Chapter 4

Preformulation studies

TABLE OF CONTENTS

PREFORMULATION STUDIES

The term "preformulation" refers to the stage of development where the physicochemical characteristics of the pharmacological substance are identified and established. Determining the drug substance's ideal formulation and delivery strategy requires thorough knowledge of the key therapeutic and physicochemical qualities. Every drug substance has inherent chemical and physical characteristics that are taken into account before pharmaceutical formulations are formulated. When designing a dosage form, this feature provides the framework for combining a drug substance with other excipients [1-4].

4.1. AUTHENTICATION OF ACTIVE INGREDIENTS

Authentication of Paclitaxel and Cyclophosphamide was done by Melting Point determination, FTIR and by determining maximum wavelength by UV visible spectrophotometer [5-7].

4.1.1 Melting Point Determination

Pure drug was placed inside a capillary tube that was sealed on one end and attached to a lab thermometer so that it was kept submerged in a liquid paraffin bath. Its melting temperature was defined as the range of temperatures between which the medication began to melt and completely melted [5, 8, 9].

4.1.2 FTIR Spectroscopy

IR-spectrum of drug was measured in the solid state by preparing a Potassium Bromide (KBr) pellet. The pure drug was previously ground individually and mixed thoroughly with KBr separately, an infrared transparent matrix at 1:100 (sample KBr) ratio. The pellets were then scanned over a wavelength range of 4000-400 cm-1 and a spectrum was obtained by using a Spectrum –II FTIR spectrometer (PerkinElmer, USA) [6, 10-15].

4.1.3 Determination of Wavelength Maxima

Fixed amount of API was dissolved in methanol and UV scan of solution was executed against the methanol as blank in UV visible spectrophotometer (Shimadzu) in between the wavelengths of 200 to 400nm [7, 16, 17].

4.2. SCREENING OF SOLID LIPIDS

In a 10 ml clear glass vial, 2.0 g solid lipid was taken and melted at temperature 10 °C above its melting point on a hot plate cum magnetic stirrer. To this melted lipid, drug was added in small increments (5 mg) with constant stirring using a magnetic bead. After addition of each increment of drug, the mixture was stirred for 15 minutes to allow complete solubilisation of drug and equilibration. Loss of transparency indicated saturation solubility of drug in the lipid [18-22].

4.3. SCREENING OF LIQUID LIPIDS/ OILS

2 ml of liquid lipid was taken in a clear glass vial and heated to 60°C. To this, drug was added in small increments (5 mg). This was then subjected to stirring to ensure thorough mixing of drug in the liquid lipid. Loss of transparency indicated saturation solubility of drug in the lipid [18-22].

4.4. SCREENING OF CO-SURFACTANTS

2 ml of co-surfactant was taken in a clear glass vial and heated to 60°C. To this, drug was added in small increments (5 mg). This was then subjected to stirring to ensure thorough mixing of drug in the co-surfactant. Loss of transparency indicated saturation solubility of drug in the co-surfactant. The precipitation of drug indicated co-surfactant is not compatible with drug [18-22].

4.5. SOLID LIPID-LIQUID LIPID COMPATIBILITY STUDIES

Solid Lipid-Liquid Lipid compatibility was evaluated by solidification test. 1.0 g of solid lipid and 1.0 g of liquid lipid was taken in clear glass vial and heated at temperature 10°C above the melting point of solid lipid on a hot plate cum magnetic stirrer and mixed using magnetic bead. After sufficient mixing, the magnetic bead was removed and the solid lipid-liquid lipid mixture was allowed to cool to room temperature (25±5°C). After cooling, mixture was observed for any droplets of oil on the surface. Similarly, solidification test was performed for the mixture of 1.0 g of solid lipid and 2.0 g of liquid lipid as well as 1.0 g of solid lipid and 3.0 g of liquid lipid [18].

4.6. SELECTION OF CO-SOLVENT

Ethanol is widely used and widely accepted co-solvent in parenteral i.v. formulations. Addition of alcohol increases the capacity of system to solubilise the hydrophobic molecules of API. Ethanol is a preferred solvent for the solubilisation of Paclitaxel and Cyclophosphamide. Therefore, ethanol was suitable as a co-solvent for the formulation of NLCs.

Acetone is also well accepted solvent due to its lower boiling point (56°C) leading to faster evaporation during compounding for NLCs. Paclitaxel and Cyclophosphamide have solubility in acetone making it a preferred co-solvent for the formulation of NLCs [23, 24].

4.7. DRUG EXCIPIENT COMPATIBILITY STUDIES

Isothermal stress testing method was used to assess the compatibility between drug-drug and drug-excipient. Briefly, a fixed amount of pure drugs and excipients were weighed separately and in combination. Individual drugs and drug-drug/drug-excipient combinations were transferred in to an appropriately labelled glass vial. Each vial was sealed properly and placed in stability chamber at 40±2°C/75±5%RH for 4 weeks for Paclitaxel-Excipient compatibility and at 25±2°C/60±5%RH for 4 weeks for Cyclophosphamide-Excipient and drug-drug compatibility.

To identify the physical instability, organoleptic parameters of samples such as colour and texture were observed initially and at the end of 4th week. To identify the chemical instability, samples were divided into two parts at the end of 4th week. First part of samples was used to record the Fourier-Transform Infrared (FT-IR) spectrum using FT-IR Spectrometer. Disappearance of absorption bands or reduction of the band intensity combined with the appearance of new bands give a clear evidence for interactions. The second part of samples were separately mixed with fixed quantity of methanol, sonicated for 5 minutes followed by filtration through 0.22 µm membrane and analysed using the developed High Performance Liquid Chromatography (HPLC) methods for API content. [25-27]

RESULTS AND DISCUSSION

4.8. AUTHENTICATION OF ACTIVE INGREDIENTS

4.8.1 Melting Point Determination

The melting point of Paclitaxel was found as 215-217°C with decomposition which is within the reported range of 213-220°C with decomposition [28, 29].

The melting point of Cyclophosphamide was found as 45°C which is in the reported range of 43-45°C [30].

4.8.2 FTIR Spectroscopy

The FTIR scan of Paclitaxel was found to match with the IR scan of reference standard [31, 32]. The FTIR spectrum of Paclitaxel is presented in Figure 4.1.

