IJPSR (2024), Volume 15, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 December 2022; received in revised form, 27 January 2024; accepted, 29 January 2024; published 01 February 2024

OPTIMIZATION OF THE FORMULATION AND *IN-VITRO* EVALUATION OF GABAPENTIN NIOSOMES FOR THE TREATMENT OF EPILEPSY

P. Rajeswari, Rohit Jain. A, Mohammed Akram and B. Anbarasan *

Department of Pharmaceutics, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai - 600116, Tamil Nadu, India.

Keywords:

Gabapentin, Niosomes, Cholesterol, Span 80, and TEM

Correspondence to Author:
Dr. B. Anbarasan

Assistant Professor,
Department of Pharmaceutics, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai - 600116, Tamil Nadu, India.

E-mail: anbarasan.b@sriramachandra.edu.in

ABSTRACT: Objective: The main purpose of this current work is to optimize the formulation and *in-vitro* evaluation of gabapentin niosomes. **Methods:** Niosomes are formulated by utilizing the thin film method. Gabapentin niosomes are prepared with cholesterol and span 80. Pre-formulation and post-formulation studies were carried out for optimized gabapentin niosomes. Such as FTIR, DSC, Zeta, Particle size, TEM, SEM, and *in-vitro* drug release studies. **Report:** Gabapentin medication pre-formulation experiments were carried out. The ultraviolet spectroscopic maximum of gabapentin was found to be 206nm. Within the concentration range of 2-10 g/ml of drug in phosphate buffer 7.4 solutions; gabapentin follows Beers law with a slope of 0.1086 x and an R^2 value of 0.9985. The particle size was determined to be round and spherical in the improved formulation F7. The particle sizes are in the 50-120mm range. Particle's external morphology/outer surface morphology were determined. **Conclusion:** The goal of this study was to optimize the formulation and *in-vitro* evaluation of gabapentin niosomes for epilepsy treatment. As a result, the current research focuses on increasing drug permeability and encapsulating the drug in niosomes vesicles for long-term central nervous system drug delivery.

INTRODUCTION: In recent years, delivering medication molecules to the target location in biological systems has become a highly specialized and sophisticated topic of pharmaceutical research in recent years. To transport the medicine to the target tissues, various innovative drug delivery systems have been identified, including liposomes, niosomes, nanoparticles, and microspheres. A drug delivery method using a distinct vesicle carrier, such as for lipid nanoparticles or niosomes, provides distinct advantages above nanoparticles, nanomaterials, and other carriers in terms of enhanced medication trapping, target site selectivity, and handling untimely drug release.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(2).594-99

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(2).594-99

Vesicular systems, such as liposomes and niosomes, are important for drug administration because they can reduce entrapped drug toxicity and change the pharmacokinetics and bioavailability of drugs [1]. When opposed to phospholipids, which are prone to oxidation, niosomes provide various advantages over liposomes, including being more stable, nontoxic, and cost-effective due to the low cost of non-ionic surfactant.

Niosomes are microscopic lamellar structures formed in aqueous environments by hydrating nonionic surfactants of the alkyl (dialkyl polyglycerol ether group most closely related to cholesterol). Drugs encapsulated in niosomes have the potential to reduce drug degradation and inactivation after administration, as well as to reduce unwanted side effects, boost medication bioavailability, and target the diseased area. GABA, or gabapentin, is a cyclohexane acetic acid, is thought to be the primary inhibitory

neurotransmitter in the mammalian central nervous system (CNS), and it shares structural similarities with GABA [2]. Gabapentin is chemically 1-(Aminomethyl) cyclohexaneacetic acid. It's a gabapentinoid, or ligand of the auxiliary 2 subunit location of some voltage-dependent calcium channels (VDCCs), and hence functions as a 2 subunit-containing VDCCs inhibitor [3]. There are two drug-binding 2 subunits, 2-1 and 2-2, and gabapentin has a similar affinity for both of them. Gabapentin binds to the 2 VDCC subunit with a high degree of selectivity. Because it is non-toxic and may pass across the blood-brain barrier, it is also used to treat anxiety and sleep disturbances. In the discipline of urology, gabapentin was initially used to treat refractory interstitial cystitis [4].

