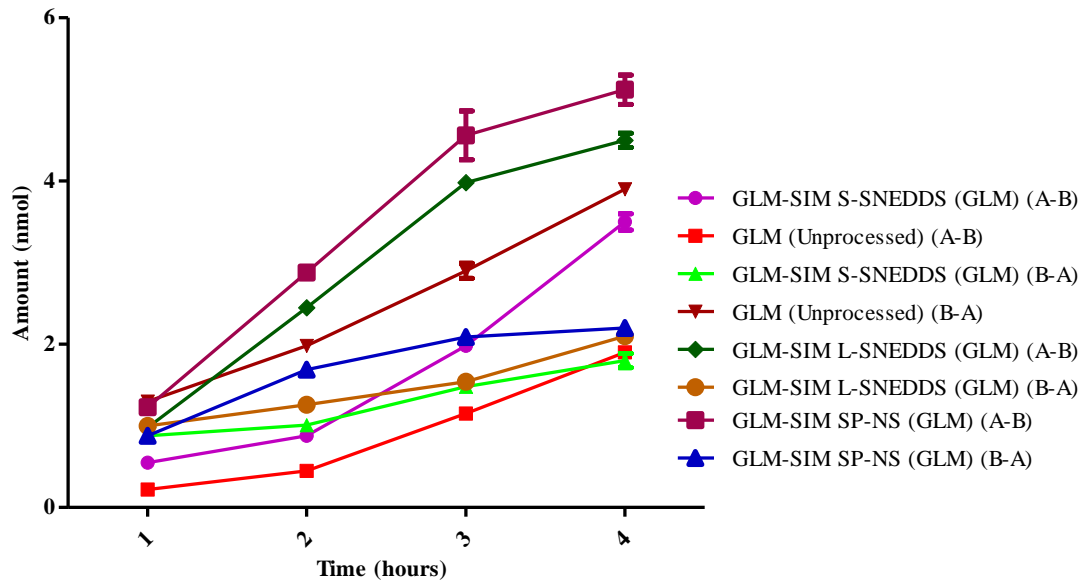
Kinetic model	GLM-SIM PM. (GLM)	GLM-SIM L-SNEDDS (GLM)	GLM-SIM S-SNEDDS (Powder) (GLM)	GLM-SIM PM. (SIM)	GLM-SIM L-SNEDDS (SIM)	GLM-SIM S- SNEDDS (Powder) (SIM)	GLM-SIM SP-NS (GLM)	GLM-SIM SP-NS (SIM)
	$r^2$	$r^2$	$r^2$	$r^2$	$r^2$	$r^2$	$r^2$	$r^2$
Zero order	0.956	0.35	0.372	0.989	0.399	0.4569	0.278	0.301
Fist order	0.963	0.857	0.870	0.983	0.930	0.906	0.680	0.791
Huguchi model	0.955	0.624	0.647	0.899	0.6732	0.7251	0.545	0.574
Hixson	0.960	0.8061	0.8098	0.985	0.7575	0.7927	0.668	0.566
Kor's peppas	0.865	0.775	0.7937	0.763	0.8185	0.8533	0.702	0.733

### ***7.11. Cellular permeability and post-cytotoxicity tests in Caco-2 cell monolayer***

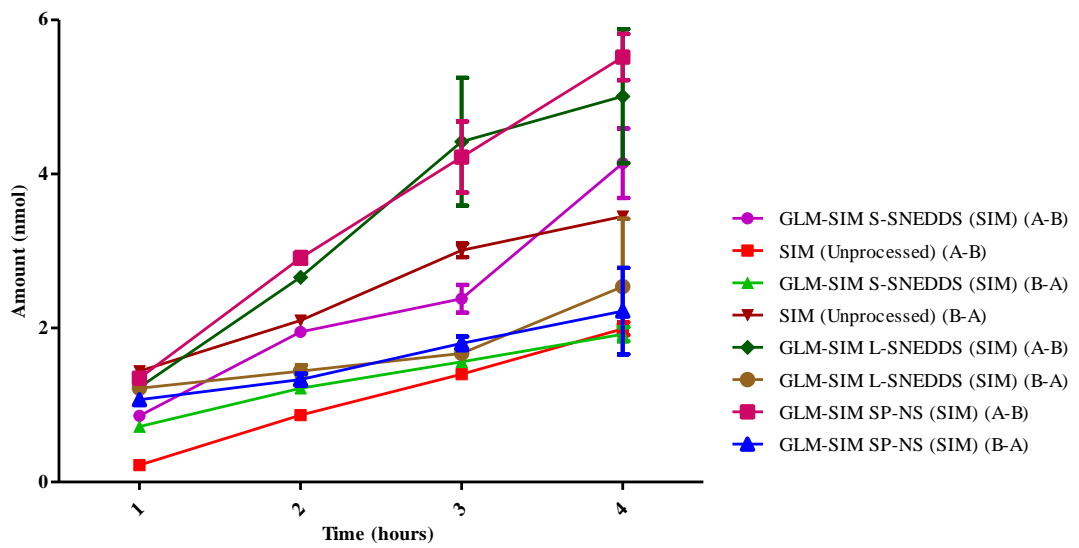
Results of GLM and SIM permeation are shown in Fig. 11A and Fig. 11B respectively. In case of GLM about 2.37, 1.84 and 2.69 folds increase in drug permeation was observed from L-SNEDDS, S-SNEDDS and SP-NS respectively as compared to their unprocessed form. While 1.85, 2.17 and 1.77 times decrease in excretion of GLM from L-SNEDDS, S-SNEDDS and SP-NS respectively were observed as compare to their unprocessed form. In case of SIM about 2.52, 2.08- and 2.78 folds increase in drug permeation was observed from L-SNEDDS, S-SNEDDS and SP-NS respectively as compare to their unprocessed form. While 1.36, 1.80, and 1.55 times decrease in excretion of drugs from L-SNEDDS, S-SNEDDS and SP-NS respectively were observed as compare to unprocessed SIM.

Hence, enhanced dissolution profile and permeability of both the drugs through SNEDDS and SP-NS is an indication of improved bioavailability of drugs when administered through oral route. In order to have better insight of oral bioavailability of both the drugs through nanoformulations, pharmacokinetic studies in rats have been conducted.

Cell viability studies carried out using MTT based assay on Caco-2 cell monolayer and results are shown in Fig. 12. The percentage cell viability of unprocessed GLM and SIM were found to be  $96.88 \pm 4.55$  and  $97.32 \pm 3.89$  respectively. S-SNEDDS and SP-NS loaded with GLM have shown cell viability  $91.54 \pm 3.90$  and  $93.45 \pm 3.98$  respectively. The percentage cell viability of S-SNEDDS and SP-NS loaded with SIM were found to be  $90.78 \pm 5.63$  and  $88.78 \pm 3.33$  respectively. The above results strongly indicated that there is no significant ( $P < 0.05$ ) difference in cell viability of nano-formulations with unprocessed forms. Cell viability results also indicated that more than 85% cells were viable after the treatment with nano formulations.

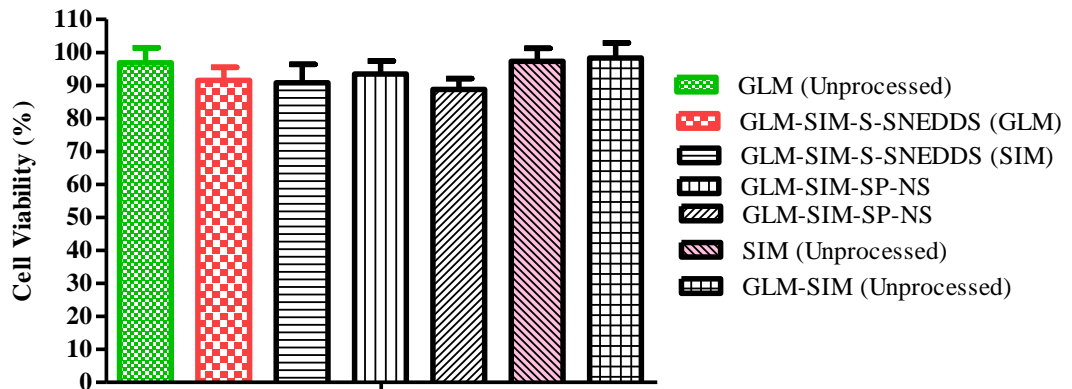


(11A)



(11B)

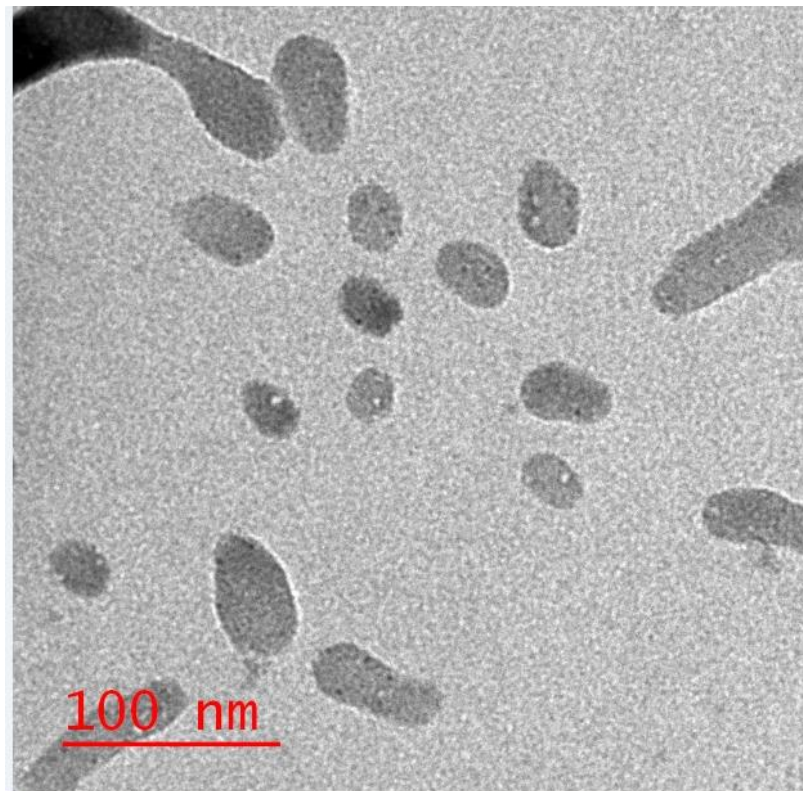
**Fig. 11:** Cell permeability (mean  $\pm$  s.d.) of A. GLM and B. SIM from nanoformulations and their unprocessed forms (n = 3).



**Fig. 12:** Cell permeability (mean  $\pm$  s.d.) of A. GLM and B. SIM from nanoformulations and their unprocessed forms (n = 3).

### 7.12. TEM analysis

The TEM image revealed spherical and unagglomerated droplets in nanometer range (Fig. 13).

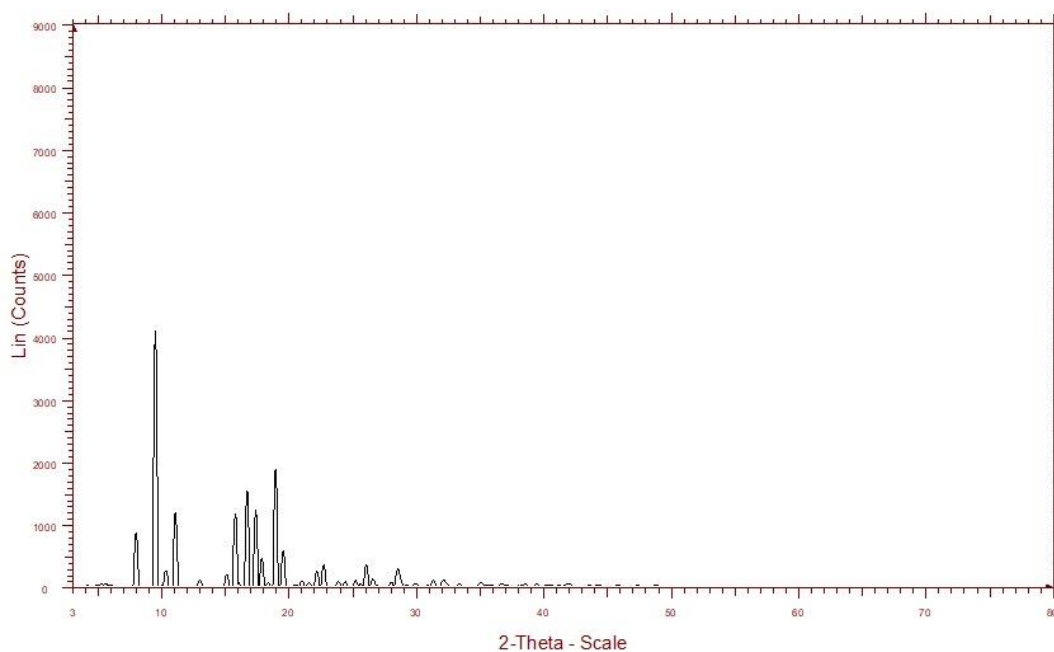


**Fig. 13:** TEM image of S-SNEDDS of SIM-GLM mixture.

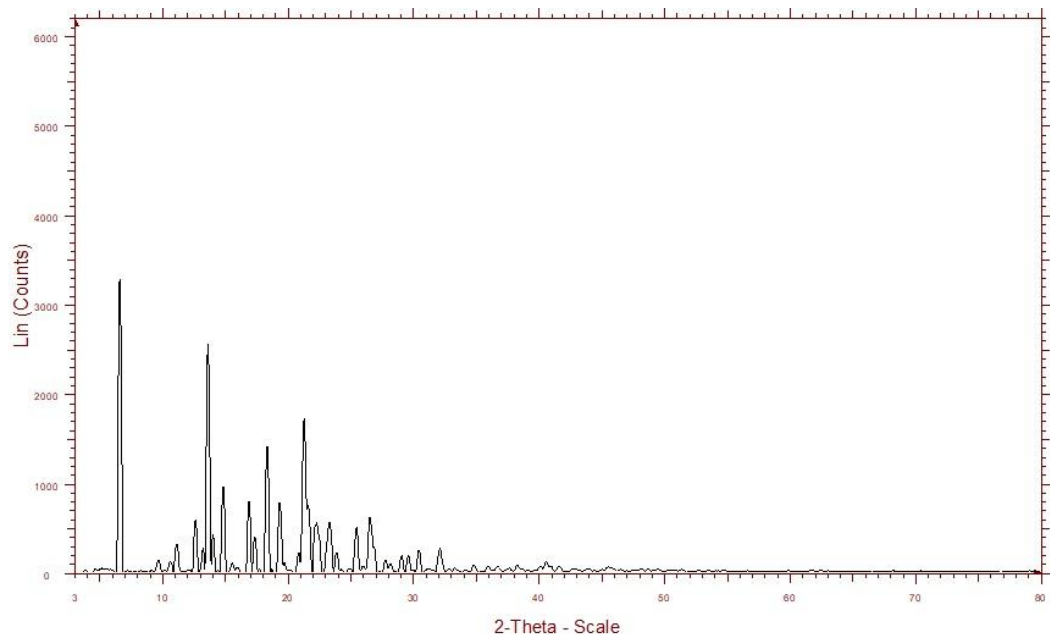


### 7.13. Powder X-ray Diffraction (PXRD) studies

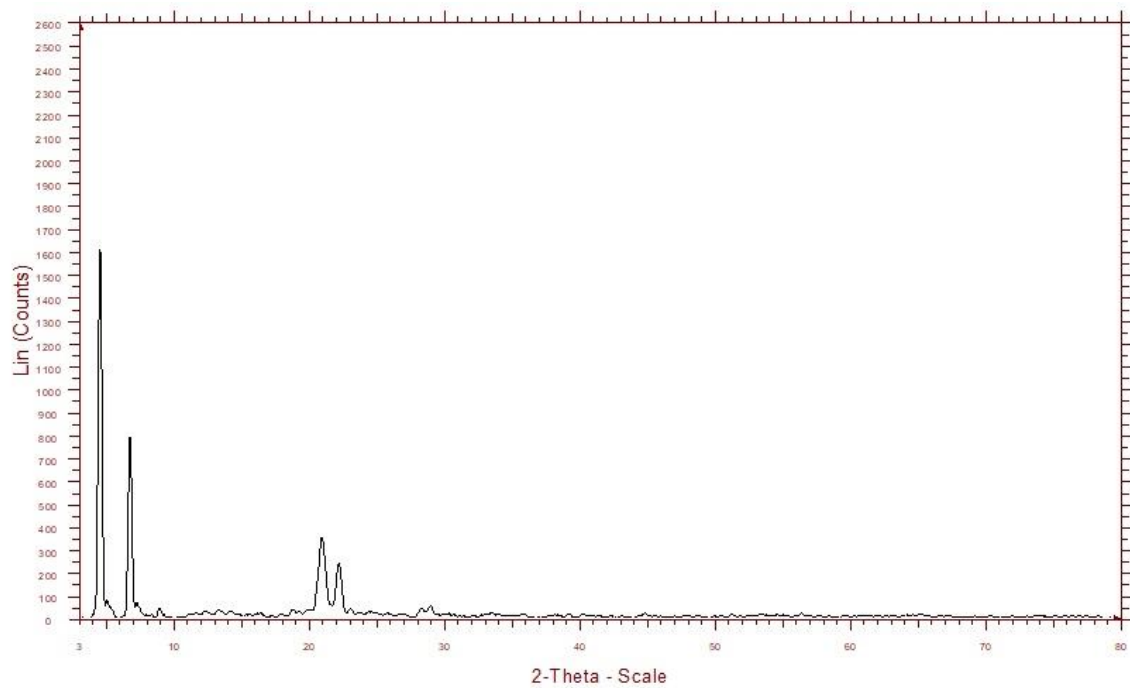
The PXRD patterns are presented in Fig. 14A- 14H. SIM and GLM had shown sharp endothermic peaks at the diffraction angles showing a typical crystalline pattern (Fig. 14A & Fig. 14B respectively). A halo pattern was observed for A-200 (Fig.14G). The S-SNEDDS formulation showed no peaks at diffraction angles, showing an amorphous pattern (Fig.14H). SLS, PVP-K30 and the SP-NS containing both the drugs showed crystalline structure (Fig. 14D-F). This indicated that in case of nanosuspension the dissolution rate got enhanced exclusively due to particle size reduction. In order to have better insight, the PXRD results were correlated with DSC studies.