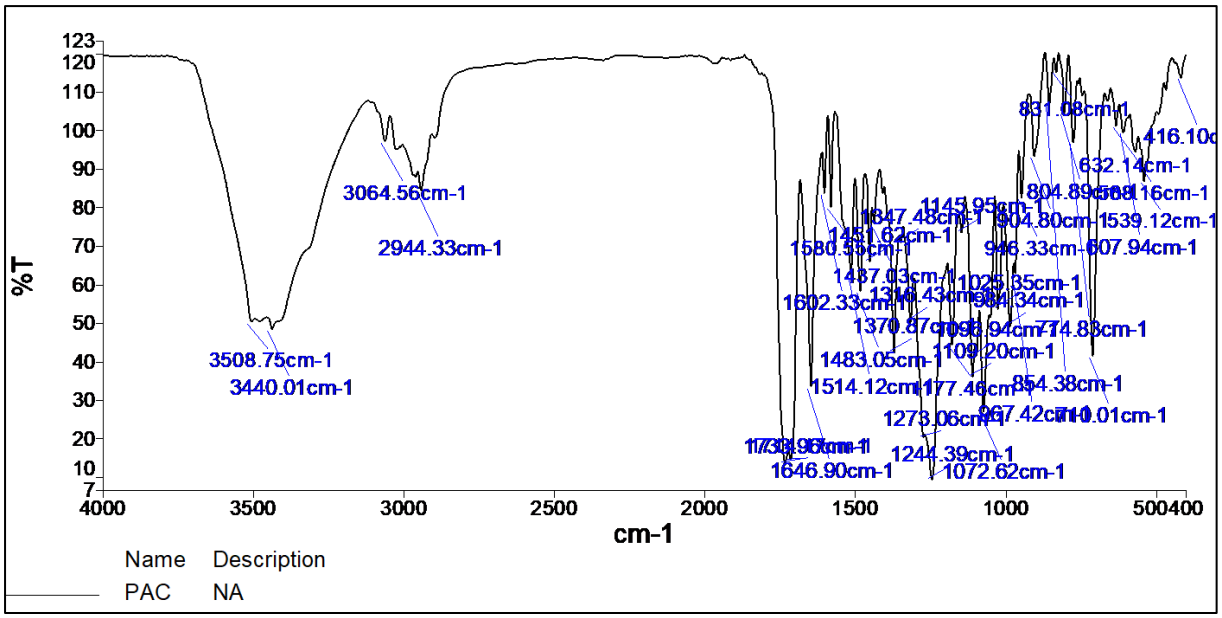

Figure 4.1: FTIR Spectrum of Paclitaxel

The FTIR scan of Cyclophosphamide API was found to match with the IR scan of reference standard [33]. The FTIR spectrum of Cyclophosphamide API is presented in Figure 4.2.

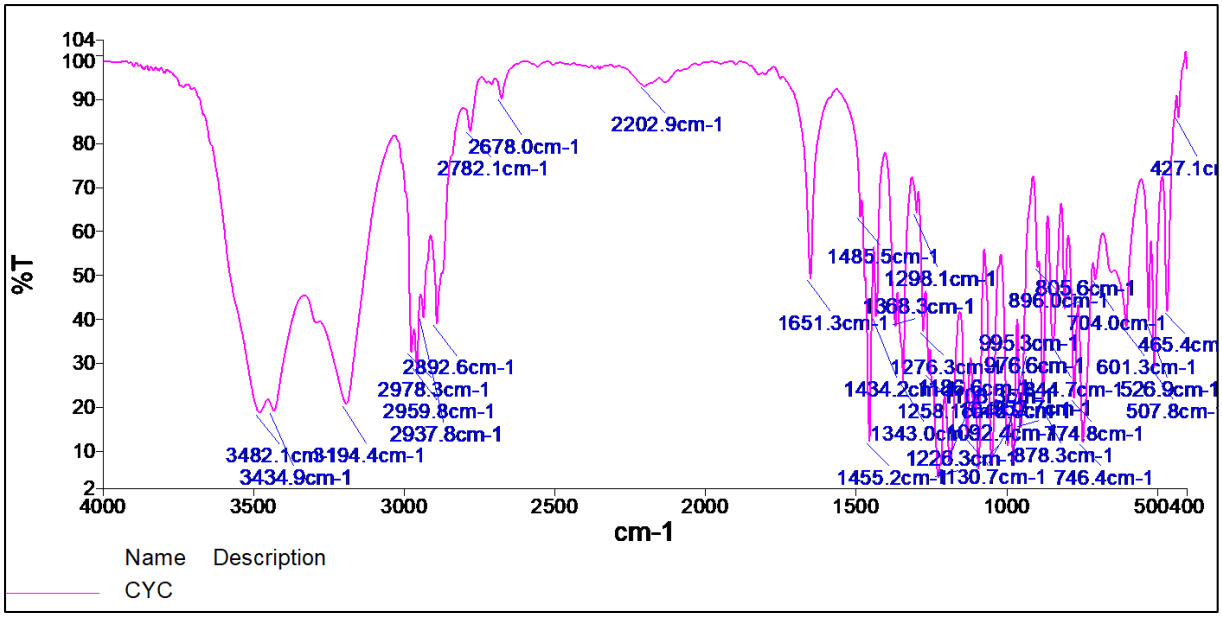

Figure 4.2: FTIR Spectrum of Cyclophosphamide

4.8.3 Determination of Wavelength Maxima

The wavelength maximum of Paclitaxel was obtained as 227nm which is in line with the reported value in the DMF of Paclitaxel. The UV scan of Paclitaxel is presented in Figure 4.3.

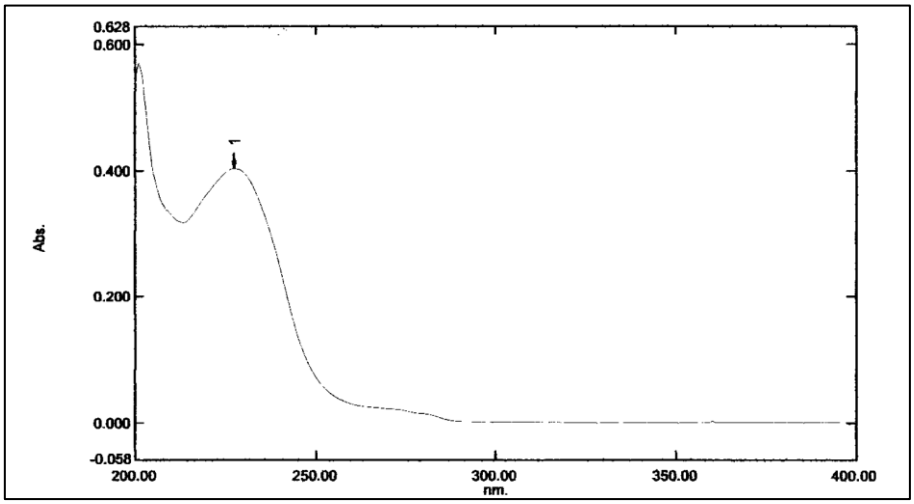

Figure 4.3: UV scan of Paclitaxel API

The wavelength maximum of Cyclophosphamide was obtained as 218nm which is in line with the reported value in DMF of Cyclophosphamide. The UV scan of Cyclophosphamide API is presented in Figure 4.4.

