FORMULATION AND EVALUATION OF LEVETIRACETAM NIOSOMES FOR IMPROVED ANTI-CONVULSANT ACTIVITY

Dissertation submitted to

The Tamilnadu Dr. M.G.R Medical University, Chennai

In partial fulfillment for the award of the Degree of

MASTER OF PHARMACY

(Pharmaceutics)

MARCH-2014

DEPARTMENT OF PHARMACEUTICS

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPPATTI ROAD,

COIMBATORE-641048

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Submitted by

MANNAM ASHOK KUMAR
Reg.No:261210906

Under the Guidance of

Dr. K.S.G. ARUL KUMARAN, M. Pharm., Ph.D.,
Head of the Department

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CERTIFICATE

This is to certify that the work as embodied in the dissertation entitled **"FORMULATION AND EVALUATION OF LEVETIRACETAM NIOSOMES FOR IMPROVED ANTI-CONVULSANT ACTIVITY"** submitted by **Reg. No: 261210906** is a bonafide work carried out by the candidate under the guidance of **Dr. K.S.G. Arulkumaran, M.Pharm., Ph.D.,** to The Tamil Nadu Dr. M.G.R. Medical University**,** Chennai, in partial fulfillment for the Degree of **Master of Pharmacy in Pharmaceutics** at the Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, Tamil Nadu during the academic year 2012-2014.

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Date:
Place: Coimbatore

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Department of Pharmaceutics.

DECLARATION

I hereby declare that this dissertation entitled **"FORMULATION AND EVALUATION OF LEVETIRACETAM NIOSOMES FOR IMPROVED ANTI-CONVULSANT ACTIVITY"** submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the award of Degree of **Master of Pharmacy in Pharmaceutics** was done by me under the institutional guidance of **Dr. K.S.G. Arulkumaran, M. Pharm., Ph.D.,** Head of the Department, Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, during the year 2012 – 2014.

Date: **MANNAM ASHOK KUMAR**

Place: Coimbatore

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"FORMULATION AND EVALUATION OF LEVETIRACETAM NIOSOMES FOR IMPROVED ANTI-CONVULSANT ACTIVITY"** Submitted By University **Reg.No:261210906** to **The Tamil Nadu Dr. M.G.R. Medical University**, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by the candidate at the department of pharmaceutics, KMCH College of Pharmacy, Coimbatore, and was evaluated by us during the academic year 2012 – 2014.

Examination Centre: KMCH College of Pharmacy, Coimbatore.

Date:

Internal Examiner **External Examiner**

Convener of Examination

KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India.

Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)
Institutional Animal Ethics committee (IAEC)

CERTIFICATE

Title of the Project	:	To compare the anti convulsant activity of Levetiracetam niosomes with a Levetiracetam plain in mice.
Proposal Number	:	KMCRET/M.Pharm / 08 /2013-14
Date received after modification (if any)	:	-NA-
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Approval date	:	21-12-2013
Animals	:	Wistar rats / Mice / Rabbit / Guinea pig
No. of animals sanctioned:	:	Male/Female - 24
Expiry date (Termination of the Project)	:	21-12-2014
Name of IAEC chairperson	:	**Dr. A. Rajasekaran**

Signature of IAEC Chairperson

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ACKNOWLEDGEMENT

My dissertation entitled **"FORMULATION AND EVALUATION OF LEVETIRACETAM NIOSOMES FOR IMPROVED ANTI-CONVULSANT ACTIVITY"** would not have been a feasible one without the grace of god almighty who gave me moral till the completion of my project.

I dedicate myself before the unfailing presence of **GOD** and constant love and encouragement given to me by my beloved **Father Koteswararao, Mother Ankalamma** and my remaining family members who deserve the credit of success in whatever work I did.

First and foremost it gives me great pleasure to record my deep sense of gratitude and indebtedness to my esteemed guide **Dr. K.S.G ARUL KUMARAN, M.Pharm., Ph.D.,** Professor, Department of Pharmaceutics, KMCH College of Pharmacy, for his constant insight, guidance, countless serenity, encouragement, support and facilities in all stages of study.