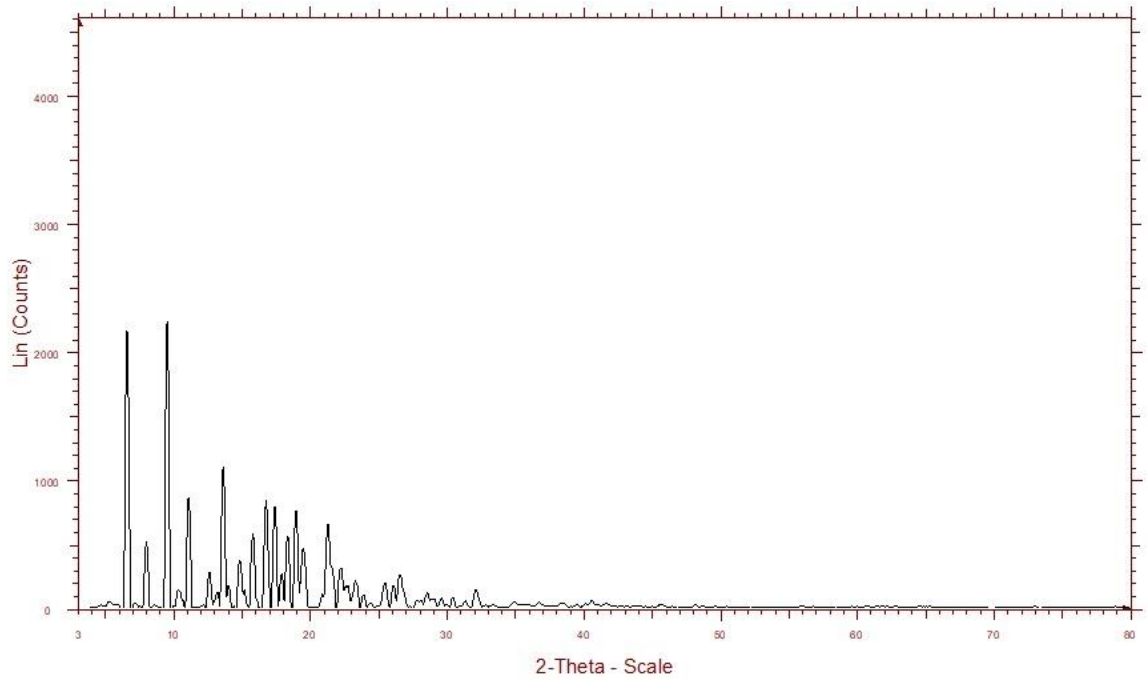
(14A) Unprocessed SIM



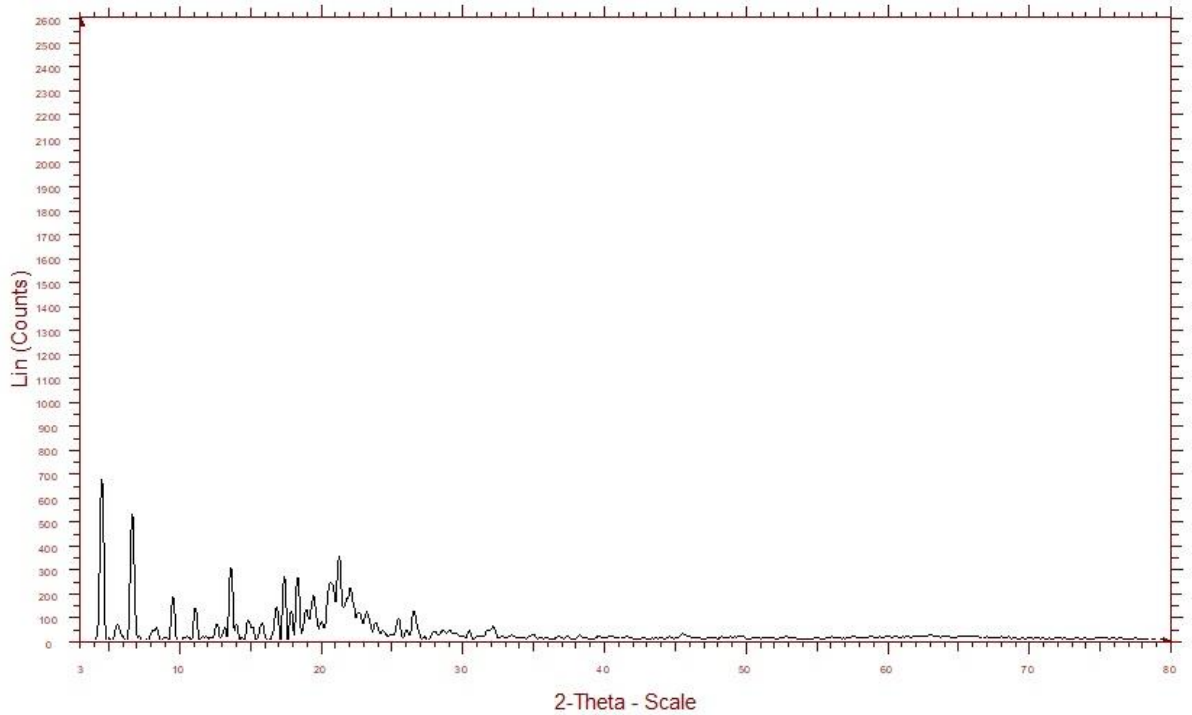
(14B) Unprocessed GLM



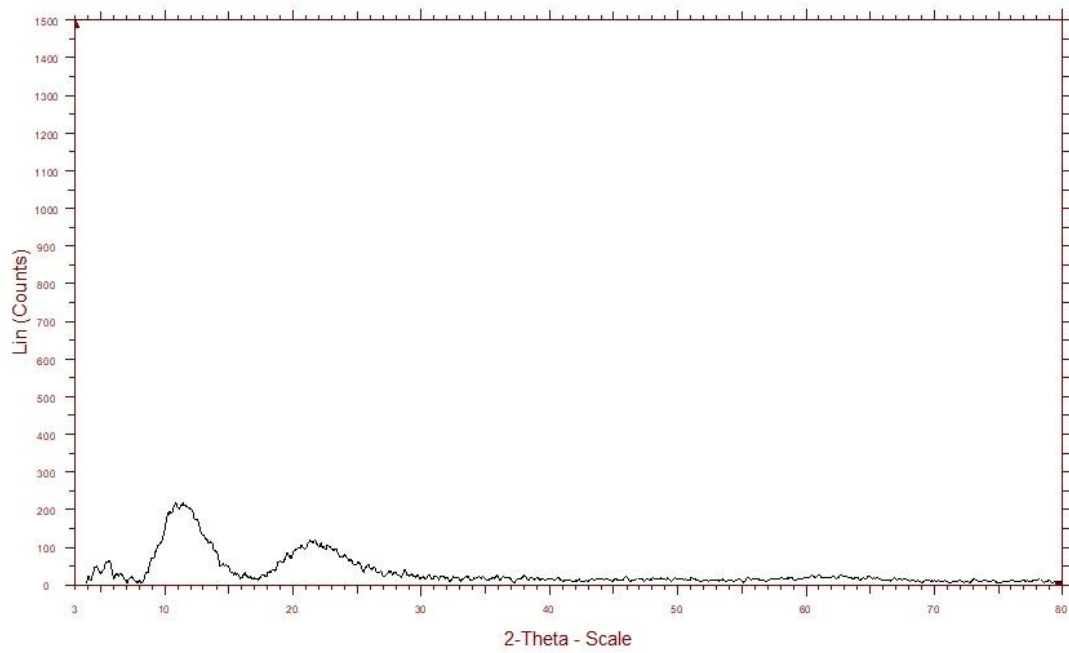
(14C) SLS



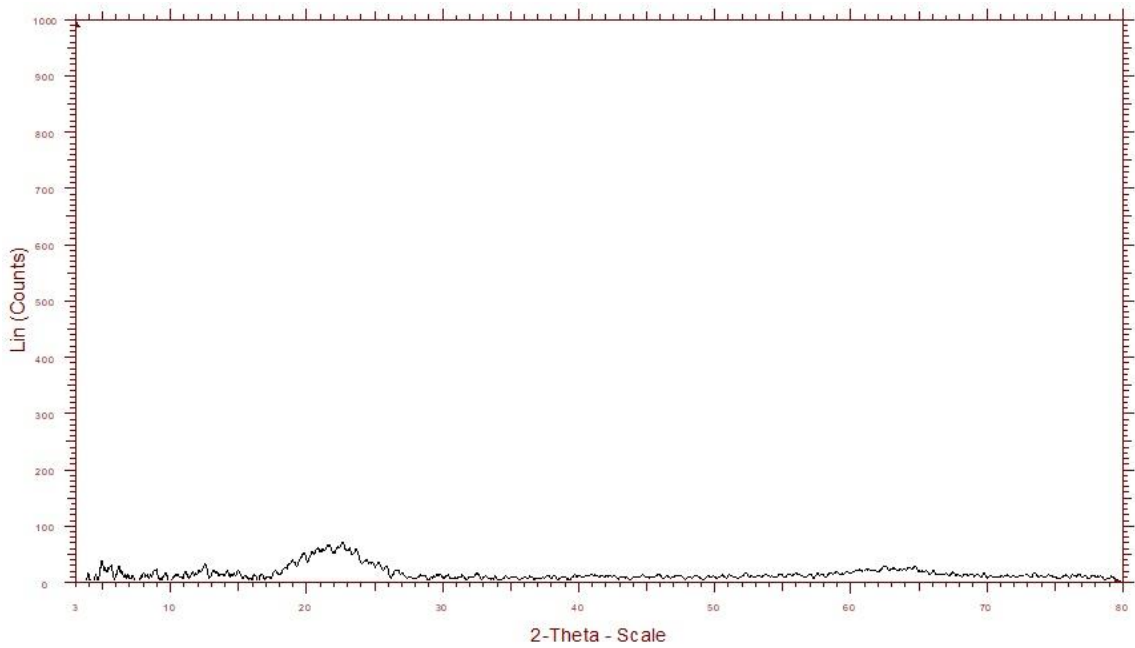
(14D) Physical mixture of SIM-GLM



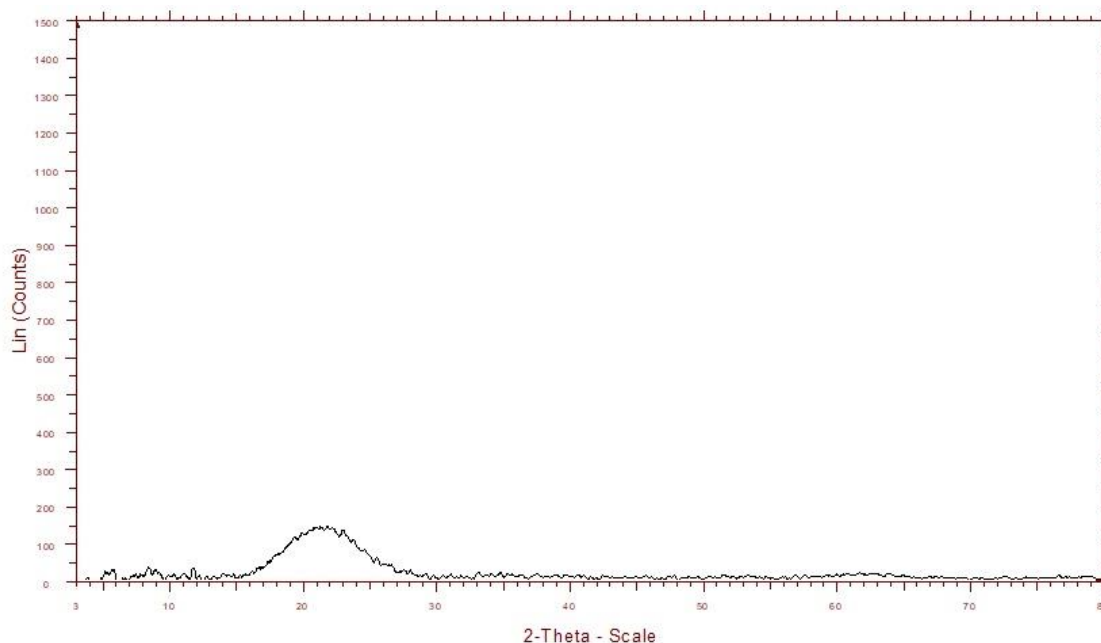
(14E) SP-NS of GLM-SIM mixture



(14F) PVP-K30



(14G) A-200

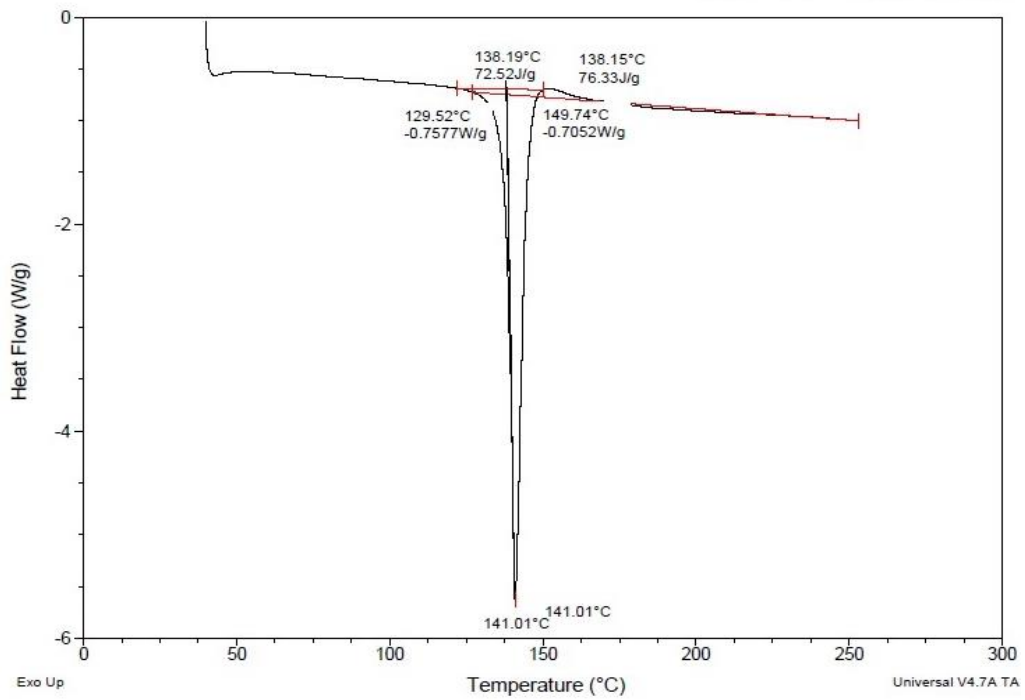


(14H) S-SNEDDS of SIM-GLM mixture

**Fig. 14:** X-ray diffraction patterns of unprocessed drugs, excipients and formulations.

#### **7.14. Differential Scanning Calorimetry (DSC) studies**

The thermograms for unprocessed SIM and GLM, SLS, PVPK-30, physical mixture of SIM and GLM, SP-NS of GLM and SIM mixture and S- SNEDDS powder are shown in Fig. 15A- 15G. The sharp endothermic peaks of unprocessed SIM and GLM were observed at 141.01°C and 210°C respectively. The sharpness of peaks revealed that both the drugs possess crystalline nature. A flat line with absence of melting endotherm was observed for A-200. This indicated that A-200 has amorphous nature, moreover the S-SNEDDS prepared using this were also found amorphous (Fig. 15G). The results indicated complete solubility of SIM and GLM in the isotropic mixture of oil and surfactant as well as complete adsorption of isotropic mixture on the porous surface of A-200 (Rajesh et al., 2018). Similar to the results of PXRD, SLS showed sharp endothermic peaks at 138.18°C (Fig.15C), PVP-K30 showed 155.02°C and 181.67°C (Fig. 15E) and SP-NS containing GLM and SIM showed sharp endothermic peaks (Fig. 15F) at 172.30°C (SIM) and 236.78°C (GLM), respectively. A little shift in melting point of both the drugs towards higher side in the SP-NS is attributed to the internal phase transformation during the formulation development.

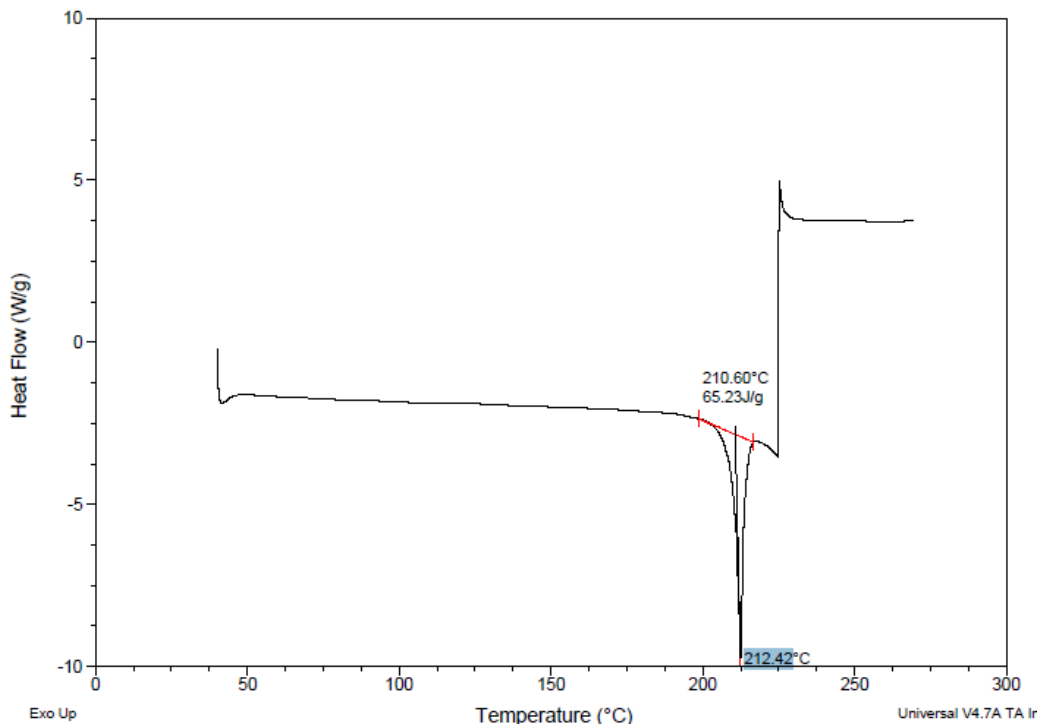


(15A) Unprocessed SIM

Sample: S2  
Size: 2.0000 mg  
Method: Ramp

DSC

File: C:\TAIData\DSC\AD Phd\LPUS2.001  
Operator: JSS  
Run Date: 22-Nov-2018 10:28  
Instrument: DSC Q200 V24.4 Build 116

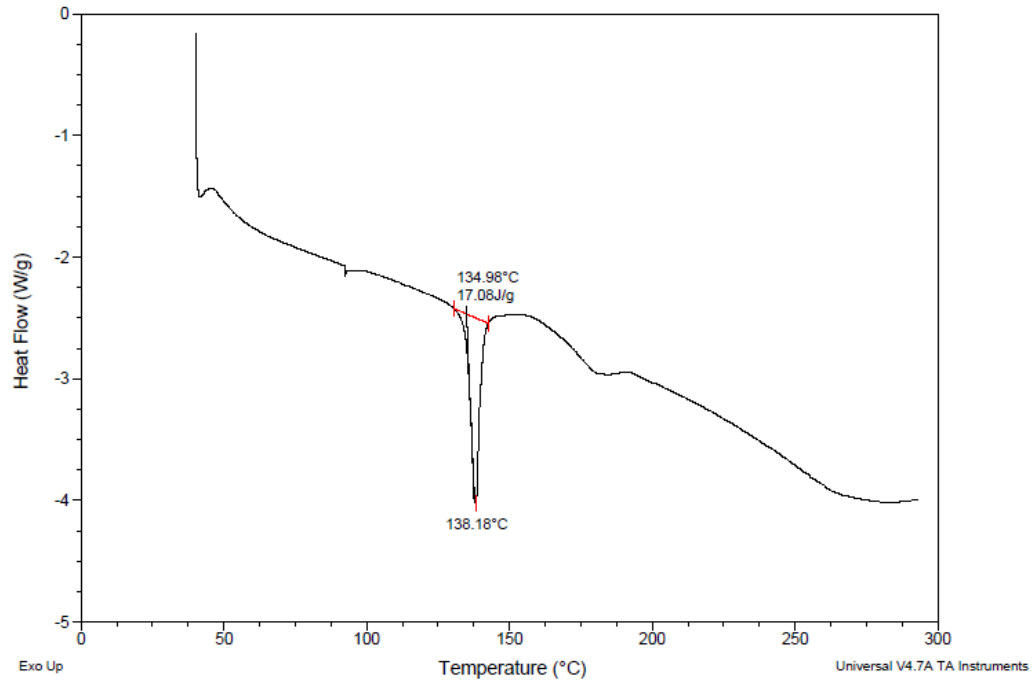


(15B) Unprocessed GLM

Sample: S3  
Size: 1.9000 mg  
Method: Ramp

DSC

File: C:\TA\Data\DSC\AD Phd\LPUS3.001  
Operator: JSS  
Run Date: 22-Nov-2018 11:41  
Instrument: DSC Q200 V24.4 Build 116

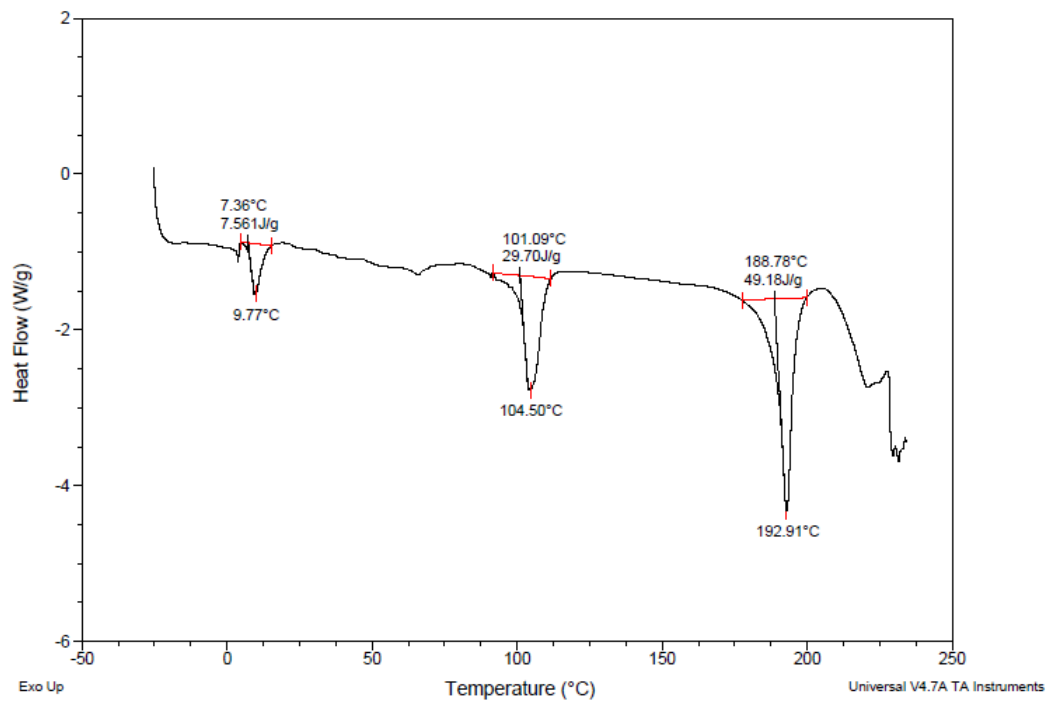


(15C) SLS

Sample: S4  
Size: 3.0000 mg  
Method: Ramp

DSC

File: C:\TA\Data\DSC\AD Phd\LPUS4.001  
Operator: JSS  
Run Date: 22-Nov-2018 09:45  
Instrument: DSC Q200 V24.4 Build 116

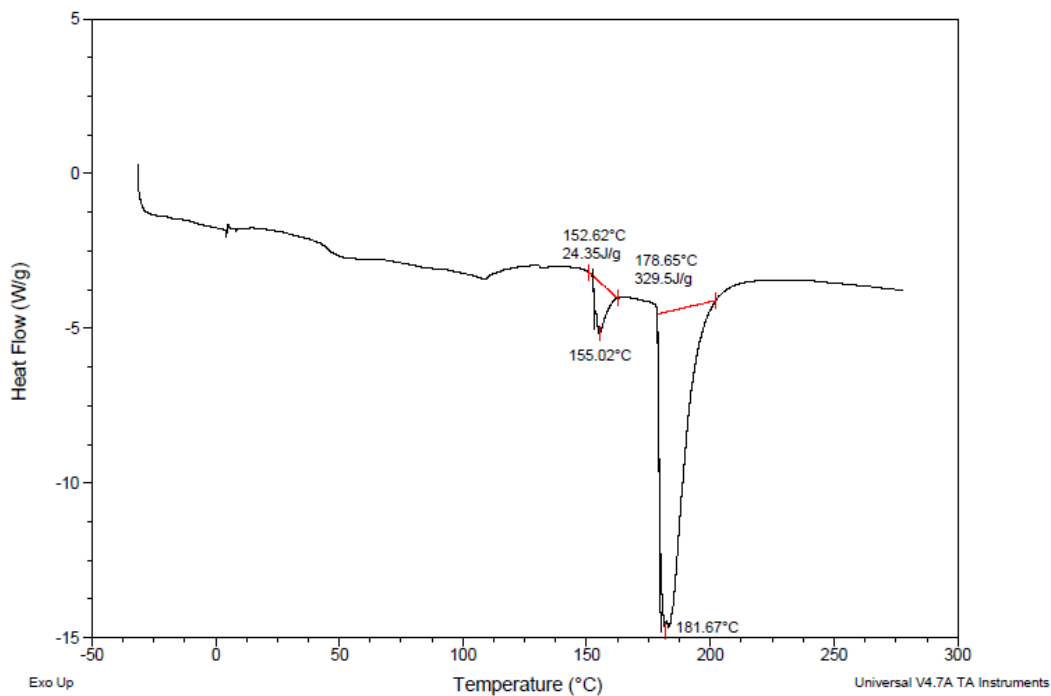


(15D) Physical mixture of GLM - SIM

Sample: S5  
Size: 2.1000 mg  
Method: Ramp

DSC

File: C:\TAData\DSC\AD Phd\LPUS5.001  
Operator: JSS  
Run Date: 22-Nov-2018 12:18  
Instrument: DSC Q200 V24.4 Build 116

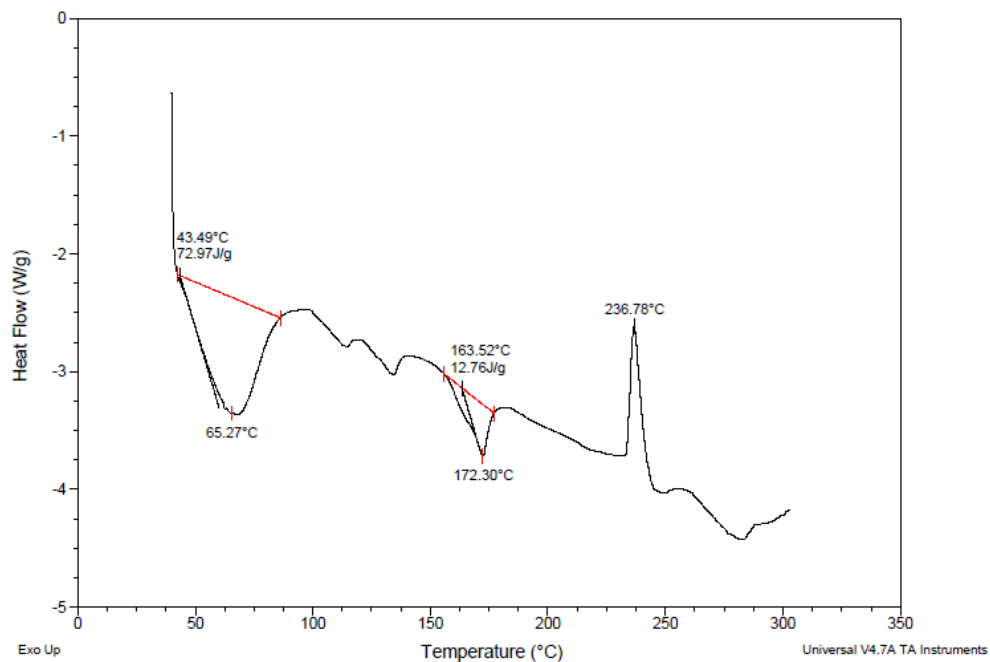


(15E) PVP – K30

Sample: S6  
Size: 2.2000 mg  
Method: Ramp

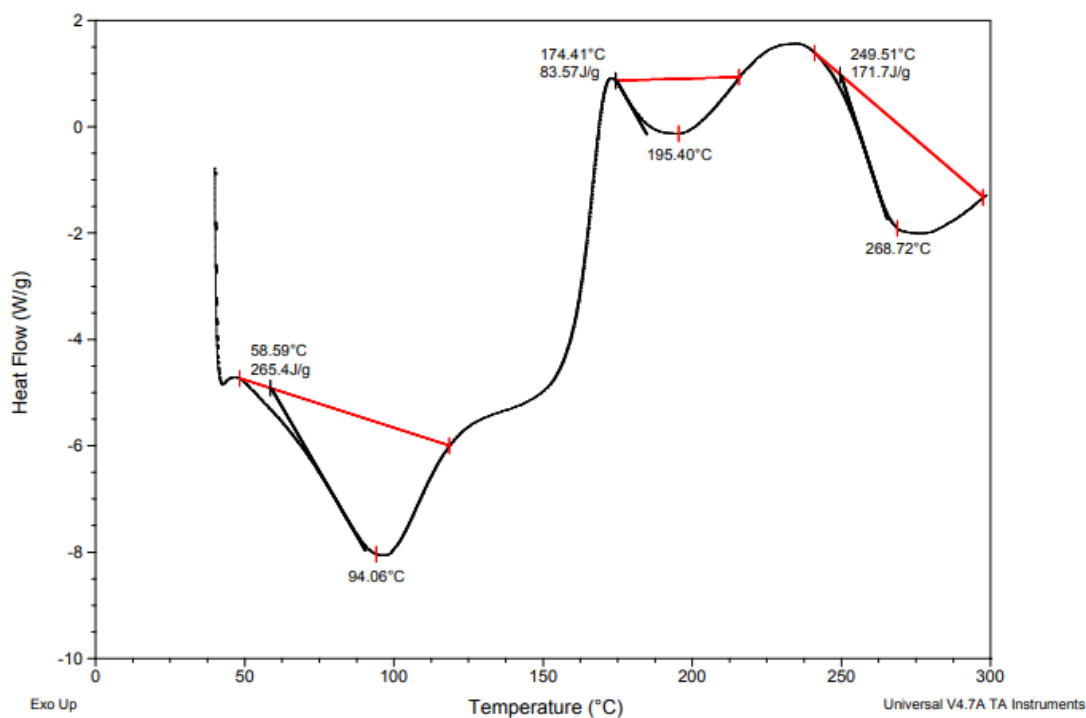
DSC

File: C:\TAData\DSC\AD Phd\LPUS6.001  
Operator: JSS  
Run Date: 22-Nov-2018 13:45  
Instrument: DSC Q200 V24.4 Build 116



(15F) SP – NS of GLM – SIM mixture



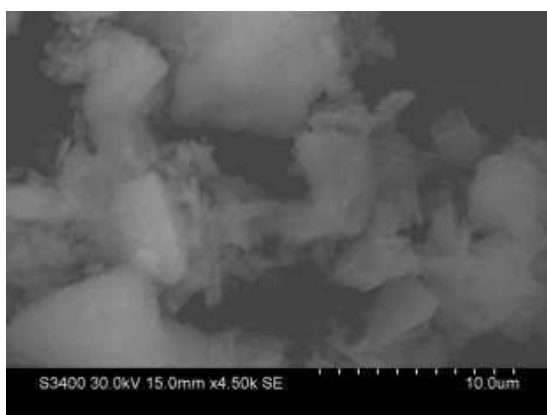


(15G). S-SNEDDS of GLM-SIM mixture

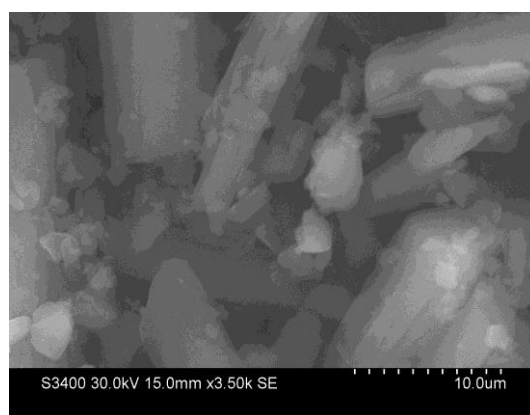
**Fig. 15:** DSC patterns of unprocessed drugs, excipients and formulations.

### 7.15. Scanning Electron Microscopy (SEM) studies

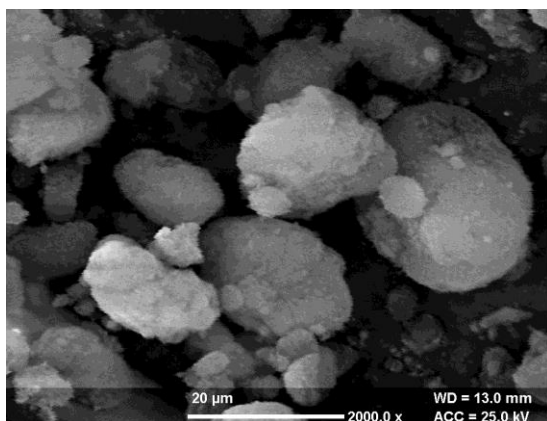
The SEM images of SIM, GLM, S-SNEDDS containing SIM-GLM and SP-NS containing SIM-GLM are shown in Fig. 16A-16D. GLM showed irregular crystalline cotton like structure (Fig.16A) and SIM appeared as flat, blade like smooth-surfaced rectangular crystals in shape with sharp irregular edges (Fig.16B). The S-SNEDDS appeared as rough-surfaced particles with porous and spherical aperture indicating that the liquid SNEDDS was absorbed or coated inside the pores of A-200 (Fig.16C). The SP-NS appeared as smooth and circular structure indicating the dispersion of drugs on the surface of carriers (i.e. SLS and PVP-K30) through spray drying (Fig.16D).



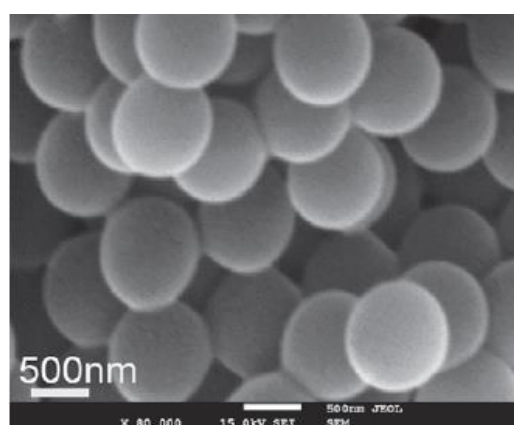
(16A)



(16B)



(16C)



(16D)

**Fig. 16:** SEM images of A. GLM; B. SIM; C. S-SNEDDS containing GLM-SIM; D. SP-NS containing GLM-SIM.

### 7.16. Stability study

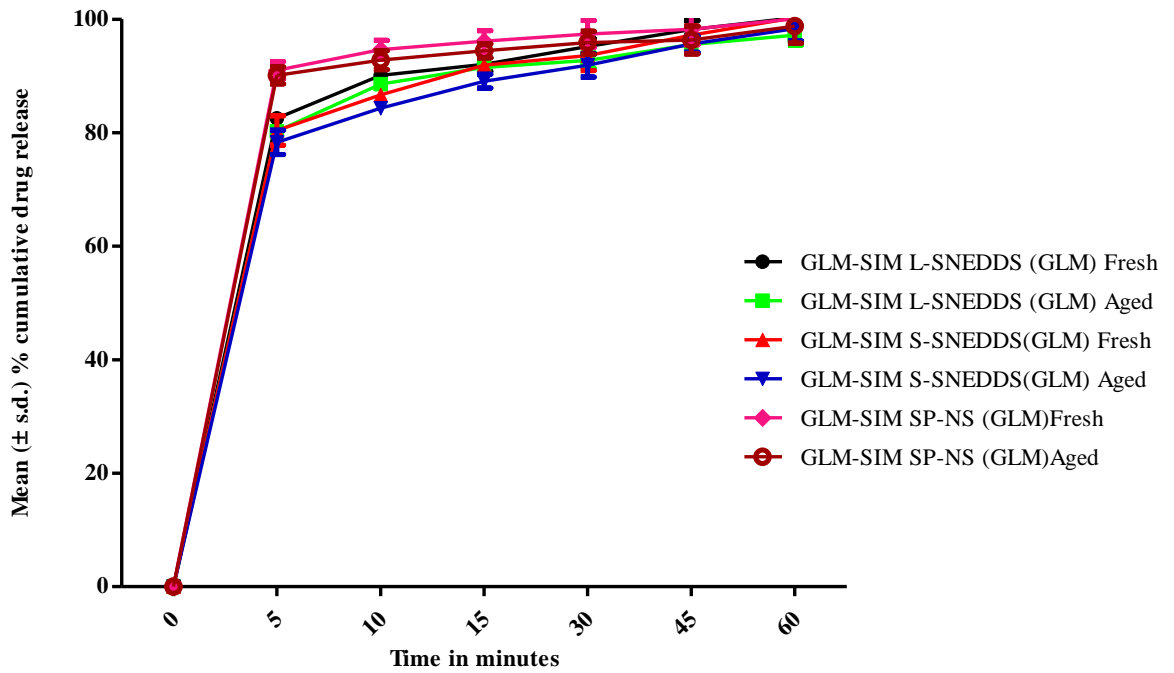
The results of stability study are given in Table 33. The study showed a slight change in the results of percentage drug loading, droplet/particle size, zeta potential and angle of repose was observed, however, these changes were not significant ( $p > 0.05$ ) in case of both the cases (i.e. samples kept at  $25 \pm 2^\circ\text{C}$  &  $60 \pm 5\% \text{R.H.}$  and  $40 \pm 2^\circ\text{C}$  &  $75 \pm 5\% \text{R.H.}$ ). Similarly, dissolution profiles of aged and fresh samples of L-SNEDDS, S-SNEDDS and SP-NS were also found to have insignificant difference in their drug release profiles (Fig.17). The p-values of fresh and aged L-SNEDDS, S-SNEDDS and SP-NS containing SIM were found to be 0.88, 0.92 and 0.95, respectively. Similarly,

p-values of fresh and aged L-SNEDDS, S-SNEDDS and SP-NS containing GLM were found to be 0.92, 0.93 and 0.95, respectively. In both the cases the p-value was found above 0.05, indicating statistically similar dissolution profiles. Further, the model independent analysis of samples indicated the f2 values for fresh and aged L-SNEDDS, S-SNEDDS and SP-NS containing SIM were found to be 54.75, 62.89 and 69.29, respectively. Similarly, the f2 values for fresh and aged L-SNEDDS, S-SNEDDS and SP-NS containing GLM were found to be 62.60, 63.91 and 70.17, respectively. These values were found above 50, indicated similar dissolution profiles.

