Formulation Development and *In-Vitro* Evaluation of Telmisartan Nanocochleates

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Running heading: Telmisartan Nanocochleates Formulations

ABSTRACT

One of the biggest issues in medication formulation is enhancing the bioavailability of medicines that are poorly water soluble. The formulation of nanocochleates is one of the most acclaimed and cutting-edge medication delivery systems for this problem. There are several strategies, such as the binary aqueous-aqueous emulsion system, the hydrogel method, the direct calcium method, and the liposome method before cochleates dialysis, but trapping methods are straightforward and affordable. The current study's goal was to create nanocochleates of the poorly water-soluble medication Telmisartan (TLM). Phosphatidylcholine and cholesterol were used to create the liposome, which was subsequently transformed into nanocochleates utilizing the trapping technique. By incorporating calcium ions into premade nanoliposomes (TLMNL), TLM-loaded nanocochleates (TLMNC) were created. Nanoliposomes were created utilizing the ethanol injection technique. Particle size, zeta potential, encapsulation effectiveness, SEM, and an in-vitro drug release investigation were all examined for the prepared nanocochleates. The results indicated that TLMNL batch F7 and TLMNCF7 had particle sizes of 318.3 nm and 187.5 nm, respectively, with encapsulation efficiencies of 84.9 percent and 89.7 percent. Both the F7 batch of nanocochleates and the TLMNL batch F7 had positive zeta potentials of

3.88 mV and -3.22 mV, respectively. When compared to normal TLM, the drug release from the nanocochleates was shown to be considerably higher. The study came to the conclusion that nanocochleates may be efficiently produced using the trapping approach with improved solubility, dissolving rate, and bioavailability.

Keywords: Telmisartan, Phosphatidylcholine, Nanoliposomes, Nanocochleates, Solubility, Bioavailability

DOI Number: 10.48047/nq.2022.20.19.NQ99194 **NeuroQuantology2022;20(19): 2301-2313**

INTRODUCTION

Cochleates are tiny, negatively charged unilamellar liposomes that condense into long, rolling microstructures consisting of many lipid bilayers. Initially discovered by Papahadjopoulos and Wilschut (Papahadjopoulos, et al. 1979), these structures were later discovered by other researchers (Goldstein and Lukaynov et al., 1997; Lee and Lukaynov et al., 1998; Lee and Carlson et al., 1999; Price and Patchan, 1991). These are solid particles with no internal aqueous space that are comprised of continuous lipid bilayer sheets that have been wrapped into a spiral shape by contact with a multicationic metal ion like Ca^{2+}. Cochleate particles are stable phospholipid-cation precipitates made up of simple, naturally occurring phospholipids like dioleylphosphatidylserine or a combination of lipids with at least phosphatidylserine (PS), phosphatidylinositol (PI), phosphatic acid (PA), and phosphatidylglycerol up to 75 percent by weight with phosphatidylcholine (PC) (Zarif et al., 2002, Reddy et al., 2010). Cochleate is the most adaptable carrier for the transport of a variety of pharmacological molecules, proteins, and peptides because to its special structure, which allows it to encapsulate both hydrophilic and hydrophobic moieties of any form and size. Additionally, they shield the entrapped molecule from lipase breakdown and severe pH, temperature, and environmental conditions (Yadav et al., 2016, Zarif et al., 2000, Yeale et al., 2013).

Delivery vehicles for nanocochleates are persistent phospholipid precipitates produced when anionic lipid vesicles interact with divalent cations (Ca^{2+} and Mg^{2+}). Calcium chloride can be added drop by drop to liposomes that have already been created to create nanocochleates. These are distinct from liposomes in that they contain a stiff, rod-shaped interior that is free of water (Zarif et al. 2000, Bothiraja et al. 2013). Due to its numerous advantages, including biocompatibility, simplicity of manufacture, fewer side effects, and increased efficacy, this lipid-based nanocochleates system has the potential to lead to the development of novel medications (Pawar et al. 2014). Encochleated molecules are less likely to oxidize as a result of non-aqueous nanocochleate structure's resistance to