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MANNAM ASHOK KUMAR

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ABBREVIATIONS USED

e.g.	Example
i.e.	That is
%	Percentage
Kg.	Kilogram
gm.	gram
mg.	Milligram
µg.	Micro gram
ml.	Milliliter
cm.	Centimeter
mm.	Millimeter
nm.	Nanometer
W/w.	Weight by weight
W/v	weight by volume
avg.	Average
hrs.	Hours
pH.	Hydrogen ion concentration
^{o}C	Degree centigrade
RH.	Relative Humidity
HCL.	Hydrochloric acid
RPM.	Revolution per minute
Abs.	Absorbance
Conc.	Concentration
Fig.	Figure
UV-VIS	Ultra violet and visible spectroscopy
FTIR	Fourier Transform Infrared spectroscopy
DSC	Differential scanning calorimetry
TEM	Transmission Electron microscopic
SEM	Scanning electron micrography
CR	Cumulative Release
IR	Immediate Release
SR	Sustained Release
USP	United State Pharmacopoeia

BP	British Pharmacopoeia
R^2	Regression coefficient
FHM	Film hydration method
REV	Reverse phase evaporation method
RES	Reticulo-endothelial system
LEV	Levetiracetam

INTRODUCTION

NIOSOMES

Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether and cholesterol with subsequent hydration in aqueous media [1].

In Niosomes, the vesicles forming amphiphile is a non-ionic surfactant such as span-60 which is usually stabilised by addition of cholesterol and small amount of anionic surfactant such as dicetyl phosphate[2].

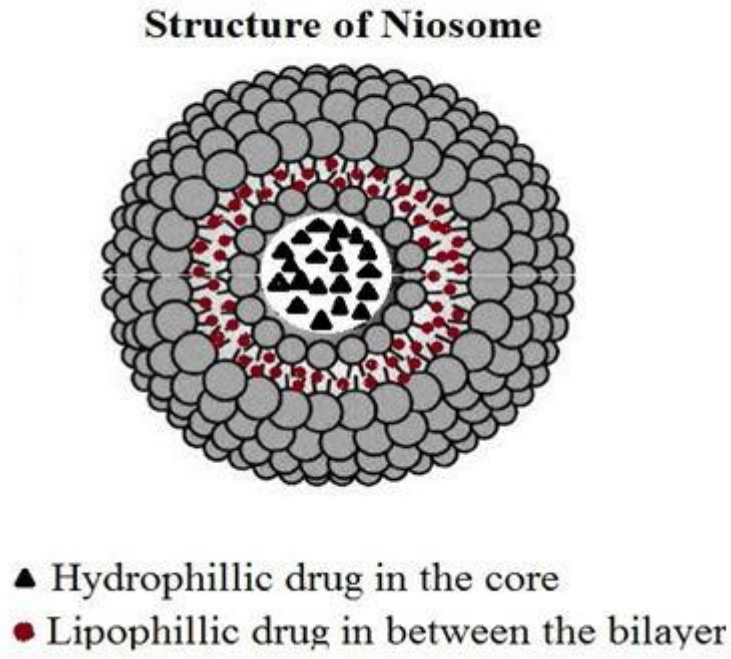


Fig 1: Structure of niosomes

ADVANTAGES OF NIOSOMES

a) Niosomes offer high patient compliance in comparison with oily dosage from. They possess an infrastructure consisting of hydrophilic, amphiphilic and lipophilic moieties together and as result can accommodate drug molecules with a

wide range as a result can accommodate drug molecules with a wide range of solubility.

b) The characteristics of the vesicle formulation are variable and controllable. Altering vesicle composition, size, lamellarity, taped volume, surface charge and concentration can control the vesicle characteristics

c) The vesicles may act as a depot, releasing the drug in a controlled manner

d) They are osmotically active and stable, as well as they increase the stability of entrapped drug.

e) Handling and storage of surfactants requires no special conditions.

f) They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs

g) They can be made to reach the site of action by oral, parenteral as well as topical routes.

PREPARATION METHODS OF NIOSOMES

A. Ether injection method[3,4]: This method provides a means of making Niosomes by slowly introducing a solution of surfactant dissolved in diethyl ether into warm water maintained at 60^{o}c. The surfactant mixture in ether is injected through 14-guage needle into an aqueous solution of material. Vaporization of ether leads to formation of single layered vesicles. Depending upon the conditions used the diameter vesicle range from 50 to 1000nm.

B. Hand shaking method (Thin film hydration technique) [4]: The mixture of vesicle forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20^{o}c) using rotator evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60^{o}c with gentle agitation. This process forms typical multilamellar Niosomes.

Thermosensitive niosomes can be prepared by evaporating the organic solvents at 60^{o}c and leaving a thin film of lipid on the wall of rotary flash evaporator

[5]. The aqueous phase containing drug is then slowly added with intermittent shaking of flask at room temperature followed by sonication.

C. Sonication [4]: In this method an aliquot of drug solution in buffer is added to the surfactant /cholesterol mixture in a 10-ml glass vial. The mixture is probe sonicated at 60^{o} c for three minutes using a sonicator with a titanium probe to yield niosomes.