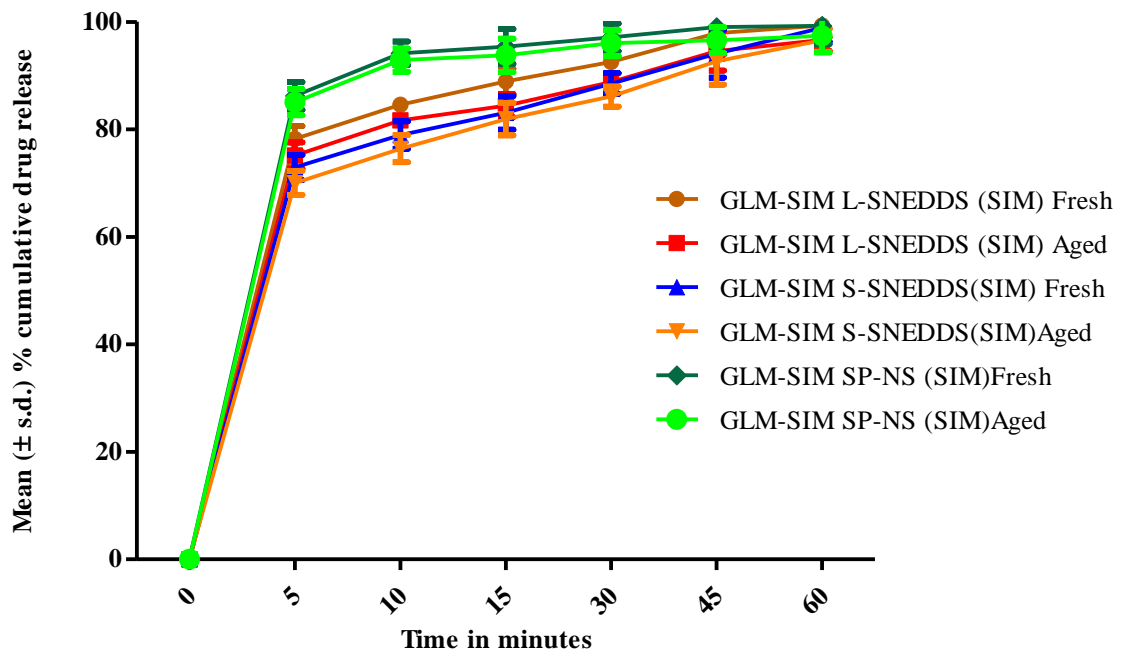
**Table 33:** Stability studies of nano- formulations

Formulation	25 ± 2°C & 60 ± 5% RH											
	% Drug loading			Mean droplet/particle size (nm)			Zeta potential (mV)			Angle of repose (°)		
	F*	A**	P Value	F	A	P Value	F	A	P Value	F	A	P Value
L-SNEDDS	GLM 94.50 ± 1.45	GLM 92.40 ± 1.67	GLM 0.08	55.63 ± 1.78	66.22 ± 1.83	0.06	-22.31 ± 1.66	-24.66 ± 1.71	0.18	NA	NA	-
	SIM 79.20 ± 1.12	SIM 75.30 ± 1.26	SIM 0.06									
	GLM 95.50 ± 1.51	GLM 93.08 ± 1.33	GLM 0.07	75.26 ± 2.138	87.76 ± 2.07	0.08	-19.54 ± 1.56	-16.88 ± 1.41	0.22	18.33 ± 1.16	22.71 ± 1.11	0.30
S-SNEDDS	SIM 92.63 ± 1.08	SIM 88.32 ± 1.17	SIM 0.05									
	GLM 95.35 ± 1.39	GLM 91.93 ± 1.62	GLM 0.07	127.40 ± 1.13	140.00 ± 1.33	0.09	-27.32 ± 2.05	-30.43 ± 1.89	0.13	29.68 ± 1.07	31.55 ± 1.11	0.50
	SIM 80.30 ± 1.33	SIM 77.21 ± 1.22	SIM 0.05									
Formulation	40 ± 2°C & 75 ± 5% RH											
	% Drug loading			Mean droplet/particle size (nm)			Zeta potential (mV)			Angle of repose (°)		
	F	A	P Value	F	A	P Value	F	A	P Value	F	A	P Value
L-SNEDDS	GLM 94.50 ± 1.45	GLM 89.64 ± 1.25	GLM 0.05	55.63 ± 1.78	74.66 ± 1.99	0.06	-22.31 ± 1.66	-25.11 ± 1.35	0.20	NA	NA	-
	SIM 79.20 ± 1.12	SIM 72.55 ± 0.87	SIM 0.05									
	GLM 95.50 ± 1.51	GLM 90.67 ± 0.98	GLM 0.06	75.26 ± 2.14	89.49 ± 2.44	0.05	-19.54 ± 1.56	23.33 ± 1.48	0.19	18.33 ± 1.16	23.56 ± 1.23	0.22
S-SNEDDS	SIM 92.63 ± 1.08	SIM 87.12 ± 1.15	SIM 0.06									
	GLM 95.35 ± 1.39	GLM 90.34 ± 1.49	GLM 0.06	127.40 ± 1.13	143.22 ± 2.19	0.06	-27.32 ± 2.05	-33.11 ± 1.88	0.21	29.68 ± 1.07	34.16 ± 1.34	0.18
	SIM 80.30 ± 1.33	SIM 72.89 ± 1.13	SIM 0.05									

F\* Fresh A\*\* Aged



(17A)



(17B)

**Fig. 17.** Dissolution studies (mean  $\pm$  s.d.) of A. GLM in fresh and aged nano formulations and B. SIM in fresh and aged nanoformulations (n= 6).

### 7.17. Bioanalytical method development and validation using HPLC

The method was found linear in the range of 50-250 ng/mL with coefficient of regression 0.9995, accurate with percentage recovery of 98.96 and precise with percentage relative standard deviation less than 2%. The retention times for ATV, GLM and SIM were found to be 3.69, 4.65 and 9.59, respectively (Fig. 18). All validation results were shown in Table 34-38.

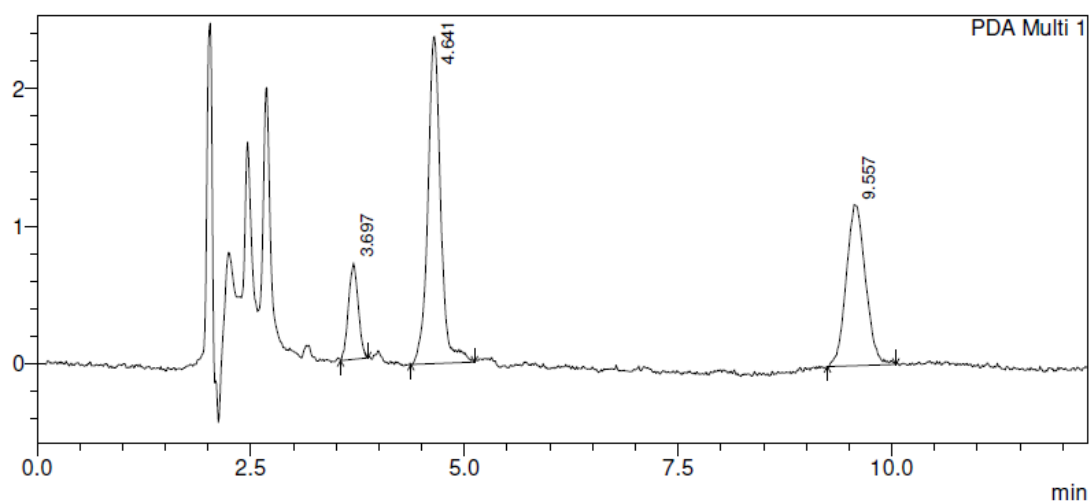


Fig. 17: Chromatogram of ATV, GLM & SIM.

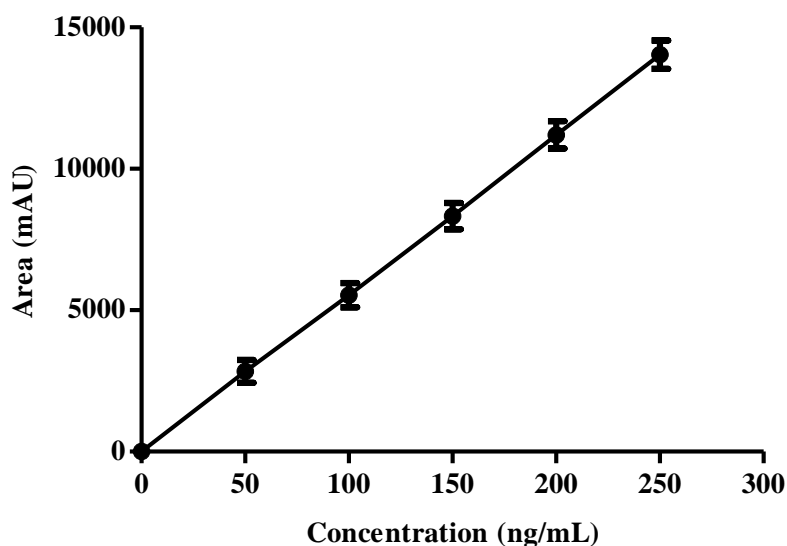
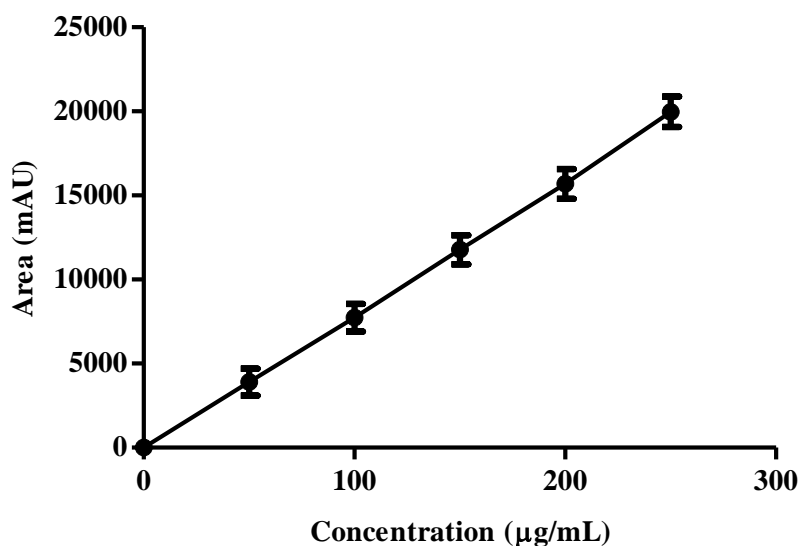


Fig. 18: Calibration plot of GLM



**Fig. 19:** Calibration plot of GLM

**Table 34:** Results of accuracy studies

Level	Conc. of standard solution (ng/mL)	Conc. of sample solution (ng/mL)	Total Conc. of solution (actual) (ng/mL)	Conc. of drug recovery from mobile phase (ng/mL) *(N=5)	% Recovery	Mean % recovery
<b>GLM</b>						
LQC	120.00	150.00	10.80	10.40 ± 1.24	96.30	98.70
MQC	150.00	150.00	12.00	11.80 ± 1.68	98.30	
HQC	180.00	150.00	13.20	13.40 ± 1.20	101.50	
<b>SIM</b>						
LQC	120.00	150.00	10.80	10.50 ± 1.31	97.20	97.93
MQC	150.00	150.00	12.00	11.70 ± 1.53	97.50	
HQC	180.00	150.00	13.20	13.10 ± 1.50	99.10	

**Table 35:** Results of precision studies for GLM

Parameters	Level	Conc. (ng/mL)	Mean (*N=6)	SD	%RSD
Repeatability (intraday precision)	LQC	120.00	5967.98	24.90	1.00
	MQC	150.00	8309.26	48.04	1.44
	HQC	180.00	18964.98	46.50	0.60
Intermediate precision (interday) Day 1	LQC	120.00	5898.23	30.10	1.25
	MQC	150.00	8505.13	56.80	1.68
	HQC	180.00	1795.73	91.40	1.20

Day 2	LQC	120.00	5933.78	46.50	1.89
	MQC	150.00	8445.44	33.90	1.01
	HQC	180.00	19103.67	71.50	0.94
Day 3	LQC	120.00	5887.62	41.30	1.39
	MQC	150.00	8329.97	52.80	1.47
	HQC	180.00	18665.47	37.70	0.48
Intermediate precision (inter analyst)					
Analyst 1	LQC	120.00	6023.51	21.20	0.90
	MQC	150.00	8337.88	58.30	1.72
	HQC	180.00	18891.22	33.50	0.43
Analyst 2	LQC	120.00	5943.56	40.20	1.65
	MQC	150.00	8434.67	44.70	1.30
	HQC	180.00	18448.74	53.20	0.70
Analyst 3	LQC	120.00	5899.58	42.20	1.73
	MQC	150.00	8376.34	32.10	0.91
	HQC	180.00	18739.77	45.50	0.59

**Table 36:** Results of precision studies for SIM

Parameters	Level	Conc. (ng/mL)	Mean (*N=6)	SD	%RSD
Repeatability (intraday precision)	LQC	120.00	6177.41	30.60	1.19
	MQC	150.00	9029.13	18.20	0.49
	HQC	180.00	11017.64	17.90	0.38
Intermediate precision (interday)					
Day 1	LQC	120.00	6095.61	30.10	1.25
	MQC	150.00	9134.65	56.80	1.68
	HQC	180.00	11314.73	92.40	1.20
Day 2	LQC	120.00	6154.55	46.50	1.89
	MQC	150.00	9066.78	34.10	1.01
	HQC	180.00	11451.35	71.50	0.94
Day 3	LQC	120.00	6174.12	41.30	1.39
	MQC	150.00	9114.32	52.80	1.47
	HQC	180.00	10998.87	37.70	0.48
Intermediate precision (inter analyst)					
Analyst 1	LQC	120.00	6203.11	36.20	1.43
	MQC	150.00	9078.93	37.40	1.02
	HQC	180.00	11773.23	20.10	0.43
Analyst 2	LQC	120.00	6178.31	41.60	1.66
	MQC	150.00	9145.65	48.90	1.35
	HQC	180.00	11524.27	60.70	1.3
Analyst 3	LQC	120.00	6109.77	20.50	0.79
	MQC	150.00	9155.67	34.60	0.97
	HQC	180.00	11754.46	57.50	1.55



**Table 37:** Robustness results of various parameters tested for GLM

Variables	Value	Concentration (ng/mL)	Peak area (mean±SD) (*N=5)	Mean of peak areas (*N=3)	Retention time (mean±SD) (*N=5)	Mean of retention times (*N=3)	% Recovery (mean±SD) (*N=5)	Mean of % recoveries (*N=3)
pH	4.30	150.00	8153.70 ± 34.10	8125.47	4.76 ± 0.01	4.77	96.40 ± 1.12	97.40
	4.50	150.00	8078.40 ± 56.70	SD = 148.10	4.78 ± 0.01	SD = 0.01	97.30 ± 1.09	SD = 1.12
	4.70	150.00	8144.30 ± 53.50	%RSD = 0.79	4.77 ± 0.01	%RSD = 0.21	98.50 ± 1.15	%RSD = 1.14
Flow rate (mL/min)	0.80	150.00	8113.60 ± 63.20	8171.63	4.77 ± 0.01	4.74	98.30 ± 1.33	99.60
	1.00	150.00	8223.50 ± 74.50	SD = 69.00	4.73 ± 0.01	SD = 0.00	101.20 ± 1.14	SD = 1.35
	1.20	150.00	8177.80 ± 69.30	%RSD = 1.12	4.73 ± 0.00	%RSD = 0.06	97.3 ± 1.57	%RSD = 1.48
Mobile phase ratio (A: B) v/v	73:27:00	150.00	8098.50 ± 53.30	8149.73	4.73 ± 0.02	4.77	98.56 ± 1.12	99.10
	75:25:00	150.00	8145.40 ± 70.10	SD = 65.30	4.76 ± 0.02	SD = 0.04	98.88 ± 1.03	SD = 1.16
	77:23:00	150.00	8205.30 ± 72.50	%RSD = 1.06	4.81 ± 0.01	%RSD = 0.93	99.87 ± 1.32	%RSD = 1.35

**Table 38:** Robustness results of various parameters tested for SIM

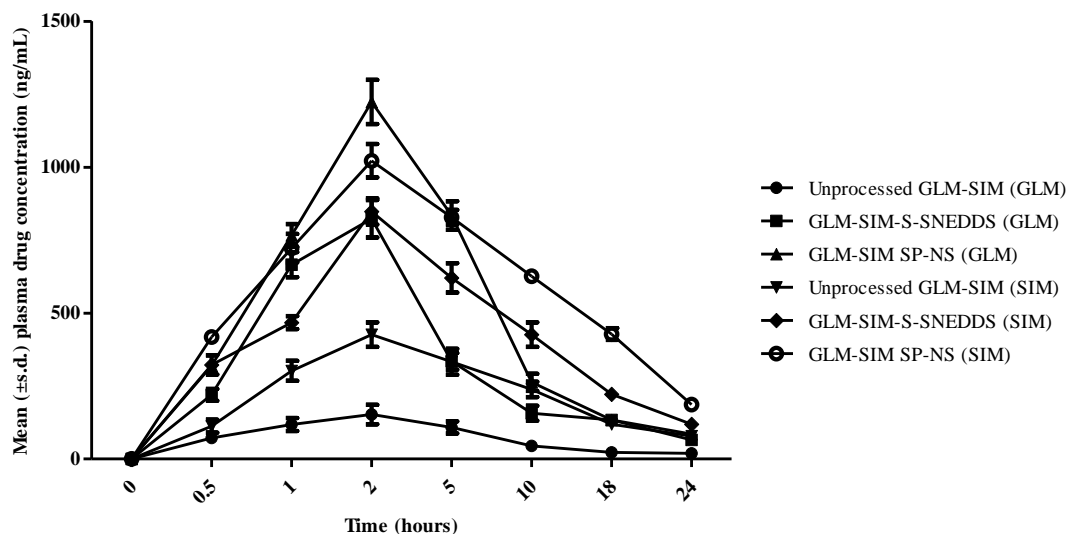
Variables	Value	Conc. (ng/mL)	Peak area (mean±SD) (*N=5)	Mean of peak areas (*N=3)	Retention time (mean±SD) (*N=5)	Mean of retention times (*N=3)	% Recovery (mean±SD) (*N=5)	Mean of % recoveries (*N=3)
pH	4.30	150.00	9189.70 ± 34.10	9148.37	9.63 ± 0.01	9.72	97.90 ± 1.03	98.60
	4.50	150.00	9067.40 ± 56.70	SD = 48.10	9.77 ± 0.01	SD = 0.01	98.40 ± 1.11	SD = 1.04
	4.70	150.00	9188.00 ± 53.50	%RSD = 0.53	9.77 ± 0.01	%RSD = 0.08	99.50 ± 0.98	%RSD = 1.05
Flow rate (mL/min)	0.80	150.00	9253.60 ± 45.20	9218.97	9.83 ± 0.01	9.76	99.70 ± 1.23	99.70
	1.00	150.00	9223.50 ± 74.50	SD = 63.00	9.72 ± .01	SD = 0.01	97.90 ± 1.15	SD = 1.20
	1.20	150.00	9179.80 ± 69.30	%RSD = 0.68	9.73 ± 0.00	%RSD = 0.06	101.40 ± 1.21	%RSD = 1.20
Mobile phase ratio (A: B) v/v	73:27:00	150.00	9198.50 ± 53.30	9176.40	9.83 ± 0.02	9.80	98.70 ± 1.12	98.90
	75:25:00	150.00	9145.40 ± 70.10	SD = 63.90	9.76 ± 0.02	SD = 0.01	99.50 ± 1.03	SD = 1.17
	77:23:00	150.00	9185.30 ± 68.50	%RSD = 0.70	9.81 ± 0.01	%RSD = 0.17	98.40 ± 1.32	%RSD = 1.17

### 7.18. Pharmacokinetic Study

Fig. 21 showed the change in mean plasma concentration of GLM and SIM after oral administration of their unprocessed form, S-SNEDDS and SP-NS to rats. The total plasma concentration of both the drugs in SNEDDS and SP-NS were higher than that of their unprocessed form. The initial plasma concentration of both the drugs in the nano formulations were significantly ( $P < 0.05$ ) higher than that of unprocessed GLM and SIM. The results of various pharmacokinetic parameters are shown in Table 39. It was observed that the  $C_{\max}$  and area under the curves (AUCs) were found highest for nanosuspensions followed by S-SNEDDS and unprocessed GLM and SIM. This indicated that oral bioavailability of the both the drugs were found to be maximum in the case of nanosuspensions as that of SNEDDS as compared to their unprocessed forms. The AUCs of nanosuspensions and SNEDDS of GLM were found to be 6.69 and 4.22-folds increment as compared to unprocessed GLM. The AUCs of nanosuspensions and SNEDDS of SIM were found to be 1.76 and 2.68 folds higher than unprocessed SIM. This study also indicated that AUCs of nanosuspensions of GLM and SIM were 1.59 and 1.52 higher than SNEDDS respectively. There was slight difference found in  $t_{1/2}$  of all the formulations and their unprocessed forms, however they were not significant.

**Table 39:** Pharmacokinetic parameters of nanoformulations and unprocessed form.

Parameter	Unit	Unprocessed GLM-SIM (GLM)	GLM-SIM-S-SNEDDS (GLM)	GLM-SIM-SP-NS (GLM)	Unprocessed GLM-SIM (SIM)	GLM-SIM-S-SNEDDS (SIM)	GLM-SIM-SP-NS (SIM)
$t_{1/2}$	h	7.03	9.13	8.59	8.92	7.98	9.69
$T_{\max}$	h	2.00	2.00	2.00	2.00	2.00	2.00
$C_{\max}$	ng/mL	152.56	823.29	1223.65	426.22	848.41	1022.23
$AUC_{0-t}$	ng/mL*h	1372.20	5749.41	9424.13	5087.55	9374.11	13738.88
$AUC_{0-\infty}$	ng/mL*h	1564.73	6608.68	10480.74	6095.94	10747.39	16352.52
MRT	h	10.61	11.24	9.62	13.45	12.03	13.99



**Fig. 20:** Mean ( $\pm$  s.d.) Plasma concentration versus time plot of drugs (n = 6).

## 7.19. Pharmacodynamics studies

### 7.19.1. Effects on body weight

Results of the average body weights of each group after giving HFD for 15 days and change in body weight after treatment was expressed in Fig. 22. Results clearly indicated that the body weight of each group was significantly increasing with HFD and decline in body weight was observed after induction of STZ till the start of treatment (16th-17th days). Immediate and significant increase in body weight was found in groups XII and XVII during the treatment. Gradual recovery was observed in groups V, VI, VII, VIII, IX, X, XI, XIII, XIV, XV and XVI as compared to normal control (GI). No recovery was observed in groups II to IV. These results also suggested the effectiveness of the formulation for improvement of loss in body weight in diabetic animals receiving treatment of drugs and nanoformulations, however, greater improvement was observed in groups XII and XVII, who received nanosuspensions and SNEDDS of GLM-SIM mixture respectively as compared to other formulations.

### 7.19.2. Effect on blood glucose level

The change in blood glucose level of different groups after receiving HFD, STZ and treatment through different formulation of GLM, SIM and GLM-SIM mixture were reported in Fig. 23. There was no change in blood glucose level (160-180 mg/dL) was

found with HFD, however, sudden increase in glucose level varying from 370-390 mg/dL were estimated administration of STZ (i.v. 35-50 mg/kg) to each group. Significant decrease in blood glucose level was observed in group XII, XVII, IX, XIV, VIII, XIII, VII, VI, XI, XVI, XV and group X as compared to experimental control. Little difference in glucose level was found in group XII as compared to normal control, which confirm the significant improvement in diabetic condition after receiving nanosuspensions of GLM-SIM mixture. This result also established the effectiveness of the formulations in given order-

Group XII>XVII>IX>XIV>VIII>XIII>VII>VI>XI>XVI>XV>X

### 7.19.3. Effect on biochemical parametrs

Table 40 showed the change in biochemical parameters such as ALP, creatinine and urea, in each group after the treatment. It is observed that there was no significant change in all the biochemical parameters (ALP, creatinine and urea) in rats of group XII as compared to normal control (Group I). Significant differences in level of these biochemical parameters were observed in groups IV-XVII as compared to experimental control (Group II). However, maximum improvements in level of biochemical parameters were observed in group XII. This result directly indicated the effectiveness of formulation received by group XII i.e. nanosuspensions of GLM-SIM mixture. We can arrange the formulations on the basis their effectiveness as-

Group XII>XVII>IX>VIII>XIV>XIII>XVI>7>XI>V>X>XV>VI>IV>III

### 7.19.4. Effect on GSH level

Fig. 24 showed the effect of treatment on GSH level of each group. Fast recovery in GSH level was found in group XII and group XVII after the treatment. No significant difference in GSH level is observed in group XII and group XVII as compared to normal control and significant difference was observed in both groups as compared to experimental control. These results confirm the effectiveness of the nanosuspensions and SNEDDS of GLM-SIM mixture.

### 7.19.5. Effect on SGOT & SGPT level

Fig. 25 showed the effect of treatment on SGOT level of each group. There was no significant in level of SGOT found in group IX, XII, XIV and group XVII as

compared to normal group (Group I). However, significant differences in SGOT level were observed in same group. These studies indicated the effectiveness of the formulation nanosuspensions and SNEDDS of GLM-SIM mixture received by groups XII and XVII, respectively. Different observations in level of SGPT were found and it was shown in Fig. 26. Level of SGPT was estimated same in group XII as in group I (normal control) and significant difference in SGPT level was observed in group XII as compared to experimental group (group II) of the . This observation clearly indicated the superiority of the formulation administered to group XII i.e. nanosuspensions of GLM-SIM mixture.

#### **7.19.6. Effect on TBARS, protein and CAT level**

Effect of treatment on TBARS, protein and CAT level of each group were expressed in Fig. 27, Fig. 28 & Fig. 29 respectively. Group XII and XVII were showing better result as compared to other groups because level of these three were found same as in group I (normal group) and significant different as compared to experimental group (Group II). These results confirm that a nanosuspension loaded with GLM-SIM mixture (Group XII) is effective as a drug carrier as compared to SNEDDS of GLM-SIM mixture (XVII).

#### **7.19.7. Effect on lipid level (TG, HDL, LDL, and total cholesterol)**

Lipid level of each group was estimated at 1<sup>st</sup> , 18<sup>th</sup> , and 46<sup>th</sup> day and results were shown in Fig.29-32. Sudden increment in TG, LDL and total cholesterol level in all groups were observed at 18<sup>th</sup> day due the high fatty diet. Immediate and significant lowering in level of these lipids was recorded in group XII and XVII after the treatment (46<sup>th</sup> day). A different trend was observed in HDL. Its level was drastically decreased in each group after high fatty diet. However, HDL level in group XII and XVII were significantly raised and found similar with group I (Normal control).