oxygen penetration. Additionally, it shields encochleated medications from biological fluid breakdown. Even if its outside layers may be exposed to abrasive environmental conditions or enzymes, such in the stomach, the inside components of the nanocochleates structure stay intact since the entire structure is likewise made up of a sequence of solid layers. They are able to encochleate materials and deliver them to the target cell's cytoplasm by targeting macrophages, which have membrane phosphatidylserine receptors (Panwar et al., 2011). The nanocochleates formulation method is particularly suitable to hydrophobic, positively and negatively charged, hydrophobic macromolecules and medicines with low oral bioavailability and fast first-pass metabolism. It is also utilized for medications like proteins and peptides that degrade at physiological pH (Bothiraja et al., 2014, Gonzalez et al., 2009).

Angiotensin-II receptor antagonist telmisartan (TLM) is used in the treatment of hypertension. According to the BCS categorization, TLM is a drug of class-II. This medication has a number of significant issues, including a poor solubility in biological fluids. TLM has an extremely low solubility in aqueous media (0.0099 mg/mL in water). Methyl benzimidazole, phenyl benzimidazole, and methyl phenyl benzoic acid are the three main functional groups in TLM. Despite being lipophilic, TLM is weakly soluble in both water and oils, according to a literature review (Log P of 7.7). The TLM has a 42–58 percent absolute bioavailability, and its biological half-life is 24 hrs. Therefore, enhancing TLM's water solubility and dissolution has therapeutic value (Kausalya et al., 2011, Bhagwat et al., 2012). Therefore, the goal of the current work was to formulate and assess TLM nanocochleates using a trapping approach to increase their solubility (Fogerite et al, 1998, Zarif et al., 2002, Bhosale et al., 2013).

One of the biggest issues in medication formulation is enhancing the bioavailability of medicines that are poorly water soluble. The current study's objective was to create nanocochleates of the poorly water soluble medication TLM. The optimized formulations were characterized for solubility profile, drug entrapment efficiency, particle size, morphology, differential scanning calorimetry, *in vitro* release study, and zeta potential.

MATERIALS AND METHODS

Chemicals

TLM was procured from Vasudha Chemicals Pvt. Ltd, Navi Mumbai, India and hydrogenated soy phosphatidylcholine and cholesterol was obtained from Glenmark Pvt. Ltd, Sinner, India as a gift sample. The reagents and solvents that were utilized were all of analytical grade. The entire experiment was conducted with double-distilled water.

Instruments

Differential Scanning Calorimeter (Mettler, STAR SW 12.10, UK), Probe Sonicator (Sonics), UV Spectrophotometer (Shimadzu 1800, Japan), Zetasizer (Malvern Instruments Ltd. 3000 SM, UK), Transmission Electron Microscope (Zeiss EM 109, USA), Scanning Electron Microscopy (Joel JSM-848, Japan), centrifuge (Remi R-303, India), freeze dryer (Chirst, Alpha 1-2 LDplus, USA), and Weighing Balance (Shimadzu AUW220D, Japan) were employed in this study.

Solubility Study

By dissolving excess amounts of TLM in several solvents (10 mL), including ethanol, methanol, and water, the solubility of TLM was ascertained. The stirring was place in a water bath shaker for two hours at a temperature of 37°C±0.5°C using a vortex mixer. After centrifuging the sample at 3000 rpm for 15 min, the supernatant was filtered through a 0.45 µm membrane filter, diluted with phosphate buffer pH 6.8, and spectrophotometrically analyzed at 234 nm (Aggarwal et al., 2014).

Differential Scanning Calorimetry

The enthalpy and temperature scales were calibrated using standards made of indium. A little portion of the sample (about 5 mg) was hermetically sealed in an aluminium pan and put into the DSC apparatus. Indium was used as the reference to micro calibrates the DSC temperature. After heating the sample at a rate of 10°C/min from 25°C to 300°C, the DSC spectra were captured. In order to keep the environment inert, nitrogen gas was purged at a flow rate of 50 mL/min (Pawar et al. 2014).

