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# Preformulation Studies of Atenolol Cardiovascular Lipid Nanocarrier Transferosome

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**KEYWORDS**

Drug, Heart, Preformulation, Transferosome

**ABSTRACT:**

Physicochemical characteristics of drugs are assessed in preformulation studies in order to optimize the formulation parameters of novel drug delivery systems. To identify suitable carriers for lipid nanocarrier formulation, we first determined the solubility of atenolol in various solvents. UV-Visible spectrophotometry was used to construct a calibration curve for accurately quantifying atenolol concentrations. Atenolol was analyzed by FTIR to assess any chemical interactions between it and the components of the lipid nanocarrier, allowing insight into the formulation's stability and compatibility. Lastly, DSC was employed to determine the optimal processing conditions for atenolol and lipid nanocarrier by examining their thermal behavior and compatibility. Atenolol is more soluble in lipid-based carriers, which facilitates its incorporation into transferosomes. It was possible to measure atenolol in lipid nanocarrier formulations accurately thanks to the linearity of the calibration curve over a wide concentration range. According to the FTIR analysis, atenolol and lipid nanocarrier components have no significant chemical interactions, which ensures formulation stability. An optimization of formulation parameters was enabled by DSC analysis, which confirmed atenolol compatibility with the lipid nanocarrier.

## Introduction

There are two types of drug carriers: solid lipid nanoparticles (SLNs) and nanostructured lipid carriers [1, 2]. A NLC carrier is arranged spatially with solid lipids and liquid lipids. As solid and liquid lipids are arranged spatially alternatively, drug loading efficiency

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is enhanced, as is the ability to overcome the crystallinity of the lipid matrix, which easily crystallizes solid lipids [3]. NLC consists of chains and blocks of lipids that are combined during storage to form amorphous particles that cannot be reconstituted [4, 5]. The method prevents the ejection of drugs during storage. Solutions, suspensions, and ointments can be carried on NLC in place of conventional carriers.

Mousellar colloidal drug carriers (NLCs) are nanoparticle dispersions composed of particles ranging in size from 10 nanometers to 500 nanometers. To produce a good NLC matrix, solid lipids must be mixed with liquid lipids [6-8]. The melting point of NLC will be lower because oil is present, compared to the melting point of SLN. Solid lipids and liquid lipids can be mixed in NLC up to 95%. In contrast to other lipid carriers, NLC are not spherical as shown in Figure 1, they are usually seen in blocks or chains. Surfactants bind solid lipid particles to liquid lipid droplets, which are embedded within the solid particles [9, 10].

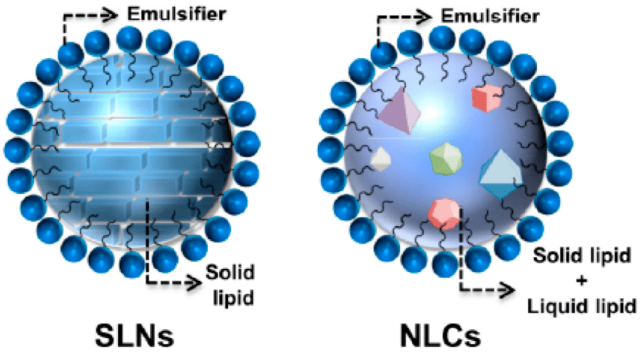


**Figure 1:** Structures of SLNs and NLCs

## Materials and Methods

### Materials

Atenolol purchased from aurobindo pvt Ltd. Hyderabad. Compritol 888AT0, Acconon C-44 EP/NF, Dynasan 114, Softigen, Witepsol H32, Stearic acid, Poloxamer, Cholesterol, Tween-20, Tween-80, Span-20, Span-80, Poly sorbate 20,Poly sorbate 80, Manittol, PVA, Polyvinylpyrolidine, DMSO, PEG, KH2PO4, NaOH, Methanol (HPLC Grade),Ammonium Acetate, Formic Acid, Tri fluro acetate, Perchloroacetic acid, ACN.