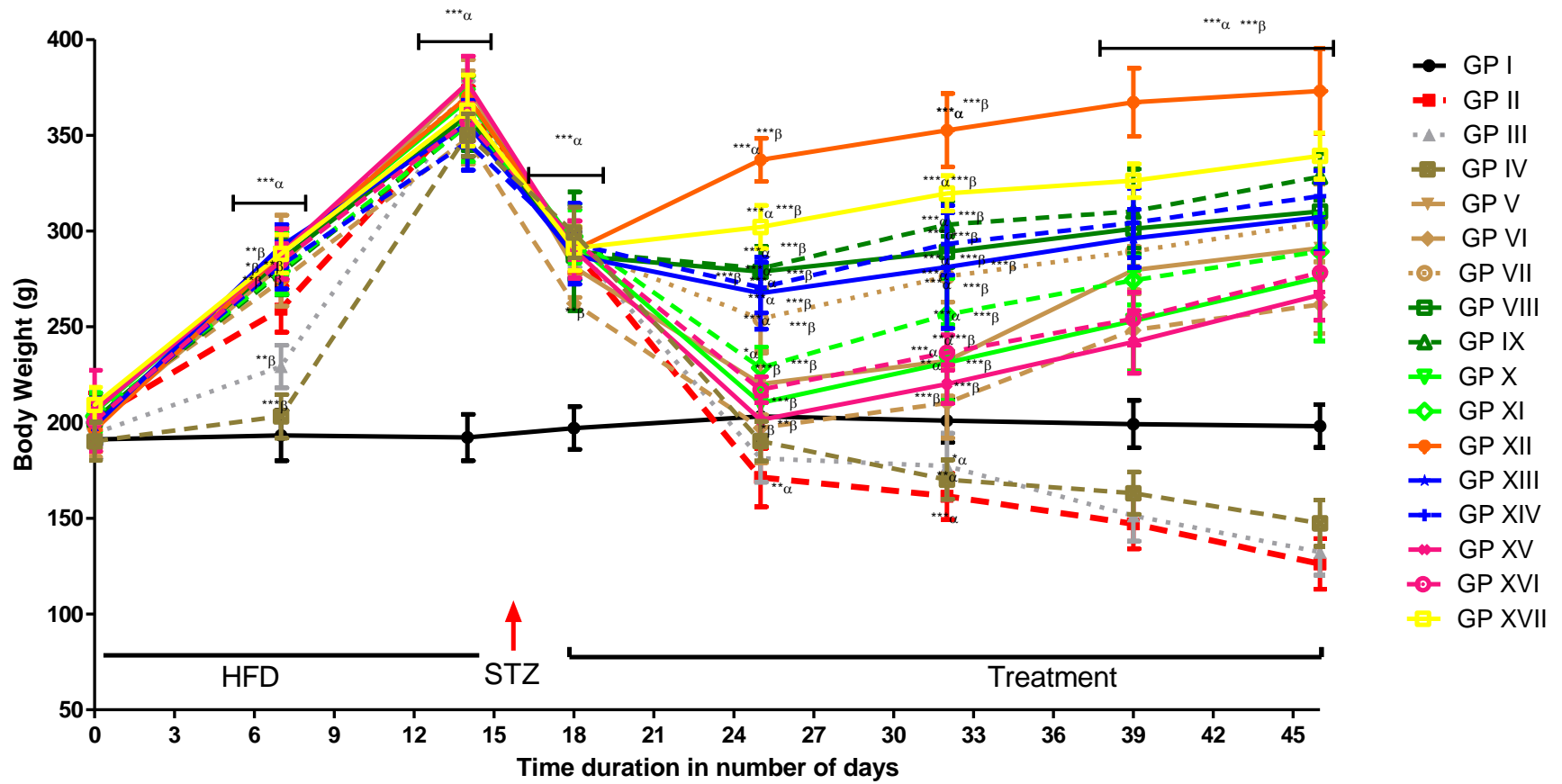
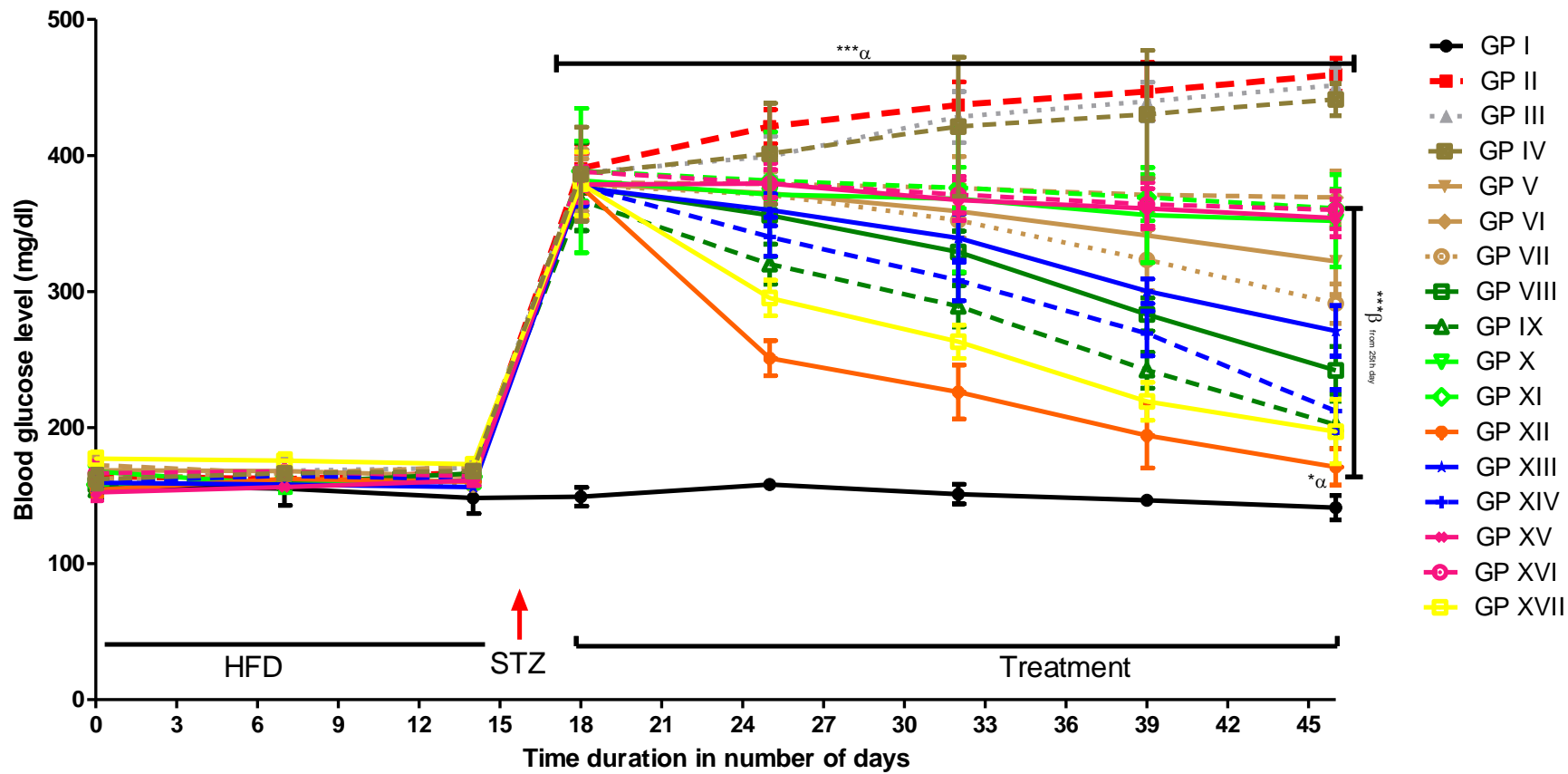


Fig. 21: Change in total body weight of each group

α- comparison with group I (normal control), β- comparison with group II (experimental control), \*- less difference (p<0.05). \*\*- more difference (p<0.001), \*\*\*- significant difference (p<0.0001)



**Fig. 22:** Effect of treatment on Blood glucose level of each group

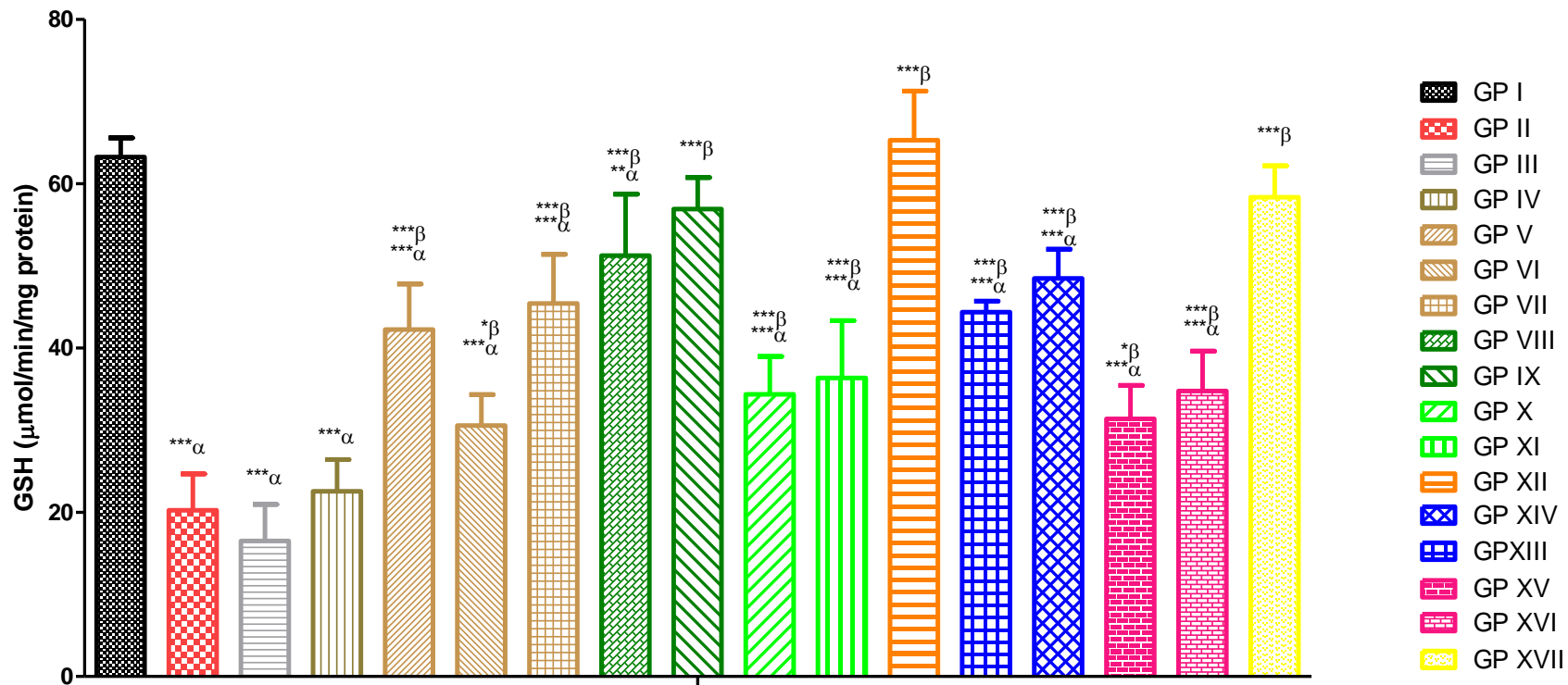
$\alpha$ - comparison with group I (normal control),  $\beta$ - comparison with group II (experimental control), \*- less difference ( $p < 0.05$ ). \*\*- more difference ( $p < 0.001$ ), \*\*\*- significant difference ( $p < 0.0001$ )

**Table 40:** Biochemical Parameters of each group.

Groups	ALP (U/dL)	Creatinine (mg/dL)	Urea (mg/dL)
GP 1	32.18±3.16	0.25±0.09,	22.45±3.32
GP 2	92.56±2.56 *** $\alpha$	0.94±0.05, *** $\alpha$	58.56±4.48 *** $\alpha$
GP 3	83.44±3.78 *** $\alpha$ , * $\beta$	0.90±0.68, *** $\alpha$	51.66±2.45 *** $\alpha$ , ** $\beta$
GP 4	78.16±2.00 *** $\alpha$ , *** $\beta$	0.86±0.08, *** $\alpha$	57.11±3.35 *** $\alpha$
GP 5	67.12±2.98 *** $\alpha$ , *** $\beta$	0.75±0.04, *** $\alpha$	38.55±2.22 *** $\alpha$ , *** $\beta$
GP 6	72.21±3.00 *** $\alpha$ , *** $\beta$	0.81±0.09, *** $\alpha$	42.03±3.31 *** $\alpha$ , *** $\beta$
GP 7	62.78±4.22 *** $\alpha$ , *** $\beta$	0.77±0.04, *** $\alpha$	37.21±1.26 *** $\alpha$ , *** $\beta$
GP 8	51.04±2.25 *** $\alpha$ , *** $\beta$	0.46±0.07, ** $\beta$	31.12±3.12 *** $\alpha$ , *** $\beta$
GP 9	46.62±4.56 *** $\alpha$ , *** $\beta$	0.39±0.02, *** $\beta$	29.43±2.22 ** $\alpha$ , *** $\beta$
GP 10	67.34±1.09 *** $\alpha$ , *** $\beta$	0.55±0.08, * $\beta$	38.39±1.98 *** $\alpha$ , *** $\beta$
GP 11	65.11±3.19 *** $\alpha$ , *** $\beta$	0.51±0.05, ** $\beta$	34.53±2.13 *** $\alpha$ , *** $\beta$
GP 12	40.01±3.49 *** $\beta$	0.29±0.07, *** $\beta$	25.22±0.67 *** $\beta$
GP 13	58.20±5.54 *** $\alpha$ , *** $\beta$	0.50±0.02, ** $\beta$	34.54±3.31 *** $\alpha$ , *** $\beta$
GP 14	52.53±3.76 *** $\alpha$ , *** $\beta$	0.45±0.01, ** $\beta$	33.30±2.11 *** $\alpha$ , *** $\beta$
GP 15	70.56±6.98 *** $\alpha$ , *** $\beta$	0.60±0.06	34.65±3.22 *** $\alpha$ , *** $\beta$
GP 16	61.78±3.46 *** $\alpha$ , *** $\beta$	0.54±0.08, * $\beta$	32.22±1.18 *** $\alpha$ , *** $\beta$
GP 17	44.56±5.12 *** $\alpha$ , *** $\beta$	0.33±0.05, *** $\beta$	28.19±3.26 * $\alpha$ , *** $\beta$

$\alpha$ - comparison with group I (normal control),  $\beta$ - comparison with group II (experimental control), \*- less difference (p<0.05). \*\*- more difference (p<0.001), \*\*\*- significant difference (p<0.0001)

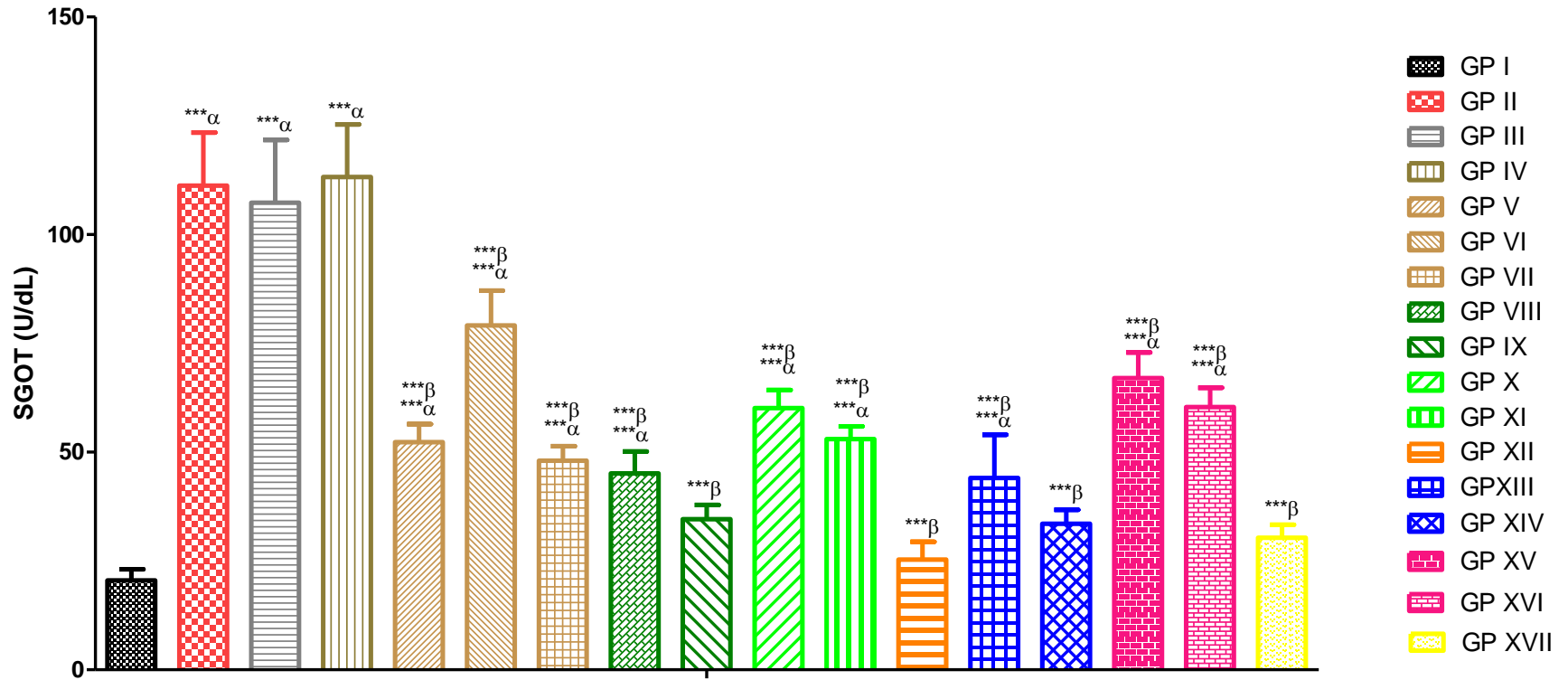




**Fig. 23:** Effect of treatment on GSH level of each group

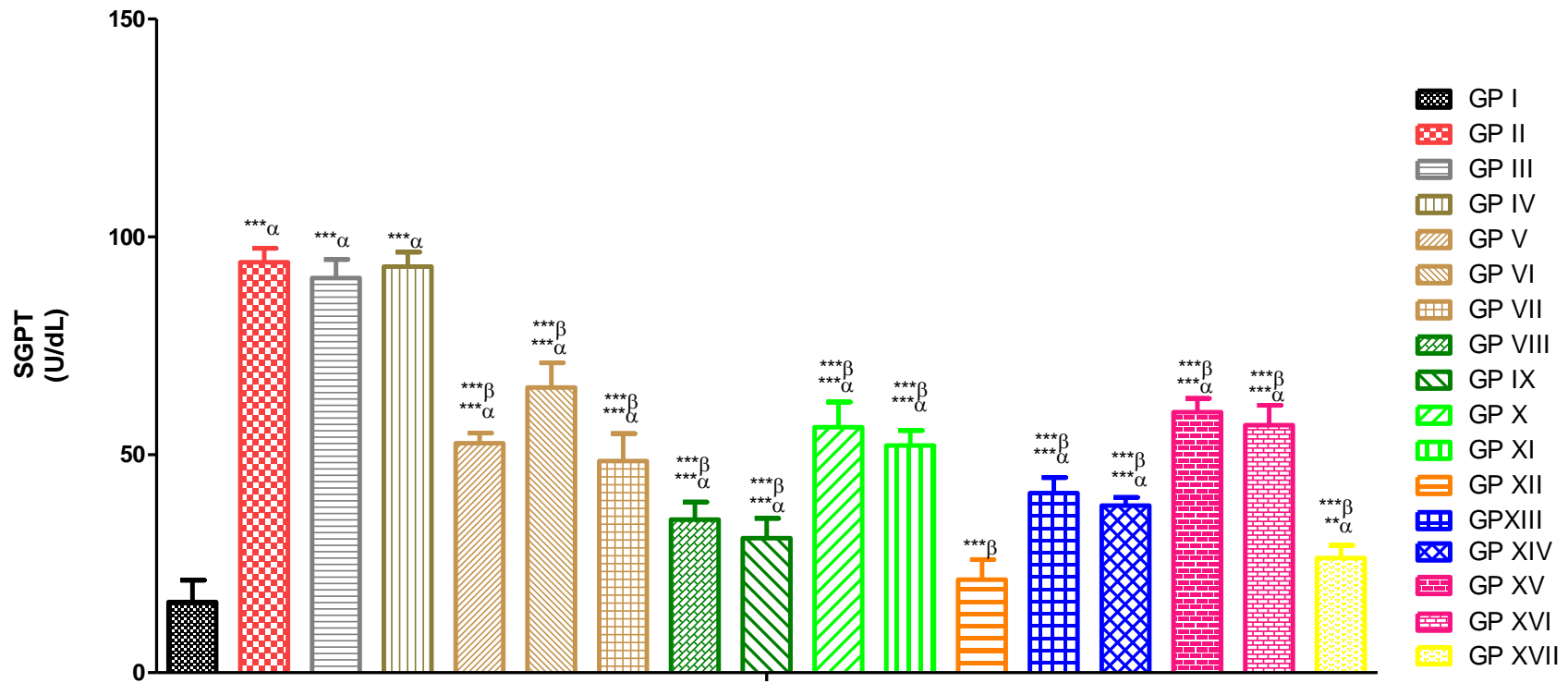
α- comparison with group I (normal control), β- comparison with group II (experimental control), \*- less difference (p<0.05). \*\*- more difference (p<0.001), \*\*\*- significant difference (P<0.001)

(p<0.0001)



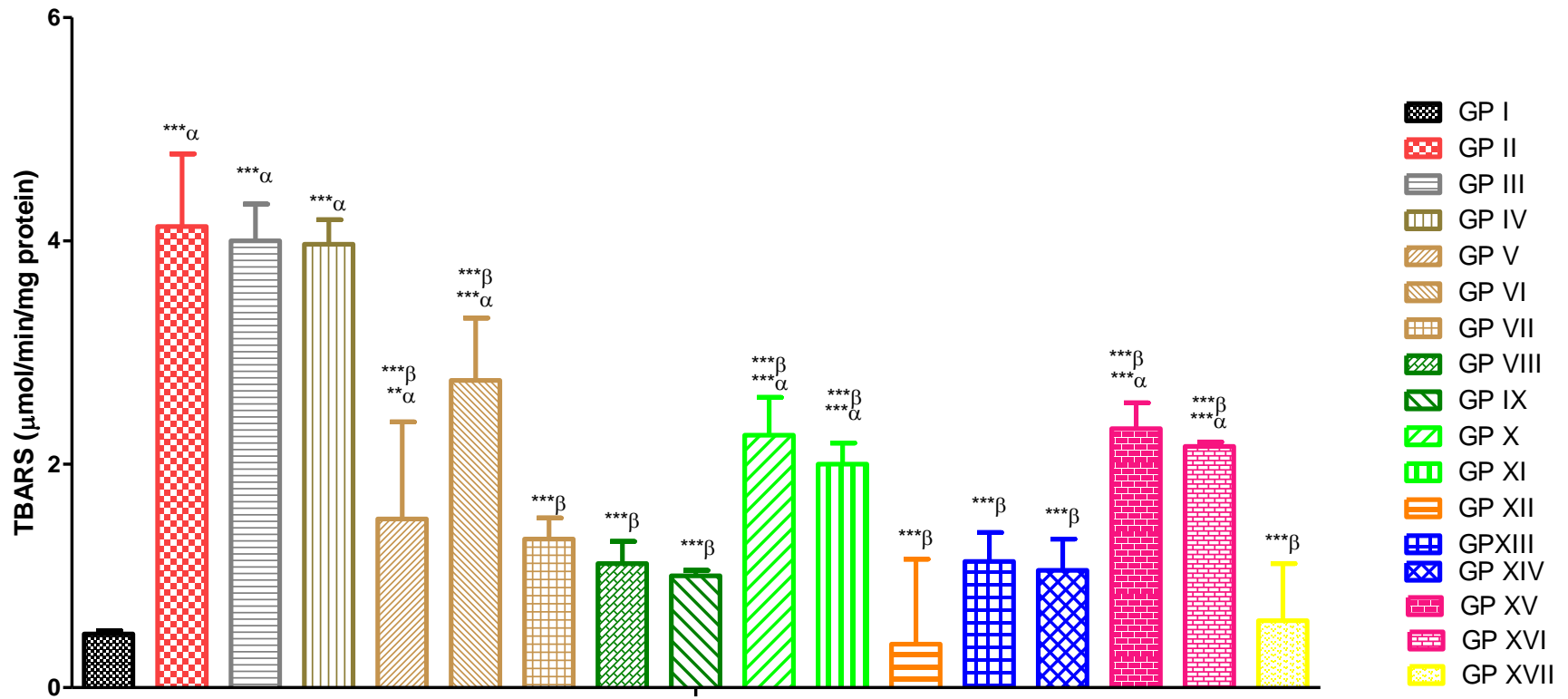
**Fig. 24:** Effect of treatment on SGOT level of each group

α- comparison with group I (normal control), β- comparison with group II (experimental control), \*- less difference (p<0.05). \*\*- more difference (p<0.001), \*\*\*- significant difference (p<0.0001)



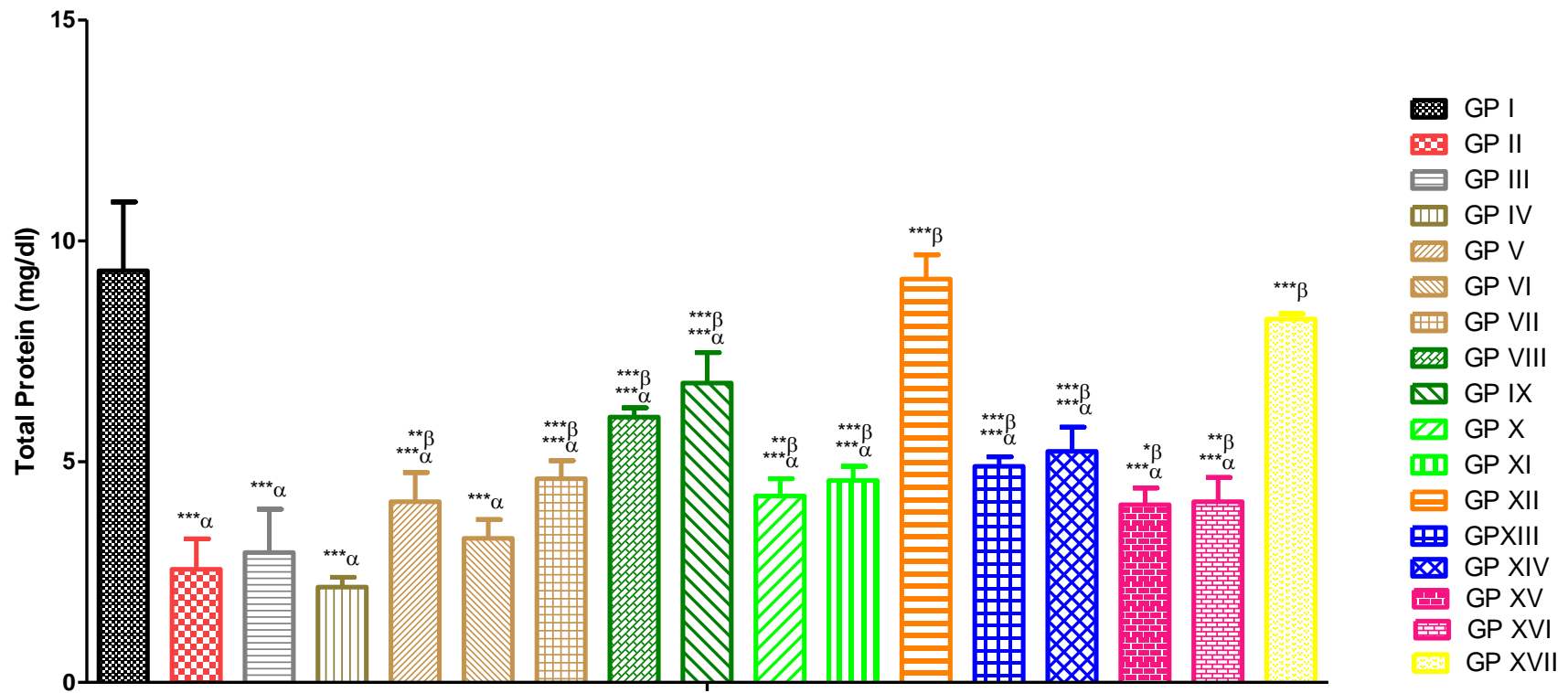
**Fig. 25:** Effect of treatment on SGPT level of each group