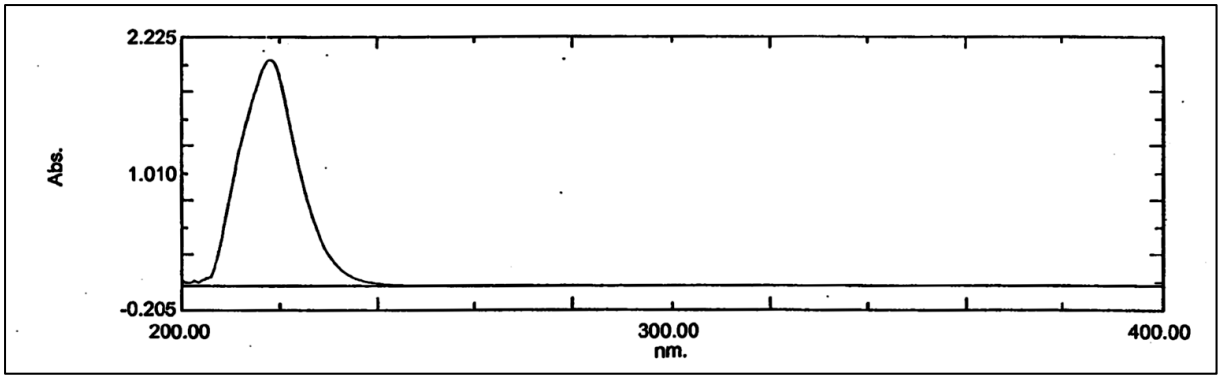

Figure 4.4: UV scan of Cyclophosphamide API

4.9. SCREENING OF SOLID LIPIDS

The solubility of Paclitaxel was tested in various solid lipids and Paclitaxel was found to have highest solubility in Stearic acid and PEG-100 Stearate. Further the solubility of Cyclophosphamide was evaluated in Stearic acid and PEG-100 Stearate. Cyclophosphamide was found to have higher solubility in PEG-100 Stearate and therefore, PEG-100 Stearate was selected as solid lipid for the preparation of nanostructured lipid carriers [34]. The results of solubility of Paclitaxel in different solid lipids are presented in Figure 4.5.

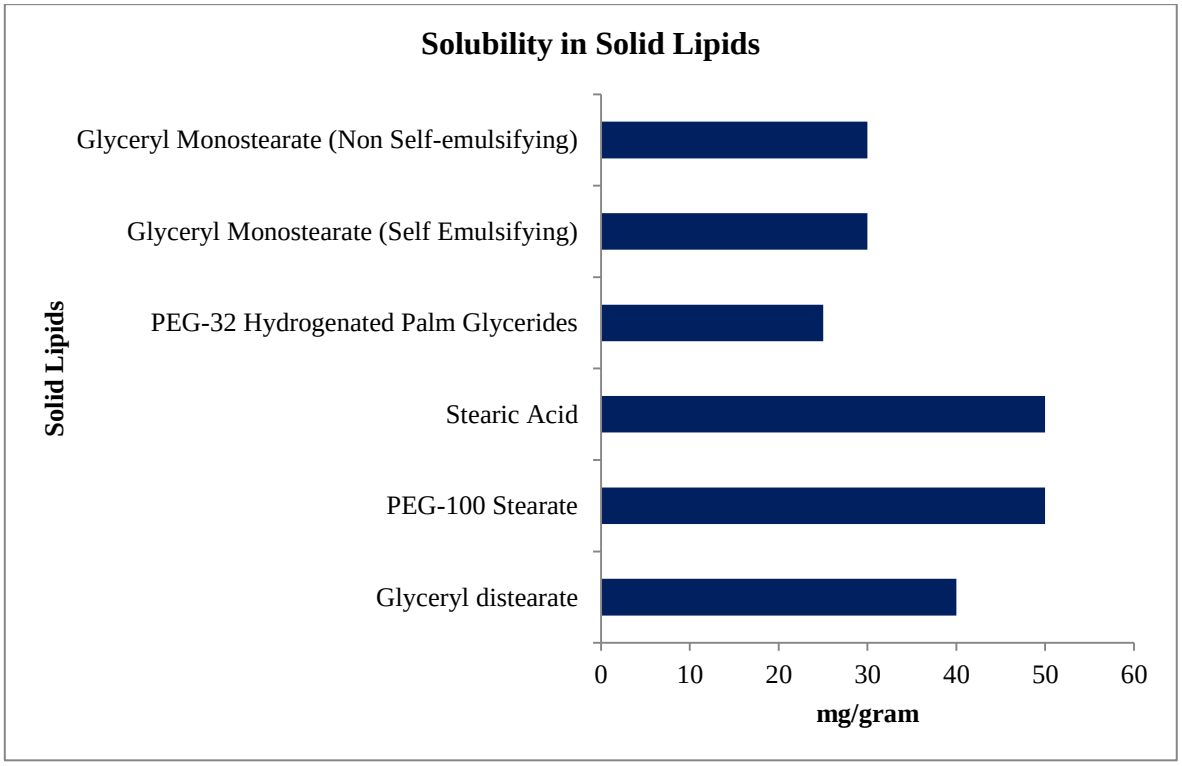

Figure 4.5: Solubility of Paclitaxel in different solid lipids

4.10. SCREENING OF LIQUID LIPIDS/ OILS

The solubility of Paclitaxel was tested in various liquid lipids/ oils and Paclitaxel was found to have highest solubility in PEG-8 Caprylic/Capric Glycerides. As Paclitaxel was found to have highest solubility in PEG-8 Caprylic/Capric Glycerides, same liquid lipids/ oils were further evaluated to check the solubility of Cyclophosphamide. Cyclophosphamide was also found to have very good solubility in PEG-8 Caprylic/Capric Glycerides. PEG-8 Caprylic/Capric Glycerides was selected as liquid lipid for the manufacturing of nanostructured lipid carriers and microemulsion [34]. Though, clove oil was having highest solubility, it was not selected as clove oil is not recommended for intravenous use [35]. The results of solubility of Paclitaxel in different liquid lipids/ oils are presented in Figure 4.6.