Preparation of Nanoliposomes

The modified ethanol injection approach was used to create TLM-loaded nanoliposomes (TLMNL) (Ghanbarzadeh et al., 2013, Minghui et al., 2007). Increasing the quantity of TLM (15 mg, 20 mg, and 25 mg) allowed for the maximum amount of TLM to be loaded into the nanoliposomes, which were then tested for particle size and encapsulation effectiveness. When TLM concentration was raised from 15 mg (TLMNL1) to 20 mg, the size of the nanoliposomes grew (TLMNL2). However, increasing the dose further (TLMNL3) to 25 mg led to poor encapsulation performance. The greatest quantity that could be placed into the nanoliposomes was set at 20 mg since the formulation TLMNL2 demonstrated high encapsulation efficiency. The nanoliposomes TLMNL2 were selected to generate the nanocochleate. In 2 mL of ethanol, cholesterol, TLM, and certain concentrations of phosphatidylcholine were dissolved before being heated to the phase transition temperature (40°C) of PC (**Table 1**). The phosphate buffer pH 6.8 was infused with the aforementioned ethanolic solution (10 mL drop by drop) while being stirred for 30 min at 40 percent amplification. The phosphate

buffer pH 6.8 was added to the final volume of TLM-loaded nanoliposomes solution to bring it up to 10 mL after the ethanol had completely evaporated. To get a TLMNL suspension, the aforementioned combination was filtered via a 0.45 µm membrane filter.

Table 1. Design of Trial Batches for Liposomes Formulation.

Batch	Component	F1	F2	F3	F4	F5	F6	F7
Drug (mg)	Telmisartan	20	20	20	20	20	20	20
Solvent (mL)	Ethanol	2	2	2	2	2	2	2
Lipids (Ratio)	Phosphatidylcholine: Cholesterol	1:1	2:1	3:1	3.7:1	5:1	6.5:1	7.5:1
Hydration Medium (mL)	Phosphate Buffer pH 6.8	10	10	10	10	10	10	10

Evaluation of Nanoliposomes

Drug Entrapment Efficiency

The absorbance of TLM solutions (concentration of 2 mg/mL to 10 mg/mL in phosphate buffer pH 6.8) was measured at 234 nm to produce a calibration curve (R^2 = 0.9997). Using centrifugation at 3000 rpm for 30 mins at 4°C to separate non-encapsulated TLM from TLMNL solution, the effectiveness of nanoliposome encapsulation was assessed (Shaikh et al., 2012). The entrapped medication was released from the sediment nanoliposomes using ethanol, which was then appropriately diluted with phosphate buffer pH 6.8. The absorbance at 234 nm was then measured, and the encapsulation efficiency was calculated using the calibration curve. The percent encapsulation efficiency was calculated using equation 1,

$$\text{Entrapment efficiency} = \frac{\text{Amount of TLM present in Nanoliposomes or Nanocochleates}}{Total\ amount\ of\ TEL\ present} \times 100 \ldots\ldots\ldots \quad (1)$$

In-vitro release study of Telmisartan nanoliposomes

Dialysis bag diffusion techniques were used to conduct an in vitro release study of TLM, TLMNL, and TLMNC in phosphate buffer pH 6.8 and compare the results to free TLM (Bothiraja et al., 2014). A formulation containing 2 mg of TLM or 2 mg TLM dispersion in a 50 mL release medium was added to a dialysis bag before being hermetically sealed and submerged. The system was continuously stirred at 100 rpm/min while being maintained at 37°C±0.5°C. To maintain sink conditions, samples were taken out and replaced with new medium at predefined intervals. Using a UV spectrophotometer set at 234 nm, the

absorbance of TLM in solution was calculated (Pawar et al., 2014).

Determination of particle size and zeta potential

The laser diffraction method was used to measure the size of TLMNL at a 90° scattering angle. The samples were mixed with distilled water to measure the average particle size. At a temperature of 25°C, the zeta potential was assessed using laser Doppler electrophoretic mobility studies.

Surface morphology

Transmission electron microscopy was used to study the morphology of the TLMNL. A drop of the diluted sample was applied on a copper grid that had been coated with carbon to create a thin liquid layer in order to produce the TEM sample. Following the removal of extra solution, the sample was analyzed and captured on camera using a transmission electron microscope at an accelerating voltage of 80 kV.