α- comparison with group I (normal control), β- comparison with group II (experimental control), \*- less difference (p<0.05). \*\*- more difference (p<0.001), \*\*\*- significant difference (p<0.0001)



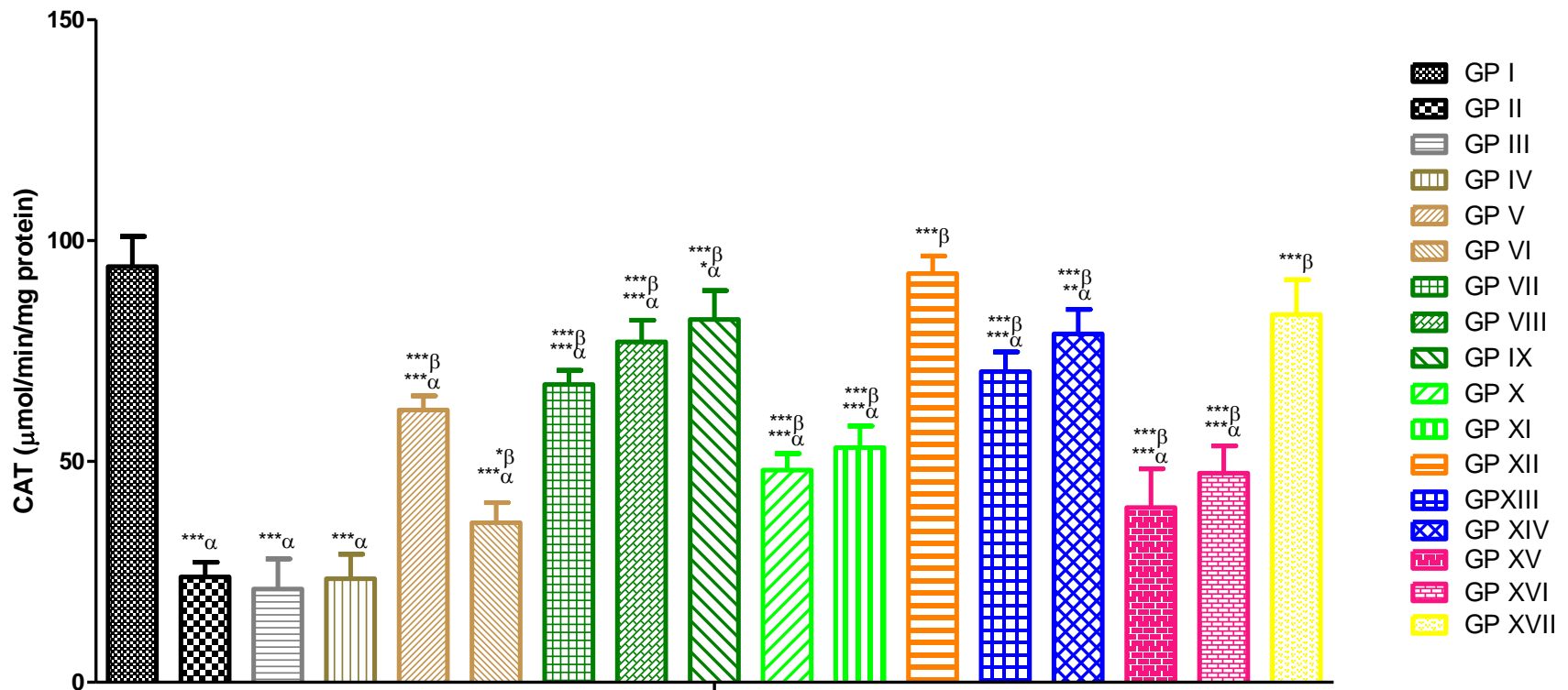
**Fig. 26:** Effect of treatment on TBARS level of each group

α- comparison with group I (normal control), β- comparison with group II (experimental control), \*- less difference. \*\*- more difference, \*\*\*-significant difference



**Fig. 27:** Effect of treatment on total protein level of each group

$\alpha$ - comparison with group I (normal control),  $\beta$ - comparison with group II (experimental control), \*- less difference ( $p < 0.05$ ). \*\*- more difference ( $p < 0.001$ ), \*\*\*- significant difference ( $p < 0.0001$ )



**Fig. 28:** Effect of treatment on CAT level of each group

α- comparison with group I (normal control), β- comparison with group II (experimental control), \*- less difference (p<0.05). \*\*- more difference (p<0.001), \*\*\*- significant difference (p<0.0001)

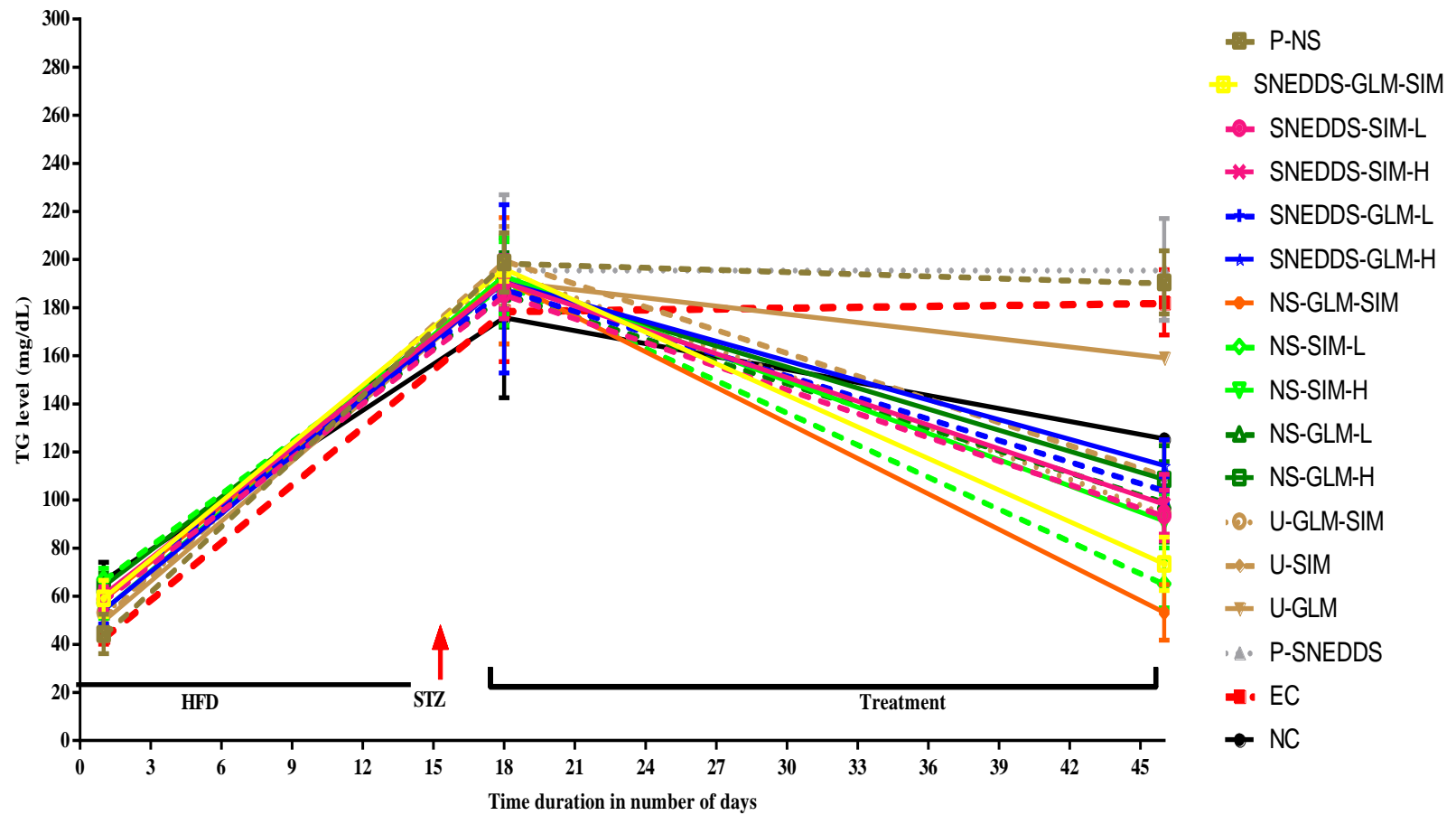


Fig. 29: Effect of treatment on TG level of each group

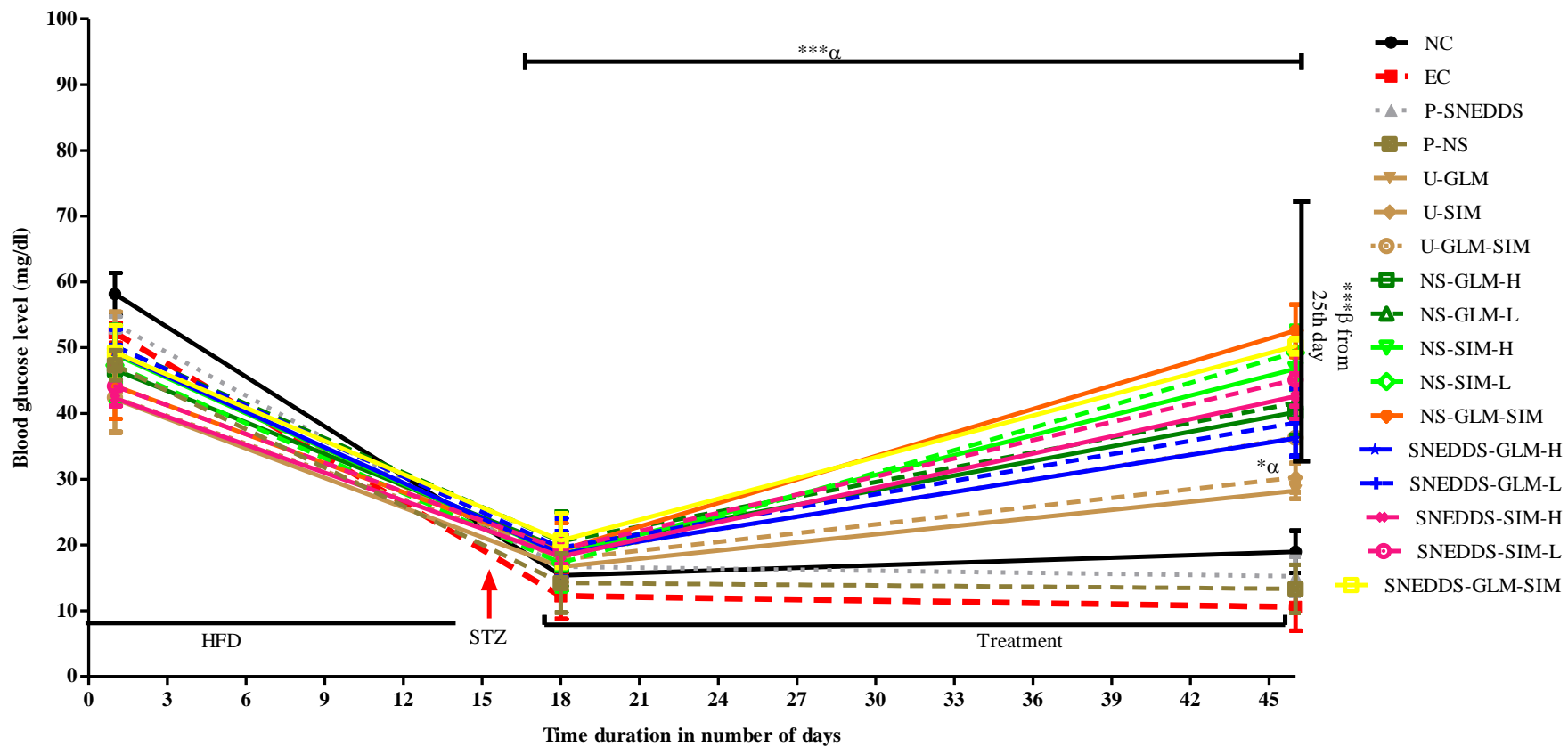


Fig. 30: Effect of treatment on HDL level of each group



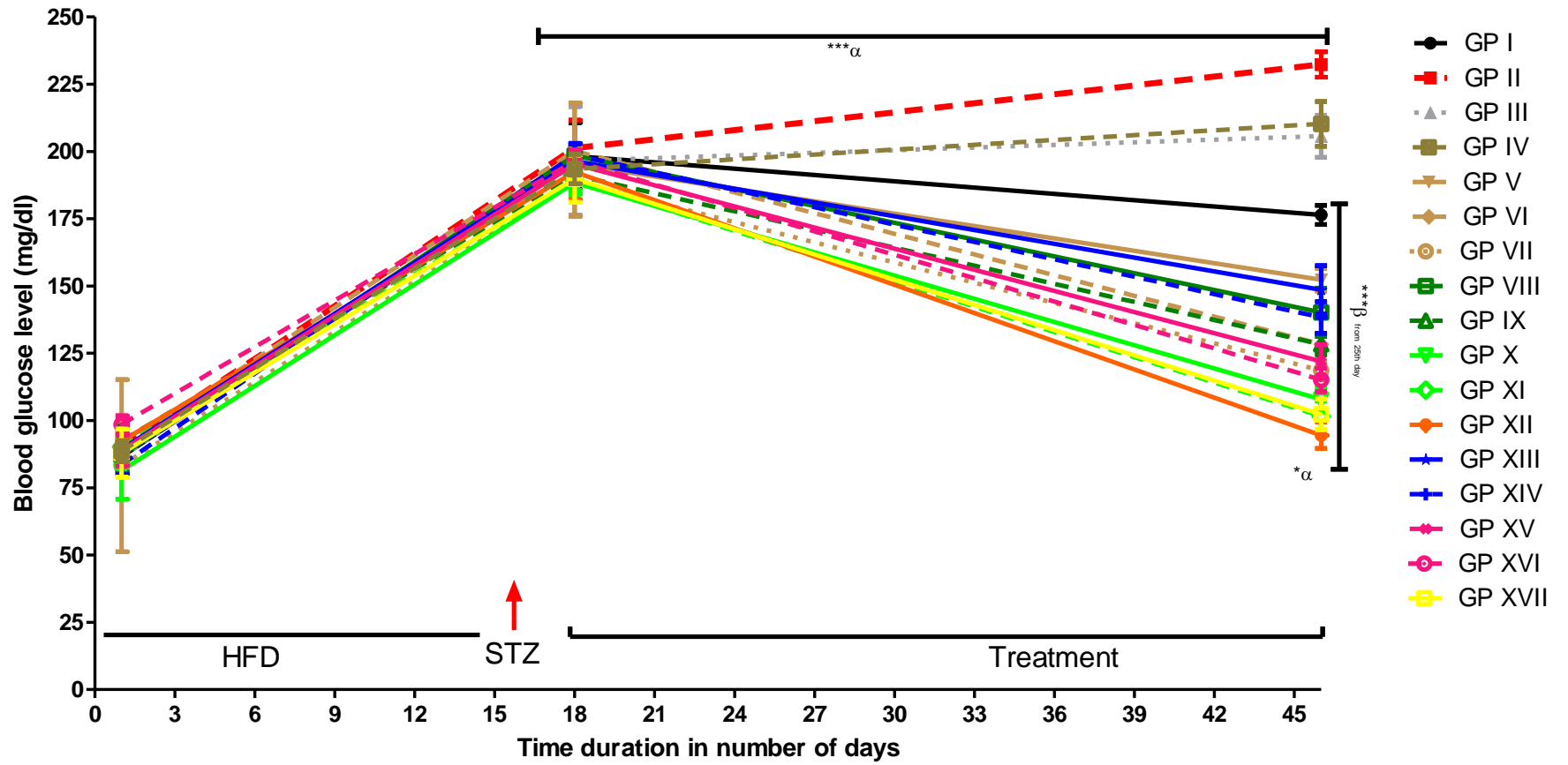


Fig. 31: Effect of treatment on LDL level of each group

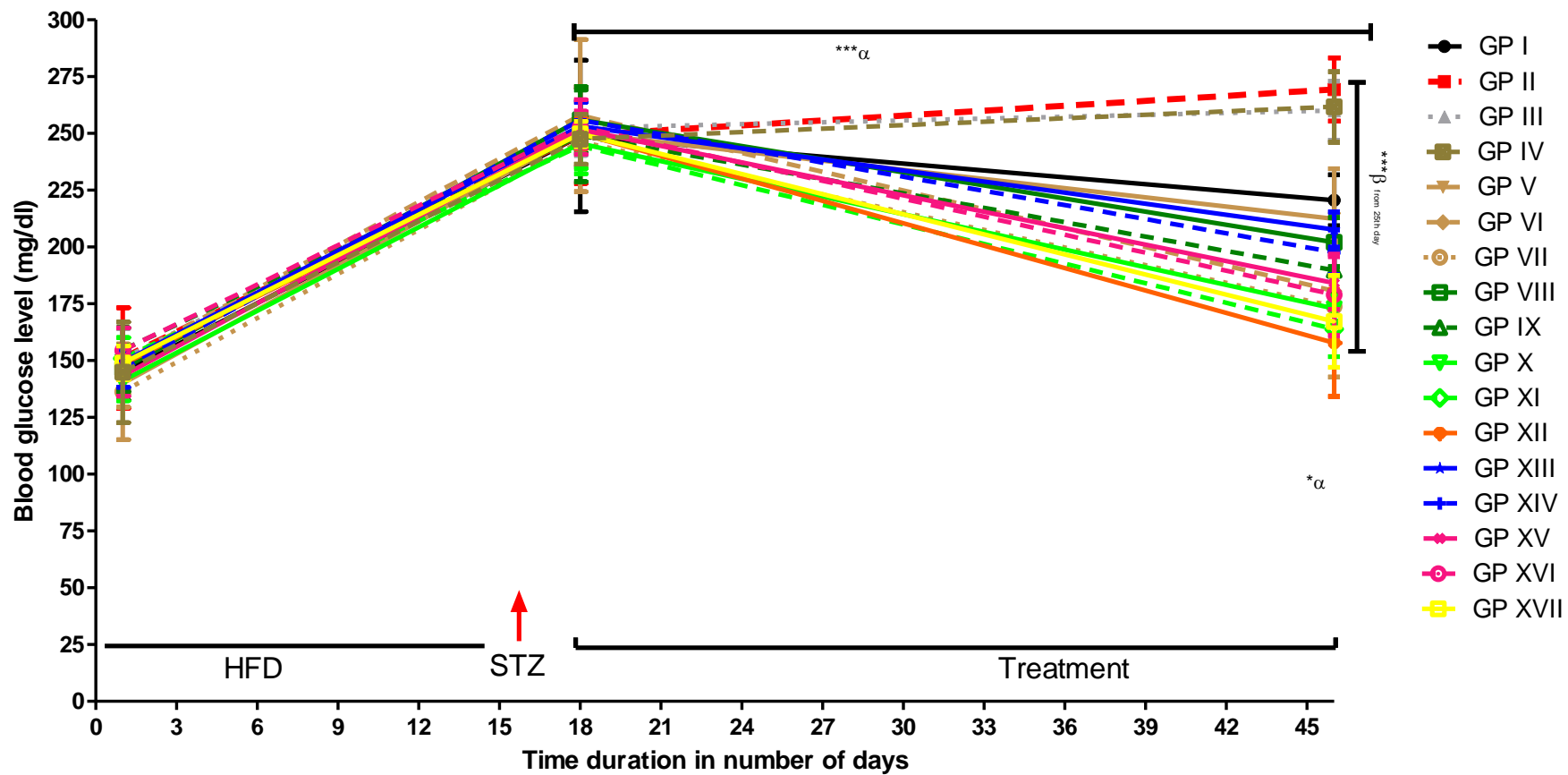


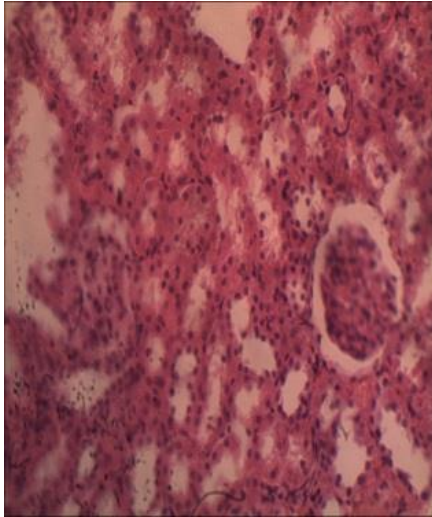
Fig. 32: Effect of treatment on total cholesterol level of each group

#### **7.19.7. Histopathological studies**

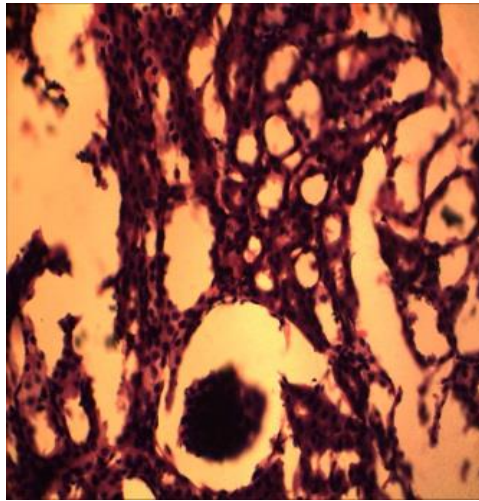
The images of kidney and liver sections of rats of different groups are shown in Fig.28 and 29. In case of NC rats normal tissue architecture was observed. The rats of EC, P-SNEDDS, P-NS complete cellular damage. The cellular architecture was found to be improved in case of rats receiving U-GLM, U-SIM, U-GLM-SIM, NS-GLM-L, NS-GLM-H, NS-SIM-L, NS-SIM-H, NS-GLM-SIM, SNEDDS-GLM-H, SNEDDS-GLM-L, SNEDDS-SIM-L, SNEDDS-SIM-H and SNEDDS-GLM-SIM. The recovery was found in the following increasing order:

U-SIM < U-GLM < U-GLM-SIM < SNEDDS-SIM-L < NS-SIM-L < SNEDDS-SIM-H < NS-SIM-H < SNEDDS-GLM-L < NS-GLM-L < SNEDDS-GLM-H < NS-GLM-H < SNEDDS-GLM-SIM < NS-GLM-SIM

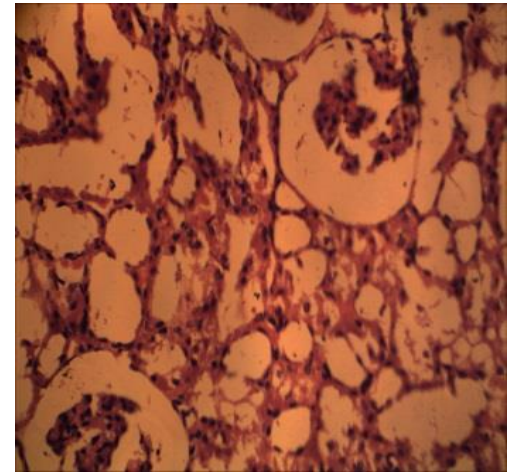
The results clearly indicated that the nanosuspension containing both the drugs have shown better recovery of cells as compared to their SNEDDS formulations.