Bio-analytical technique approval incorporates every one of the methodologies and checks needed to demonstrate the dependability of a strategy for quantitative assurance of grouping of an analyte. For logical strategy approval, the US-FDA has given a few rules in ICH [5].

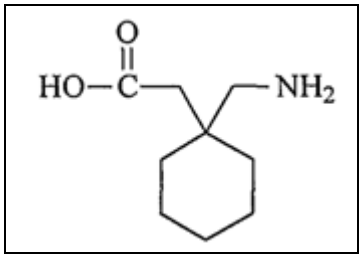

FIG. 1: CHEMICAL STRUCTURE OF GABAPENTIN [6]

MATERIALS AND METHODS:

Chemical and Reagents: The Standard drug of Gabapentin was collected as a gift sample from Madras Pharmaceuticals Pvt Ltd. Span-80,20 from SD fine Pvt Ltd, cholesterol from loba chemie pvt ltd. Methanol AR grades were procured from nice laboratories.

Pre-formulation Studies:

Drug Excipients Incompatibility Studies: FT-IR Studies ensured physical compatibility studies. The study's drug sample, drug, excipient, and formulation mixes were chosen. The spectra were acquired by utilizing a pellet press to make potassium bromide pellets in a dry environment. The spectrum of the crude drug and the drug-excipient possible combinations were examined to rule out incompatibility concerns (FTIR 4100 JASCO).

DSC Studies: A thermal analysis operating system was attached to a differential scanning calorimeter (DSC, Model 2920, TA Instruments). Under nitrogen purge, approximately 2–6 mg of the sample was heated from room temperature to 250 degrees C at varied rates in a crimped aluminum pan or an open aluminum pan [7].

Solubility Studies: The equilibrium solubility of Gabapentin in various vehicles can be used to screen excipients. An extra amount of Gabapentin was added to each excipient's 1 ml using a cyclo mixer, both components were combined for 5 minutes in a vial (Remi Mumbai, India). The mixtures were centrifuged at 10000 rpm in a 12C micro-centrifuge. Gabapentin was extracted in methanol after the supernatant was separated. (Perkin Elmark Lamda 35)UV visible spectrophotometer set to 206nm was used to determine the drug content [8].

Zeta Potential Studies: The zeta sizer NanoZS-90 was used to determine the mean particle size for the formulations (Malvern Instruments Limited). A proper dilution with filtered water (0.5 m) filter was used to take the reading at a 90^{o} angle to the incident beam of 25 degree C [9].

Preparation of Niosomes by Thin Film Method [10]: Thin film hydration was used to create the niosomal formulations. The niosomal formulations were made by thin film hydration. Gabapentin, nonionic surfactant, and cholesterol were carefully weighed and dissolved in 10 mL of solvent mixture (Chloroform: Methanol 2:1 ratios) in a round bottom flask. The thin films were hydrated with 10ml of phosphate buffered saline pH 7.4 produced under decreased pressure in a rotary flash evaporator, and the flask was continuously rotating at 60 degrees C at varied revolutions per minute (RPMs). The same process was used to make empty niosomes but without the gabapentin.

Evaluation Parameters for Gabapentin Niosomes:

Optical Microscopic Analysis: The vesicles that were formed were examined using photo-electron microscopy. A small amount of dispersion was placed on a glass slide and studied using a Leica Optical Microscope at various magnification levels to check for vesicle structure (10X and 40X).

TEM Analysis: The internal morphology of the produced vesicle was studied using TEM (FEG JAPAN). The sample was created by diluting a mixture in a 1:5 proportion and depositing it on a plate covered in carbon on one side. 20L of sample was added to the copper plate and dried overnight [11].

SEM Analysis: A scanning electron microscope was used to examine the surface morphology of the produced niosomes (SEM). The solid portion of the niosomal formulation was then fixed on screw-shaped stubs using double-sided carbon adhesive tape. The samples were coated with platinum in an ion sputter chamber under vacuum in an argon atmosphere, then analyzed at a 15000v accelerating voltage [12].

Particle size Distribution: Laser diffraction (Malvern Particle Size Analyzer) was used to determine the particle size distribution of the niosomal formulation, and the mean vesicular diameter was estimated [13].