### Methods

**Solubility studies:**

It was determined that atenolol dissolved in distilled water and solid- lipid such as dynasan, acconon, cholesterol, Stearic acid, compritol, witepsol and beeswax [11]. NLC formulations are evaluated based on solid lipids and liquid lipids determined through this study. After it has been dissolved in 10 ml of lipid, it is allowed to reach equilibrium by being dissolved in 10 ml of lipid. Using mg/ml as a measure of solubility, drugs were calculated to be soluble in lipids.

**Calibration curve**

**Preparation of buffer solution:**

**Preparation of NaOH (0.2 M Sodium Hydroxide):**

A solution consisting of 40 to 60% sodium hydroxide should be prepared by dissolving it in water. This method involves siphoning off the clear supernatant liquid and diluting it with carbon dioxide free water that contains 8.0g of NaOH in 1000 ml of water, and then standardizing the solution [12].

**Preparation of Potassium Di hydrogen Phosphate (0.2 M $KH_2PO_4$):**

Dilute 27.218 g of $KH_2PO_4$ with one liter of $H_2O$.

**Preparation of Buffer solution: Phosphate Buffer (pH 7.4)**

In a 200 ml volumetric flask, 50 ml of Potassium Di hydrogen Phosphate and 39.1 ml of Sodium hydroxide should be added along with distilled water for a total volume of 200 ml. Prepare 1000ml of phosphate buffer by adding 0.25 liter of 0.2M Potassium Di hydrogen

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Phosphate and 195.5ml of 0.2M NaOH, and then top it off with distilled water.

**Calibration Curve of Atenolol**

**Stock solution Preparation:**

Make a stock solution of 1mg/ml or 1000g/ml by accurately weighing 100mg Atenolol, slightly dissolved in 10ml methanol then adding 0.1 liter of buffer solution of pH 7.4

**Preparation of serial dilution for calibration curve:**

λ max for calibration was analyzed between 200 – 400 nm range, and it was to be 224 nm. A calibration curve was constructed from serial dilutions such as 2, 4, 6, 8, 10, 12 microgram/ml concentration (concentration ranges of (2-34µg/ml)) may obey the Beer's law. Using this λmax, UV absorbances are measured for each diluted sample. An absorbance calibration graph was constructed between concentrations in µg/ml and their respective concentrations in nm.

**FTIR analysis**

A FTIR study was conducted to determine whether Atenolol interacts chemically with other ingredients where are used in this formulation like lipids & surfactants. In this study, Potassium Bromide pellet has been used to disperse NLC & a pure drug (Atenolol). Atenolol (0.2%) and KBr are ground together and the sample is then compressed by moving the handle of the mini KBr pellet press. NLC dispersion was kept in a centrifuge for 40 minutes at 15000 RPM. The centrifuge tube's supernatant liquid was sucked out using a syphon, and the remaining liquid was collected and left to dry at 50°C to remove any extra water [13]. By stacking dried NLC dispersion between potassium bromide pellets and scanning it over range of 4000 to 500 $cm^{-1}$ wave number using OPUS Spectrum software integrated into a FTIR instrument (Bruker, Germany), the dispersion was scanned over 4000 to 500 $cm^{-1}$, with a resolution of 4 $cm^{-1}$. We ensured reproducible contact between samples and crystal holders for scanning by placing them on a sample stage with a force gauge of 100 N.

**Analysis of Differential Scanning Calorimetry**

The loss on drying of sample was determined using Differential Scanning Calorimetric studies. Reports about the purity of drugs, their compatibility with excipients, and the crystallinity of NLC formulations can be produced. DSC studies were carried out using the DSC 70 (Schimadzu model) instrument for pure drugs (Atenolol) and drug loaded NLC dispersion. Samples were roughly weighed at 5 mg and heating range from 20ºc to 200°c @ 20°c/g at a rate of 20°c/g for writing effluent gas. The melting range was determined as an exothermic or endothermic peak.