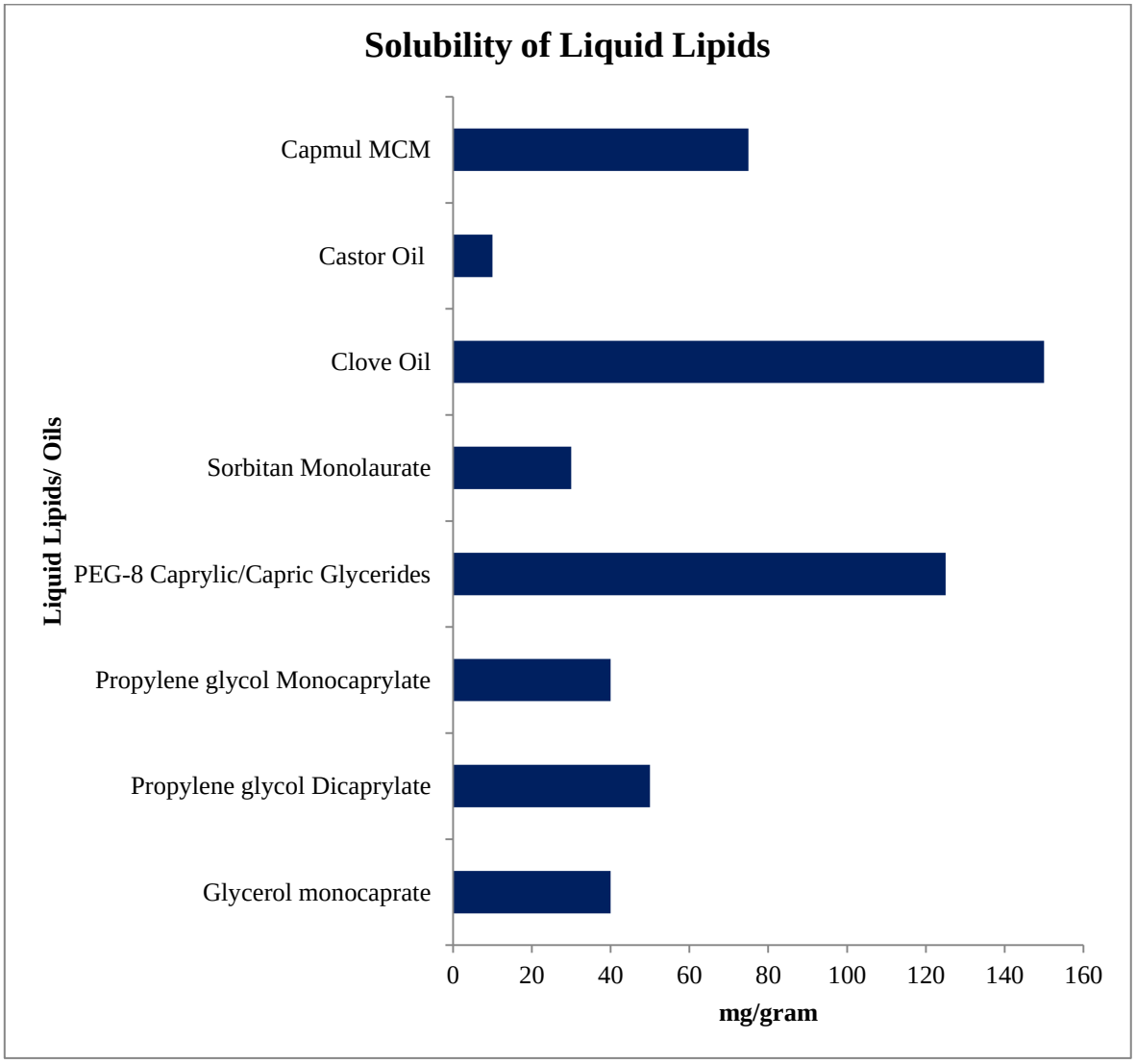

Figure 4.6: Solubility of Paclitaxel in different liquid lipids/ oils

4.11. SCREENING OF CO-SURFACTANTS

Paclitaxel was found to have highest solubility of more than 100mg/gram in PEG-400. Hence, same co-surfactant was further evaluated to check the solubility of Cyclophosphamide. Cyclophosphamide was also found to have very good solubility of more than 250mg/gram in PEG-400. Paclitaxel's solubility in PEG-400 is largely due to hydrophobic interactions between the drugs and the polyethylene chains, which help to dissolve the drugs more effectively than in other co-surfactants. These interactions are crucial because Paclitaxel is very poorly soluble in water, necessitating the use of solvents like PEG-400 to enhance its solubility for therapeutic applications [36-38].

4.12. SOLID LIPID-LIQUID LIPID COMPATIBILITY STUDIES

PEG-100 Stearate and PEG-8 Caprylic/Capric Glycerides mixtures were observed to be compatible with each other at 1:1, 1:2 and 1:3 ratio with no droplets on the surface of solidified mixture and as well as on the filter paper. Hence, mixture of PEG-100 Stearate and PEG-8 Caprylic/Capric Glycerides was selected for formulation of nanostructured lipid carriers [39-40].

4.13. DRUG EXCIPIENT COMPATIBILITY STUDIES

[A] Physical Stability

There was no change in the physical description for all the mixtures after 4 weeks as compared to the Initial physical description. Based on the physical appearance, it is clear that both the drugs are compatible with all the excipients.

[B] Compatibility by FTIR

FTIR scan of drug alone versus the drug-drug and drug-excipient mixtures showed no change in the spectral peaks (Figure 4.7 and Figure 4.8). No significant changes in the distinct bands of the drug substance in drug-drug and drug-excipient mixtures were observed when compared to drug substance alone at the end of 4 weeks. For Paclitaxel, carbonyl (C-O) stretching at 1734cm^{-1}, C-O stretching of amide group at 1645cm^{-1} and ester (C-O) stretching at 1245cm^{-1} are distinct bands. Similarly, C-O stretching of amide group at around 1650cm^{-1}, C–Cl stretching at around 800cm^{-1} and secondary amine group stretching at 746cm^{-1} are distinct bands for Cyclophosphamide. No change in these distinct bands for

Paclitaxel and Cyclophosphamide were observed. The results are presented in Table 4.1 and Table 4.2.

