# Chapter 4
# Preformulation study

Dermal Delivery of Protein/Peptide Based Antimicrobial to Treat Secondary Infection in Psoriasis and Eczema

## 4.1 Introduction

Preformulation study is one of the critical prerequisites in the preparation of any drug delivery systems. It provides the info required to describe the nature of an API and provide a basis for the drug-excipients mixture and selection in the dosage form. Preformulation investigations are designed to recognize the physicochemical properties and excipients that may affect the method of manufacture, formulation design, and pharmacokinetic properties of the resulting product. The present investigation includes the solubility screening of drugs (Omiganan and DPK-060) in different lipids (solid and liquid lipids) and surfactants, along with the excipient compatibility studies with peptides.

## 4.2 Materials and Instruments

### 4.2.1 Materials

**Table 4.1 List of materials**

| **Materials & Reagents** | **Manufacturers** |
|---|---|
| Omiganan | S-Biochem, Kerala, India (Custom synthesis) |
| DPK-060 | S-Biochem, Kerala, India (Custom synthesis) |
| Methanol (A.R. & HPLC Grade) | Spectrochem Pvt. Ltd., Mumbai |
| Steric acid | Sigma Aldrich, USA |
| Compritol 888 ATO | Gattefosse, Mumbai |
| Glyceryl monostearate | Gattefosse, Mumbai |
| Miglyol- 840 | Gattefosse, Mumbai |
| Precirol-ATO-5 | Gattefosse, Mumbai |
| Oleic acid | Gattefosse, Mumbai |
| Labrasol | Gattefosse, Mumbai |
| Transcutol HP | Gattefosse, Mumbai |
| Lauroglycol 90 | Gattefosse, Mumbai |
| Lauroglycol FCC | Gattefosse, Mumbai |
| Cremophor RH 40 | BASF Chemical Company, USA |
| Campul MCM | Abitec Corporation, USA |

| | |
|---|---|
| Captex® 200 P | Abitec Corporation, USA |
| Isopropyl myristate | Loba Chemie Pvt. Ltd., India |
| Dipalmitoylphosphatidylcholine (DPPC) | Lipoid GmBH, Germany |
| Hydrogenatedsoyphosphatidylcholine (HSPC) | Lipoid GmBH, Germany |
| Cholesterol | Sigma Aldrich, USA |
| Egg lecithin | Lipoid GmBH, Germany |
| Soya lecithin | Lipoid GmBH, Germany |
| PEG 400 | MP Biomedicals Pvt. Ltd., Mumbai |
| Distilled water | Prepared In-house |

### 4.2.2 Instruments

**Table 4.2 List of instruments**

| **Equipment** | **Manufacturer** |
|---|---|
| Digital Weighing Balance | Shimadzu, Japan |
| RP-HPLC with UV Detector (gradient) | Agilent OpenLab CDS EZChrom, India |
| FT-IR spectrophotometer | Shimadzu 8400S, Japan |
| Mass spectrophotometer | Agilent-6125B, India |
| Vortex mixer | Spinix, Japan |
| Incubator Thermo-shaker | Bombay Lab. Services, Mumbai |
| Centrifuge | Remi equipments, India |

## 4.3 Methodology

### 4.3.1 Melting point determination

In this method, Omiganan/DPK-060 was filled into a capillary tube (one end was closed) and tied to the thermometer and ensure that it remains dipped in a liquid paraffin [1]. The temperature range at which the Omiganan/DPK-060 starts melting and complete melting was recorded.

### 4.3.2 Drug authentication by mass spectrometry

Agilent 6125-B mass spectrophotometer was used for analysis. Briefly, nebulizer gas flow was used at 1.5L/min along with CDL temperature of 250 ºC and CDL voltage of -20V. Electrospray ionization (ESI) was completed by applying a voltage of +4.5kV. Samples were prepared by dissolving 5 mg of Omiganan/DPK-060 in water: acetonitrile (50:50) solution, and the mass spectra were recorded for Omiganan and DPK-060. The sample infusion flow rate was 0.2mL/min.

### 4.3.3 Screening of lipids/oils and surfactants based on solubility

Solubility is defined as the ability of the solute to dissolve in a solvent. It is evaluated in terms of the maximum amount of solute dissolved in a solvent at equilibrium. [2]. To determine the solubility of Omiganan and DPK-060 in liquid lipids/oils and surfactants, an extra amount of the Omiganan/DPK-060 was transferred to the vials comprising 500 mg of liquid lipids/oils and subjected for vortexing for 10 min. This mixture was then incubated in a shaker at 50 rpm for 48 h at 37ºC. The resulting mixture was then centrifuged for 10 min at 3000 rpm to separate the excess amount of Omiganan/DPK-060, and the supernatant was diluted with a water-methanol mixture. The amount of Omiganan/DPK-060 solubilized in oils/liquid lipids, and surfactants was calculated using the developed HPLC method.