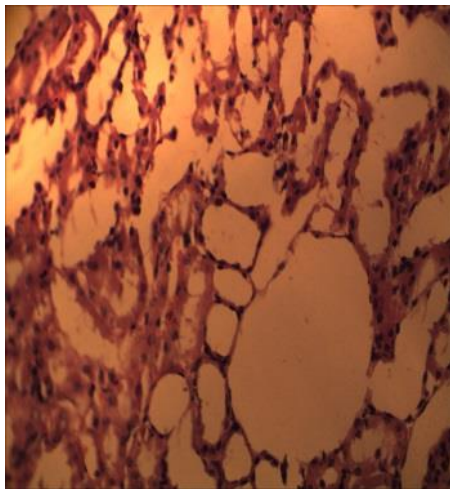
NC



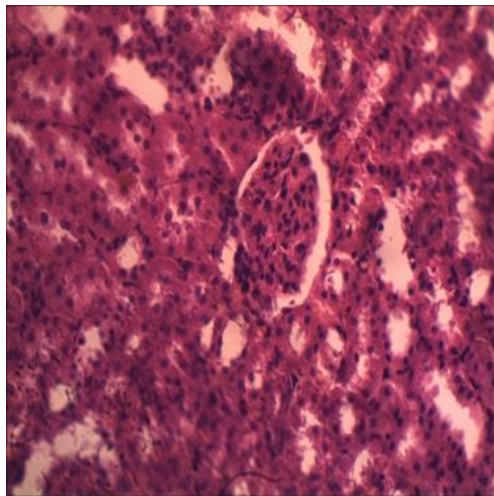
EC



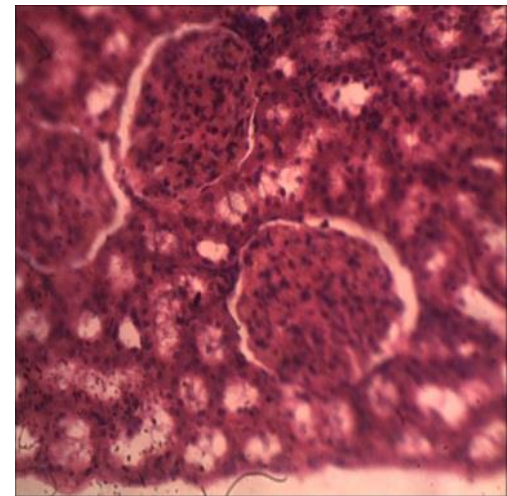
P-SNEDDS



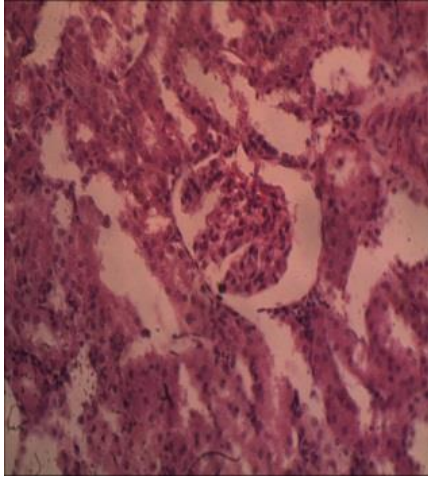
P-NS



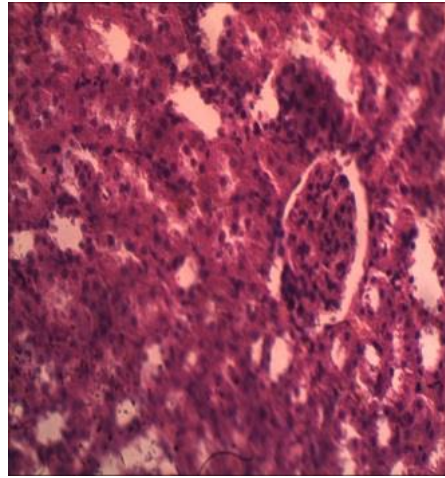
U-GLM



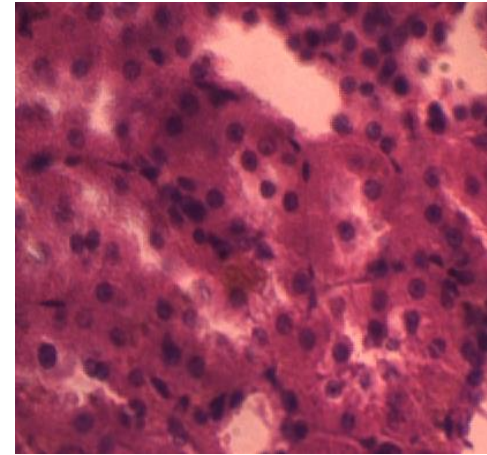
U-SIM



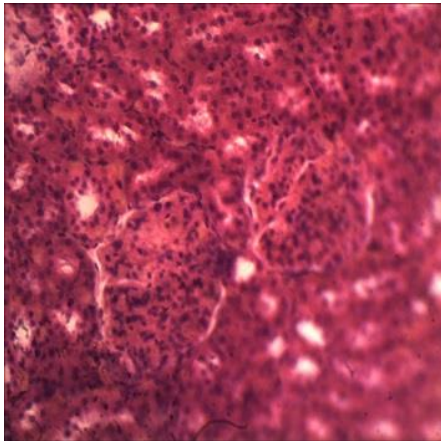
U-GLM-SIM



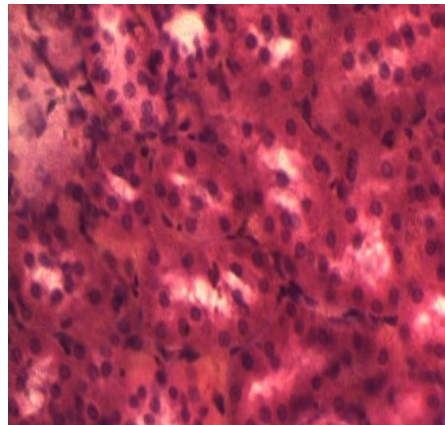
NS-GLM-H



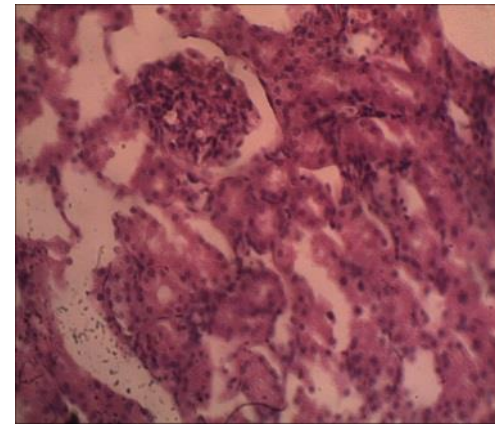
NS-GLM-L



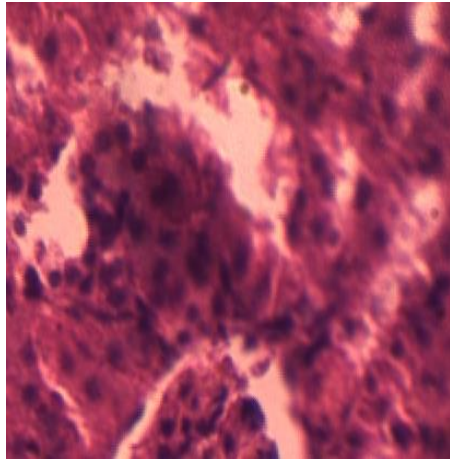
NS-SIM-H



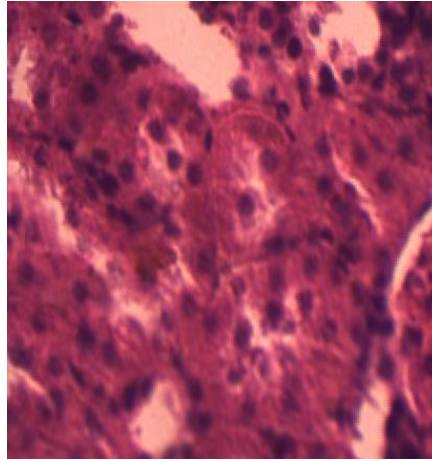
NS-SIM-L



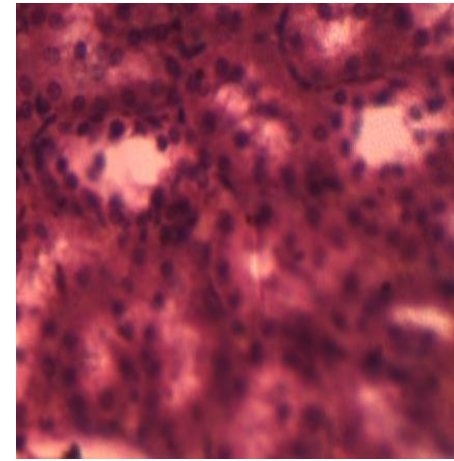
NS-GLM-SIM



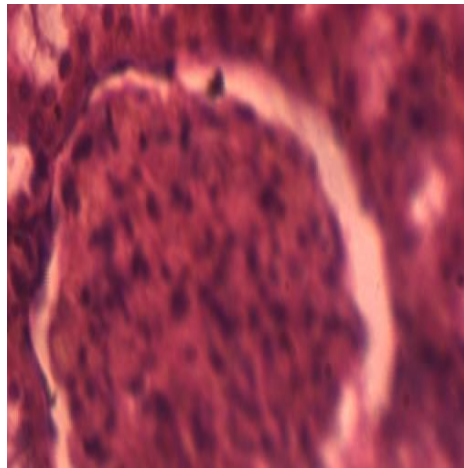
SNEDDS-GLM-H



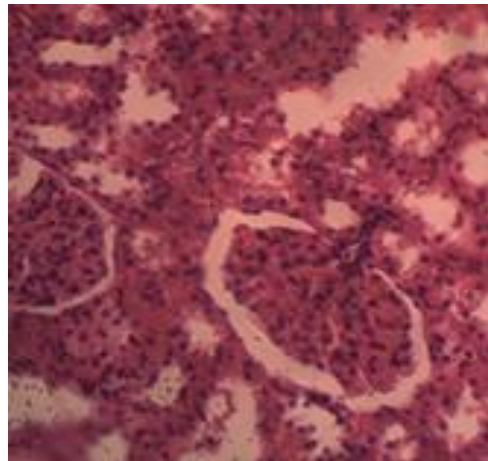
SNEDDS-GLM-L



SNEDDS-SIM-H

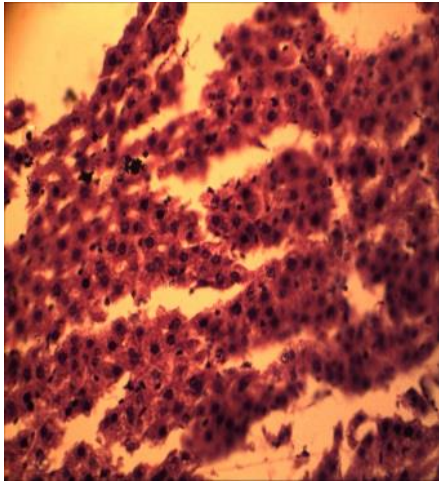


SNEDDS-SIM-L

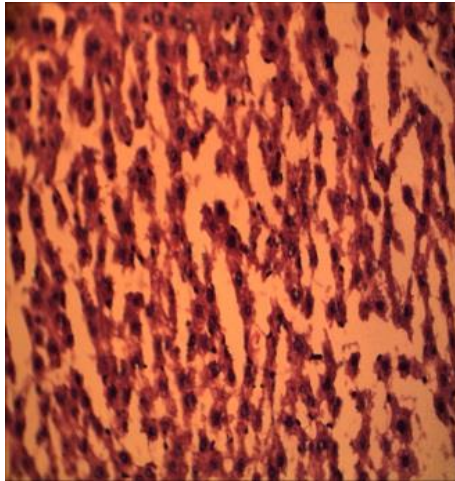


SNEDDS-GLM-SIM

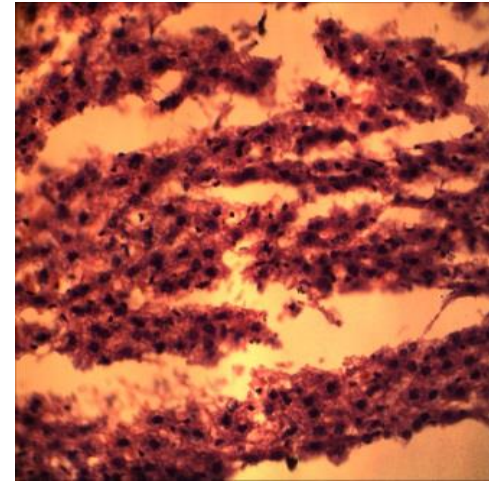
**Fig. 33:** Histological sections of kidney.



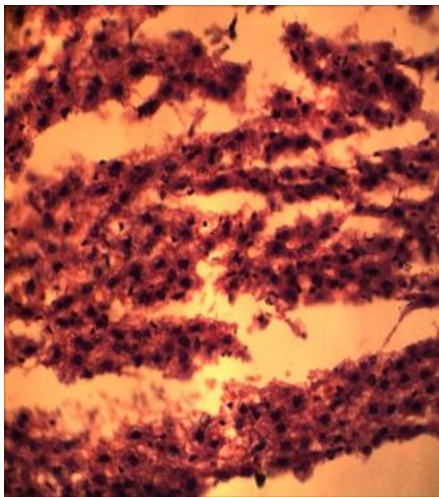
NC



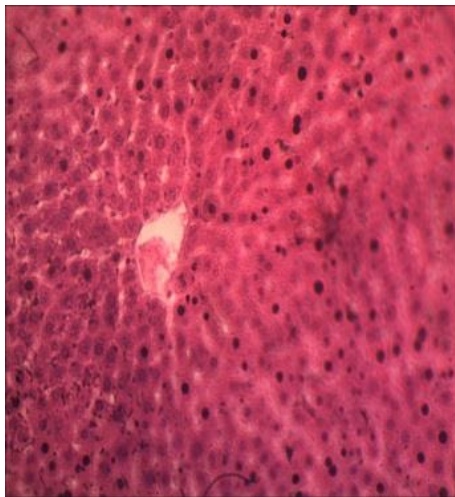
EC



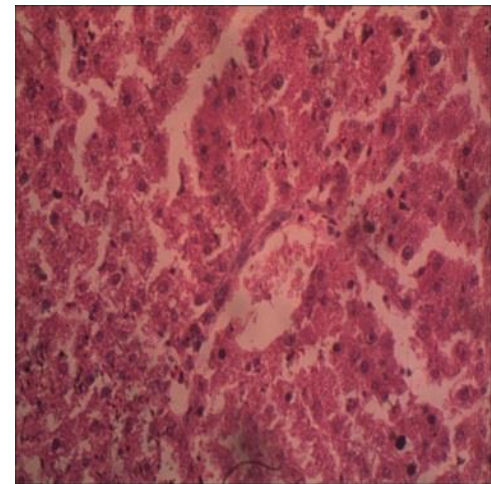
P-SNEDDS



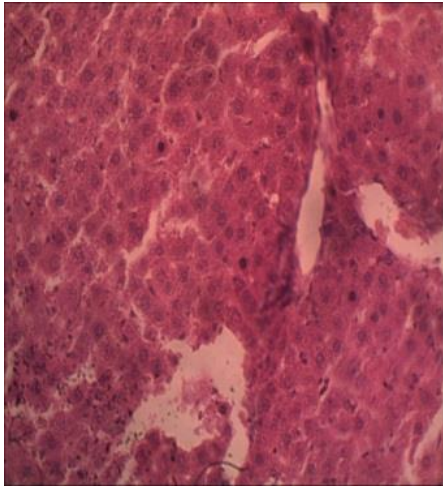
P-NS



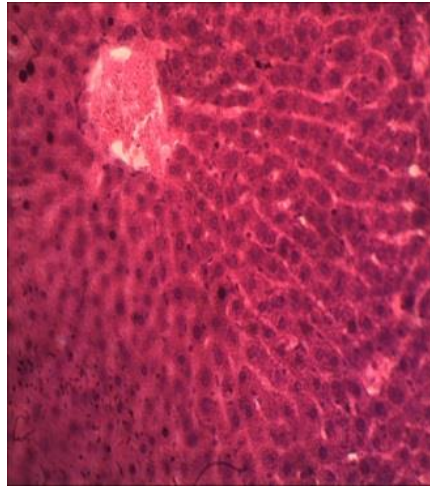
U-GLM



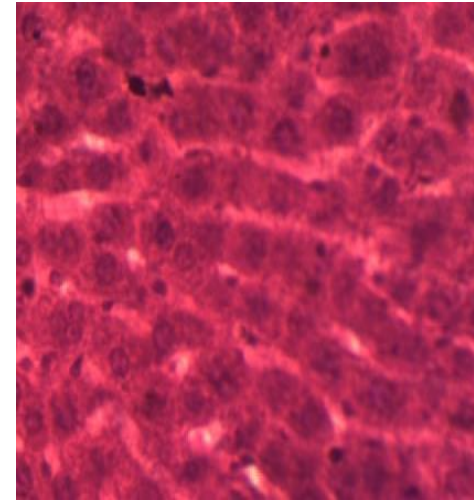
U-SIM



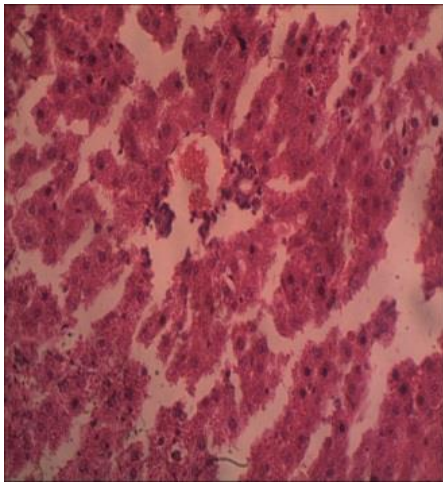
U-GLM-SIM



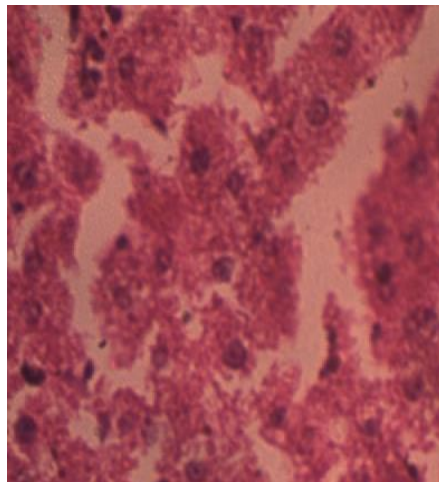
NS-GLM-H



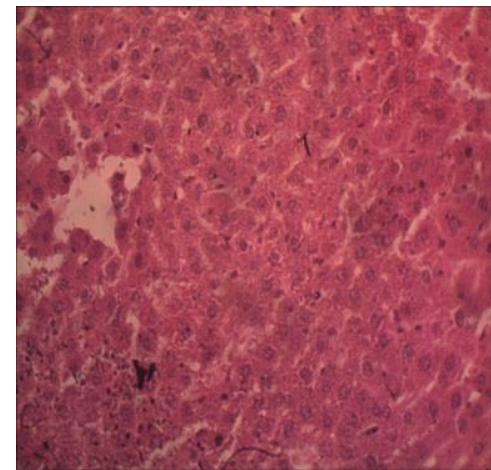
NS-GLM-L



NS-SIM-H

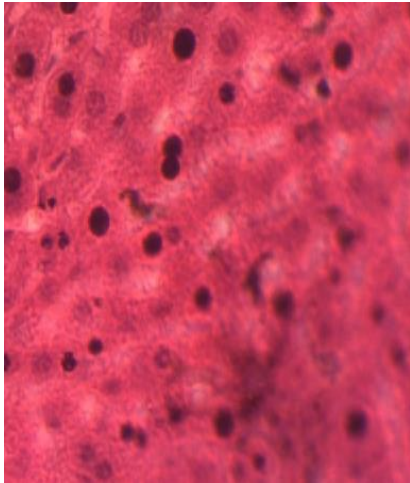


NS-SIM-L

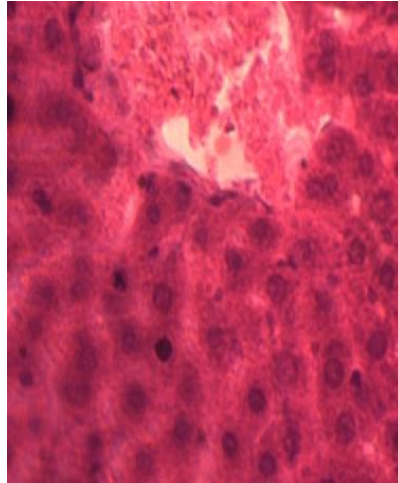


NS-GLM-SIM

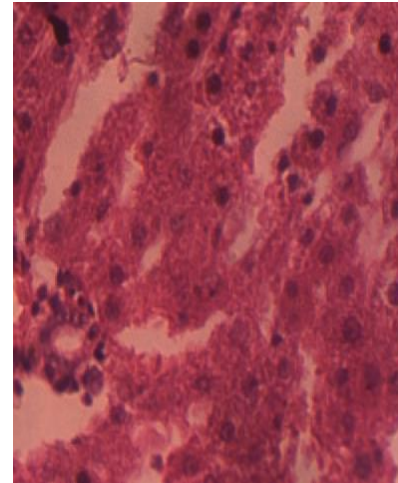




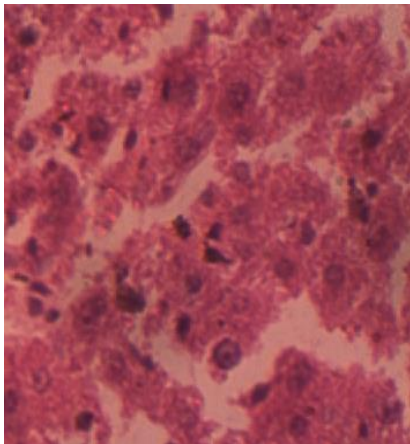
SNEDDS-GLM-H



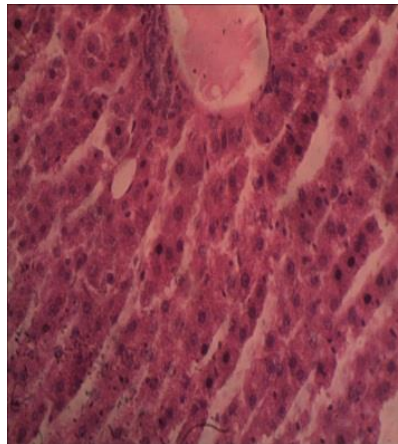
SNEDDS-GLM-L



SNEDDS-SIM-H



SNEDDS-SIM-L



SNEDDS-GLM-SIM

**Fig. 34:** Histological sections of liver.

## **8. CONCLUSION**

The study was initiated with an aim to investigate the most effective nanotechnology approach between SNEDDS and nanosuspension to improve the oral bioavailability of GLM and SIM upon co-administration. The nanosuspension was successfully prepared by LAP and optimized using BBD. The SNEDDS were prepared and optimized using ternary phase diagram, thermodynamic, centrifugation and cloud point studies. The stability study indicated that the prepared formulations were stable. Both the prepared formulations indicated significant enhancement in dissolution rate, oral bioavailability and improvement in the biochemical parameters including the histopathology of liver and kidney of rat undergone treatment as compared to their unprocessed form (U-GLM and U-SIM). This indicated that the drugs were found to be more effective when they are delivered through nanocarriers. It is important to note that the combination of both the drugs was found to be more effective than their individual doses. The in-vivo results indicated that nanosuspension containing both the drugs (NS-GLM-SIM) was found more efficacious than that of their SNEDDS formulation (SNEDDS-GLM-SIM). The positive results of pre-clinical studies indicated towards exploration of these formulations for clinical trials so that the research should reach in the form of product behind bedside of patients suffering from T2DM.

## 9. REFERENCES

- A Rahman, M., Mujahid, M., Hussain, A., 2016. Self-emulsifying Pellets Prepared by Extrusion/Spheronization: In vitro/In vivo Evaluation. *Recent patents on drug delivery & formulation* 10, 245-252.
- Abbaspour, M., Jalayer, N., Makhmalzadeh, B.S., 2014. Development and evaluation of a solid self-nanoemulsifying drug delivery system for loratadin by extrusion-spheronization. *Advanced pharmaceutical bulletin* 4, 113.
- Abdalla, A., Klein, S., Mäder, K., 2008. A new self-emulsifying drug delivery system (SEDDS) for poorly soluble drugs: characterization, dissolution, in vitro digestion and incorporation into solid pellets. *European journal of pharmaceutical sciences* 35, 457-464.
- Abdalla, A., Mäder, K., 2007. Preparation and characterization of a self-emulsifying pellet formulation. *European Journal of Pharmaceutics and Biopharmaceutics* 66, 220-226.
- Abdelbary, G., Amin, M., Salah, S., 2013. Self nano-emulsifying simvastatin based tablets: design and in vitro/in vivo evaluation. *Pharmaceutical development and technology* 18, 1294-1304.
- Agrawal, A.G., Kumar, A., Gide, P.S., 2015. Self emulsifying drug delivery system for enhanced solubility and dissolution of glipizide. *Colloids and Surfaces B: Biointerfaces* 126, 553-560.
- Ahmed, A.M., 2002. History of diabetes mellitus. *Saudi medical journal* 23, 373-378.
- Akkar, A., Müller, R.H., 2003. Intravenous itraconazole emulsions produced by SolEmuls technology. *European Journal of Pharmaceutics and Biopharmaceutics* 56, 29-36.
- Albertini, B., Sabatino, M.D., Melegari, C., Passerini, N., 2015. Formulation of spray congealed microparticles with self-emulsifying ability for enhanced glibenclamide dissolution performance. *Journal of microencapsulation* 32, 181-192.
- Alinaghi, A., Tan, A., Rao, S., A Prestidge, C., 2015. Impact of solidification on the performance of lipid-based colloidal carriers: oil-based versus self-emulsifying systems. *Current drug delivery* 12, 16-25.

- Arunachalam, K., Parimelazhagan, T., 2013. Antidiabetic activity of *Ficus amplissima* Smith. bark extract in streptozotocin induced diabetic rats. *Journal of ethnopharmacology* 147, 302-310.
- Attama, A., Mpamaugo, V., 2006. Pharmacodynamics of piroxicam from self-emulsifying lipospheres formulated with homolipids extracted from *Capra hircus*. *Drug delivery* 13, 133-137.
- Attama, A., Nzekwe, I., Nnamani, P., Adikwu, M., Onugu, C., 2003. The use of solid self-emulsifying systems in the delivery of diclofenac. *International journal of pharmaceutics* 262, 23-28.
- Bakhle, S.S., Avari, J.G., 2015. Development and characterization of solid self-emulsifying drug delivery system of cilnidipine. *Chemical and Pharmaceutical Bulletin* 63, 408-417.
- Balakrishnan, P., Lee, B.-J., Oh, D.H., Kim, J.O., Lee, Y.-I., Kim, D.-D., Jee, J.-P., Lee, Y.-B., Woo, J.S., Yong, C.S., 2009. Enhanced oral bioavailability of Coenzyme Q10 by self-emulsifying drug delivery systems. *International journal of pharmaceutics* 374, 66-72.
- Balakumar, K., Raghavan, C.V., Abdu, S., 2013. Self nanoemulsifying drug delivery system (SNEDDS) of rosuvastatin calcium: design, formulation, bioavailability and pharmacokinetic evaluation. *Colloids and Surfaces B: Biointerfaces* 112, 337-343.
- Barnett, A., 2006. DPP-4 inhibitors and their potential role in the management of type 2 diabetes. *International journal of clinical practice* 60, 1454-1470.
- Basalious, E.B., Shawky, N., Badr-Eldin, S.M., 2010. SNEDDS containing bioenhancers for improvement of dissolution and oral absorption of lacidipine. I: development and optimization. *International journal of pharmaceutics* 391, 203-211.
- Beg, S., Sandhu, P.S., Batra, R.S., Khurana, R.K., Singh, B., 2015. QbD-based systematic development of novel optimized solid self-nanoemulsifying drug delivery systems (SNEDDS) of lovastatin with enhanced biopharmaceutical performance. *Drug delivery* 22, 765-784.
- Beg, S., Swain, S., Singh, H.P., Patra, C.N., Rao, M.B., 2012. Development, optimization, and characterization of solid self-nanoemulsifying drug delivery systems of valsartan using porous carriers. *AAPS PharmSciTech* 13, 1416-1427.

- Bodmeier, R., McGinity, J., 1988. Solvent selection in the preparation of poly (DL-lactide) microspheres prepared by the solvent evaporation method. *International journal of pharmaceutics* 43, 179-186.
- Brand, W., 2019. Nanosuspension of natural materials and preparation method thereof. Google Patents.
- Cervera, A., Wajcberg, E., Sriwijitkamol, A., Fernandez, M., Zuo, P., Triplitt, C., Musi, N., DeFronzo, R.A., Cersosimo, E., 2008. Mechanism of action of exenatide to reduce postprandial hyperglycemia in type 2 diabetes. *American Journal of Physiology-Endocrinology and Metabolism* 294, E846-E852.
- Chai, F., Sun, L., Ding, Y., Liu, X., Zhang, Y., Webster, T.J., Zheng, C., 2016. A solid self-nanoemulsifying system of the BCS class IIb drug dabigatran etexilate to improve oral bioavailability. *Nanomedicine* 11, 1801-1816.
- Chan, H.-K., Kwok, P.C.L., 2011. Production methods for nanodrug particles using the bottom-up approach. *Advanced drug delivery reviews* 63, 406-416.
- Charman, W.N., Porter, C.J., 1996. Lipophilic prodrugs designed for intestinal lymphatic transport. *Advanced drug delivery reviews* 19, 149-169.
- Chavan, R.B., Modi, S.R., Bansal, A.K., 2015. Role of solid carriers in pharmaceutical performance of solid supersaturable SEDDS of celecoxib. *International journal of pharmaceutics* 495, 374-384.
- Chen, A., Shi, Y., Yan, Z., Hao, H., Zhang, Y., Zhong, J., Hou, H., 2015. Dosage form developments of nanosuspension drug delivery system for oral administration route. *Current pharmaceutical design* 21, 4355-4365.
- Chen, Y., Chen, C., Zheng, J., Chen, Z., Shi, Q., Liu, H., 2011. Development of a solid supersaturable self-emulsifying drug delivery system of docetaxel with improved dissolution and bioavailability. *Biological and Pharmaceutical Bulletin* 34, 278-286.
- Chistyakov, B., 2001. 6. Theory and practical application aspects of surfactants, *Studies in Interface Science*. Elsevier, pp. 511-618.
- Cho, W., Kim, M.-S., Kim, J.-S., Park, J., Park, H.J., Cha, K.-H., Park, J.-S., Hwang, S.-J., 2013. Optimized formulation of solid self-microemulsifying sirolimus delivery systems. *International journal of nanomedicine* 8, 1673.

Christiansen, M.L., Holm, R., Kristensen, J., Kreilgaard, M., Jacobsen, J., Abrahamsson, B., Müllertz, A., 2014. Cinnarizine food-effects in beagle dogs can be avoided by administration in a Self Nano Emulsifying Drug Delivery System (SNEDDS). *European Journal of Pharmaceutical Sciences* 57, 164-172.

Cui, S., Zhao, C., Chen, D., He, Z., 2005. Self-microemulsifying drug delivery systems (SMEDDS) for improving in vitro dissolution and oral absorption of Pueraria lobata isoflavone. *Drug development and industrial pharmacy* 31, 349-356.

Czajkowska-Kośnik, A., Szekalska, M., Amelian, A., Szymańska, E., Winnicka, K., 2015. Development and evaluation of liquid and solid self-emulsifying drug delivery systems for atorvastatin. *Molecules* 20, 21010-21022.

Dash, R.N., Mohammed, H., Humaira, T., Ramesh, D., 2015. Design, optimization and evaluation of glipizide solid self-nanoemulsifying drug delivery for enhanced solubility and dissolution. *Saudi Pharmaceutical Journal* 23, 528-540.

Date, A.A., Nagarsenker, M., 2007. Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for cefpodoxime proxetil. *International journal of pharmaceutics* 329, 166-172.

Date, A.A., Patravale, V., 2004. Current strategies for engineering drug nanoparticles. *Current opinion in colloid interface science* 9, 222-235.

Debuigne, F., Cuisenaire, J., Jeunieu, L., Masereel, B., Nagy, J.B., 2001. Synthesis of nimesulide nanoparticles in the microemulsion epikuron/isopropyl myristate/water/n-butanol (or isopropanol). *Journal of colloid and interface science* 243, 90-101.

Devraj, R., Williams, H.D., Warren, D.B., Mohsin, K., Porter, C.J., Pouton, C.W., 2013. In vitro assessment of drug-free and fenofibrate-containing lipid formulations using dispersion and digestion testing gives detailed insights into the likely fate of formulations in the intestine. *European Journal of Pharmaceutical Sciences* 49, 748-760.

Dixit, R., Nagarsenker, M., 2008. Self-nanoemulsifying granules of ezetimibe: design, optimization and evaluation. *European journal of pharmaceutical sciences* 35, 183-192.

Dressman, J.B., Amidon, G.L., Reppas, C., Shah, V.P., 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. *Pharmaceutical research* 15, 11-22.