D. Micro fluidization [6]: Micro fluidisation is a recent technique used to prepare unilamellar vesicles of defined size distribution. This method is based on submerged jet principle in which to fluidized streams interact at ultra high velocity, in precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheet long a common front is arranged such that the energy supplied to the system remains within the area of Niosomes formation. The result is a greater uniformity, smaller size and better reproducibility of Niosomes formed.

E. Multiple membrane extrusion method [6]: Mixture of surfactant, cholesterol and dicetyl phosphate in chloroform is made into thin film by evaporation. The film is hydrated with aqueous drug solution and the resultant suspension extruded through polycarbonate membranes, which are placed in series for up to 8 passages. It is a good method for controlling niosomes size.

F. Reverse Phase Evaporation Technique (REV) [5]: In this method the cholesterol and surfactant in 1:1 ratio are dissolved in a mixture of ether and chloroform. An aqueous phase containing drug is added to this and the resulting two phases are sonicated at 4-5^{o}c. The clear gel is formed is further sonicated after the addition of small amount of phosphate buffered saline (PBS). The organic phase is removed at 40^{o}c under low pressure. The resulting viscous Niosomes suspension is diluted with PBS and heated on water bath at 60 c for 10 min to yield Niosomes.

G.Trans membrane p^{H} gradient(inside acidic)Drug Uptake Process (remote Loading) [7]: Surfactant and cholesterol are dissolved in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask. The film is hydrated with 300 mM citric acid (p^{H}4) by vortex mixing. The multi lamellar vesicle are frozen and thawed three times and sonicated. To this niosomal suspension aqueous solution containing 10 mg /ml of drug is added and

vortexed. The p^H of sample is then raised to 7-7.2 with 1M disodium phosphate. The mixture is later heated at 60°c for 10 minutes to give niosomes.

H. The "Bubble" Method [8]: It is noval technique for the one step preparation of liposomes and Niosomes without the use of organic solvents. The bubbling unit consists of round bottomed flask with three necks positioned in water bath to control the temperature. Water cooled reflex and thermometer is positioned in the first and second neck and nitrogen supply through the third neck. Cholesterol and surfactant are dispersed together in this buffer (p^H 7.4) at 70°c, the dispersion mixed for 15 seconds with high shear homogenizer Are immediately afterwards "bubbled" at 70°c using nitrogen gas.

I. Formation of Niosomes from proniosomes [9]: Another method of producing Niosomes is to coat a water-soluble carrier such as sorbitol with surfactant. The result of the coating process is a dry formulation. In which each water-soluble particle is covered with a thin film of dry surfactant. This preparation is termed "proniosomes". The niosomes are recognised by the addition of aqueous phase at T>Tm and brief agitation.

T= Temperature.

Tm= mean phase transition temperature.

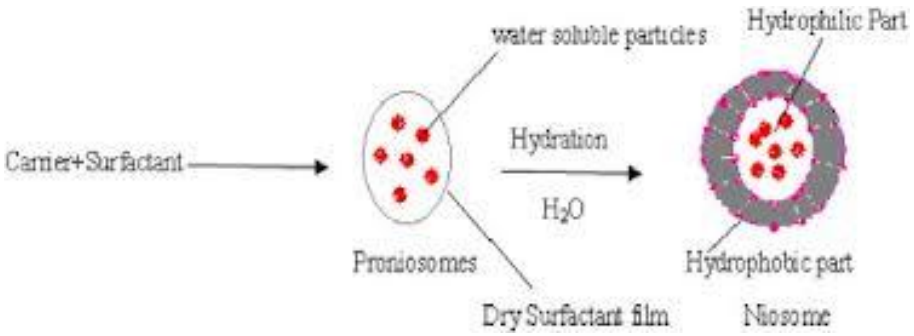


Fig 2: Figure showing the formation of niosomes from proniosomes

Table1:

Brief example of some drugs incorporated into niosomes using different methods

Method of preparation	Drug incorporated
Ether injection	Sodium stibogluconate Doxorubicin
Hand shaking	Methotrexate Doxorubicin
Sonication	9-desglycinamide 8-arginine Vasopressin Oestradiol

SEPERATION OF UNENTRAPPED DRUG

The removal of unentrapped from the vesicles can be accomplished by various techniques, which include:

1. Dialysis [8]: The aqueous nisomal dispersion is dialyzed in a dialysis tubing against phosphate buffer or normal saline or glucose solution.
2. Gel Filtration [9,10]: The unentrapped drug is removed by gel filtration of niosomal dispersion through a cephadex-G-50 column and elution with phosphate buffered saline or normal saline.
3. Centrifugation [11,12]: The niosomal suspension is centrifuged and the supernatant is separated. The pellet is washed and then resuspended to obtain a niosomal suspension from unentrapped drug.