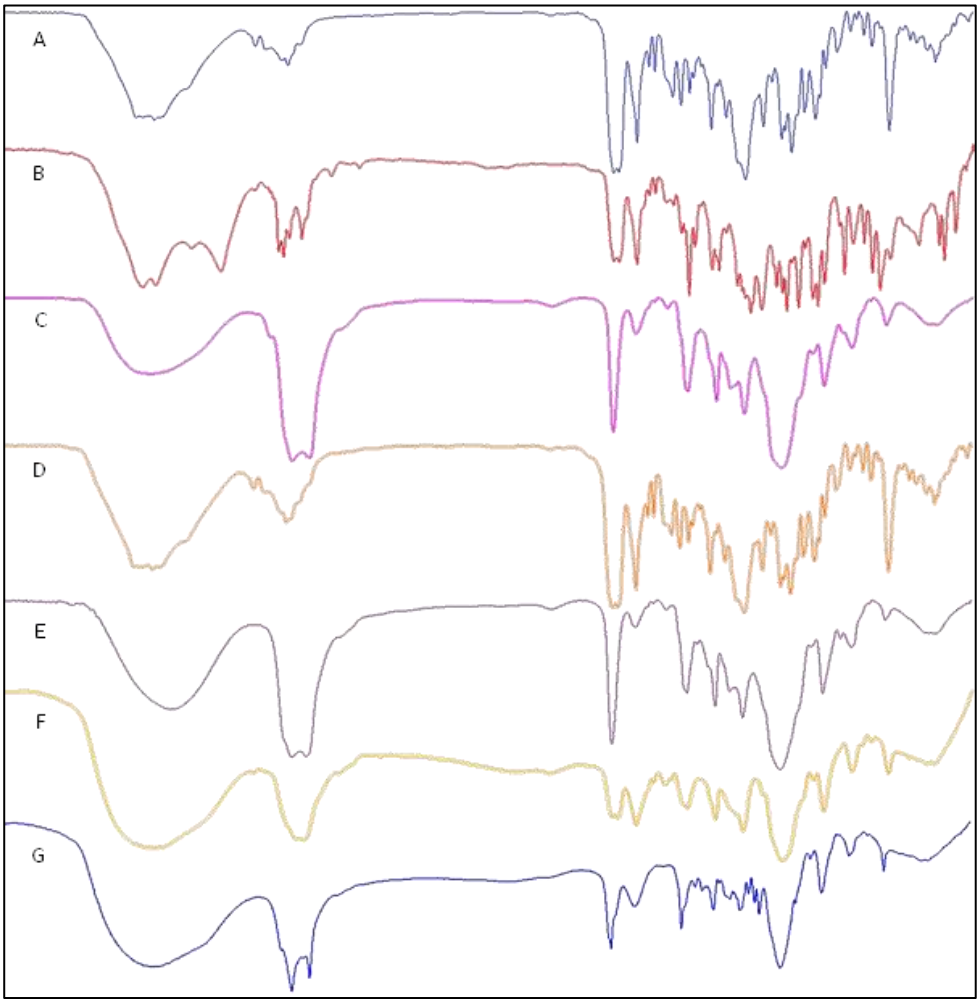

Figure 4.7: FTIR Spectra of Paclitaxel – Excipient Compatibility studies [A] Paclitaxel pure drug [B] Paclitaxel + Cyclophosphamide [C] Paclitaxel + Cremophor EL [D] Paclitaxel + Soluplus [E] Paclitaxel + Acconon MC8-2 [F] Paclitaxel + PEG-400 [G] Paclitaxel + Softemul 165

Table 4.1: FTIR Spectra Interpretation for Drug-Drug and Drug-Excipient compatibility studies of Paclitaxel [41]

Sr. No.	Description of Mixture	Distinct bands observed in FTIR		
		Carbonyl (C–O) stretching	C-O stretching of Amide group	Ester (C–O) stretching
A	Paclitaxel pure drug	1733.96	1646.90	1244.39
B	Paclitaxel + Cyclophosphamide	1734.92	1648.14	1246.90
C	Paclitaxel + Cremophor EL	1733.61	1650.22	1247.57
D	Paclitaxel + Soluplus	1734.32	1646.79	1244.61
E	Paclitaxel + Acconon MC8-2	1735.07	1647.66	1249.24
F	Paclitaxel + PEG 400	1735.40	1646.43	1249.09
G	Paclitaxel + Softemul 165	1730.95	1643.10	1253.59