Preparation of Nanocochleates

Under a vortex, 50 µL of calcium chloride solution (0.1 M) were dropped into the produced nanoliposome suspension (Bothiraja et al., 2014). The production of nanocochleates instantly caused the TLMNL phase to become turbid. The aqueous phase in the structure of nanocochleates was tightly constrained, and the calcium ions were crucial for the stability of the system. Through partial membrane dehydration and cross-linking of opposing phospholipid molecules, calcium plays a key role in bringing closed phospholipid bilayers together. Calcium keeps the drug-exclusion bridge in place (Zarif et al., 2005).

Evaluation of nanocochleates

Drug Entrapment Efficiency

The centrifuge tube made of polypropylene received 1 mL of TLMNC. The nanocochleates pellet was added 100 l of EDTA (pH 9.5) to enable the opening of the nanocochleates into nanoliposomes and the release of TLM after the tube had been centrifuged at 3000 rpm for 30 minutes at 4°C (Zarif et al. 2002). After adding 1 mL of ethanol, the mixture was vortexed. The resultant clear solution had a phosphate buffer pH of 6.8 and was appropriately diluted before having its absorbance measured at 234 nm. Equation 1 was used to compute the percent encapsulation efficiency and to quantify the free TLM content in the supernatant.

In-vitro release study

Using dialysis bag diffusion techniques, an in vitro release study of TLMNC was carried out in phosphate buffer pH 6.8 and compared with free TLM (Bothiraja et al. 2014). A formulation containing 2 mg of TLM in dissolving media was added, hermetically sealed, and submerged in 50 mL of release medium in a dialysis bag. The system was continuously stirred at 100 rpm/min while being maintained at 37°C±0.5°C. Samples were taken every hour for 24 hrs, sink conditions were maintained, and samples were examined with a UV-Visible spectrophotometer at a wavelength of 234 nm.

Determination of particle size and zeta potential

The laser diffraction method was used to calculate the size of TLMNC. At a scattering angle of 90°C, measurements of

particle size were made. The samples were mixed with distilled water to measure the average particle size. At a temperature of 25°C, the zeta potential was assessed using laser Doppler electrophoretic mobility studies.

Surface morphology

The morphology and surface characteristics of TLMNC were visualized using SEM. The nanocochleates were first dried under vacuum and were glued to aluminium stab and gold coated under Argon atmosphere. The coated nanocochleates were finally characterized for surface morphology (scale bar was 25000x magnification).

Lyophilization of Optimized batch of Telmisartan nanocochleates

The mannitol (5 percent W/V solution) was added in nanocochleates suspension (Batch F7) as a cryoprotectant to avoid lysis of nanocochleates. The sample was first subjected to deep freezing in the deep freezer for 2 hr. Further, it was primary-dried at -30°C to -40°C for 1 hr and for secondary dried at -54°C to -60°C for 4 hr in a freeze dryer.

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Differential Scanning Calorimetry

***In vitro* release study of lyophilized telmisartan nanocochleates**

TLM was released from the nanocochleates *in vitro* using a dissolving procedure in phosphate buffer at pH 6.8. In a 900 mL release medium, a formulation equal to 20 mg of TLM lyophilized powder was added. The sample was withdrawn and refilled with the same amount of fresh medium to maintain the sink conditions, and the entire system was held at 37°C±0.5°C with 100 rpm/min of continuous magnetic stirring. The UV-Vis spectrophotometer was used to calculate the amount of TLM that was absorbed by the solution.