CHARECTERISATION OF NIOSOMES

a. **Entrapment efficiency**: After preparing niosomal dispersion, unentrapped drug is separated by dialysis, centrifugation, or gel filtration as described above the drug remind entrapped in Niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% triton x-100 and analysing the resultant solution by appropriate assay method for the drug. Where,

Entrapment efficiency (EF) = (Amount entrapped total amount) x 100

b. **Vesicular diameter:** Niosomes, similar to liposomes, assumes spherical shape and so their diameter can be determined using light microscopy, photon-correlation microscopy and freeze fracture electron microscopy.

Freeze thawing [6] (keeping vesicles suspension at-20^{o}c for 24 hours and then heating to ambient temperature) of Niosomes increase the vesicle diameter, which might be attributed to fusion of vesicles during the cycle.

c. ***In vitro* release**: A method in vitro release rate study includes the use of dialysis tubing. A dialysis sac is washed and soaked in distilled water. The vesicle suspension is pippeted into a bag made up of tubing and sealed. The bag containing the vesicles is placed in 200 ml of buffer solution in a 250 ml beaker with constant shaking at 25^{o}c or 37^{o}c. At various time intervals, the buffer is analysed for the drug content by an appropriate essay method [9].

Factors affecting vesicles size, entrapment efficiency and release characteristics:

a. **Drug:** Entrapment of drug in Niosomes increases vesicle size, probably by intraction of solute with surfactant head groups, increase in the charge and mutual repulsion of the surfactant bilayers, thereby increasing vesicle size [9,12]. In polyoxyethylene glycol (peg) coated vesicles, some drug is entrapped in the long peg chains, thus reducing the tendency to increase the size [11]. The hydrophilic lypophylic balance of the drug effects degree of entrapment.

b. **Amount and type of surfactant:** The mean size of niosomes increases proportionally with increase in the HLB of surfactants like Span 85 (HLB 1.8) to

Span 20 (HLB 8.6) because the surface free energy decreases with an increase in hydrophobicity of surfactant [9].

The bilayers of the vesicles are either in the so-called liquid state or in gel state, depending on the temperature, the type of lipid or surfactant and the presence of other components such as cholesterol. In the gel state, alkyl chains are present in a well ordered structure, and in the liquid state, the structure of the bilayers is more disordered. The surfactants and lipids are characterized by the gel-liquid base transition temperature (TC) [9].Phase transition temperature (TC) of surfactant also affects entrapment efficiency i.e. Span 60 having higher TC, provides better entrapment.

c. **Cholesterol content and charge:** inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency [9]. In general, the action of cholesterol is two folds; on one hand, cholesterol increases the chain order of liquids-state bilayers and on the other, cholesterol decreases the chain orders by gel state bilayers. At a high cholesterol concentration, the gel state is transformed to a liquid-ordered phase.

An increase in cholesterol content of the bilayers resulted in a decrease in the release rate of encapsulated material and therefore an increase of rigidity of the bilayers obtained [12,13,14]. Presence of charge tends to increase the interlamellar distance between successive bilayers in multilamellar vesicle structure and leads to greater overall entrapped volume.

d. **Methods of preparations:** Hand shaking method forms vesicles with greater diameter (0.35-13 nm) compared to the ether injection method (50-1000 nm) [6].

Small sized niosomes can be produced by reverse phase evaporation (REV) method [5,15]. Microfluidization [6] method gives greater uniformity and small size vesicles.

e. **Resistance to osmotic stress:** Addition of a hypertonic salt solution to a suspension of niosomes brings about reduction in diameter. In hypotonic salt solution, there is initial slow release slight swelling of vesicle probably due to

inhibition eluting fluid from vesicles, followed by faster released, which may be due to mechanical loosening of vesicles structure under osmotic stress [1,16].

APPLICATIONS OF NIOSOMES

Niosomal drug delivery is potentially applicable to many pharmacological agents for their actions against various diseases. Some of their therapeutic applications are discussed below.

1) Targeting on bioactive agents

a. **To reticulo-endothelial system (RES):** The cells of RES preferentially take up the vesicles. The uptake of niosomes by the cells is also by circulating serum factors known as opsonins , which mark them for clearance such localized drug accumulation has however, been exploited in treatment of animal tumors to metastasize to the liver and the spleen and in parasitic infestation of liver [1].

b. **To organs other than RES**: It has been suggested that carrier system can be directed to specific site in the body by use of antibodies [17]. Immunoglobulins seem to bind quite readily to the lipids surface, thus offering a convenient means for targeting drug carrier [18]. Many cells posses the intrinsic abilities to recognise and bind particular carbohydrates determinants and these can be exploited to direct carrier system to particular cell.