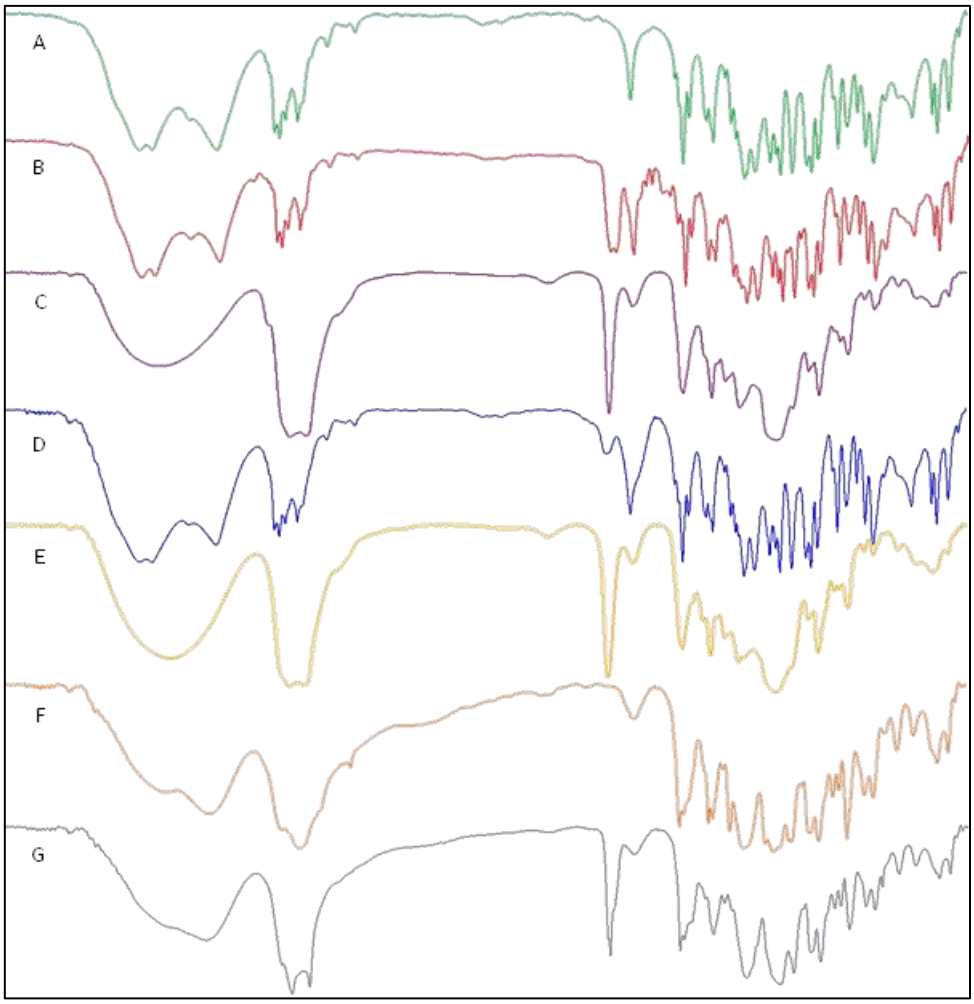

Figure 4.8: FTIR Spectra of Cyclophosphamide – Excipient Compatibility studies [A] Cyclophosphamide pure drug [B] Paclitaxel + Cyclophosphamide [C] Cyclophosphamide + Cremophor EL [D] Cyclophosphamide + Soluplus [E] Cyclophosphamide + Acconon MC8-2 [F] Cyclophosphamide + PEG-400 [G] Cyclophosphamide + Softemul 165

Table 4.2: FTIR Spectra Interpretation for Drug-Drug and Drug-Excipient compatibility studies of Cyclophosphamide [42]

Sr. No.	Description of Mixture	Distinct bands observed in FTIR (cm^{-1})		
		C-O stretching of Amide group	C-Cl stretching	Secondary amine stretching
A	Cyclophosphamide pure drug	1651.58	844.64	746.35
B	Paclitaxel + Cyclophosphamide	1648.14	846.42	746.59
C	Cyclophosphamide + Cremophor EL	1643.94	841.08	742.97
D	Cyclophosphamide + Soluplus	1650.34	844.76	746.43
E	Cyclophosphamide + Acconon MC8-2	1643.38	839.12	744.62
F	Cyclophosphamide + PEG 400	1639.60	842.53	745.01
G	Cyclophosphamide + Softemul 165	1643.19	840.79	744.71

[C] Compatibility by HPLC

The assay of drug alone versus the drug-drug and drug-excipient mixtures was found to be within the range of 98.0 to 102.0% as analyzed by HPLC. No shift in the retention time of Paclitaxel or Cyclophosphamide was observed for drug substances present in drug-excipient mixtures. Thus, it was concluded that all the selected excipients were compatible with Paclitaxel and Cyclophosphamide. The overlays of the chromatograms are presented in Figure 4.9.

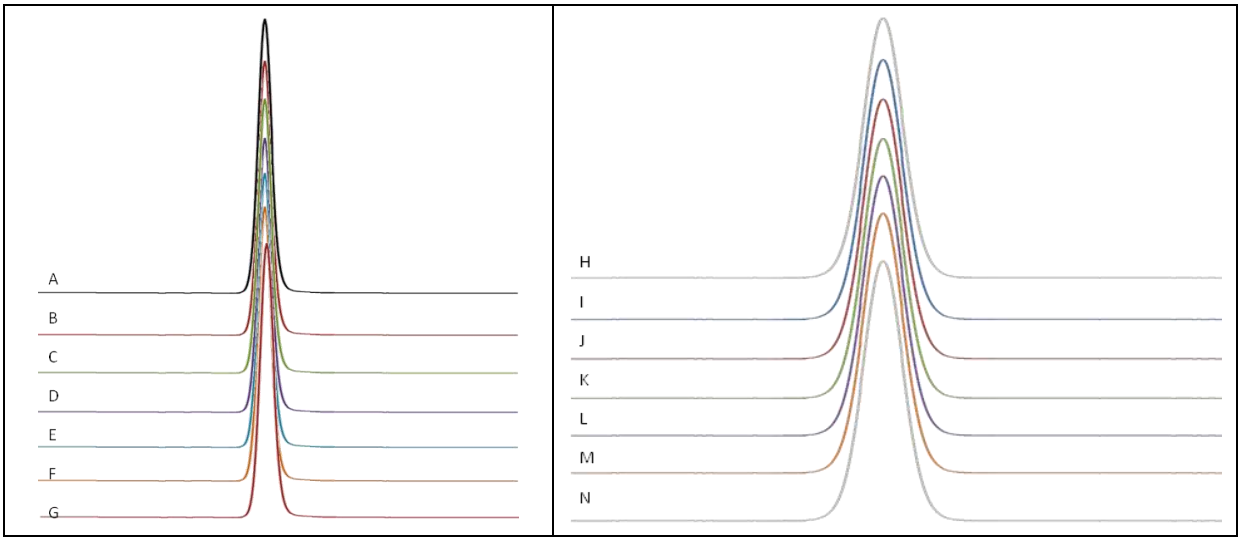

Figure 4.9: Overlay of Chromatograms for Drug-Drug and Drug-Excipient compatibility studies [A] CYC pure drug [B] CYC in CYC + Cremophor EL mixture [C] CYC in CYC + Soluplus mixture [D] CYC in CYC + Acconon MC8-2 mixture [E] CYC in CYC + PEG-400 mixture [F] CYC in CYC + Softemul 165 mixture [G] CYC in PAC pure drug + CYC pure drug mixture [H] Paclitaxel pure drug [I] PAC in PAC + Cremophor EL mixture [J] PAC in PAC + Soluplus mixture [K] PAC in PAC + Acconon MC8-2 mixture [L] PAC in PAC + PEG-400 mixture [M] PAC in PAC + Softemul 165 [N] PAC in PAC pure drug + CYC pure drug

REFERENCES

1. Bharate SS, Vishwakarma RA. Impact of preformulation on drug development. Expert Opin Drug Deliv. 2013 Sep;10(9):1239-57. doi: 10.1517/17425247.2013.783563. Epub 2013 Mar 27. PMID: 23534681.