**Results and Discussion**

**The characterisation of drugs**

**Table 1:** Solubility of Atenolol in Solid Lipid

| Solvent and Solid Lipid | Atenolol Solubility (mg/ml) |
|---|---|
| Water | 29 |
| Chloroform | 8 |
| Dynasan | 30 |
| Cholesterol | 33 |
| Acconon | 22 |
| Witepsol | 52 |
| Stearic acid | 30 |
| Compritol | 46 |
| Bees wax | 23 |

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| | |
|---|---|
| **Melting point (°C)** | 159 |
| **Particle size in diameter(µm)** | 34 |

Atenolol showed a high water solubility, showing 30mg/ml, as well as a high solubility in lipids, particularly Witepsol H32 (52mg/ml) and Compritol (46mg/ml). The soluble concentration of atenolol in Softigen was 65 mg/ml. Tables 5.2 and 5.3 present the results.

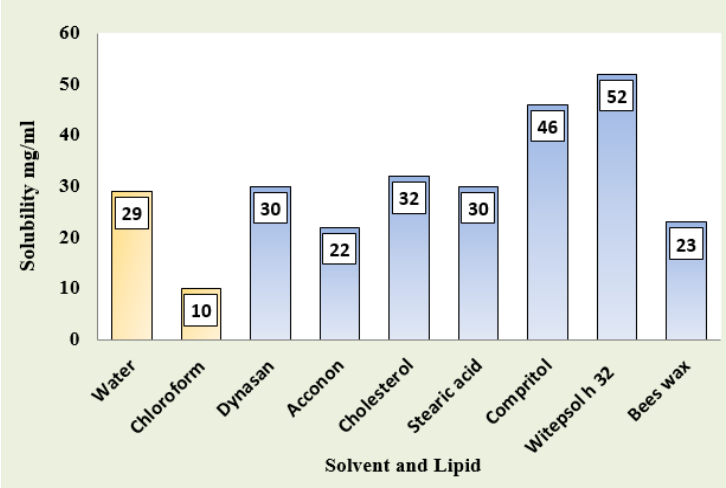


**Figure 2:** Solubility of Atenolol in various Solid lipids

**Table 2:** Liquid Lipid Solubility of Atenolol

| Liquid Lipid | Atenolol Solubility (mg/ml) |
|---|---|
| Olive oil | 34 |
| Softigen | 65 |
| Soyabean oil | 23 |

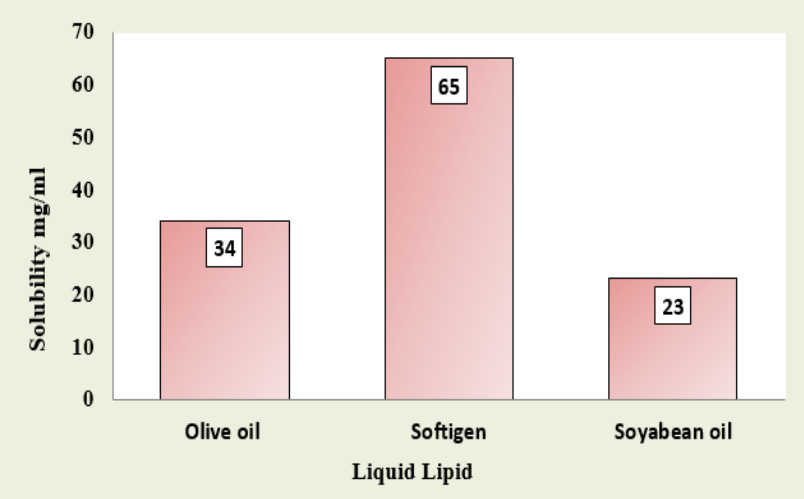


**Figure 3:** Solubility of Atenolol in various Liquid lipids

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**Calibration curve preparation**