In selecting solid lipid, 100 mg of several solid lipids (Compritol 888, Precirol ATO-5, Glyceryl monostearate, and stearic acid) were placed in a water-bath in glass vials at 80ºC for 2 h. Further, Omiganan/DPK-060 was added to these lipids in increments in glass vials. The maximum amount of the Omiganan/DPK solubilized in a specific lipid was evaluated visually (clear solution formation of molted lipid) [3, 4].

### 4.3.4 Drug-excipients compatibility study by FT-IR

IR spectrums of Omiganan and DPK-060 were recorded in the solid-state by the potassium bromide (KBr) pellet method. The pure Omiganan/DPK-060 and drug-excipients mixture was grounded into a fine powder and mixed thoroughly with KBr. The KBr pellets were prepared by putting 10-12 metric tons of pressure in a motorized pellet press. Subsequently, these pellets were scanned in the 4000-400 $cm^{-1}$ wave range and

spectrum were recorded by using an FTIR spectrometer-430 (Shimadzu 8400S, Japan). The drug-excipient ratio used for the compatibility study was shown in Table 4.3.

**Table 4.3 Mixture components and their ratio for drug-excipient compatibility assessment**

| Mixture components | Weight ratio | | | | |
|---|---|---|---|---|---|
| | **Omiganan NLC** | **Omiganan liposomes** | **Omiganan lotion** | **DPK-060 NLC** | **DPK-060 lotion** |
| Omiganan | 1 | 1 | 1 | - | - |
| DPK-060 | - | - | - | 1 | 1 |
| Compritol 888ATO | - | - | - | 4 | - |
| Precirol-ATO-5 | 3 | - | - | - | - |
| Egg lecithin | - | - | - | 2 | - |
| Soya lecithin | - | 2 | - | - | - |
| DPPC | - | 4 | - | - | - |
| HSPC | - | 4 | - | - | - |
| Cholesterol | - | 2 | - | - | - |
| Ceteareth-25 | - | - | 2.5 | - | 2.5 |
| Steareth-10 | - | - | 0.3 | - | 0.3 |
| PVP K30 | - | - | 2 | - | 2 |

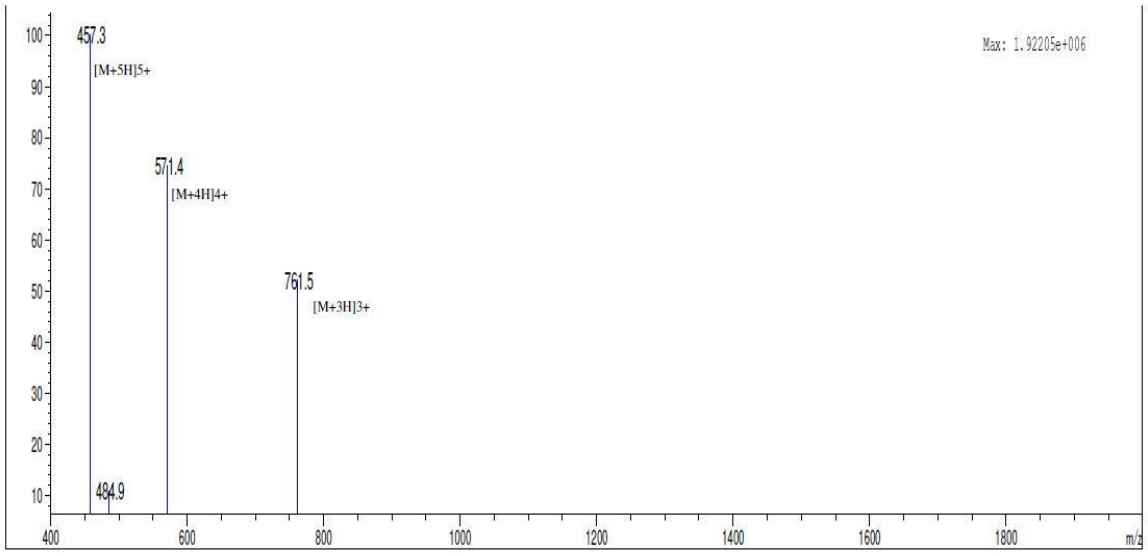
## 4.4 Results and Discussion

### 4.4.1 Melting point determination

The melting point of Omiganan and DPK-060 was found to be 214-220 ºC and 245-250 ºC by capillary tube method, respectively.