2. Ahirwar, K., & Shukla, R. (2023). Preformulation Studies: A Versatile Tool in Formulation Design. IntechOpen. doi: 10.5772/intechopen.110346

3. Sadeghi-Oroumiyeh, A., Valizadeh, H., & Zakeri-Milani, P. (2021). Determination of Paclitaxel Solubility and Stability in the Presence of Injectable Excipients. Pharmaceutical Chemistry Journal, 55(9), 983+. https://link.gale.com/apps/doc/A691222222/AONE?u=anon~8316a08e&sid=googleScholar&xid=ddf0dfd9

4. Henchoz, Y., Bard, B., Guillarme, D., Carrupt, P.-A., Veuthey, J.-L., & Martel, S. (2009). Analytical tools for the physicochemical profiling of drug candidates to predict absorption/distribution. Analytical and Bioanalytical Chemistry, 394(3), 707–729. doi:10.1007/s00216-009-2634-y

5. YrjöRoos, Melting and glass transitions of low molecular weight carbohydrates, Carbohydrate Research, Volume 238, 1993,Pages 39-48, ISSN 0008-6215, https://doi.org/10.1016/0008-6215(93)87004-C.

6. Górniak A, Złocińska A, Trojan M, Pęcak A, Karolewicz B. Preformulation Studies of Ezetimibe-Simvastatin Solid Dispersions in the Development of Fixed-Dose Combinations. Pharmaceutics. 2022 Apr 22;14(5):912. doi: 10.3390/pharmaceutics14050912. PMID: 35631498; PMCID: PMC9147300.

7. Alhamhoom Y, Honmane SM, Hani U, Osmani RAM, Kandasamy G, Vasudevan R, Paramshetti S, R Dudhal R, K Kengar N, Charde MS. Study of Formulation and Process Variables for Optimization of PiroxicamNanosuspension Using 32 Factorial Design to Improve Solubility and In Vitro Bioavailability. Polymers (Basel). 2023 Jan 17;15(3):483. doi: 10.3390/polym15030483. PMID: 36771784; PMCID: PMC9919943.

8. Tetko IV, Sushko Y, Novotarskyi S, Patiny L, Kondratov I, Petrenko AE, Charochkina L, Asiri AM. How accurately can we predict the melting points of drug-like compounds? J Chem Inf Model. 2014 Dec 22;54(12):3320-9. doi: 10.1021/ci5005288. Epub 2014 Dec 9. PMID: 25489863; PMCID: PMC4702524.

9. Mao F, Kong Q, Ni W, Xu X, Ling D, Lu Z, Li J. Melting Point Distribution Analysis of Globally Approved and Discontinued Drugs: A Research for Improving the Chance of

Success of Drug Design and Discovery. Chemistry Open. 2016 Mar 21;5(4):357-68. doi: 10.1002/open.201600015. PMID: 27547646; PMCID: PMC4981057.

10. Foschi M, Marziale M, Biancolillo A. Advanced Analytical Approach Based on Combination of FT-IR and Chemometrics for Quality Control of Pharmaceutical Preparations. Pharmaceuticals (Basel). 2022 Jun 18;15(6):763. doi: 10.3390/ph15060763. PMID: 35745682; PMCID: PMC9228031.

11. Ye Q, Meng X. Highly efficient authentication of edible oils by FTIR spectroscopy coupled with chemometrics. Food Chem. 2022 Aug 15;385:132661. doi: 10.1016/j.foodchem.2022.132661. Epub 2022 Mar 8. PMID: 35299015.

12. Dhanikula AB, Panchagnula R. Fluorescence anisotropy, FT-IR spectroscopy and 31-P NMR studies on the interaction of paclitaxel with lipid bilayers. Lipids. 2008 Jun;43(6):569-79. doi: 10.1007/s11745-008-3178-1. Epub 2008 May 6. PMID: 18458975.

13. Wen, Z., Jincheng, W. Preparation and Characterization of a Cyclophosphamide-Core PAMAM Dendritic Montmorillonite. Silicon 10, 483–493 (2018). https://doi.org/10.1007/s12633-016-9478

14. Khodabakhsh F, Bourbour M, Yaraki MT, Bazzazan S, Bakhshandeh H, Ahangari Cohan R, Tan YN. pH-Responsive PEGylated Niosomal Nanoparticles as an Active-Targeting Cyclophosphamide Delivery System for Gastric Cancer Therapy. Molecules. 2022 Aug 24;27(17):5418. doi: 10.3390/molecules27175418. PMID: 36080186; PMCID: PMC9457647.

15. Jorgelina Cussa, Juliana M. Juarez, Marcos B. Gomez Costa & Oscar A. Anunziata (2024) Successful controlled release of cyclophosphamide from tert-butylamine-functionalized large-pore SBA-15, Composite Interfaces, DOI: 10.1080/09276440.2024.2331329

16. Andreeva M, Niedmann PD, Binder L, Armstrong VW, Meden H, Binder M, Oellerich M. A simple and reliable reverse-phase high-performance liquid chromatographic procedure for determination of paclitaxel (taxol) in human serum. Ther Drug Monit. 1997 Jun;19(3):327-32. doi: 10.1097/00007691-199706000-00014. PMID: 9200775.

17. Andreeva M, Niedmann PD, Binder L, Armstrong VW, Meden H, Binder M, Oellerich M. A simple and reliable reverse-phase high-performance liquid chromatographic procedure for determination of paclitaxel (taxol) in human serum. Ther Drug Monit. 1997 Jun;19(3):327-32. doi: 10.1097/00007691-199706000-00014. PMID: 9200775.

18. Sharma, A., & Baldi, A. (2018). Nanostructured Lipid Carriers: A Review. Journal of Developing Drugs, 7 (2), 1-12.

19. Wang, W., Chen, L., Huang, X., & Shao, A. (2016). Preparation and Characterization of Minoxidil Loaded Nanostructured Lipid Carriers. PharmSciTech .

20. Shah, K. A., Date, A. A., Joshi, M. D., & Patravale, V. B. (2007). Solid lipid nanoparticles (SLN) of tretinoin: Potential in topical delivery. International Journal of Pharmaceutics, 163-171.

21. Design, preparation and in vitro characterizations of fluconazole loaded nanostructured lipid carriers. Brazilian Journal of Pharmaceutical Sciences , 1-14.