**Atenolol**

**Table 3:** Atenolol standard curve in 7.4 pH phosphate buffer (224 nm)

| Concentration (µg/ml) | UV absorbance (nm) |
|---|---|
| 0 | 0 |
| 2 | 0.030 |
| 4 | 0.054 |
| 6 | 0.079 |
| 8 | 0.104 |
| 10 | 0.128 |
| 12 | 0.152 |

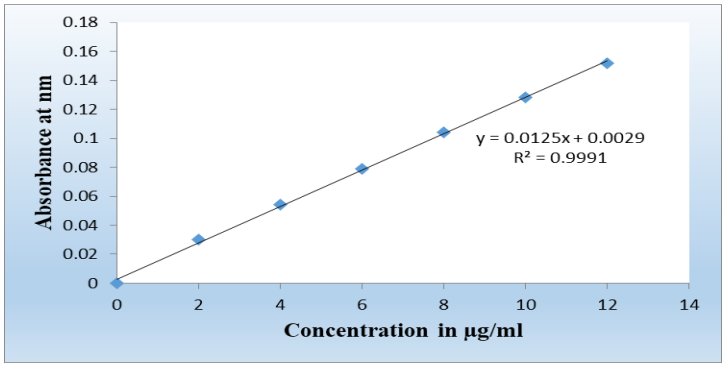


**Figure 4:** Calibration curve of Atenolol in pH 7.4 phosphate buffer at 224 nm

The range of concentrations of 2–34 µg/ml (as per Beer's law) as we generated a calibration curve using pH 7.4 phosphate buffer at 224 nm. The serial dilution are prepared from the stock solution by serial dilutions such as 2, 4, 6, 8, 10,and 12 µg/ml concentration. The correlation and linearity are outstanding, and the r2 value is 0.999 in pH 7.4 phosphate buffer with levels ranging from 2 to 12 µg/ml. A summary of the results can be found in table 1.

**The Compatibility Studies are as follows:**

A FTIR and DSC study was conducted to determine the drug and excipient compatibility