Entrapment Efficiency: The formulation's entrapment efficiency was tested with samples centrifuged with 2 ml of the suspension for 60 minutes at 14,000 rpm while maintaining a temperature of 4 °C to separate nanoparticles from unentrapped medication. Using a UV-visible spectrophotometer, the free drug content in the residual liquid was evaluated at 206nm [14].

Drug Content: By pouring 10 mg of niosomal dispersal in a bottom flask and then applying phosphate buffer pH 7.4 to bring the volume up to the required volume, the concentration of niosomes was determined. After 1ml of the solution was withdrawn and diluted up to 10ml with phosphate buffer pH7:4, the amount of drug present was measured at 265nm using a UV spectrophotometer.

***In-vitro* Release Studies:** Niosomal dispersion was released in vitro using a dialysis bag (Himedia dialysis membrane of molecular weight cut off 12,000-14,000). The niosomal preparation was deposited in a donor compartment of a dialysis bag with an effective length of 5 cm. As a receptor compartment, the dialysis bag was poured into a beaker and added to 100 ml of pH 7.4 phosphate buffer saline (PBS). The warmth of the receptor compartment was maintained around 37°C±1°C, and it was stirred at a 1°C/min rate [15].

Stability Studies: Drug leaching from niosomes during storage was investigated using stability tests. The ability of vesicles to retain medication was tested by storing the chosen niosomal suspension in sealed glass vials at 4 degrees C 2 degrees C, 25 degrees C 2 degrees C for 8 weeks. Periodically, samples were taken and examined for aggregation, drug entrapment, and residual drug content [16].

RESULTS AND DISCUSSION:

Drug-excipient Incompatibility Studies: ATR-FTIR spectra of gabapentin, cholesterol, and nonionic surfactants are shown in **Fig. 2**. The gabapentin niosomal formulation revealed similar peaks, indicating that the medication and the excipient have no interaction. C-H Stretching 2830cm-1, O=C-H Stretching 2650cm-1, N=C=N Stretching 2260cm-1, C=O Stretching 1600cm-1, C=N Stretching 1310cm-1, CH2 Rocking 760cm-1 were found in pure gabapentin, and O-H Stretching 3461cm-1, =C-H bending 3100cm-1, C=O Stretching.

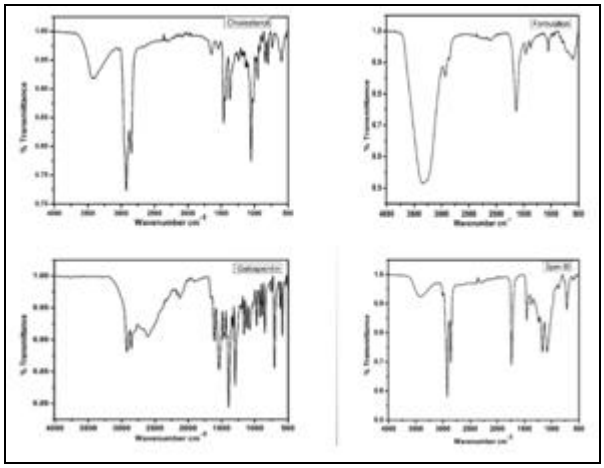

FIG. 2: FTIR SPECTRUM OF GABAPENTIN PURE, FORMULATION, CHOLESTEROL, AND SPAN-80

DSC Analysis: The interaction was studied by DSC as shown in **Fig. 3** which represents a non-invasive analytical technique. DSC analysis was carried out for gabapentin, span 80, cholesterol gabapentin loaded niosomes. The DSC thermogram of gabapentin showed a sharp endothermic peak at 177.2°C which corresponds to the melting of the drug. Span 60 and cholesterol showed endotherms at 59.10°C and 148.8°C. DSC thermograms of

gabapentin-loaded niosomal formulation interestingly exhibited endotherms at 117.3°C. When niosomes were loaded with gabapentin, the cholesterol endotherm shifted from 148.8°C to 117.3°C, indicating an increase in the phase transition temperature of niosomes. These findings point to a strong drug-bilayer interaction, which could explain the increased trapping of gabapentin in niosomal formulations and long-term drug release.