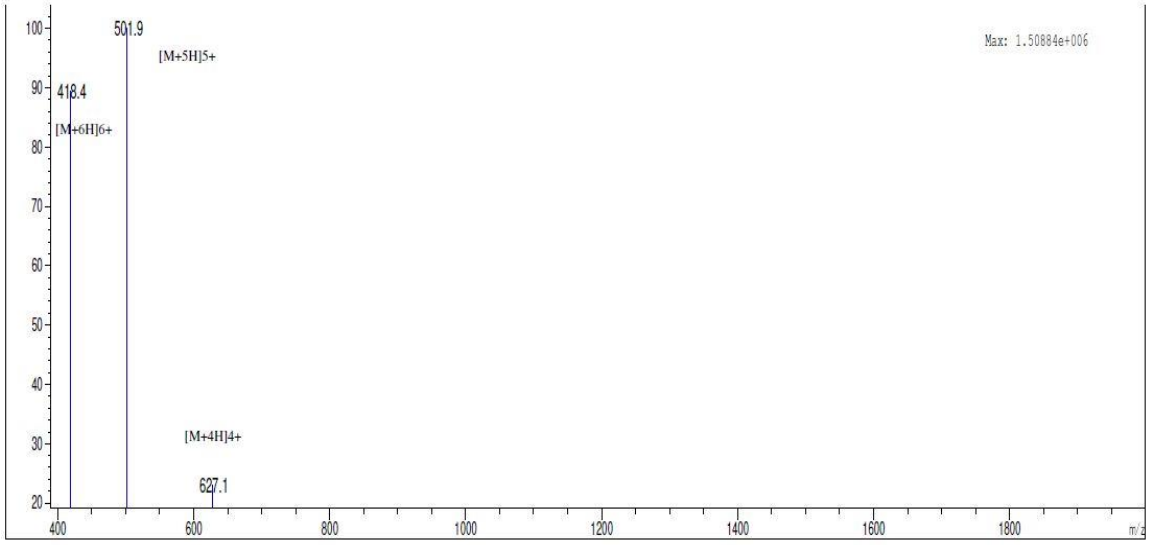
### 4.4.2 Drug authentication by mass spectrometry

The mass spectra of Omiganan and DPK-060 are shown in Fig. 4.1 and 4.2, respectively. Results demonstrated that the molecular weight of Omiganan and DPK-060 was found to be the same as available in the literature.




| | | | | | |
|---|---|---|---|---|---|
| Sample Description | | Instrument | Agilent-6125B | | |
| Analyzed date: | 2018-07-20 | Probe: | ESI | Probe Bias: | +4.5kv |
| Analyst: | Dr.AR-SBio | Nebulizer Gas Flow: | 1.5L/min | Detector: | 1.5kv |
| Sample: | Pep-156 | CDL: | -20.0v | T. Flow: | 0.2ml/min |
| M.W.: | 2281.68 | CDL Temp.: | 250 °C | B. Conc.: | 50%H2O/50%ACN |

**Figure 4.1 Mass spectra of Omiganan**




| | | | | | |
|---|---|---|---|---|---|
| Sample Description | | Instrument | Agilent-6125B | | |
| Analyzed date: | 2018-07-19 | Probe: | ESI | Probe Bias: | +4.5kv |
| Analyst: | Dr.AR-SBio | Nebulizer Gas Flow: | 1.5L/min | Detector: | 1.5kv |
| Sample: | Pep-155 | CDL: | -20.0v | T. Flow: | 0.2ml/min |
| M.W.: | 2504.85 | CDL Temp.: | 250 °C | B. Conc.: | 50%H2O/50%ACN |

**Figure 4.2 Mass spectra of DPK-060**

### 4.4.3 Screening of lipids/oils and surfactants based on solubility

The solubility of Omiganan and DPK-060 in the oils, surfactants and solid lipids is demonstrated in Fig. 4.3 and 4.4, respectively. From the results, Precirol-ATO-5, Miglyol 840, Tween 80, and PEG 400 were selected as solid lipid, liquid lipid/oil, and surfactant, respectively, to formulate Omiganan loaded nano-lipid constructs because of the higher drug solubilization potential of these excipients. Compritol 888 ATO, Miglyol 840, and Tween 80 were selected as solid lipid, liquid lipid/oil, and surfactant, respectively, to formulate DPK-060 loaded nano-lipid constructs.