RESULTS AND DISCUSSION

Solubility study of Telmisartan

The solubility of TLM in ethanol and water was investigated. The solubility of TLM in water and ethanol was found to be 0.0099 mg/mL and 0.0297 mg/mL, respectively. TLM had significantly higher solubility in ethanol as compared to other solvents

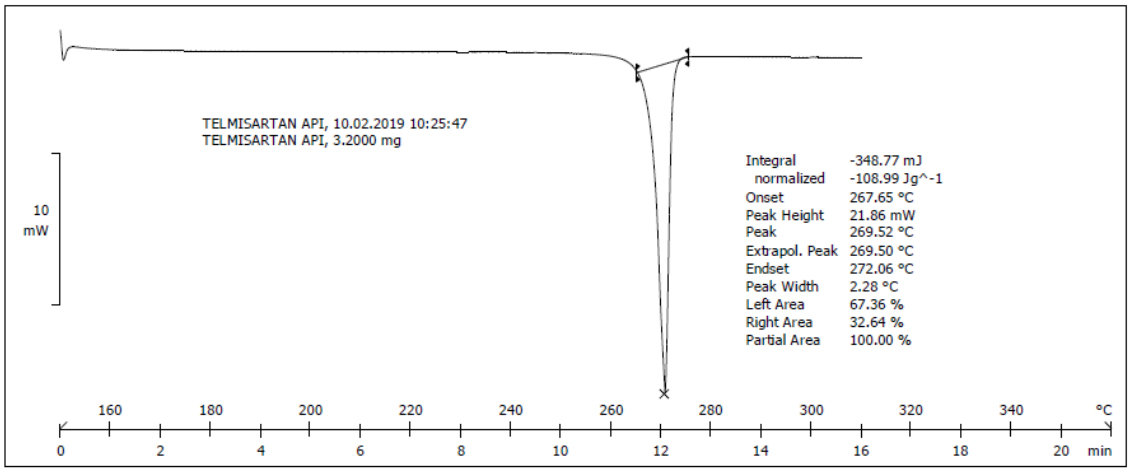

Figure 1. DSC analysis of Telmisartan.

Change in heat capacity that occurs at the glass transition temperature, TLM characterized the presence of endothermic peak which is corresponding to the melting point 267°C-272°C (**Figure 1**). The onset of the peak is found to be at 267°C.

Drug Entrapment Efficiency

The amount of medication integrated into liposomes as a percentage of the total amount of drug utilized is how encapsulation efficiency is measured. The EE percent for all batches was shown to be between 35.3 to 86.3 percent (**Table 2**). In the instance of the EE percent, the composition factors were determined to be significant, and both variables showed a strong association. As indicated in **Table 2**, the percentage of EE was likewise observed to rise with an increase in the PC:CH ratio. The percentage of entrapment effectiveness of liposomes improves from 77.2 percent to 84.9 percent and from 86.3 percent to 89.7 percent for batches F6 and F7, respectively, after the manufacture of nanocochleates. However, the high lipophilicity of the medication may be the cause of the observed high EE percent.

Table 2. Encapsulation Efficiency of liposome and nanocochleate batches.

Batch	% Entrapment Efficiency of liposomes	% Entrapment Efficiency of nanocochleates
F1	35.3	39.1
F2	40.5	45.7
F3	48	51.3
F4	59.9	59.8
F5	65.4	73.1
F6	77.2	84.9
F7	86.3	89.7

***In vitro* release study of TLM dispersion, TLMNL, TLMNC, TLM lyophilized powder**

In vitro release of the TLM in TLM dispersion, TLMNL, TLMNC, and TLM lyophilized powder was investigated and compared (**Figure 2**). On the basis of percentage entrapment efficiency, the F7 liposomes and nanocochleate batch subjected to the *in vitro* drug release study. The TLM dispersion showed only 28.24 percent release after 24 hrs. However, the result revealed that F6 and F7 batch of liposomes showed 72.70 percent and 83.98 percent drug release within 24 hrs. These formulations had good drug release as compared to other batches within 24 hrs. Therefore, batch F7 is used to prepare the nanocochleates and other evaluation studies.