2. Neoplasia: Doxorubicin, the anthracyclic antibiotic with broad spectrum antitumor activity, shows a dose – dependent irreversible cardiotoxic effect. Niosomal delivery of this drug to mice bearing S-180 tumor increased their life span and decreased the rate of proliferation of sarcoma [19]. Niosomal entrapment increased the half-life of the drug, prolonged its circulation and altered its metabolism. Intra venous administration of methotrexate entrapped in niosomes to S-180 tumor bearing mice resulted in total regression of tumor and also higher plasma level and slower elimination [21,20].

3. Leishmaniasis: Niosomes can be used for targeting of drug in the treatment of diseases in which the infecting organism resides in the organ of reticulo –

endothelial system. Leishmaniasis is such a disease in which parasite invades cells of liver and spleen. The commonly prescribed drugs are antimonials, which are related to arsenic, and at high concentration the damage the heart, liver and kidney.

4. **Delivery of peptide drugs:** Yoshida *et al* [14] investigated oral delivery of 9-desglycinamide, 8-arginine vasopressin entrapped in niosomes in an in vitro intestinal loop model and reported that stability of peptide increased significantly

5. **Immunological applications of niosomes:** Niosomes have been used for studying the nature of the immune response provoked by antigens. Brewer and Alexander [22] have reported niosomes as potent adjuvant in terms of immunological selectivity, low toxicity and stability.

6. **Niosomes as carriers for Hemoglobin:** Niosomes can be used as carrier for hemoglobin. Niosomal suspension shows a visible spectrum of superimposable on to that of free hemoglobin. Vesicles are permeable to oxygen and hemoglobin dissociation curve can be modified similarly to non-encapsulated haemoglobin[23,24].

7. **Transdermal delivery of drugs by niosomes:** Slow penetration of drug through skin is the major drawback of transdermal route of delivery an increase in the penetration rate has been achieved by transdermal delivery of drug incorporated in niosomes Jayraman *et al* [25] has studied the topical delivery of erythromycin from various formulations including niosomes or hairless mouse. From the studies, and confocal microscopy, it was seen that non-ionic vesicles could be formulated to target pilosebaceous glands.

8. **Sustained release:** Azmin *et al* [26] suggested the role of liver as a depot for methotrexate after niosomes are taken up by a liver cell. Sustained release action of niosomes can be applied to drugs with low therapeutic index and low water solubility since those could be maintained in the circulation via niosomal encapsulation.

9. **Localized drug action:** Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and low penetrability through

epithelium and connective tissue keeps the drug localised at the site of administration.

Localized drug action results in enhancements of efficacy of potency of the drug and at the same time reduces its systemic toxic effects. (Eg.) Antimonials encapsulated within niosomes are taken up by mononuclear cells resulting in localization of drug, increase in potency and hence decrease both in dose and toxicity[8,27].

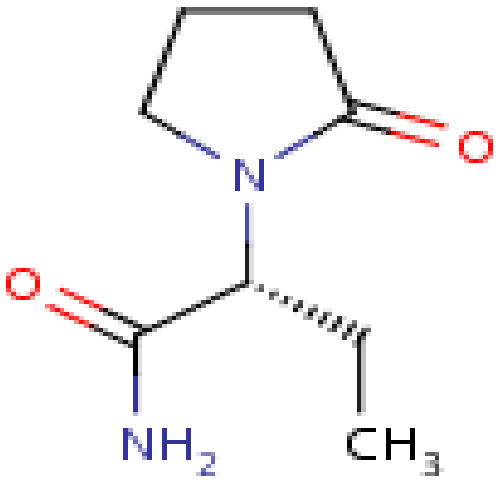
DRUG PROFILE

Name : Levetiracetam

Brand Name : Keppra

Levetiracetam is an anticonvulsant medication used to treat epilepsy.

Structure Levetiracetam :



IUPAC Name : (2R)-2-(2-oxopyrrolidin-1-yl)butanamide

Chemical Formula : $C_8H_{14}N_2O_2$

Molecular weight : 170.209g/mol

Half life : 6-8 hr

Solubilty : Very soluble in water, Freely soluble in Chloroform, Freely soluble in Methanol, Soluble in Ethanol, Sparingly soluble in acetonitrile.

Categoties : Anticonvulsants

Nootropic Agents

Available Form : 250 mg Tablets, 500 mg tablets.