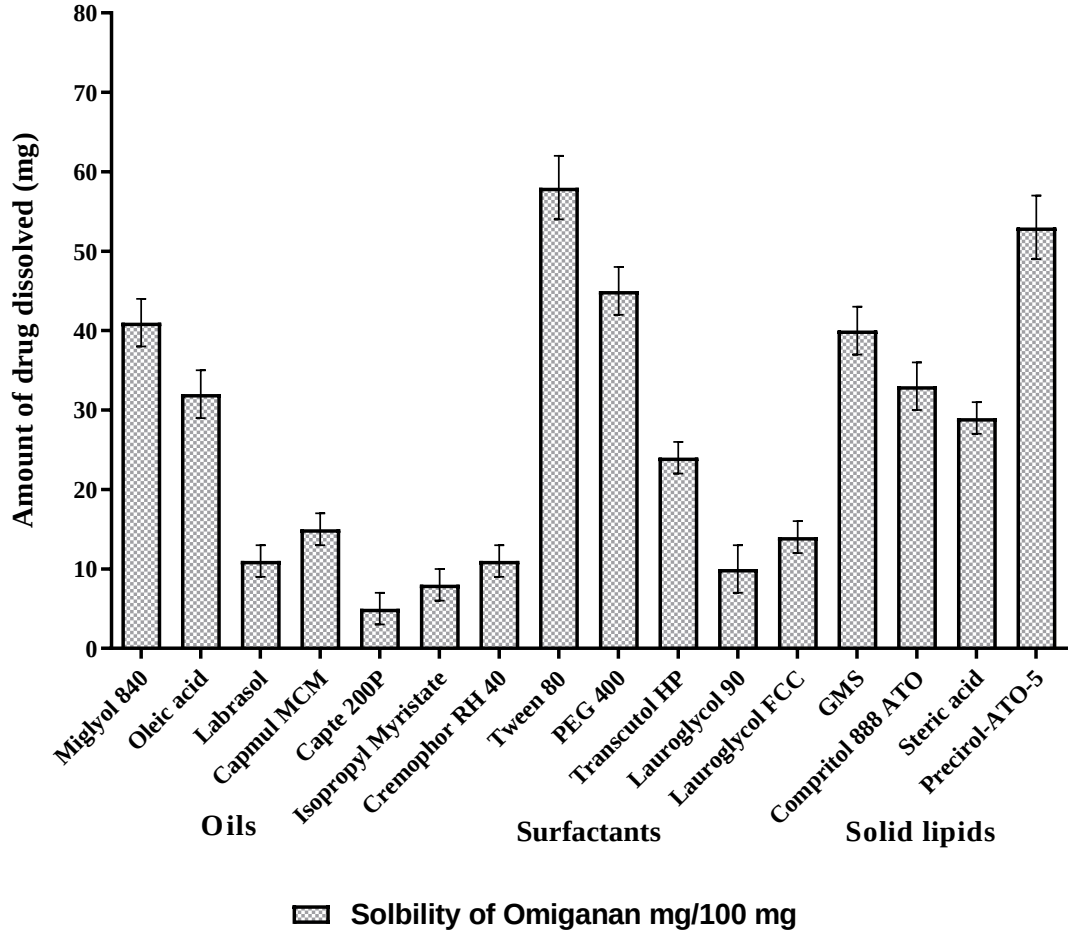



**Figure 4.3 Solubility of Omiganan in various excipients**

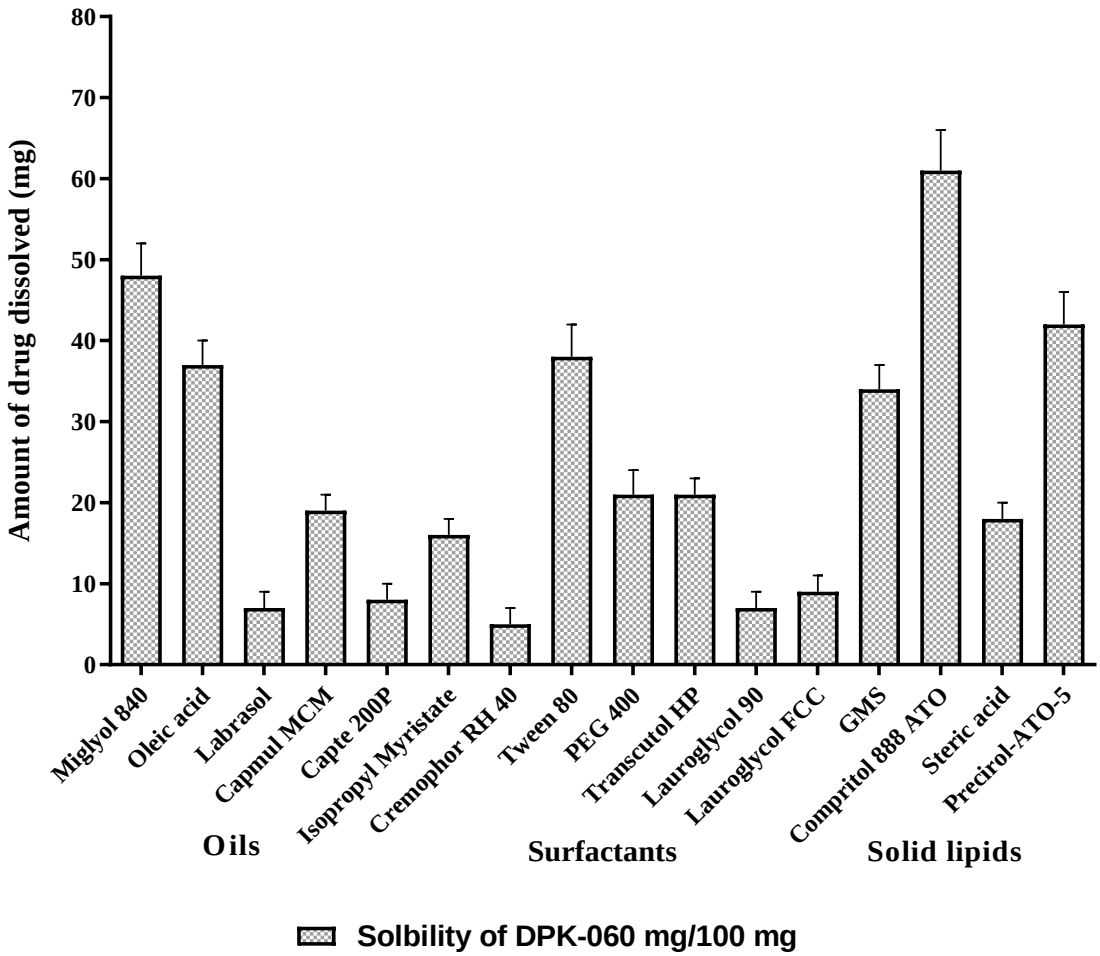



**Figure 4.4 Solubility of DPK-060 in various excipients**

### 4.4.4 Drug-Excipients Compatibility Study by FT-IR

The IR spectra of Omiganan and drug-excipients are shown in Fig. 4.5-4.8. The results (IR spectra) demonstrated the compatibility of Omiganan with the excipients used. Similarly, the compatibility was also found between the DPK-060 and excipients and is shown in Fig. 4.9-4.10