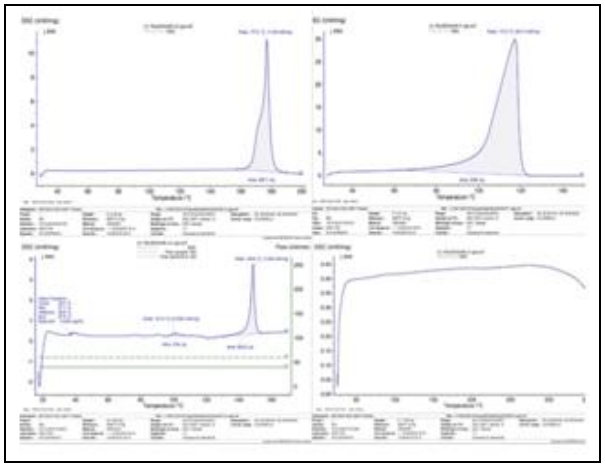

FIG. 3: DSC SPECTRUM OF GABAPENTIN PURE, FORMULATION, CHOLESTEROL AND SPAN80

Zeta Potential Analysis: To allow efficient cell interaction and promote intracellular delivery of niosome a high positive zeta potential is needed. A high absolute zeta potential value signifies a high electric surface charge on the drug-loaded niosome, which can cause strong repellent forces among particles to prevent it from aggregation Zeta potential value of gabapentin noisome is -14.3mv shown in **Fig. 4.**

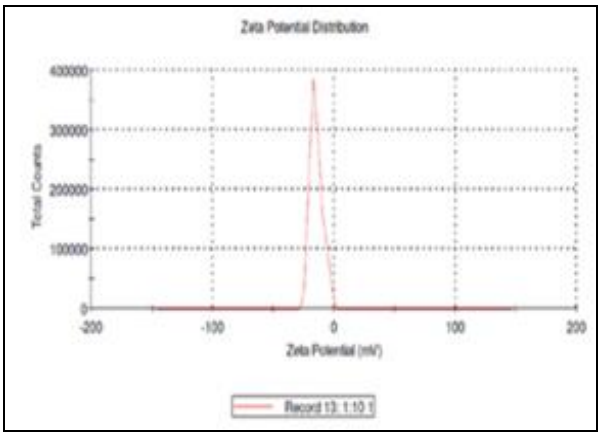

FIG. 4: ZETA POTENTIAL OF THE FORMULATION

Optical Microscopic Study: The optimized formulation was characterized by using optical microscopy and the formations of vesicles were clear due to the lower CPP (Critical Packing Parameter) value. The results are shown in the **Fig. 5.**

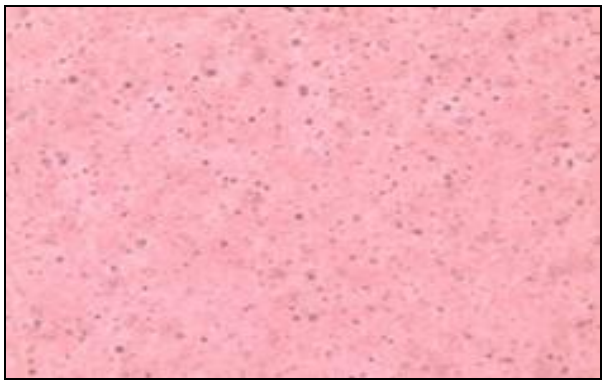

FIG. 5: OPTICAL MICROSCOPY OF FORMULATION

SEM Analysis: SEM image of prepared noisome formulation shows the coating of surfactant cholesterol mixture on drug particles. Most of the vesicles are spherical and have discrete sharp boundaries with a size range of 20 - 150nm.