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**FTIR Analysis:**

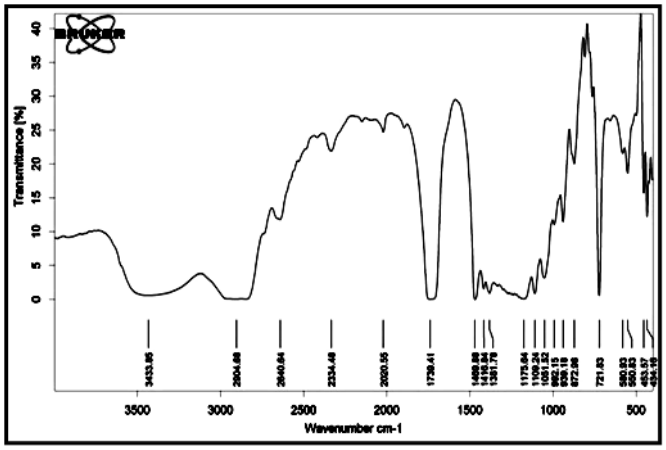


**Figure 5:** FTIR spectra of Atenolol pure drug

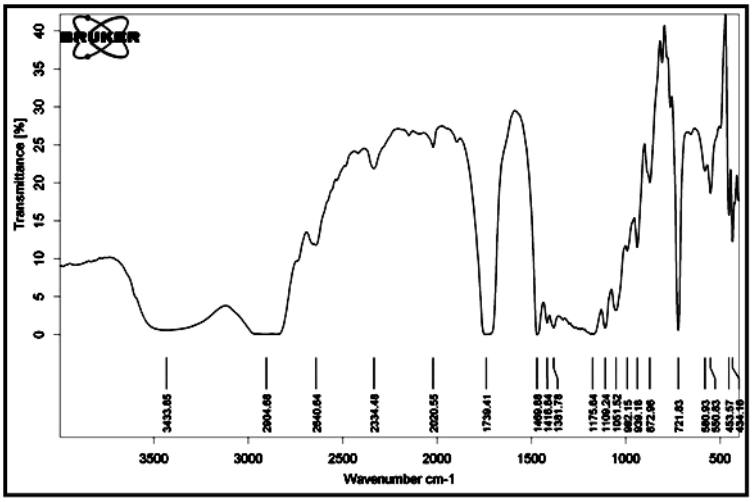


**Figure 6:** FTIR spectra of Witepsol solid lipid

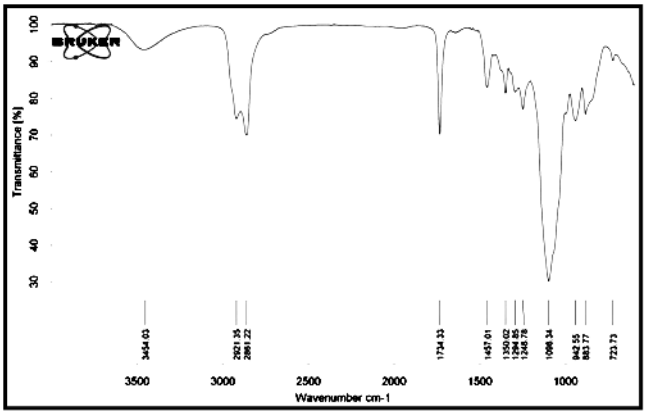


**Figure 7:** FTIR spectra of Softigen liquid lipid

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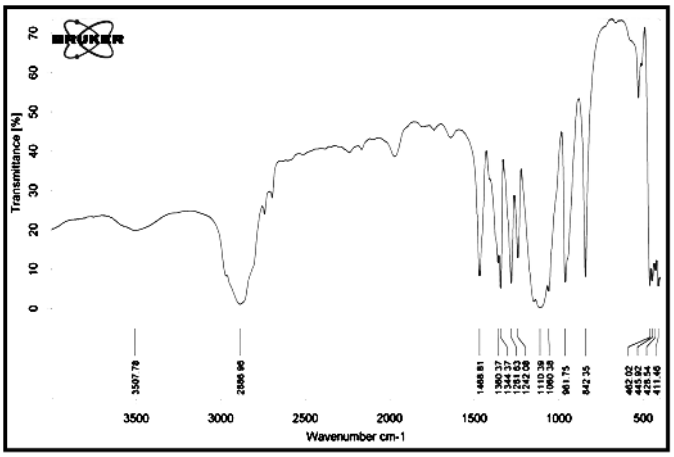