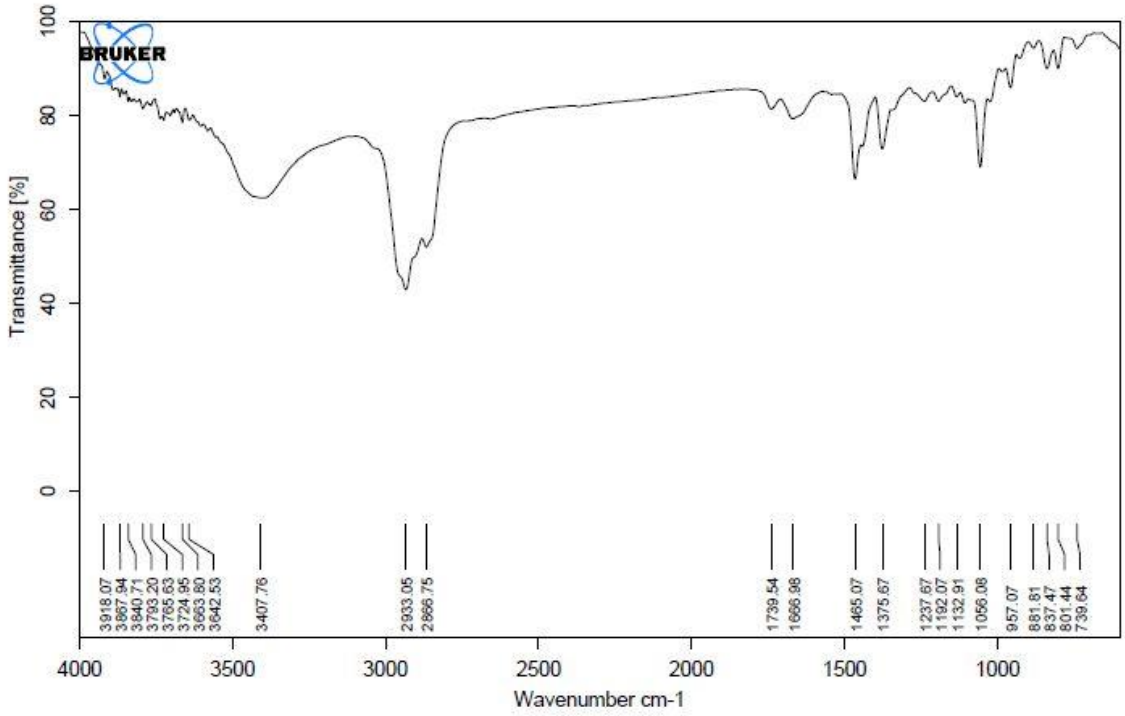



**Figure 4.5 FT-IR spectra of Omiganan**

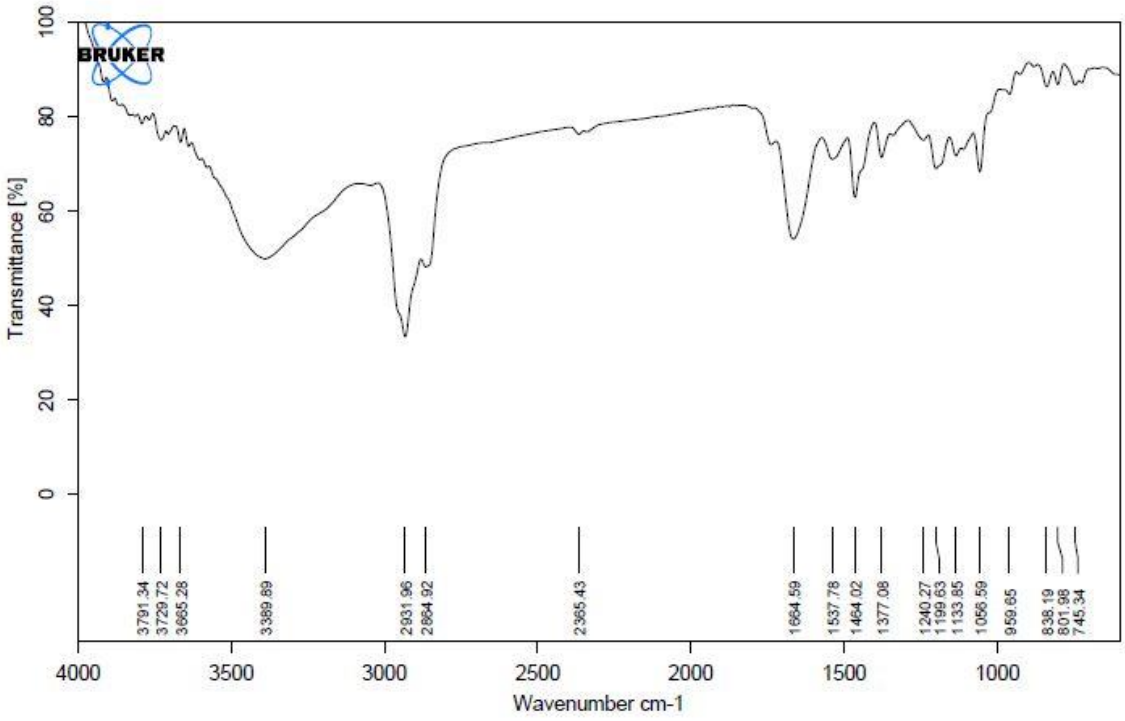



**Figure 4.6 FT-IR spectra of Omiganan + lipids mixture (for liposomes)**

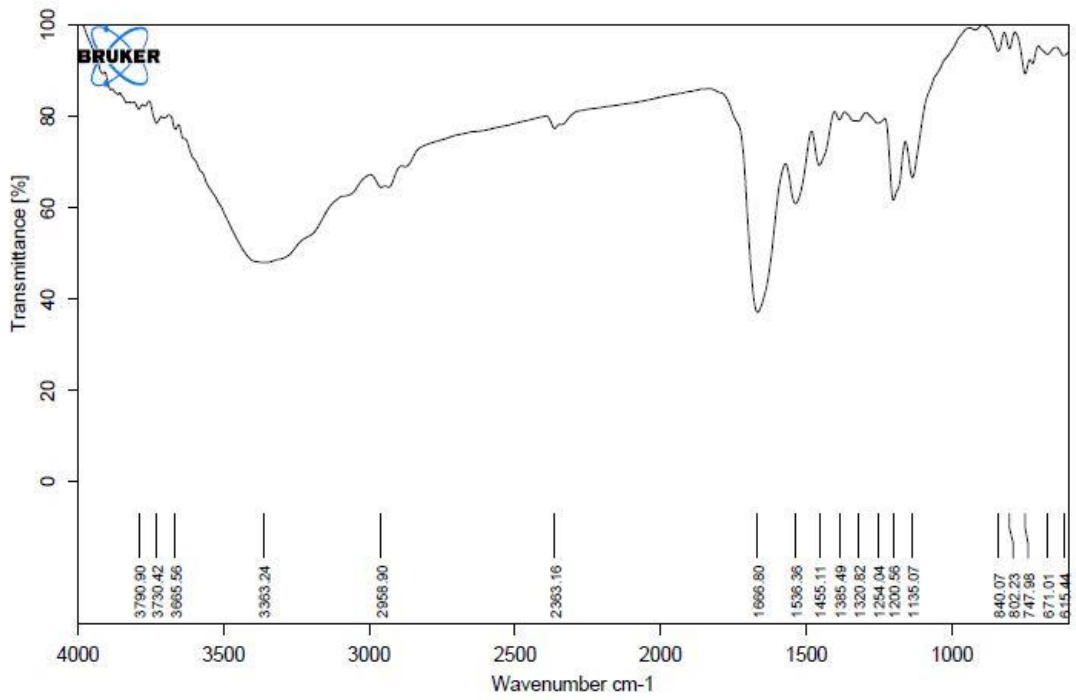



**Figure 4.7 FT-IR spectra of Omiganan + lipids mixture (for NLCs)**

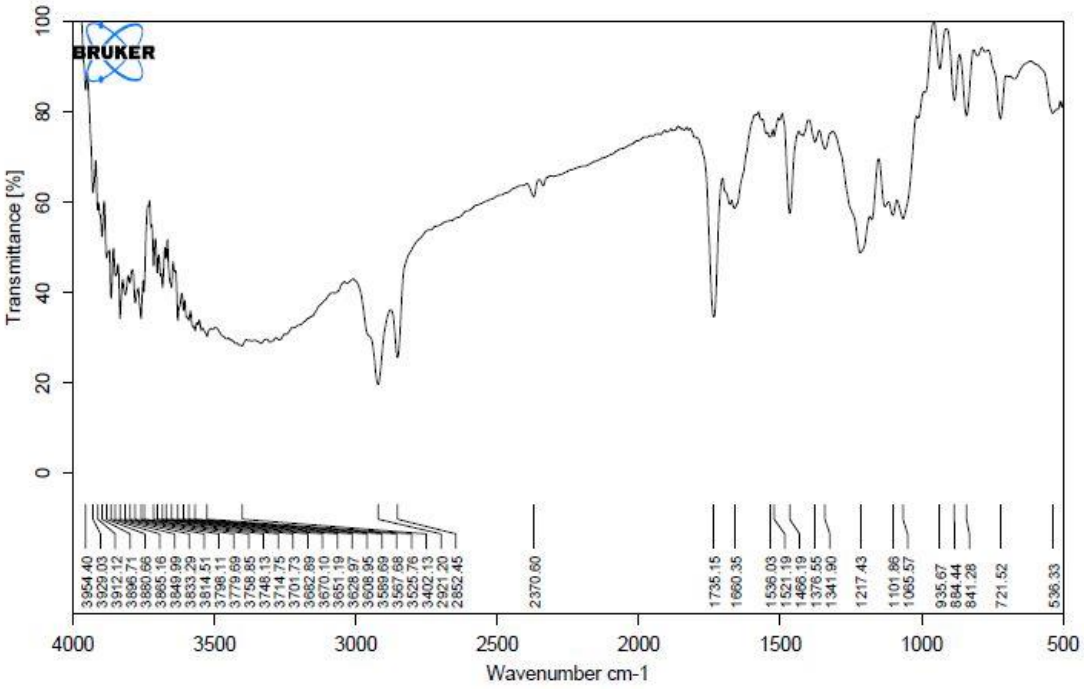