The appearance of noisome vesicles in the scanning electron micrograph is smooth, which indicates a thin and uniform coating over the drug. Based on the scale of the micrograph, no significant change in the size of particles is seen. The observation clearly shows that there is no aggregation between the particles, due to surfactant coating as shown in the **Fig. 6.**

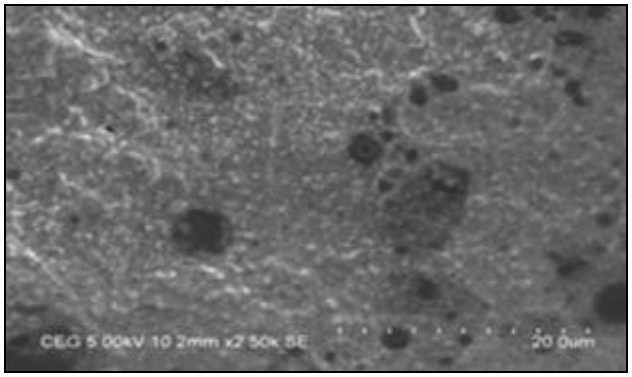

FIG. 6: SEM IMAGE OF FORMULATION F11

TEM Analysis: The optical formulation shows the particle size uniform and discrete in size exhibited in round and spherical and the size was found to be around 20-150nm as shown in the above **Fig. 7.** The observation clearly shows that there is no aggregation between the particles, due to surfactant coating

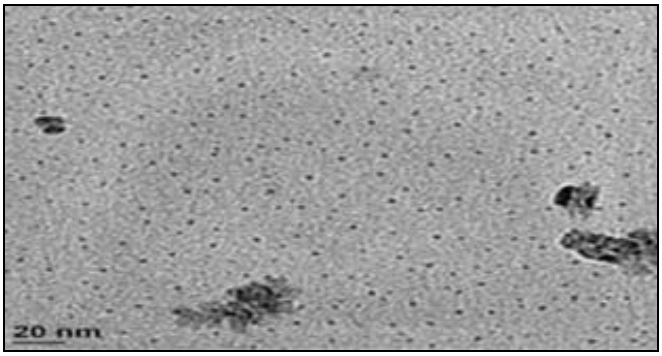

FIG. 7: TEM IMAGE OF FORMULATION F11

Particle size Distribution: The average particle size of gabapentin niosomes is 130.7nm. As shown in **Fig. 8** which indicates the niosomes is stable.

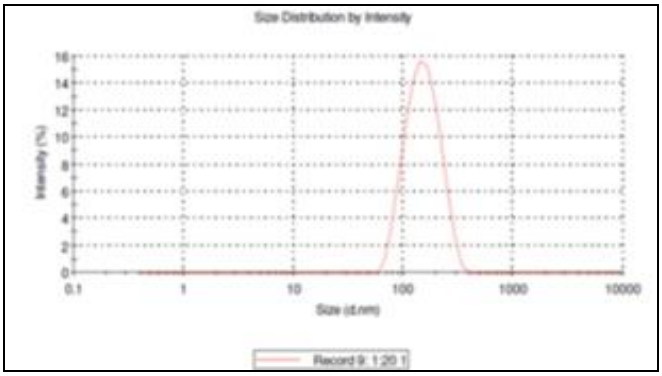

FIG. 8: PARTICLE SIZE DISTRIBUTION OF F11

Entrapment Efficiency: As seen in the graph above, the percentage EE of all formulations was determined to be between 30.45 and 80.65 percent. The effect of surfactant concentration on entrapment efficiency is significant in all formulations. The greatest entrapment efficiency is 80.65% in Formulation F11. **Fig. 9** depicts the results, which were tabulated.

FIG. 9: ENTRAPMENT EFFICIENCY OF OPTIMIZED FORMULATIONS

Drug Content: The niosomes prepared in this study revealed 80.03% – 97.03% drug content. As shown in **Fig. 10**, in the formulations of niosomes F11 formulation shows a better drug content of 97.03%.

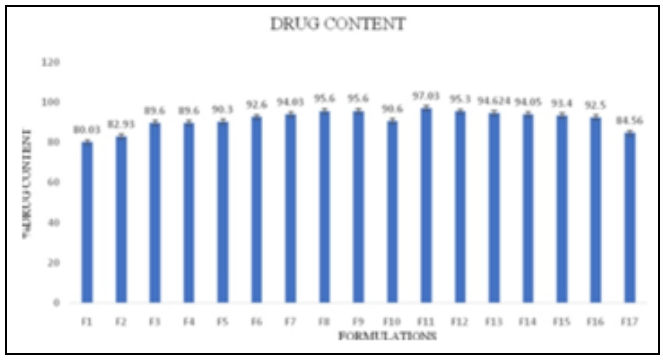

FIG. 10: DRUG CONTENT OF OPTIMIZED FORMULATIONS

***In-vitro* Release Studies:** Niosomal formulations are a type of niosomal formulation. The rate of drug release is dependent on the % of drug entrapment efficiency, according to experimental research. The F11 formulation (span 80, C/S; 10:120 -48.189 percent for about 8 hours) had a longer release time than the others (17.17 percent to 41.987 percent for about 8 hours) **Fig. 11** & **12.**