Du, J., Li, X., Zhao, H., Zhou, Y., Wang, L., Tian, S., Wang, Y., 2015. Nanosuspensions of poorly water-soluble drugs prepared by bottom-up technologies. *International journal of pharmaceutics* 495, 738-749.

Eaimtrakarn, S., Rama Prasad, Y., Ohno, T., Konishi, T., Yoshikawa, Y., Shibata, N., Takada, K., 2002. Absorption enhancing effect of labrasol on the intestinal absorption of insulin in rats. *Journal of drug targeting* 10, 255-260.

El-Zahaby, S.A., AbouGhaly, M.H., Abdelbary, G.A., El-Gazayerly, O.N., 2018. Zero-order release and bioavailability enhancement of poorly water soluble Vinpocetine from self-nanoemulsifying osmotic pump tablet. *Pharmaceutical development and technology* 23, 900-910.

Elgart, A., Cherniakov, I., Aldouby, Y., Domb, A.J., Hoffman, A., 2013. Improved oral bioavailability of BCS class 2 compounds by self nano-emulsifying drug delivery systems (SNEDDS): the underlying mechanisms for amiodarone and talinolol. *Pharmaceutical research* 30, 3029-3044.

Femminella, G.D., Bencivenga, L., Petraglia, L., Visaggi, L., Gioia, L., Grieco, F.V., De Lucia, C., Komici, K., Corbi, G., Edison, P., 2017. Antidiabetic drugs in Alzheimer's disease: Mechanisms of action and future perspectives. *Journal of diabetes research* 2017.

Fong, J.C., Kao, Y.-S., Tsai, H.-y., Chiou, Y.-Y., Chiou, G.-Y., 2004. Synergistic effect of endothelin-1 and cyclic AMP on glucose transport in 3T3-L1 adipocytes. *Cellular signalling* 16, 811-821.

Franceschinis, E., Santomaso, A., Benda, L., Perissutti, B., Voinovich, D., Realdon, N., 2015. Influence of process variables on the properties of simvastatin self-emulsifying granules obtained through high shear wet granulation. *Powder technology* 274, 173-179.

Gamal, W., Fahmy, R.H., Mohamed, M.I., 2017. Development of novel amisulpride-loaded solid self-nanoemulsifying tablets: preparation and pharmacokinetic evaluation in rabbits. *Drug development and industrial pharmacy* 43, 1539-1547.

Gao, P., Morozowich, W., 2006. Development of supersaturatable self-emulsifying drug delivery system formulations for improving the oral absorption of poorly soluble drugs. *Expert opinion on drug delivery* 3, 97-110.

Gao, P., Rush, B.D., Pfund, W.P., Huang, T., Bauer, J.M., Morozowich, W., Kuo, M.S., Hageman, M.J., 2003. Development of a supersaturable SEDDS (S-SEDDS) formulation of paclitaxel with improved oral bioavailability. *Journal of pharmaceutical sciences* 92, 2386-2398.

Garg, V., Gupta, R., Kapoor, B., Singh, S.K., Gulati, M., 2016. Application of self-emulsifying delivery systems for effective delivery of nutraceuticals, *Emulsions*. Elsevier, pp. 479-518.

Garg, V., Kaur, P., Gulati, M., Singh, S.K., Kumar, B., Pandey, N.K., Yadav, A.K., Kumar, R., Kuppusamy, G., De, A., 2019a. Coadministration of Polypeptide-k and Curcumin Through Solid Self-Nanoemulsifying Drug Delivery System for Better Therapeutic Effect Against Diabetes Mellitus: Formulation, Optimization, Biopharmaceutical Characterization, and Pharmacodynamic Assessment. *Assay and drug development technologies* 17, 201-221.

Garg, V., Kaur, P., Gulati, M., Singh, S.K., Kumar, B., Pandey, N.K., Yadav, A.K., Kumar, R., Kuppusamy, G., De, A., 2019b. Coadministration of Polypeptide-k and Curcumin Through Solid Self-Nanoemulsifying Drug Delivery System for Better Therapeutic Effect Against Diabetes Mellitus: Formulation, Optimization, Biopharmaceutical Characterization, and Pharmacodynamic Assessment. *Assay and drug development technologies*.

Garg, V., Kaur, P., Singh, S.K., Kumar, B., Bawa, P., Gulati, M., Yadav, A.K., 2017. Solid self-nanoemulsifying drug delivery systems for oral delivery of polypeptide-k: formulation, optimization, in-vitro and in-vivo antidiabetic evaluation. *European Journal of Pharmaceutical Sciences* 109, 297-315.

Grahek, R., Kocevar, K., Novak, S., Homar, M., Rozman, P., Klancar, U., 2014. Nanosuspension of abiraterone acetate. WO2014009436.

Guardado-Mendoza, R., Prioleta, A., Jiménez-Ceja, L.M., Sosale, A., Folli, F., 2013. The role of nateglinide and repaglinide, derivatives of meglitinide, in the treatment of type 2 diabetes mellitus. *Archives of medical science: AMS* 9, 936.



Gugulothu, D., Pathak, S., Suryavanshi, S., Sharma, S., Patravale, V., 2010. Self-microemulsifying suppository formulation of  $\beta$ -artemether. *Aaps Pharmscitech* 11, 1179-1184.

Guideline, I.H.T., Guideline for Residual Solvents Q3C (R3). ICH Steering Committee, Step 4.

Gumaste, S.G., Dalrymple, D.M., Serajuddin, A.T., 2013. Development of solid SEDDS, V: compaction and drug release properties of tablets prepared by adsorbing lipid-based formulations onto Neusilin® US2. *Pharmaceutical research* 30, 3186-3199.

Hörter, D., Dressman, J., 2001. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Advanced drug delivery reviews* 46, 75-87.

Hu, X., Lin, C., Chen, D., Zhang, J., Liu, Z., Wu, W., Song, H., 2012. Sirolimus solid self-microemulsifying pellets: formulation development, characterization and bioavailability evaluation. *International journal of pharmaceutics* 438, 123-133.

I Jethara, S., D Patel, A., R Patel, M., S Patel, M., R Patel, K., 2015. Recent survey on nanosuspension: a patent overview. *Recent patents on drug delivery & formulation* 9, 65-78.

Inugala, S., Eedara, B.B., Sunkavalli, S., Dhurke, R., Kandadi, P., Jukanti, R., Bandari, S., 2015. Solid self-nanoemulsifying drug delivery system (S-SNEDDS) of darunavir for improved dissolution and oral bioavailability: in vitro and in vivo evaluation. *European Journal of Pharmaceutical Sciences* 74, 1-10.

Inzucchi, S.E., 2018. Personalizing glucose-lowering therapy in patients with type 2 diabetes and cardiovascular disease. *Endocrinology and Metabolism Clinics* 47, 137-152.

Iosio, T., Voinovich, D., Grassi, M., Pinto, J.F., Perissutti, B., Zacchigna, M., Quintavalle, U., Serdoz, F., 2008. Bi-layered self-emulsifying pellets prepared by co-extrusion and spheronization: Influence of formulation variables and preliminary study on the in vivo absorption. *European Journal of Pharmaceutics and Biopharmaceutics* 69, 686-697.

Iosio, T., Voinovich, D., Perissutti, B., Serdoz, F., Hasa, D., Grabnar, I., Dall'Acqua, S., Zara, G.P., Muntoni, E., Pinto, J., 2011. Oral bioavailability of silymarin phytocomplex formulated as self-emulsifying pellets. *Phytomedicine* 18, 505-512.

Jacobs, C., Kayser, O., Müller, R., 2001. Production and characterisation of mucoadhesive nanosuspensions for the formulation of bupravaquone. *International journal of pharmaceutics* 214, 3-7.

Jain, A.K., Thanki, K., Jain, S., 2014a. Solidified self-nanoemulsifying formulation for oral delivery of combinatorial therapeutic regimen: part I. Formulation development, statistical optimization, and in vitro characterization. *Pharmaceutical research* 31, 923-945.

Jain, A.K., Thanki, K., Jain, S., 2014b. Solidified self-nanoemulsifying formulation for oral delivery of combinatorial therapeutic regimen: part II in vivo pharmacokinetics, antitumor efficacy and hepatotoxicity. *Pharmaceutical research* 31, 946-958.

Jaiswal, P., Aggarwal, G., Harikumar, S.L., Singh, K., 2014. Development of self-microemulsifying drug delivery system and solid-self-microemulsifying drug delivery system of telmisartan. *International journal of pharmaceutical investigation* 4, 195.

Jerry, R., Chisholm, D., 2004. Thiazolidinediones-mechanisms of action. *Aust Prescr* 27, 67-70.

Josef, E., Bianco-Peled, H., 2013. Sponges carrying self-microemulsifying drug delivery systems. *International journal of pharmaceutics* 458, 208-217.

Joshi, S.R., Standl, E., Tong, N., Shah, P., Kalra, S., Rathod, R., 2015. Therapeutic potential of  $\alpha$ -glucosidase inhibitors in type 2 diabetes mellitus: an evidence-based review. *Expert opinion on pharmacotherapy* 16, 1959-1981.

Juhnke, M., Berghausen, J., Timpe, C., 2010. Accelerated formulation development for nanomilled active pharmaceutical ingredients using a screening approach. *Chemical Engineering & Technology* 33, 1412-1418.

Kallakunta, V.R., Bandari, S., Jukanti, R., Veerareddy, P.R., 2012. Oral self emulsifying powder of lercanidipine hydrochloride: formulation and evaluation. *Powder Technology* 221, 375-382.

- Kanaujia, P., Ng, W.K., Tan, R.B., 2014. Solid self-emulsifying drug delivery system (S-SEDDS) for improved dissolution rate of fenofibrate. *Journal of microencapsulation* 31, 293-298.
- Kang, J.H., Oh, D.H., Oh, Y.-K., Yong, C.S., Choi, H.-G., 2012. Effects of solid carriers on the crystalline properties, dissolution and bioavailability of flurbiprofen in solid self-nanoemulsifying drug delivery system (solid SNEDDS). *European Journal of Pharmaceutics and Biopharmaceutics* 80, 289-297.
- Kang, M.J., Jung, S.Y., Song, W.H., Park, J.S., Choi, S.-U., Oh, K.T., Choi, H.-K., Choi, Y.W., Lee, J., Lee, B.-J., 2011. Immediate release of ibuprofen from Fujicalin®-based fast-dissolving self-emulsifying tablets. *Drug development and industrial pharmacy* 37, 1298-1305.
- Kaul, K., Tarr, J.M., Ahmad, S.I., Kohner, E.M., Chibber, R., 2013. Introduction to diabetes mellitus, *Diabetes*. Springer, pp. 1-11.
- Kaur, P., Singh, S.K., Garg, V., Gulati, M., Vaidya, Y., 2015. Optimization of spray drying process for formulation of solid dispersion containing polypeptide-k powder through quality by design approach. *Powder technology* 284, 1-11.
- Kayser, O., Olbrich, C., Yardley, V., Kiderlen, A., Croft, S., 2003. Formulation of amphotericin B as nanosuspension for oral administration. *International journal of pharmaceutics* 254, 73-75.
- Ke, Z., Hou, X., Jia, X.-b., 2016. Design and optimization of self-nanoemulsifying drug delivery systems for improved bioavailability of cyclovirobuxine D. *Drug design, development and therapy* 10, 2049.
- Keohane, K., Rosa, M., Coulter, I.S., Griffin, B.T., 2016. Enhanced colonic delivery of ciclosporin A self-emulsifying drug delivery system encapsulated in coated minispheres. *Drug development and industrial pharmacy* 42, 245-253.
- Khan, M.Z.I., Raušl, D., Zanoški, R., Zidar, S., Mikulčić, J.H., Krizmanić, L., Eškinja, M., Mildner, B., Knežević, Z., 2004. Classification of loratadine based on the biopharmaceutics drug classification concept and possible in vitro–in vivo correlation. *Biological and Pharmaceutical Bulletin* 27, 1630-1635.
- Khursheed, R., Singh, S.K., Wadhwa, S., Kapoor, B., Gulati, M., Kumar, R., Ramanunni, A.K., Awasthi, A., Dua, K., 2019. Treatment strategies against diabetes: Success so far and challenges ahead. *European journal of pharmacology*, 172625.

Kim, D.W., Kwon, M.S., Yousaf, A.M., Balakrishnan, P., Park, J.H., Kim, D.S., Lee, B.-J., Park, Y.J., Yong, C.S., Kim, J.O., 2014. Comparison of a solid SMEDDS and solid dispersion for enhanced stability and bioavailability of clopidogrel napadisilate. *Carbohydrate polymers* 114, 365-374.

Kim, K.S., Yang, E.S., Kim, D.S., Kim, D.W., Yoo, H.H., Yong, C.S., Youn, Y.S., Oh, K.T., Jee, J.-P., Kim, J.O., 2017. A novel solid self-nanoemulsifying drug delivery system (S-SNEDDS) for improved stability and oral bioavailability of an oily drug, 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol. *Drug delivery* 24, 1018-1025.

King, H., Aubert, R.E., Herman, W.H., 1998. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. *Diabetes care* 21, 1414-1431.

Koga, K., Kusawake, Y., Ito, Y., Sugioka, N., Shibata, N., Takada, K., 2006. Enhancing mechanism of Labrasol on intestinal membrane permeability of the hydrophilic drug gentamicin sulfate. *European journal of pharmaceutics and biopharmaceutics* 64, 82-91.

Kohli, K., Chopra, S., Dhar, D., Arora, S., Khar, R.K., 2010. Self-emulsifying drug delivery systems: an approach to enhance oral bioavailability. *Drug discovery today* 15, 958-965.

Kommuru, T., Gurley, B., Khan, M., Reddy, I., 2001. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. *International journal of pharmaceutics* 212, 233-246.

Kreuter, J., 2001. Nanoparticulate systems for brain delivery of drugs. *Advanced drug delivery reviews* 47, 65-81.

Krupa, A., Jachowicz, R., Kurek, M., Figiel, W., Kwiecień, M., 2014. Preparation of solid self-emulsifying drug delivery systems using magnesium aluminometasilicates and fluid-bed coating process. *Powder technology* 266, 329-339.

Krupa, A., Szłek, J., Jany, B.R., Jachowicz, R., 2015. Preformulation studies on solid self-emulsifying systems in powder form containing magnesium aluminometasilicate as porous carrier. *AAPS PharmSciTech* 16, 623-635.

Kumar, B., Garg, V., Singh, S., Pandey, N.K., Bhatia, A., Prakash, T., Gulati, M., Singh, S.K., 2018. Impact of spray drying over conventional surface adsorption technique for improvement in micromeritic and biopharmaceutical characteristics of

self-nanoemulsifying powder loaded with two lipophilic as well as gastrointestinal labile drugs. *Powder technology* 326, 425-442.

Lei, Y., Lu, Y., Qi, J., Nie, S., Hu, F., Pan, W., Wu, W., 2011. Solid self-nanoemulsifying cyclosporin A pellets prepared by fluid-bed coating: preparation, characterization and in vitro redispersibility. *International journal of nanomedicine* 6, 795.

Li, L., Yi, T., Lam, C., 2013. Effects of spray-drying and choice of solid carriers on concentrations of Labrasol® and Transcutol® in solid self-microemulsifying drug delivery systems (SMEDDS). *Molecules* 18, 545-560.

Liu, P., Rong, X., Laru, J., van Veen, B., Kiesvaara, J., Hirvonen, J., Laaksonen, T., Peltonen, L., 2011. Nanosuspensions of poorly soluble drugs: preparation and development by wet milling. *International journal of pharmaceutics* 411, 215-222.

Liversidge, G.G., Conzentino, P., 1995. Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats. *International journal of pharmaceutics* 125, 309-313.

Liversidge, G.G., Cundy, K.C., Bishop, J.F., Czekai, D.A., 1992. Surface modified drug nanoparticles. Google Patents.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *Journal of biological chemistry* 193, 265-275.

Lv, L.-Z., Tong, C.-Q., Lv, Q., Tang, X.-J., Li, L.-M., Fang, Q.-X., Yu, J., Han, M., Gao, J.-Q., 2012. Enhanced absorption of hydroxysafflor yellow A using a self-double-emulsifying drug delivery system: in vitro and in vivo studies. *International journal of nanomedicine* 7, 4099.

Ma, H., Chu, M., Itagaki, K., Xin, P., Zhou, X., Zhang, D., Wang, Y., Fu, J., Sun, S., 2014. Formulation and in vitro Characterization of a novel Solid lipid-based drug delivery system. *Chemical and Pharmaceutical Bulletin* 62, 1173-1179.

Ma, H., Zhao, Q., Wang, Y., Guo, T., An, Y., Shi, G., 2012. Design and evaluation of self-emulsifying drug delivery systems of *Rhizoma corydalis decumbentis* extracts. *Drug development and industrial pharmacy* 38, 1200-1206.

Mahesh, K.V., Singh, S.K., Gulati, M., 2014. A comparative study of top-down and bottom-up approaches for the preparation of nanosuspensions of glipizide. *Powder technology* 256, 436-449.

Malaysia, P., 2012. Optimization of spray drying process parameters of Piper betle L.(Sirih) leaves extract coated with maltodextrin. *Journal of Chemical and Pharmaceutical Research* 4, 1833-1841.

Mandawgade, S.D., Sharma, S., Pathak, S., Patravale, V.B., 2008. Development of SMEDDS using natural lipophile: application to  $\beta$ -artemether delivery. *International journal of pharmaceutics* 362, 179-183.

Mekjaruskul, C., Yang, Y.-T., Leed, M.G., Sadgrove, M.P., Jay, M., Sripanidkulchai, B., 2013. Novel formulation strategies for enhancing oral delivery of methoxyflavones in *Kaempferia parviflora* by SMEDDS or complexation with 2-hydroxypropyl- $\beta$ -cyclodextrin. *International journal of pharmaceutics* 445, 1-11.

Merisko-Liversidge, E., Liversidge, G.G., 2011. Nanosizing for oral and parenteral drug delivery: a perspective on formulating poorly-water soluble compounds using wet media milling technology. *Advanced drug delivery reviews* 63, 427-440.

Milović, M., Simović, S., Lošić, D., Dashevskiy, A., Ibrić, S., 2014. Solid self-emulsifying phospholipid suspension (SSEPS) with diatom as a drug carrier. *European Journal of Pharmaceutical Sciences* 63, 226-232.

Modasiya, M., Lala, I., Prajapati, B., Patel, V., Shah, D., 2009. Design and characterization of fast disintegrating tablets of piroxicam. *International Journal of PharmTech Research* 1, 353-357.

Mohsin, K., Shahba, A., Alanazi, F., 2012. Lipid based self emulsifying formulations for poorly water soluble drugs-an excellent opportunity. *Ind J Pharm Edu Res* 46, 88-96.

Möschwitzer, J., Achleitner, G., Pomper, H., Müller, R.H., 2004. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. *European Journal of Pharmaceutics and Biopharmaceutics* 58, 615-619.

Möschwitzer, J., Müller, R.H., 2006. New method for the effective production of ultrafine drug nanocrystals. *Journal of nanoscience and nanotechnology* 6, 3145-3153.

Mosharraf, M., Nyström, C., 1995. The effect of particle size and shape on the surface specific dissolution rate of microsized practically insoluble drugs. *International journal of pharmaceutics* 122, 35-47.

Müller, R.H., Benita, S., Bohm, B., 1998. Emulsions and nanosuspensions for the formulation of poorly soluble drugs. CRC Press.

Müller, R.H., Jacobs, C., Kayser, O., 2001. Nanosuspensions as particulate drug formulations in therapy: rationale for development and what we can expect for the future. *Advanced drug delivery reviews* 47, 3-19.

Mustapha, O., Kim, K.S., Shafique, S., Kim, D.S., Jin, S.G., Seo, Y.G., Youn, Y.S., Oh, K.T., Lee, B.-J., Park, Y.J., 2017. Development of novel cilostazol-loaded solid SNEDDS using a SPG membrane emulsification technique: Physicochemical characterization and in vivo evaluation. *Colloids and Surfaces B: Biointerfaces* 150, 216-222.

Nagaraju, P., Krishnachaithanya, K., Srinivas, V., Padma, S., 2010. Nanosuspensions: A promising drug delivery systems. *Int J Pharm Sci Nano* 2, 679-684.

Nasr, A., Gardouh, A., Ghonaim, H., Abdelghany, E., Ghorab, M., 2016a. Effect of oils, surfactants and cosurfactants on phase behavior and physicochemical properties of self-nanoemulsifying drug delivery system (SNEDDS) for irbesartan and olmesartan. *International Journal of Applied Pharmaceutics* 8, 13-24.

Nasr, A., Gardouh, A., Ghorab, M., 2016b. Novel solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of olmesartan medoxomil: Design, formulation, pharmacokinetic and bioavailability evaluation. *Pharmaceutics* 8, 20.

Nazzal, S., Khan, M.A., 2006. Controlled release of a self-emulsifying formulation from a tablet dosage form: Stability assessment and optimization of some processing parameters. *International journal of pharmaceutics* 315, 110-121.

Nekkanti, V., Karatgi, P., Prabhu, R., Pillai, R., 2010. Solid self-microemulsifying formulation for candesartan cilexetil. *Aaps Pharmscitech* 11, 9-17.

Newton, M., Petersson, J., Podczek, F., Clarke, A., Booth, S., 2001. The influence of formulation variables on the properties of pellets containing a self-emulsifying mixture. *Journal of pharmaceutical sciences* 90, 987-995.

Nikolakakis, I., Malamataris, S., 2014. Self-Emulsifying pellets: Relations between kinetic parameters of drug release and emulsion Reconstitution—Influence of formulation variables. *Journal of pharmaceutical sciences* 103, 1453-1465.

Ochman, A.R., Lipinski, C.A., Handler, J.A., Reaume, A.G., Saporito, M.S., 2012. The Lyn kinase activator MLR-1023 is a novel insulin receptor potentiator that elicits

a rapid-onset and durable improvement in glucose homeostasis in animal models of type 2 diabetes. *Journal of Pharmacology and Experimental Therapeutics* 342, 23-32.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry* 95, 351-358.

Onoue, S., Uchida, A., Kuriyama, K., Nakamura, T., Seto, Y., Kato, M., Hatanaka, J., Tanaka, T., Miyoshi, H., Yamada, S., 2012. Novel solid self-emulsifying drug delivery system of coenzyme Q10 with improved photochemical and pharmacokinetic behaviors. *European Journal of Pharmaceutical Sciences* 46, 492-499.

Palla, B., Shah, D., 2002. Stabilization of high ionic strength slurries using surfactant mixtures: molecular factors that determine optimal stability. *Journal of colloid and interface science* 256, 143-152.

Parekh, V.J., Desai, N.D., Shaikh, M.S., Shinde, U.A., 2017. Self nanoemulsifying granules (SNEGs) of meloxicam: preparation, characterization, molecular modeling and evaluation of in vivo anti-inflammatory activity. *Drug development and industrial pharmacy* 43, 600-610.

Parmar, K., Patel, J., Sheth, N., 2015. Self nano-emulsifying drug delivery system for Embelin: Design, characterization and in-vitro studies. *asian journal of pharmaceutical sciences* 10, 396-404.

Parmar, N., Singla, N., Amin, S., Kohli, K., 2011. Study of cosurfactant effect on nanoemulsifying area and development of lercanidipine loaded (SNEDDS) self nanoemulsifying drug delivery system. *Colloids and Surfaces B: Biointerfaces* 86, 327-338.

Patel, G., Shelat, P., Lalwani, A., 2016. Statistical modeling, optimization and characterization of solid self-nanoemulsifying drug delivery system of lopinavir using design of experiment. *Drug delivery* 23, 3027-3042.

Patel, P.V., Patel, H.K., Panchal, S.S., Mehta, T.A., 2013. Self micro-emulsifying drug delivery system of tacrolimus: formulation, in vitro evaluation and stability studies. *International journal of pharmaceutical investigation* 3, 95.

Patel, S., Jani, G., Patel, M., 2012. Solid self emulsifying drug delivery system: An emerging dosage form for poorly bioavailable drugs. *Inventi Rapid: NDDS* 4, 1-7.

Patel, V.R., Agrawal, Y., 2011. Nanosuspension: An approach to enhance solubility of drugs. *Journal of advanced pharmaceutical technology & research* 2, 81.



- Patil, P., Joshi, P., Paradkar, A., 2004. Effect of formulation variables on preparation and evaluation of gelled self-emulsifying drug delivery system (SEDDS) of ketoprofen. *AAPS PharmSciTech* 5, 43-50.
- Patil, P., Paradkar, A., 2006. Porous polystyrene beads as carriers for self-emulsifying system containing loratadine. *AAPS PharmSciTech* 7, E199-E205.
- Patravale, V., Date, A.A., Kulkarni, R., 2004. Nanosuspensions: a promising drug delivery strategy. *Journal of pharmacy and pharmacology* 56, 827-840.
- Paun, J., Tank, H., 2012. Nanosuspension: An emerging trend for bioavailability enhancement of poorly soluble drugs. *Asian Journal of Pharmacy and Technology* 2, 157-168.
- Pecoits-Filho, R., Perkovic, V., 2018. Are SGLT2 inhibitors ready for prime time for CKD? *Clinical Journal of the American Society of Nephrology* 13, 318-320.
- Peters, K., Muller, R., 1996. Nanosuspensions for the oral application of poorly soluble drugs, *Proceeding European Symposium on Formulation of Poorly-Available Drugs for Oral Administration*, APGI, Paris.
- Piero, M., Nzaro, G., Njagi, J., 2015. Diabetes mellitus-a devastating metabolic disorder. *Asian journal of biomedical and pharmaceutical sciences* 5, 1.
- Porter, C.J., Pouton, C.W., Cuine, J.F., Charman, W.N., 2008. Enhancing intestinal drug solubilisation using lipid-based delivery systems. *Advanced drug delivery reviews* 60, 673-691.
- Pouton, C.W., 2000. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *European journal of pharmaceutical sciences* 11, S93-S98.
- Pouton, C.W., Porter, C.J., 2008. Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. *Advanced drug delivery reviews* 60, 625-637.
- Prabhakar, P., Kumar, A., Doble, M., 2014. Combination therapy: a new strategy to manage diabetes and its complications. *Phytomedicine* 21, 123-130.
- Prabhakar, P.K., Doble, M., 2008. A target based therapeutic approach towards diabetes mellitus using medicinal plants. *Current Diabetes Reviews* 4, 291-308.

- Qi, X., Qin, J., Ma, N., Chou, X., Wu, Z., 2014. Solid self-microemulsifying dispersible tablets of celastrol: Formulation development, characterization and bioavailability evaluation. *International journal of Pharmaceutics* 472, 40-47.
- Qi, X., Wang, L., Zhu, J., Hu, Z., Zhang, J., 2011. Self-double-emulsifying drug delivery system (SDEDDS): a new way for oral delivery of drugs with high solubility and low permeability. *International journal of pharmaceutics* 409, 245-251.
- Rabinow, B.E., 2004. Nanosuspensions in drug delivery. *Nature reviews Drug discovery* 3, 785.
- Raghavan, S., Trividic, A., Davis, A., Hadgraft, J., 2000. Effect of cellulose polymers on supersaturation and in vitro membrane transport of hydrocortisone acetate. *International journal of pharmaceutics* 193, 231-237.
- Rahman, M.A., Hussain, A., Hussain, M.S., Mirza, M.A., Iqbal, Z., 2013. Role of excipients in successful development of self-emulsifying/microemulsifying drug delivery system (SEDDS/SMEDDS). *Drug development and industrial pharmacy* 39, 1-19.
- Rajesh, S.Y., Singh, S.K., Pandey, N.K., Sharma, P., Bawa, P., Kumar, B., Gulati, M., Jain, S.K., Gowthamarajan, K., Singh, S., 2018. Impact of various solid carriers and spray drying on pre/post compression properties of solid SNEDDS loaded with glimepiride: in vitro-ex vivo evaluation and cytotoxicity assessment. *Drug development and industrial pharmacy* 44, 1056-1069.
- Ramasahayam, B., Eedara, B.B., Kandadi, P., Jukanti, R., Bandari, S., 2015. Development of isradipine loaded self-nano emulsifying powders for improved oral delivery: in vitro and in vivo evaluation. *Drug development and industrial pharmacy* 41, 753-763.
- Rao, S.V.R., Shao, J., 2008. Self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of protein drugs: I. Formulation development. *International journal of pharmaceutics* 362, 2-9.
- Rao, S.V.R., Yajurvedi, K., Shao, J., 2008. Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of protein drugs: III. In vivo oral absorption study. *International journal of pharmaceutics* 362, 16-19.
- Rashid, R., Kim, D.W., Abid Mehmood Yousaf, O.M., ud Din, F., Park, J.H., Yong, C.S., Oh, Y.-K., Youn, Y.S., Kim, J.O., Choi, H.-G., 2015. Comparative study on

solid self-nanoemulsifying drug delivery and solid dispersion system for enhanced solubility and bioavailability of ezetimibe. *International journal of nanomedicine* 10, 6147.