**Figure 4.8 FT-IR spectra of Omiganan and lipids mixture (for lotion)**

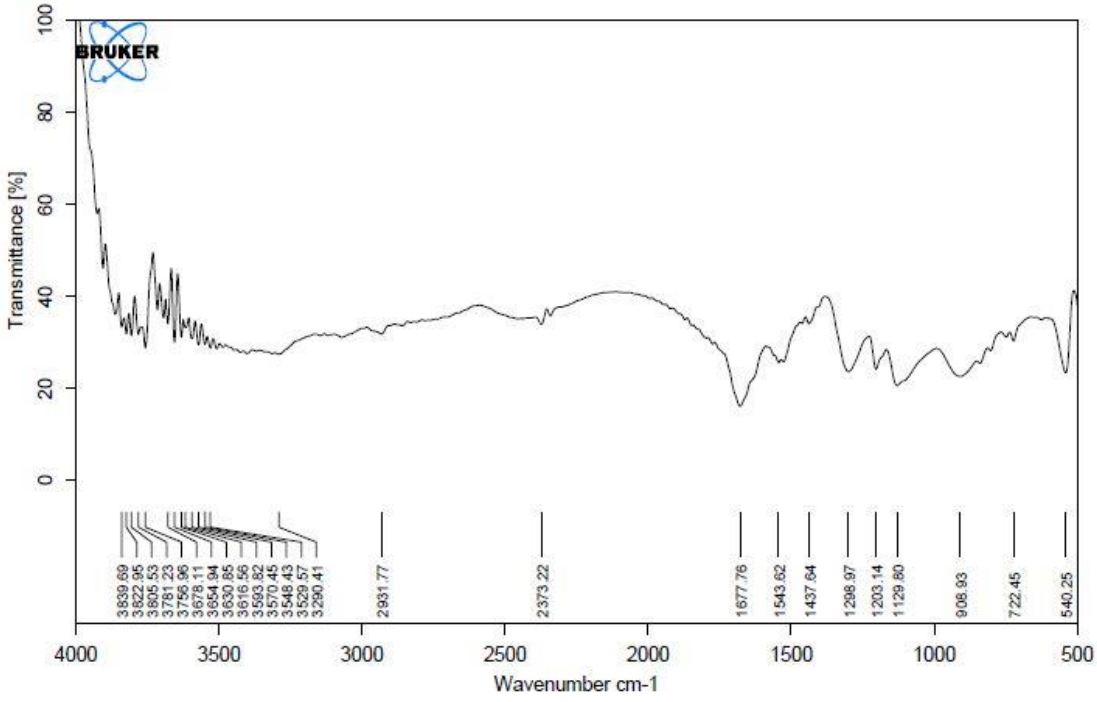



**Figure 4.9 FT-IR spectra of DPK-060**

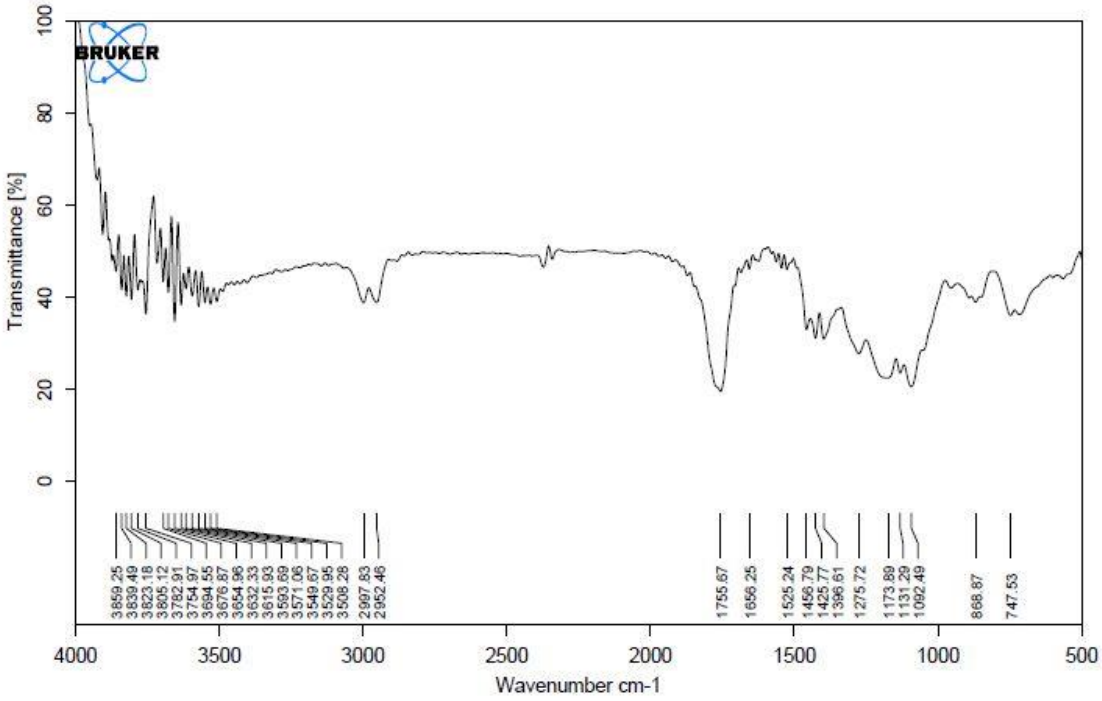



**Figure 4.10 FT-IR spectra of DPK-060 + lipids mixture (NLCs)**

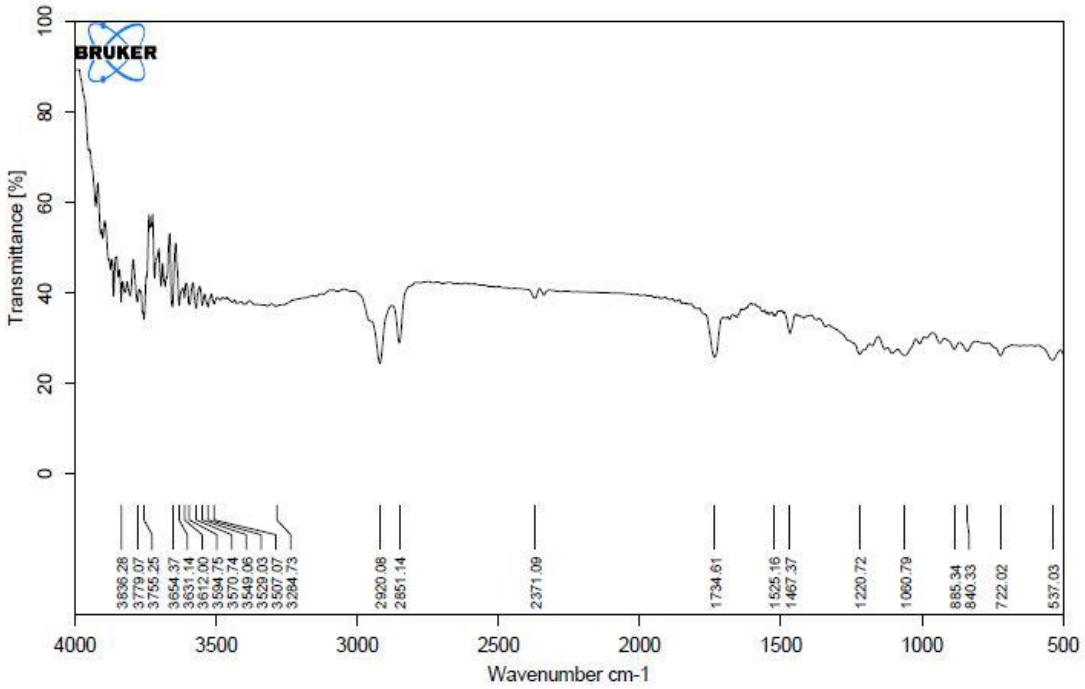



**Figure 4.11 FT-IR spectra of DPK-060 and lipids mixture (for lotion)**

## 4.5 References

1. Prajapati, D.R., K.R. Patel, and M.R. Patel, *DESIGN AND DEVELOPMENT OF FLOATING IN SITU GEL OF TAPENTADOL HCl.* Indo American Journal of Pharmaceutical Research, 2016. **6**(4): p. 5162-5174.
2. Zhou, L., et al., *Development of a high throughput equilibrium solubility assay using miniaturized shake-flask method in early drug discovery.* Journal of pharmaceutical sciences, 2007. **96**(11): p. 3052-3071.
3. Shete, H. and V. Patravale, *Long chain lipid based tamoxifen NLC. Part I: preformulation studies, formulation development and physicochemical characterization.* International journal of pharmaceutics, 2013. **454**(1): p. 573-583.
4. Ranpise, N.S., S.S. Korabu, and V.N. Ghodake, *Second generation lipid nanoparticles (NLC) as an oral drug carrier for delivery of lercanidipine hydrochloride.* Colloids and Surfaces B: Biointerfaces, 2014. **116**: p. 81-87.