**Figure 8:** FTIR spectra of Poloxamer

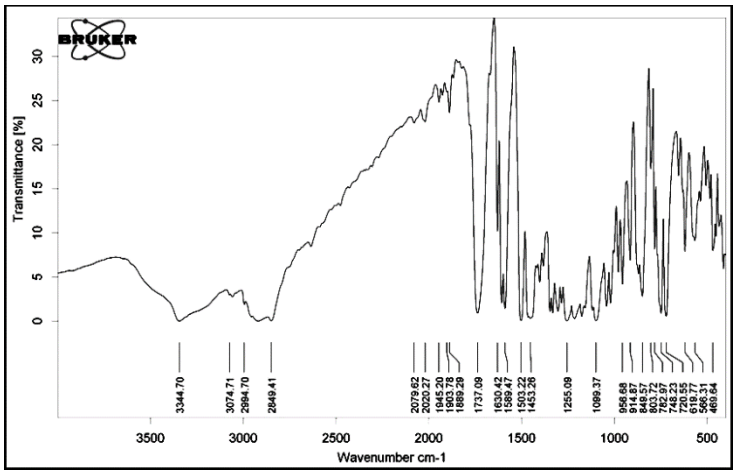


**Figure 9:** FTIR spectra of physical mixture of Atenolol pure drug + excipients

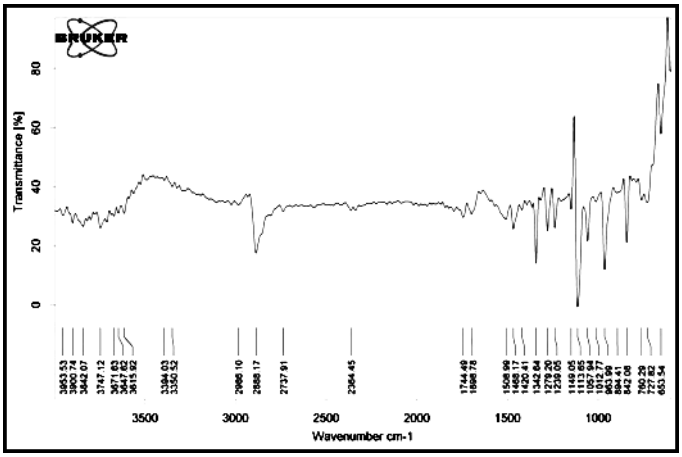


**Figure 10:** FTIR spectra of Atenolol NLC

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The primary functional units are -OH (stretching) was found to be at 3336.84 $cm^{-1}$ and 2921.83 $cm^{-1}$. –NH (Stretching) was found to be at 1333.33 $cm^{-1}$, C-O shown wavenumbers at 1451.21 $cm^{-1}$ and OH groups at 3337.84 $cm^{-1.}$ C-H (Stretching shows 1255.00 $cm^{-1}$ and bending shows of 852.28 $cm^{-1}$) in the atenolol drug respectively. N-H (stretching) shows the wavenumbers was to be 3351.50 $cm^{-1}$, 1507.96 $cm^{-1}$, stretching of O-H was found to be 1343.62 $cm^{-1}$, stretching of C-O group of 1238.04 $cm^{-1}$, and C-O (bending) was found to be 843.38 $cm^{-1}$ in the atenolol NLC Some functional groups may intersect in NLCs due to the strong lipid encapsulation of atenolol.

**Analysis of DSC data**

DSC evaluation was carried for preparations of atenolol and NLC in order to evaluate the compatibility of the drug and excipients [14]. A nano-encapsulated particle's polymeric effect can also be determined using this technique.

**Table 4:** DSC thermograms of pure drug and NLC formulation melting points

| Samples | Melting Point in ° C |
|---|---|
| **Atenolol** | 171.10 |
| **Atenolol NLC** | 177.10 |