PHARMACOLOGY

Mechanism of action

The precise mechanism(s) by which levetiracetam exerts its antiepileptic effect is unknown. The antiepileptic activity of levetiracetam was assessed in a number of animal models of epileptic seizures. Levetiracetam did not inhibit single seizures induced by maximal stimulation with electrical current or different chemoconvulsants and showed only minimal activity in submaximal stimulation and in threshold tests. Protection was observed, however, against secondarily generalized activity from focal seizures induced by pilocarpine and kainic acid, two chemoconvulsants that induce seizures that mimic some features of human complex partial seizures with secondary generalization. Levetiracetam also displayed inhibitory properties in the kindling model in rats, another model of human complex partial seizures, both during kindling development and in the fully kindled state. The predictive value of these animal models for specific types of human epilepsy is uncertain. Levetiracetam is thought to stimulate synaptic vesicle protein 2A (SV2A), inhibiting neurotransmitter release.

PHARMACOKINETICS

Absorption

Rapidly and almost completely absorbed after oral administration (99%). Peak plasma concentrations occurring in about an hour following oral administration in fasted subjects.

Metabolism

The major metabolic pathway of levetiracetam (24% of dose) is an enzymatic hydrolysis of the acetamide group. No CYP450 metabolism detected.

Excretion

Sixty-six percent (66%) of the dose is renally excreted unchanged. The metabolites have no known pharmacological activity and are renally excreted. The mechanism of excretion is glomerular filtration with subsequent partial tubular.

Toxicity

Side effects include aggression, agitation, coma, drowsiness, reduced consciousness, slowed breathing.

Table No2: Pharmacokinetic profile of Levetiracetam

Pharmacokinetic Characters	**Levetiracetam**
bioavailability (%)	~ 100%
protein binding (%)	<10%
Volume of distribution(L/Kg)	Not Available
% metabolized	24%
Elimination $T^{1/2}$ (hr)	6-8hrs

EXCIPIENT PROFILE

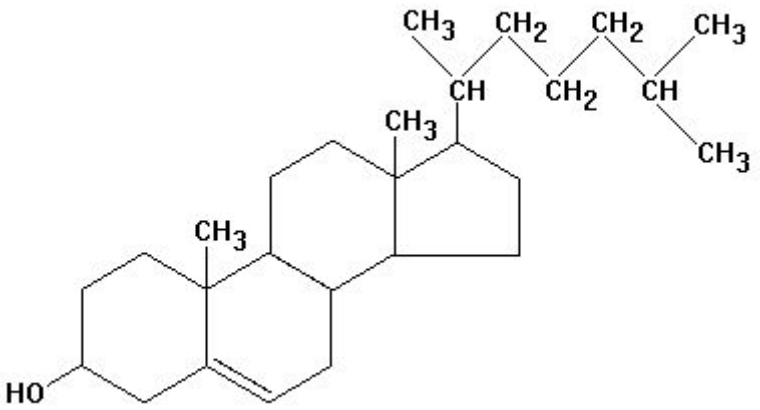
CHOLESTEROL

Name: Cholesterol

Description:

The principal sterol of all higher animals, distributed in body tissues, especially the brain and spinal cord, and in animal fats and oils.

Structure of Cholesterol:



Chemical Formula : $C_{27}H_{46}$

Molecular weight : 386.6535 g/mol

Melting point : 148.5^{o}c

Boiling point : 360^{o}c

Water solubility : 0.095 mg/L (at 30^{o}c)

SPAN 40

Synonym **: Sorbitan monopalmitate (Span 40)**

Chemical Formula **:** $C_{22}H42O_6$

Molecular weight **:** 402.57 g/mol

Structure of Span 40:

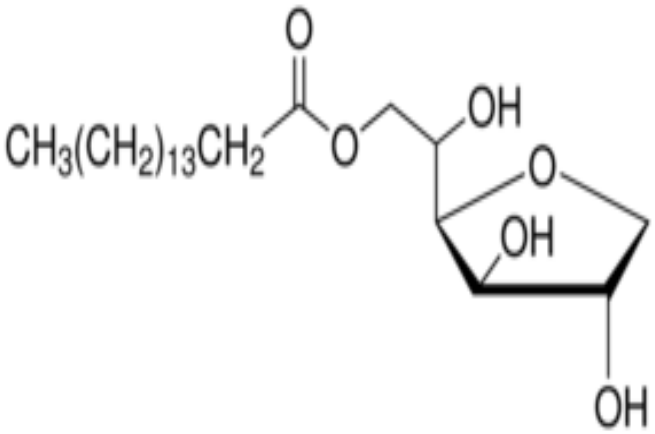


Table 3:

Properties of Span 40

Related Categoties	Biochemical and Reagents, Cleaners, Detergents.
Description	Non-ionic
Melting point	46-47^{o}c(lit)
HLB value	6.7±10

SPAN 60

Synonym : **Sorbitane monostearate, sorbitan stearate**

Chemical Name : Span 60

Chemical Formula : $C_{24}H_{46}O_6$

Description : Span 60 is a Sorbitane monostearate then is used as a non-ionic detergent.