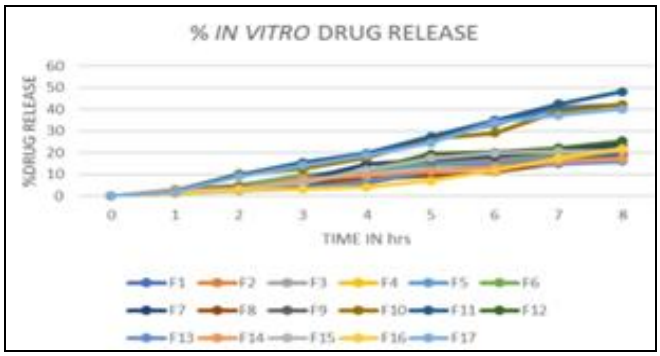

FIG. 11: COMPARISON OF *IN-VITRO* RELEASE STUDIES OF SPAN 80 NIOSOMES WITH DIFFERENT RATIOS

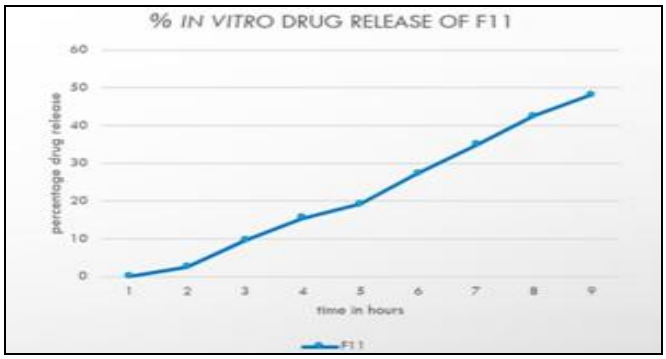

FIG. 12: *IN-VITRO* RELEASE STUDIES FOR F11

Stability Studies: The entrapment efficiency of the drug in the niosomal dispersion was estimated immediately after the preparation and after every

month for three months **Fig. 13**. The drug leakage from the vesicles was at least at 4ºC and 25ºC. This could be due to surfactant and lipid phase transitions, which cause vesicle leakage at extremely high temperatures during storage. As a result, the niosomes could be stored at both refrigeration and ambient temperature.

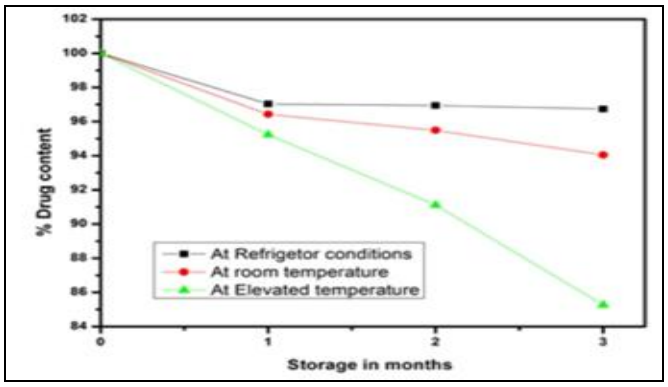

FIG. 13: STABILITY STUDIES OF GABAPENTIN NIOSOMES FORMULATION F11

CONCLUSION: The present study aimed to be optimization of the formulation and *in-vitro* evaluation of gabapentin niosomes for the treatment of epilepsy. Gabapentin is a highly water soluble low permeability BCS Class III drug. As a result, the current research focuses on increasing drug permeability and encapsulating the drug in niosomes vesicles for long-term central nervous drug delivery. We may deduce from this research that Gabapentin follows the Beers Law. The niosomes generated with span 80 are more niosome-like than those formed with tween 80.