22. Budai-Szucs, M. (2019). Design and Optimization of Nanostructured Lipid Carrier Containing Dexamethasone for Ophthalmic Use. MDPI Journal of Pharmaceutics, 11, 1-18.

23. Li Q, Cai T, Huang Y, Xia X, Cole SPC, Cai Y. A Review of the Structure, Preparation, and Application of NLCs, PNPs, and PLNs. Nanomaterials (Basel). 2017 May 27; 7(6):122. doi: 10.3390/nano7060122. PMID: 28554993; PMCID: PMC5485769.

24. Lee JS, Oh H, Sung D, Lee JH, Choi WI. High Solubilization and Controlled Release of Paclitaxel Using Thermosponge Nanoparticles for Effective Cancer Therapy. Pharmaceutics. 2021 Jul 27; 13(8):1150. doi: 10.3390/pharmaceutics13081150. PMID: 34452111; PMCID: PMC8398058.

25. Dave, Vivek & Haware, Rahul & Sangave, Nikhil & Sayles, Matthew &Popielarczyk, Michael. (2015). Drug-Excipient Compatibility Studies in Formulation Development: Current trends and techniques. AAPS, FDD Section Newsletter - January 2015.

26. Chidambaram M, Krishnasamy K. Drug-Drug/Drug-Excipient Compatibility Studies on Curcumin using Non-Thermal Methods. Adv Pharm Bull. 2014;4(3):309-12. doi: 10.5681/apb.2014.045. Epub 2014 Feb 7. PMID: 24754017; PMCID: PMC3992969.

27. Chidambaram M, Krishnasamy K. Drug-Drug/Drug-Excipient Compatibility Studies on Curcumin using Non-Thermal Methods. Adv Pharm Bull. 2014;4(3):309-12. doi: 10.5681/apb.2014.045. Epub 2014 Feb 7. PMID: 24754017; PMCID: PMC3992969.

28. National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 36314, Paclitaxel. Retrieved March 30, 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/Paclitaxel.

29. Zhang D, Yang R, Wang S, Dong Z. Paclitaxel: new uses for an old drug. Drug Des Devel Ther. 2014 Feb 20;8:279-84. doi: 10.2147/DDDT.S56801. PMID: 24591817; PMCID: PMC3934593.

30. National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 2907, Cyclophosphamide. Retrieved March 30, 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/Cyclophosphamide.

31. Sakhi M, Khan A, Iqbal Z, Khan I, Raza A, Ullah A, Nasir F, Khan SA. Design and Characterization of Paclitaxel-Loaded Polymeric Nanoparticles Decorated With Trastuzumab for the Effective Treatment of Breast Cancer. Front Pharmacol. 2022 Mar 14;13:855294. doi: 10.3389/fphar.2022.855294. PMID: 35359855; PMCID: PMC8964068.

32. Hiremath JG, Khamar NS, Palavalli SG, Rudani CG, Aitha R, Mura P. Paclitaxel loaded carrier based biodegradable polymeric implants: Preparation and in vitro characterization. Saudi Pharm J. 2013 Jan;21(1):85-91. doi: 10.1016/j.jsps.2011.12.002. Epub 2012 Jan 5. PMID: 23960822; PMCID: PMC3745043.

33. Govindammal M, Prasath M. Vibrational spectra, Hirshfeld surface analysis, molecular docking studies of (RS)-N,N-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide by DFT approach. Heliyon. 2020 Aug 20;6(8):e04641. doi: 10.1016/j.heliyon.2020.e04641. Erratum in: Heliyon. 2024 Feb 12;10(4):e26325. PMID: 32904270; PMCID: PMC7452535.

34. Ma P, Mumper RJ. Paclitaxel Nano-Delivery Systems: A Comprehensive Review. J Nanomed Nanotechnol. 2013 Feb 18;4(2):1000164. doi: 10.4172/2157-7439.1000164. PMID: 24163786; PMCID: PMC3806207.

35. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-. Eugenol (Clove Oil) [Updated 2019 Oct 28]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK551727/

36. Jing X, Deng L, Gao B, Xiao L, Zhang Y, Ke X, Lian J, Zhao Q, Ma L, Yao J, Chen J. A novel polyethylene glycol mediated lipid nanoemulsion as drug delivery carrier for paclitaxel. Nanomedicine. 2014 Feb;10(2):371-80. doi: 10.1016/j.nano.2013.07.018. Epub 2013 Aug 20. PMID: 23969104.

37. Sadeghi-Oroumiyeh, Ali, et al. "Determination of Paclitaxel Solubility and Stability in the Presence of Injectable Excipients." Pharmaceutical Chemistry Journal, vol. 55, no. 9, Dec. 2021, pp. 983+. Gale Academic OneFile, link.gale.com/apps/doc/A691222222/AONE?u=anon~9ca70f34&sid=googleScholar&xid=13aae33c. Accessed 30 Mar. 2024.

38. Tao Yang, Fu-De Cui, Min-Koo Choi, Hongxia Lin, Suk-Jae Chung, Chang-Koo Shim &Dae-Duk Kim (2007) Liposome Formulation of Paclitaxel with Enhanced Solubility and Stability, Drug Delivery, 14:5, 301-308, DOI: 10.1080/10717540601098799

39. Elmowafy M, Al-Sanea MM. Nanostructured lipid carriers (NLCs) as drug delivery platform: Advances in formulation and delivery strategies. Saudi Pharm J. 2021 Sep;29(9):999-1012. doi: 10.1016/j.jsps.2021.07.015. Epub 2021 Jul 21. PMID: 34588846; PMCID: PMC8463508.

40. Viegas C, Patrício AB, Prata JM, Nadhman A, Chintamaneni PK, Fonte P. Solid Lipid Nanoparticles vs. Nanostructured Lipid Carriers: A Comparative Review. Pharmaceutics. 2023 May 25;15(6):1593. doi: 10.3390/pharmaceutics15061593. PMID: 37376042; PMCID: PMC10305282.

41. Martins, K. F., Messias, A. D., Leite, F. L., &Duek, E. A. R. (2014). Preparation and characterization of paclitaxel-loaded PLDLA microspheres. Materials Research, 17(3), 650–656. doi:10.1590/s1516-14392014005000028

42. Sheikh, Setareh&Goudarzian, Nooredin. (2023). High-efficiency loading and controlled release of cyclophosphamide on graphene oxide and adsorption isotherm studies. Journal of Chemical Technology and Metallurgy. 58. 10.59957/jctm.v58i6.3.