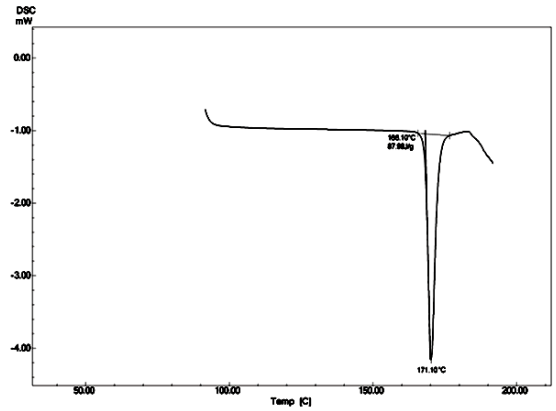


**Figure 11:** DSC thermogram of Atenolol pure drug

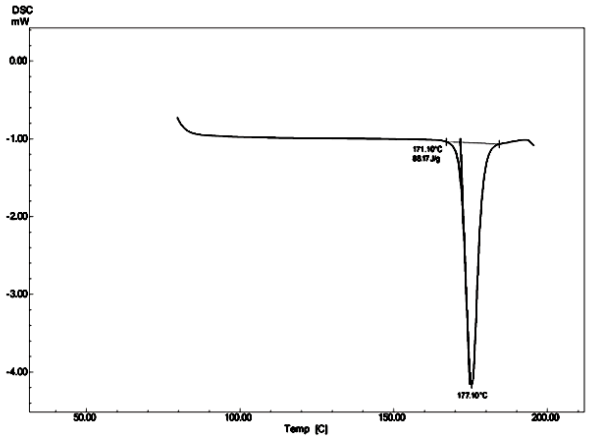


**Figure 12:** DSC thermogram of Atenolol NLC

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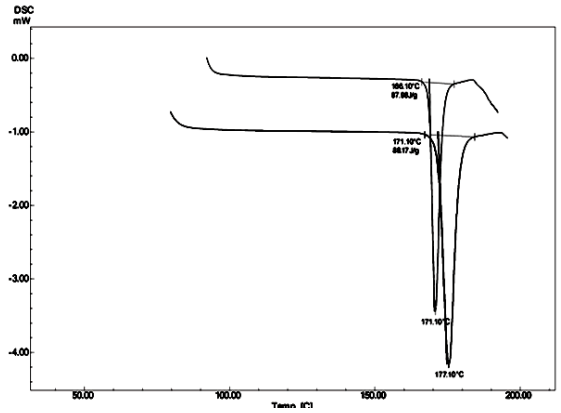


**Figure 13:** DSC overlap thermo gram of Atenolol and Atenolol NLC

A DSC thermogram of atenolol showed the following endothermic peak readings; atenolol 171.10°C; atenolol NLC thermogram shows 177.10°C as the primary melting point. Table 5.5 contains the results.

**Discussion:**

The solubility studies for atenolol cardiolipid nanocarrier transferosomes are crucial to understanding the drug's physicochemical properties and compatibility with the lipid carrier system. Because of its limited aqueous solubility, atenolol presents challenges in formulation development.

Water, organic solvents, and surfactant solutions are among the solvents used in solubility studies, providing insights into the drug's solubility profile. Moreover, these studies enhance atenolol solubility and facilitate its incorporation into lipid nanocarrier systems by selecting appropriate lipid components and surfactants. In addition, the solubility of atenolol in different media allows calculation of drug loading capacities and optimization of formulation parameters such as drug-to-lipid ratios.

It is crucial to assess drug loading efficiency and determine the concentration of drugs in a formulation using the calibration curve for atenolol. Using known concentrations of atenolol is the basis for constructing the calibration curve. An analysis of this curve allows the relationship between the analyte concentration and the detector response to be determined, which is typically linear within a given range of concentration. Furthermore, calibration curves facilitate the assessment of method validation parameters, such as linearity, accuracy, precision, and sensitivity. Quantifying atenolol in the lipid nanocarrier transferosomes requires these parameters to ensure reliability and reproducibility.

Atenolol and lipid nanocarriers can be studied physicochemically by DSC, as well as any changes induced during formulation. A DSC thermogram provides important information about a formulation's melting behavior, crystallinity, and compatibility. Atenolol and its lipid components exhibit characteristic endothermic peaks corresponding to their melting points. When peaks shift or change shape, it indicates that there has been some interaction between the drug and the lipid carriers, such as the formation of complexes or the encapsulation of the drug in the lipid matrix.

**Conclusion**

The preformulation studies of atenolol-loaded cardiovascular lipid nanocarrier transferosomes show that there is a promising potential for improved drug delivery in cardiovascular therapy using these nanocarrier transferosomes. Taking these conclusions into consideration, there seems to be a great deal of promise in the use of atenolol-loaded transferosomes for optimizing cardiovascular drug therapy, offering a novel method for improving patient outcomes by overcoming limitations associated with traditional formulations.

FTIR analysis allowed identification of individual constituents and their chemical interactions by identifying characteristic peaks corresponding to functional groups. Potential molecular interactions between atenolol and lipid components were indicated

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by shifts or changes in peak positions. A transferosomal formulation's encapsulation efficiency, stability, and release kinetics may be influenced by these interactions. Any changes in peak intensities or new peaks indicative of chemical degradation could be identified by comparing the spectra of the formulation components before and after encapsulation.

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