Rena, G., Pearson, E.R., Sakamoto, K., 2013. Molecular mechanism of action of metformin: old or new insights? *Diabetologia* 56, 1898-1906.

Renuka, S., Sachin Kumar, Gulati, M., Kaur, I., 2014. Characterization of solid state forms of glipizide. *Powder Technology* 264, 365-376.

Sah, H., 1997. Microencapsulation techniques using ethyl acetate as a dispersed solvent: effects of its extraction rate on the characteristics of PLGA microspheres. *Journal of controlled release* 47, 233-245.

Sah, H., 2000. Ethyl formate—alternative dispersed solvent useful in preparing PLGA microspheres. *International journal of pharmaceutics* 195, 103-113.

Schmitz, O., Brock, B., Rungby, J., 2004. Amylin agonists: a novel approach in the treatment of diabetes. *Diabetes* 53, S233-S238.

Seo, Y.G., Kim, D.-W., Cho, K.H., Yousaf, A.M., Kim, D.S., Kim, J.H., Kim, J.O., Yong, C.S., Choi, H.-G., 2015. Preparation and pharmaceutical evaluation of new tacrolimus-loaded solid self-emulsifying drug delivery system. *Archives of pharmacol research* 38, 223-228.

Seo, Y.G., Kim, D.H., Ramasamy, T., Kim, J.H., Marasini, N., Oh, Y.-K., Kim, D.-W., Kim, J.K., Yong, C.S., Kim, J.O., 2013. Development of docetaxel-loaded solid self-nanoemulsifying drug delivery system (SNEDDS) for enhanced chemotherapeutic effect. *International journal of pharmaceutics* 452, 412-420.

Serraton, M., Newton, M., Booth, S., Clarke, A., 2007. Controlled drug release from pellets containing water-insoluble drugs dissolved in a self-emulsifying system. *European Journal of Pharmaceutics and Biopharmaceutics* 65, 94-98.

Setthacheewakul, S., Mahattanadul, S., Phadoongsombut, N., Pichayakorn, W., Wiwattanapatapee, R., 2010. Development and evaluation of self-microemulsifying liquid and pellet formulations of curcumin, and absorption studies in rats. *European Journal of Pharmaceutics and Biopharmaceutics* 76, 475-485.

Shah, A.V., Serajuddin, A.T., 2012. Development of solid self-emulsifying drug delivery system (SEDDS) I: Use of poloxamer 188 as both solidifying and emulsifying agent for lipids. *Pharmaceutical research* 29, 2817-2832.

- Shah, V.P., Tsong, Y., Sathe, P., Liu, J.-P., 1998. In vitro dissolution profile comparison—statistics and analysis of the similarity factor,  $f_2$ . *Pharmaceutical research* 15, 889-896.
- Shahba, A.A.-W., Mohsin, K., Alanazi, F.K., 2012. Novel self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of cinnarizine: design, optimization, and in-vitro assessment. *AAPS PharmSciTech* 13, 967-977.
- Shanmugam, S., Baskaran, R., Balakrishnan, P., Thapa, P., Yong, C.S., Yoo, B.K., 2011a. Solid self-nanoemulsifying drug delivery system (S-SNEDDS) containing phosphatidylcholine for enhanced bioavailability of highly lipophilic bioactive carotenoid lutein. *European Journal of Pharmaceutics and Biopharmaceutics* 79, 250-257.
- Shanmugam, S., Park, J.-H., Kim, K.S., Piao, Z.Z., Yong, C.S., Choi, H.-G., Woo, J.S., 2011b. Enhanced bioavailability and retinal accumulation of lutein from self-emulsifying phospholipid suspension (SEPS). *International journal of pharmaceutics* 412, 99-105.
- Shao, A., Chen, G., Jiang, N., Li, Y., Zhang, X., Wen, L., Yang, F., Wei, S., 2013. Development and evaluation of self-microemulsifying liquid and granule formulations of *Brucea javanica* oil. *Archives of pharmacal research* 36, 993-1003.
- Sharma, P., Singh, S.K., Pandey, N.K., Rajesh, S.Y., Bawa, P., Kumar, B., Gulati, M., Singh, S., Verma, S., Yadav, A.K., 2018. Impact of solid carriers and spray drying on pre/post-compression properties, dissolution rate and bioavailability of solid self-nanoemulsifying drug delivery system loaded with simvastatin. *Powder Technology* 338, 836-846.
- Shazly, G., Mohsin, K., 2015. Dissolution improvement of solid self-emulsifying drug delivery systems of fenofibrate using an inorganic high surface adsorption material. *Acta Pharmaceutica* 65, 29-42.
- Singh, B., Bandopadhyay, S., Kapil, R., Singh, R., Katare, O.P., 2009. Self-emulsifying drug delivery systems (SEDDS): formulation development, characterization, and applications. *Critical Reviews™ in Therapeutic Drug Carrier Systems* 26.

Singh, B., Singh, R., Bandyopadhyay, S., Kapil, R., Garg, B., 2013. Optimized nanoemulsifying systems with enhanced bioavailability of carvedilol. *Colloids and Surfaces B: Biointerfaces* 101, 465-474.

Sinha, B., Müller, R.H., Möschwitzer, J.P., 2013. Bottom-up approaches for preparing drug nanocrystals: formulations and factors affecting particle size. *International journal of pharmaceutics* 453, 126-141.

Sodalee, K., Sapsuphan, P., Wongsirikul, R., Puttipipatkachorn, S., 2016. Preparation and evaluation of alpha-mangostin solid self-emulsifying drug delivery system. *Asian J. Pharm* 11, 225-226.

Sola, D., Rossi, L., Schianca, G.P.C., Maffioli, P., Bigliocca, M., Mella, R., Corliandò, F., Fra, G.P., Bartoli, E., Derosa, G., 2015. Sulfonylureas and their use in clinical practice. *Archives of medical science: AMS* 11, 840.

Sood, S., Jain, K., Gowthamarajan, K., 2014. Optimization of curcumin nanoemulsion for intranasal delivery using design of experiment and its toxicity assessment. *Colloids and Surfaces B: Biointerfaces* 113, 330-337.

Stremmel, W., 1988. Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein. *The Journal of clinical investigation* 82, 2001-2010.

Taha, E.I., Al-Suwayeh, S.A., Anwer, M.K., 2009. Preparation, in vitro and in vivo evaluation of solid-state self-nanoemulsifying drug delivery system (SNEDDS) of vitamin A acetate. *Journal of drug targeting* 17, 468-473.

Tang, B., Cheng, G., Gu, J.-C., Xu, C.-H., 2008a. Development of solid self-emulsifying drug delivery systems: preparation techniques and dosage forms. *Drug discovery today* 13, 606-612.

Tang, J., Sun, J., Cui, F., Zhang, T., Liu, X., He, Z., 2008b. Self-emulsifying drug delivery systems for improving oral absorption of ginkgo biloba extracts. *Drug delivery* 15, 477-484.

Tarate, B., Chavan, R., K Bansal, A., 2014. Oral solid self-emulsifying formulations: a patent review. *Recent patents on drug delivery & formulation* 8, 126-143.

Tran, P.H.-L., Tran, T.T.-D., Piao, Z.Z., Van Vo, T., Park, J.B., Lim, J., Oh, K.T., Rhee, Y.-S., Lee, B.-J., 2013. Physical properties and in vivo bioavailability in human

volunteers of isradipine using controlled release matrix tablet containing self-emulsifying solid dispersion. *International journal of pharmaceutics* 450, 79-86.

Trotta, M., Gallarate, M., Carlotti, M.E., Morel, S., 2003. Preparation of griseofulvin nanoparticles from water-dilutable microemulsions. *International journal of pharmaceutics* 254, 235-242.

Trotta, M., Gallarate, M., Pattarino, F., Morel, S., 2001. Emulsions containing partially water-miscible solvents for the preparation of drug nanosuspensions. *Journal of Controlled Release* 76, 119-128.

Truong, D.H., Tran, T.H., Ramasamy, T., Choi, J.Y., Lee, H.H., Moon, C., Choi, H.-G., Yong, C.S., Kim, J.O., 2016. Development of solid self-emulsifying formulation for improving the oral bioavailability of erlotinib. *Aaps Pharmscitech* 17, 466-473.

Van Eerdenbrugh, B., Vermant, J., Martens, J.A., Froyen, L., Van Humbeeck, J., Augustijns, P., Van den Mooter, G., 2009. A screening study of surface stabilization during the production of drug nanocrystals. *Journal of pharmaceutical sciences* 98, 2091-2103.

Van Speybroeck, M., Williams, H.D., Nguyen, T.-H., Anby, M.U., Porter, C.J., Augustijns, P., 2012. Incomplete desorption of liquid excipients reduces the in vitro and in vivo performance of self-emulsifying drug delivery systems solidified by adsorption onto an inorganic mesoporous carrier. *Molecular pharmaceutics* 9, 2750-2760.

Vyas, M., Galani, V.J., 2010. In vivo and in vitro drug interactions study of glimepride with atorvastatin and rosuvastatin. *Journal of Young Pharmacists* 2, 196-200.

Wadhwa, J., Asthana, A., Gupta, S., Shilkari Asthana, G., Singh, R., 2014. Development and optimization of polymeric self-emulsifying nanocapsules for localized drug delivery: design of experiment approach. *The Scientific World Journal* 2014.

Wang, L., Dong, J., Chen, J., Eastoe, J., Li, X., 2009. Design and optimization of a new self-nanoemulsifying drug delivery system. *Journal of colloid and interface science* 330, 443-448.

Wang, Z., Sun, J., Wang, Y., Liu, X., Liu, Y., Fu, Q., Meng, P., He, Z., 2010. Solid self-emulsifying nitrendipine pellets: preparation and in vitro/in vivo evaluation. *International journal of pharmaceutics* 383, 1-6.

Weerapol, Y., Limmatvapirat, S., Nunthanid, J., Sriamornsak, P., 2014. Self-nanoemulsifying drug delivery system of nifedipine: impact of hydrophilic–lipophilic balance and molecular structure of mixed surfactants. *AAPS pharmscitech* 15, 456-464.

WHO, 2016. Global report on diabetes.

Xia, D., Gan, Y., Cui, F., 2014. Application of precipitation methods for the production of water-insoluble drug nanocrystals: production techniques and stability of nanocrystals. *Current pharmaceutical design* 20, 408-435.

Yadav, S.K., Mishra, S., Mishra, B., 2012. Eudragit-based nanosuspension of poorly water-soluble drug: formulation and in vitro–in vivo evaluation. *AAPS PharmSciTech* 13, 1031-1044.

Yadollahi, R., Vasilev, K., Simovic, S., 2015. Nanosuspension technologies for delivery of poorly soluble drugs. *Journal of Nanomaterials* 2015, 1.

Yao, J., Lu, Y., Zhou, J.P., 2008. Preparation of nobiletin in self-microemulsifying systems and its intestinal permeability in rats. *Journal of Pharmacy & Pharmaceutical Sciences* 11, 22-29.

Yi, T., Wan, J., Xu, H., Yang, X., 2008. A new solid self-microemulsifying formulation prepared by spray-drying to improve the oral bioavailability of poorly water soluble drugs. *European Journal of Pharmaceutics and Biopharmaceutics* 70, 439-444.

You, J., Li, Q.-p., Han, X., Yu, Y.-w., Yang, M.-s., 2005. A novel formulation design about water-insoluble oily drug: preparation of zedoary turmeric oil microspheres with self-emulsifying ability and evaluation in rabbits. *International journal of pharmaceutics* 288, 315-323.

Zhang, B., Lu, W., Chen, W., 2008a. Study on self-microemulsifying drug delivery system of Jiaotai Pill active components. *Zhong yao cai= Zhongyaocai= Journal of Chinese medicinal materials* 31, 1068-1071.

Zhang, P., Liu, Y., Feng, N., Xu, J., 2008b. Preparation and evaluation of self-microemulsifying drug delivery system of oridonin. *International journal of pharmaceutics* 355, 269-276.

Zidan, A.S., Aljaeid, B.M., Mokhtar, M., Shehata, T.M., 2015. Taste-masked orodispersible tablets of cyclosporine self-nanoemulsion lyophilized with dry silica. *Pharmaceutical development and technology* 20, 652-661.



***Publications from current research work***

1. Parth Sharma , Sachin Kumar Singh, **Narendra Kumar Pandey**, Sarvi Yadav Rajesh , Palak Bawa , Bimlesh Kumar , Monica Gulati , Saurabh Singh , Surajpal Verma , Ankit Kumar Yadav, SheetuWadhwa, Subheet Kumar Jain , Kuppusamy Gowthamarajan, Adil Hussain Malik, Suksham Gupta, Rubiya Khursheed. Impact of solid carriers and spray drying on pre/post-compression properties, dissolution rate and bioavailability of solid selfnanoemulsifying drug delivery system loaded with simvastatin. Powder Technology 338 (2018) 836–846. (**Impact Factor – 3.33**)
2. Singh, S.K., Vaidya, Y., Gulati, M., Bhattacharya, S., Garg, V., **Pandey, N.K.** Nanosuspension: Principles, perspectives and practices. Current Drug Delivery, 2016, 13, 1222-1246. (**Impact Factor – 2.5**)
3. Sarvi Yadav Rajesh, Sachin Kumar Singh, **Narendra Kumar Pandey**, Parth Sharma, Palak Bawa, Bimlesh Kumar, Monica Gulati, Subheet Kumar Jain, Kuppusamy Gowthamarajan & Saurabh Singh. Impact of various solid carriers and spray drying on pre/post compression properties of solid SNEDDS loaded with glimepiride: in vitro-ex vivo evaluation and cytotoxicity assessment. DDIP 2018; 44 (7) 1056-1069. (**Impact Factor – 2.29**)
4. **Narendra Kumar Pandey**, Sachin Kumar Singh, Dipanjoy Ghosh, Rubiya Khursheed, Rajan Kumar, Bhupinder Kapoor, Bimlesh Kumar, Ankit Awasthi. Method development and validation for simultaneous estimation of glimepiride and simvastatin by using reversed phase high-performance liquid chromatography. RJPT, Feb. 2020: Vol:13No:02 (**Accepted**)

*Publications from allied research work*

1. Souvik Mohanta, Sachin Kumar Singh, Bimlesh Kumar, Monica Gulati, Jivan Jyoti, Sananda Som, Sakshi Panchal, Indu Melkani, Mayukh Banerjee, Shubham Kumar Sinha, Rubiya Khursheed, Ankit Kumar Yadav, Vishu Verma, Rajan Kumar, Deep Shikha Sharma, Adil Hussain Malik, **Narendra Kumar Pandey**, Sheetu Wadhwa. Solidification of liquid Modified Apple Polysaccharide by its adsorption on solid porous carriers through spray drying and evaluation of its potential as binding agent for tablets. *International Journal of Biological Macromolecules* 120 (2018) 1975–1998. (**Impact Factor – 4.7**)
2. Souvik Mohanta, Sachin Kumar Singh, Bimlesh Kumar, Monica Gulati, Rajesh Kumar, Ankit Kumar Yadav, Sheetu Wadhwa, Jivan Jyoti, Sananda Soma, Kamal Dua, **Narendra Kumar Pandey**. Efficacy of co-administration of modified apple polysaccharide and probiotics in guar gum-Eudragit S100 based mesalamine mini tablets: A novel approach in treating ulcerative colitis. *International Journal of Biological Macromolecules* 126 (2019) 427–435. (**Impact Factor – 4.7**)
3. Jyoti J, Anandhakrishnan NK, Singh SK, Kumar B, Gulati M, Gowthamarajan K, Kumar R, Yadav AK, Kapoor B, **Pandey NK**, Som S, Mohanta S, Melkani I, Khursheed R, Narang R. A three-pronged formulation approach to improve oral bioavailability and therapeutic efficacy of two lipophilic drugs with gastric lability *Drug Delivery and Translational Research* (2019) 9:848–865. (**Impact Factor – 3.49**)
4. Bimlesh Kumar , Varun Garg , Saurabh Singh, **Narendra Kumar Pandey**, Amit Bhatia, T. Prakash, Monica Gulati, Sachin Kumar Singh. Impact of spray drying over conventional surface adsorption technique for improvement in micromeritic and biopharmaceutical characteristics of self-nanoemulsifying powder loaded with two lipophilic as well as gastrointestinal labile drugs. *Powder Technology* 2018; 326: 425–442. (**Impact Factor – 3.33**)
5. **Narendra Kumar Pandey**, Hans Raj Sehla, Varun Garg, Tejasvi Gaur, Bimlesh Kumar, Sachin Kumar Singh, Monica Gulati, K. Gowthamarajan,

Palak Bawa, Sarvi Yadav Rajesh, Parth Sharma, and Rakesh Narang. Stable Co-crystals of Glipizide with Enhanced Dissolution Profiles: Preparation and Characterization. *AAPS PharmSciTech* 2017; 18(7):2454-2465. **(Impact Factor – 2.68)**

6. Sharma A, Kumar B, Singh SK, Gulati M, Vaidya Y, Sadotra M, Rathee H, Ghai D, Malik AH, Yadav AK, Maharshi P, Bawa P, Rajesh SY, Sharma P, **Pandey NK**, Mohant S. In vitro and in vivo pharmacokinetic evaluation of guar gum-Eudragit® S100 based colon-targeted spheroids of sulfasalazine co-administered with probiotics. *Curr Drug Deliv.* 2017 Dec 7. doi: 10.2174/1567201815666171207165059. **(Impact Factor – 2.5)**
7. Garg V, Kaur P, Gulati M, Singh SK, Kumar B, **Pandey NK**, Yadav AK, Kumar R, Gowthamarajan K, De A, Puttappa N, Wadhwa. Co-administration of polypeptide-k and curcumin through solid self-nanoemulsifying drug delivery system for better therapeutic effect against diabetes mellitus: Formulation, optimization, biopharmaceutical characterization and pharmacodynamic assessment. *Assay and Drug Development Technologies*, 2019 17(4):201-22. **(Impact Factor – 1.44)**
8. Melkani I, Kumar B, Panchal S, Singh SK, Singh A, Gulati M, Gill SBS, Jyoti J, **Pandey NK**, Kumar S, Subedi B. Comparison of sildenafil, fluoxetine and its co-administration against chronic constriction injury induced neuropathic pain in rats: An influential additive effect. *Neurological Research*, 2019, 1-8. **(Impact Factor – 1.44)**
9. Som S, Singh SK, Khatik GL, Kapoor B, Gulati M, K Gowthamarajan, Anandhakrishnan NK, Kumar B, Yadav AK, Kumar R, Singh PK, Khursheed R, Kumar R, **Pandey NK**, Jyoti J, Mohanta S, Porwal O. Quality by design-based crystallization of curcumin crystals using liquid anti-solvent precipitation: Micromeritic, biopharmaceutical and stability aspects. *Assay and Drug Development Technologies*, 2019, doi: 10.1089/adt.2018.913. **(Impact Factor – 1.44)**

10. Jasmine Kaur, Palak Bawa, Sarvi Yadav Rajesh, Parth Sharma, Deepak Ghai, Jivan Jyoti, Sananda Som, Souvik Mohanta, Harish Rathee, Adil Hussain Malik, Sachin Kumar Singh, Bimlesh Kumar, Monica Gulati, **Narendra Kumar Pandey**, Varun Garg, Ankit Kumar Yadav, Rakesh Narang. Formulation of curcumin nanosuspension using box-behnken design and study of impact of drying techniques on its powder characteristics. AJPCR 2017, doi: 10.22159/ajpcr.2017.v10s4.21335. (**SJR – 0.28**)
11. Prabhjot Kaur, Varun Garg, Palak Bawa, Roopesh Sharma, Sachin Kumar Singh\*, Bimlesh Kumar, Monica Gulati, **Narendra Kumar Pandey**, Rakesh Narang, Sheetu Wadhwa, Souvik Mohanta, Jivan Jyoti, Sananda Som. Formulation, systematic optimization, in vitro, ex vivo, and stability assessment of transethosome based gel of curcumin. AJPCR 2016;11(2):41-47. (**SJR – 0.28**)
12. Bimlesh Kumar, Varun Garg, Amarjeet Singh, **Narendra Kumar Pandey**, Saurabh Singh, Sakshi Panchal, Indu Melkani, Raji Manzi Axel, Souvik Mohanta, Jivan Jyoti, Sananda Som, Monica Gulati, Amit Bhatia, T. Prakash, Sachin Kumar Singh. Investigation and optimization of formulation parameters for self-nanoemulsifying delivery system. AJPCR 2018; 11(2): 12-18. (**SJR – 0.28**)
13. Abhishek Chatterjee<sup>1</sup>, Dileep Singh Baghel<sup>1</sup>, Amit Mittal<sup>1</sup>, Saurabh Singh, Bimlesh Kumar, **Narendra Kumar Pandey**, Sachin Kumar Singh, Tamilvanan S, Shruti Chopra, Amit Bhatia, Anand Kumar Chaudhary. In vitro anti-inflammatory and antioxidant activities of hinguleswara rasabased herbomineral formulations. AJPCR 2018; 11(2): 24-27. (**SJR – 0.28**)
14. Saurabh Singh, Bimlesh Kumar, **Narendra Kumar Pandey**, Barinder Kaur, Arun Kumar, Dileep Singh Baghel, Malti G. Chauhan, Sachin Kumar Singh. Synthesis of copper nanoparticles using ascorbic acid and cetyl trimethyl ammonium bromide. AJPCR 2018; 11(2):62-64. (**SJR – 0.28**)
15. Pinakin Pandya, **Narendra Kumar Pandey**, Sachin Kumar Singh, Manish Kumar. Formulation and characterization of ternary complex of poorly soluble

- Duloxetine hydrochloride. *Journal of Applied Pharmaceutical Science* 5 (06); 2015: 088-096. (**SJR – 0.23**)
16. Jagadish Muthyala, Sachin Kumar Singh, Monica Gulati, Bimlesh Kumar, Harish Rathee, Deepak Ghai, Jasmine Kaur, **Narendra Kumar Pandey**, Ankit Kumar Yadav, Renuka. Systematic Development and Characterization of Liquisolid Compacts of Atorvastatin-Glipizide Binary Mixture to Achieve Enhanced Dissolution and Stability Profile. *Asian Journal of Pharmaceutics*, Oct-Dec 2016, (Suppl)10(4), S2. (**SJR-0.20**)
  17. Ranjith Anishetty, Sachin Kumar Singh, Varun Garg, Ankit Kumar Yadav, Monica Gulati, Bimlesh Kumar, **Narendra Kumar Pandey**, Rakesh Narang, Amit Mittal. Discriminatory potential of biphasic medium over compendial and Biorelevant medium for assessment of dissolution behavior of tablets Containing meloxicam nanoparticles. *Asian J Pharm Clin Res*, Vol 9, Issue 4, 2016, 1-12. (**SJR – 0.20**)
  18. Pemba Lahmo, Sachin Kumar Singh\*, Varun Garg, Peddi Maharshi, Ankit Kumar Yadav, Monica Gulati, Bimlesh Kumar, **Narendra Kumar Pandey**, Prabhjot Kaur, Rakesh Narang, Amit Mittal. Influence of formulation parameters on dissolution rate enhancement of piroxicam using liquisolid technique. *Asian J Pharm Clin Res*, Vol 9, Issue 4, 2016, 1-6. (**SJR – 0.20**)
  19. **Narendra Pandey**, Bimlesh Kumar, Sachin Kumar Singh, Parth Sharma, Yadav Sarvi, Raghvendra G. Investigation of influence of shape on drug loading and entrapment efficiency of nanoparticles. *Journal of Pharmacy Research* (2017):850-855. (**SJR – 0.18**)
  20. Priyabrata Mohanta, **Narendra K. Pandey**, Deepak N. Kapoor, Sachin K. Singh, Yadav Sarvi, Parth Sharma. Development of surfactant-based nanocarrier system for delivery of an antifungal drug. *Journal of Pharmacy Research* 2017; 11 (9) : 1153-1158 (**SJR – 0.18**)
  21. Bimlesh Kumar, Adil Hussain Malik, Parth Sharma, Harish Rathee, T. Prakash, Amit Bhatia, Monica Gulati, **Narendra Kumar Pandey**, Saurabh Singh Baghel, Sachin Kumar Singh. Validated reversed-phase high-performance

liquid chromatography method for simultaneous estimation of curcumin and duloxetine hydrochloride in tablet and self-nanoemulsifying drug delivery systems. Journal of Pharmacy Research 2017; 11 (1): 1166-1178. (SJR – 0.18)

22. Pandya Pinakin, **Narendra Kumar Pandey**, Sachin Kumar Singh, Varun Garg. MCC SANAQ® burst: A unique carrier for formulation of sublingual tablets. Int.J. PharmTech Res. 2016,9(1),pp 15-22. (SJR – 0.15)
23. Jasleen Kaur, Surajpal Verma, **Narendra Kumar Pandey**, Parth Sharma, Shruti Chopra, Shyam Baboo, Indu Bala. A mechanistic approach of qbd for the preparation and evaluation of immediate release tablet containing mycophenolate mofetil and prednisolone. International Journal of Pharmaceutical Quality Assurance 2016; 7(4); 98-106.
24. Saurabh Singh, Sachin Kumar Singh, Malti G. Chauhan, Bimlesh Kumar, **Narendra Kumar Pandey**, Barinder Kaur, Arun Kumar, Souvik Mohanta, Monica Gulati, Sheetu Wadhwa, Ankit Kumar Yadav, Pankaj Kumar Singh, Yogita Kumari, Gurmandeep Kaur, Rubiya Khursheed and A. Clarrisse . Quality by design-based optimization of formulation and process variables for controlling particle size and zeta potential of spray dried incinerated copper nanosuspension. Recent Innovation in Chemical Engineering 2019; 12(3):248-260. (SJR – 0.13)
25. Ankit Kumar Yadav, Varun Garg<sup>1</sup>, Monica Gulati, Parikshit Bansal, Kompal Bansal, Puneet Kaur, Sachin Kumar Singh, Amit Mittal, Rakesh Narang, Bimlesh Kumar, **Narendra Kumar Pandey**, Sheetu Wadhwa, Gopal Lal Khatik, Mayukh Banerjee, Souvik Mohanta, Shubham Sinha, Jivan Jyoti, Sananda Som, Bhupinder Kapoor, Saurabh Singh. Design and performance verification of newly developed disposable static diffusion cell for drug diffusion/permeability studies. Asian J Pharm Clin Res 2018; 11 (2): 1-7. (SJR – 0.20)

### ***Presentation***

1. Presented poster on “Method development for simultaneous estimation of glimepiride and simvastatin by using reversed-phase high-performance liquid chromatography” in national conference PHYTON 2018 and **secured third position**.
2. Presented poster on “Nanosuspension: New possibilities for poorly soluble drugs” in International Conference of Pharmacy 2017.



**LOVELY  
PROFESSIONAL  
UNIVERSITY**

*Transforming Education Transforming India*

## Division of Academic Affairs

LPU/DAA/EC/160204/023

Dated: 4<sup>th</sup> Feb 2016.

Narendra Kumar Pandey  
Q. No 7 CT 89, Obra Colony  
Sonbhadra, Uttar Pradesh  
India, Pin-231219.

Subject: Letter of Candidacy for Ph.D.

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. degree on 12<sup>th</sup> Oct 2015 by accepting your thesis research proposal titled:

"Nanotechnology mediated co-formulation of Simvastatin and Glimepiride", supervised by Dr. Sachin kumar Singh, Associate Professor, at Lovely Professional University, Phagwara, Punjab.

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. degree students of the University, and amendments, if any, made from time to time.

We wish you the very best in completing your thesis research requirements in the near future. Please do not hesitate to contact us in case you have questions about the rules and regulations of the University.

  
Signature