ACKNOWLEDGEMENT: The authors would express their thanks to the Department of Pharmaceutics, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai-600116 Tamil Nadu, India for providing the encouragement facilities to complete this work.

CONFLICT OF INTEREST: The authors declare that No conflict of interest among us.

REFERENCES:

1. Aldawsari MF, Khafagy ES, Alotaibi HF & Abu Lila AS: Vardenafil-Loaded Bilosomal Mucoadhesive Sponge for Buccal Delivery: Optimization, Characterization, and In Vivo Evaluation. Polymers (Basel) 2022; 14.
2. Tony RM: Green Bio-Analytical Study of Gabapentin in Human Plasma Coupled with Pharmacokinetic and Bioequivalence Assessment Using UPLC-MS/MS. Separations 2023; 10.
3. Panebianco MV, Al-Bachari S, Hutton JL & Marson AG: Gabapentin add-on treatment for drug-resistant focal epilepsy. Cochrane database Syst. Rev 2021; 1: CD001415.
4. Khizer Z: Personalised 3D-Printed Mucoadhesive Gastroretentive Hydrophilic Matrices for Managing Overactive Bladder (OAB). Pharmaceuticals 2023; 16: 1–20.
5. Phillips SJ, Oliveto A, Mancino MJ & Hendrickson HP: Development and validation of a rapid liquid chromatography/tandem mass spectrometry method to quantitate gabapentin and buprenorphine in human serum. Rapid Commun. Mass Spectrom 2021; 35: 9104.
6. WO2006090208A1.pdf.
7. Moin A: Formulation, characterization, and cellular toxicity assessment of tamoxifen-loaded silk fibroin nanoparticles in breast cancer. Drug Deliv 2021; 28: 1626–1636.
8. Aldawsari MF, Khafagy ES, Moglad EH & Selim Abu Lila A: Formulation optimization, *in-vitro* and *in-vivo* evaluation of niosomal nanocarriers for enhanced topical delivery of cetirizine. Saudi Pharm J 2023; 31: 101734.
9. Shete MB, Deshpande AS & Shende PK: Nanostructured lipid carrier-loaded metformin hydrochloride: Design, optimization, characterization, assessment of cytotoxicity and ROS evaluation. Chem. Phys. Lipids 2023; 250: 105256.
10. Thabet Y, Elsabahy M & Eissa NG: Methods for preparation of niosomes: A focus on thin-film hydration method. Methods 2022; 199: 9–15.
11. Zaid Alkilani A: Formulation and evaluation of azithromycin-loaded niosomal gel: optimization, *in-vitro* studies, rheological characterization, and cytotoxicity study. ACS Omega 2022; 7: 39782–39793.
12. Hegdekar NY, Priya S, Shetty SS & Jyothi D: Formulation and Evaluation of Niosomal Gel Loaded with Asparagus racemosus Extract for Anti-inflammatory Activity. Indian J Pharm Educ Res 2023; 57: 63–74.
13. Bhattacharya S: Preparation and Evaluation of Diclofenac Sodium Niosomes Using Round Bottom Flask Method. Asian J Pharm 2020; 14: 188–194.
14. Khafagy ES, Almutairy BK & Abu Lila AS: Tailoring of Novel Bile Salt Stabilized Vesicles for Enhanced Transdermal Delivery of Simvastatin: A New Therapeutic Approach against Inflammation. Polymers (Basel) 2023; 15.
15. Gugleva V: Formulation and evaluation of hybrid niosomal *in-situ* gel for intravesical co-delivery of curcumin and gentamicin sulfate. Pharmaceutics 2022; 14: 1–23.
16. Barot T, Rawtani D & Kulkarni P: Development, characterization and *in-vitro in-vivo* evaluation of Farnesol loaded niosomal gel for applications in oral candidiasis treatment. Heliyon 2021; 7: 07968.

How to cite this article:
Rajeswari P, Jain AR, Akram M and Anbarasan B: Optimization of the formulation and *in-vitro* evaluation of gabapentin niosomes for the treatment of epilepsy. Int J Pharm Sci & Res 2024; 15(2): 594-99. doi: 10.13040/IJPSR.0975-8232.15(2).594-99.