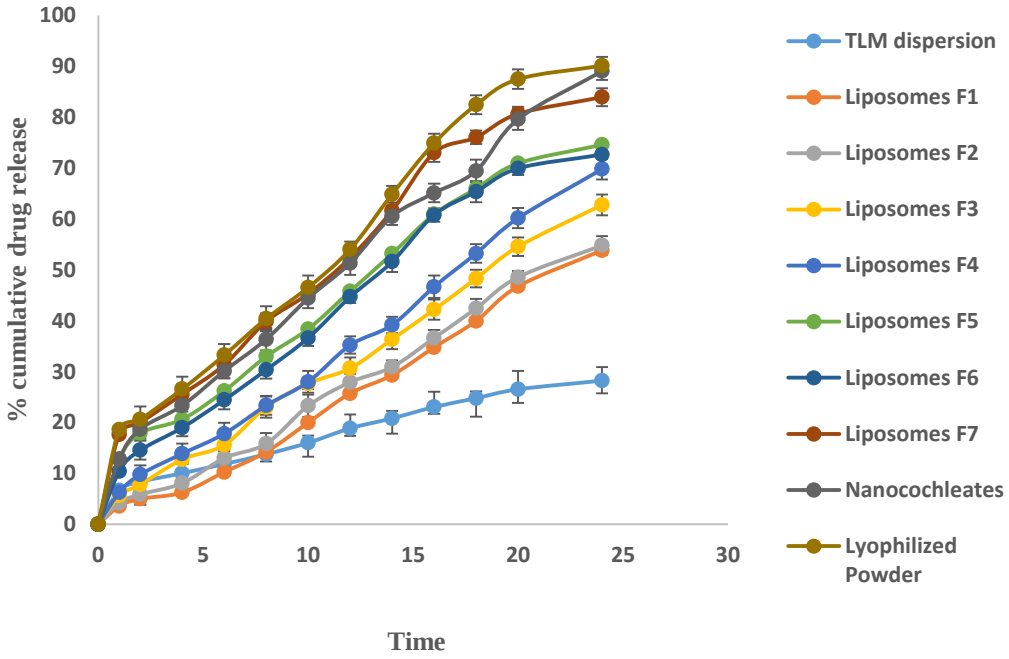

Figure 2. *In vitro* release study of TLM dispersion, liposomes, nanocochleates, and lyophilized powder.

Particle size and Zeta Potential

The optimized TLMNL batch F7 showed particle size of 318.3 nm with an encapsulation efficiency of 86.3 percent. Whereas, the TLMNC batch F7 showed particle size of 187.5 nm (**Figure 3a** and **Figure 3c**) with an encapsulation efficiency of 89.7 percent. The other batches show highest particle size as compare to batch F7 due to decrease concentration of lipid. TLMNL The nanocochleates in batch F7 had a negative zeta potential of -3.22 mV and a positive zeta potential of 3.88 mV, respectively (**Figure 3b** and **Figure 3d**). The zeta potential was determined to be in the region of 30 mV, indicating improved formulation stability.