Structure of Span 60:

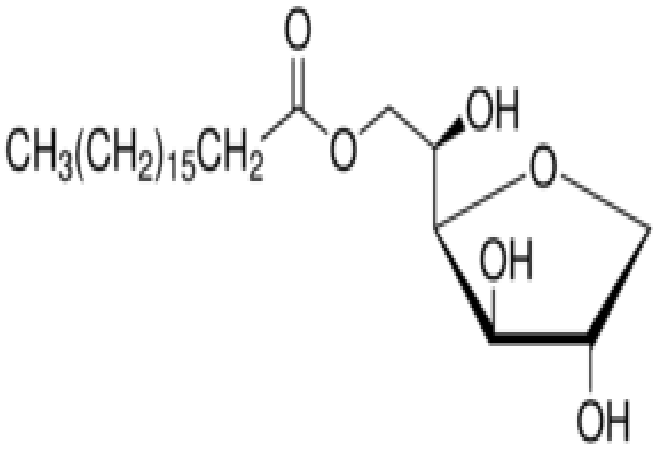


Table 4:

Properties of Span 60

Related Categories	Biochemical and Reagents, Cleaners, Detergents, Detergents N toZ,Nonionic.
Description	Non-ionic
HLB value	4.7

Application:

Span 60 has been used in a study to assess encapsulation of doxorubicin in niosomes as a route to tumor targeting. It has also been used in a study to investigate the use of non-ionic surfactants as contrast agents for use in diagnostic ultrasounds.

LITERATURE REVIEW

- **Raja K. *et al*[29]** Formulated and evaluated the Maltodextrin based Proniosomal Drug Delivery System containing Anti-diabetic (Glipizide) drug to avoid many of the problems associated with aqueous niosome dispersions and problems of physical stability (aggregation, fusion, leaking) could be minimized. Maltodextrin-based proniosomes are a potentially scalable method for producing niosomes for delivery of hydrophobic or amphiphilic drugs. The optimized formulation developed using the desirability approach produced high drug encapsulation efficiency and sustained anti-diabetic activity in rats following oral administration.

- **Rajesh Z. Mujoriya**, **Kishordhamonder *et* al[30]** Niosomes drug delivery system. Niosomes are thoughts to be better candidate's drug delivery as compared to liposomes due to various factors like cost, stability etc. Various type of drug deliveries can be possible using niosomes like targeting, ophthalmic, topical, parentral, etc. Niosomes are thoughts to be better candidate's drug delivery as compared to liposomes due to various factors like cost, stability etc. Various type of drug deliveries can be possible using niosomes like targeting, ophthalmic, topical, parentral, etc.

- **Anchal Sankhyan and Pravin Pawar[31]** Recent Trends in Niosome as Vesicular Drug Delivery System. The article focuses on various advantages of vesicular systems (niosomes) to develop the effective delivery system to achieve maximum effective concentration. Niosomes, nonionic surfactant vesicles with lamellar structure which may be unilamellar and multilamellar serve to be efficient in providing these required advantages. Niosomes are novel drug delivery system which offers a large number of advantages over other conventional and vesicular delivery systems. From the above compilation of work it can be concluded that niosomes have suitability for encapsulating a varied variety of drugs and also the benefits offered by niosomes are also widely exploited. Niosomes have evolved for treatment of many dreadful diseases efficiently with reduced side effects and better patient compliance. Thus niosomes present itself as a versatile tool in therapeutics.

- **P. Prabhu *et al*** [32] Prepared and evaluated the niosomes of brimonidine tartrate as ocular drug delivery system by film hydration method and evaluated for photomicroscopic characteristics, entrapment efficiency, *in vitro, ex- in vitro* drug release, in vivo intra ocular pressure lowering activity. The *in vitro and ex- in vitro* drug release studies showed that, there was slow and prolonged release of drug from all the formulations and followed zero order kinetics.

- **Sabarikumar K. *et al*[33]** Performed bioavailability enhancement of aceclofenac niosomes containing surfactants and cholesterol. The aim of the study was to develop and evaluate niosomal formulation of Aceclofenac in order to improve its bioavailability. The results of *in vitro* drug release studies showed that formulation (ANF-3) has better modified and extended over release of drug (92.19%) for 72hrs. ANF-3 emerged as the most satisfactory formulation in so far as its properties were concerned.

- **N. Pavala Rani *et al*** [34] Formulated and evaluated rifampicin and gatifloxacin niosomes on logarithmic-phase cultures of *Mycobacterium tuberculosis*. The bactericidal activities of the niosomal formulation were studied by BACTEC radiometric method using the resistant strains (RF 8554) and sensitive strains (H37Rv) of *Mycobacterium tuberculosis* which showed greater inhibition and reduced growth index. Niosomes of rifampicin and gatifloxacin were prepared by lipid hydration technique using rotary flash evaporator. The *in vitro* release study showed that 98.98% and 97.74% of release of rifampicin and gatifloxacin niosomes respectively.