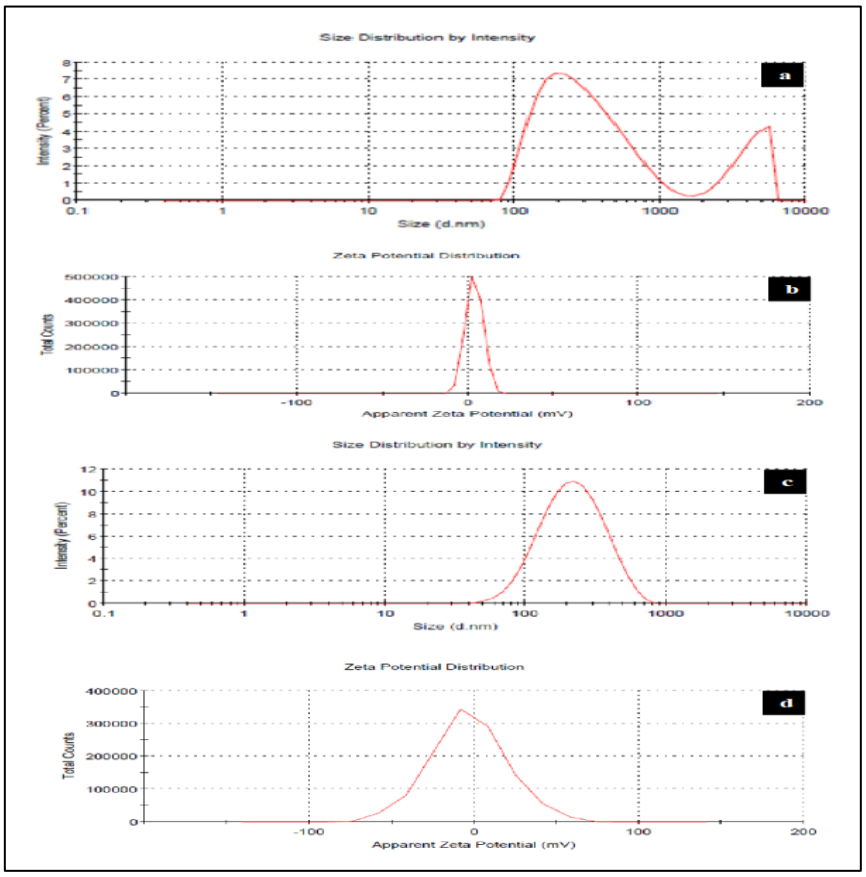

Figure 3. Zeta potential and particle size: **a)** Particle size of Batch F7TLMNL; **b)** Zeta Potential of Batch F7TLMNL; **c)** Particle size of Batch F7TLMNC; and **d)** Zeta Potential of Batch F7 TLMNC.

Surface morphology

TLM nanoliposomes' surface morphology (batch F7) exhibited uniform unilamellar, discrete, and round structure (**Figure 4a** and **Figure 4b**), whereas nanocochleates displayed tubular rod-shaped and were claimed to have no internal aqueous space (**Figure 4c**).

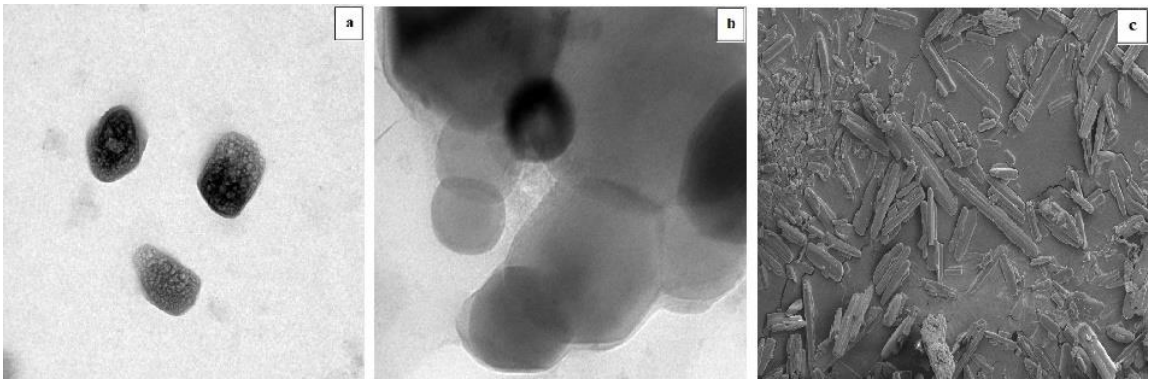

Figure 4. Microphotographs: a) TEM image of liposomes showing size of 0.5 µm; b) TEM image of liposomes depicting size of 100 nm; and c) SEM image of telmisartan-loaded nanocochleates.

CONCLUSION

The natural lipophilic antihypertensive bioactive TLM was successfully transported as nanocochleates in the current experiment employing the trapping method methodology using phosphatidylcholine, cholesterol, and calcium ions. The created nanocochleates showed improved TLM encapsulation effectiveness and sustained release. The rate at which the medication dissolved was greatly increased by nanocochleates. Amorphization, which is known to promote drug solubility and dissolution rate, better wettability, and the significant reduction in particle size to the nano range were all factors that contributed to the higher dissolving rate. Due to their innately low water solubility and bioavailability, additional BCS class-II drugs may also experience oral delivery issues and benefit from this method.

ACKNOWLEDGMENT

The authors appreciate the free sample of Telmisartan from Vasudha Chemicals Pvt. Ltd. in Navi Mumbai. Additionally, the authors are grateful to Glenmark Pvt. Ltd., Sinner, India, for donating a sample of cholesterol and hydrogenated soy phosphatidylcholine.

FUNDING

No agency provided any funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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