- **Darwish, I.A. *et al*** [35] Evaluated crown ether based niosomes as cation containing and cation sensitive drug delivery systems. The release of the fluorescent marker rhodamine B from PCE/CHOL niosomes was slightly increased by the addition of calcium ions but remained unaffected by the addition of sodium ions. This is thought to be due to a slightly greater ease of divalent cation chelation by the crown ether head groups when compared to the monovalent cation, the latter of which are more highly solvated in aqueous solution. This is the first study on the effect of ions on the release properties of crown ether based niosomes. These systems may be developed as cation containing or cation sensitive release systems for drug delivery and other industrial uses.

- **Ahmed N. Allam *et al*** [36]Formulated and evaluated the Acyclovir niosomes for ophthalmic use. Niosomes are prepared using two different methods namely: film hydration method (FHM) and reverse phase evaporation method (REV). The study includes qualitative ocular irritation testing. The data obtained from corneal permeation studies showed that the cumulative amount permeated from most niosomal formulations was lower than that from drug solution, except with those corresponding to cholesterol: surfactant molar ratio 1:1.

- **Vijay D. Wagh *et al*** [37] Formulated and evaluated itraconazole niosomes drug delivery system and its antimycotic activity against *Candida albicans*. Formulated niosomes were evaluated for vesicle size, entrapment efficiency, drug release, skin permeation, and antimycotic activity. Itraconazole niosomes were having larger zone of inhibition than marketed formulation when activity was checked against *C. albicans*.

- **Vyasjigar* *et al*** [38] Formulated and evaluated of topical niosomal gel of erythromycin in various parameter. Erythromycin is macrolide antibiotic used commonly for the treatment of acne either single (or) in combination. The finding of this investigation have conclusively demonstrated that encapsulation of Erythromycin in to niosomal gel formulatin improves skin retention which may be reflected, based on prior hypothesis, as improved therapeutic response and reduce adverse symptoms.

- **V. Lokeswara Babu *et al*** [39] Formulated and evaluated by using different Novel Drug Delivery System (NDDS) which are used for targeting drugs to different organ system and for controlled/sustained release of drug from the dosage form Niosomal Drug Delivery System is suitable for encapsulating toxic anti cancer drugs, anti-infective drugs,anti AIDS drugs etc.

- **Loscher W *et al*** [40] Antiepileptogenic effect of the novel anticonvulsant levetiracetam in the kindling model of temporal lobe epilepsy. We have previously shown that novel anticonvulsant levetiracetam exerts potent anticonvulsant activity against both focal and generalized seizures in fully amygdale-kindled rats, i.e., a model of temporal lobe epilepsy. We determined the pharmokokinetics of the drug after i.p. injection. Levetiracetam hada relative short half-life 2-3 hr.when rats were treated with

levetiracetam during kindling acquisition at daily i.p. doses of 13,27 or 54 mg/kg, the drug dose dependently suppressed the increase in seizure severity and duration induced by repeated amygdale stimulation. Adverse effect were not observed at any dose of levetiracetam tested in kindled rats.

- **Amish Ashvinkumar Dangi *et al*** [41] Formulation and Evaluation of Colon Targeted Drug Delivery System of Levetiracetam using Pectin as Polymeric Carrier: The aim of the present work was to develop and evaluate colon specific sustained release tablet using levetiracetam (LEV), microbially degradable polymeric carrier (pectin), coating material and matrix forming polymers. Pectin, drug and physical mixture were evaluated for incompatibility study by Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). All the batches of matrix tablet (F1-F4) were subjected for *in-vitro* dissolution in various simulated gastric fluids for suitability for colon specific drug delivery system. The studies confirmed that, the designed formulation could be used potentially for colon delivery by controlling drug release in stomach and the small intestine. The prepared tablets met the compendia limits in terms of physiochemical parameters and dissolution studies. As a result, colon delivery of levetiracetam appeared to be a promising alternative to traditional drug administration routes.

- **Balaiah *et al*** [42] Formulation Development and *In-Vitro* Characterization of Oral Levetiracetam: Levetiracetam is a second-generation antiepileptic agent useful in the treatment of partial onset and myoclonic seizures, which has short plasma half-life of 7 ± 1 hour in adults along with bitter taste and faint odor. Preformulation studies were carried out to rule out any drug-polymer interactions by DSC technique. In the in vitro release studies initial burst release was observed from all the formulations. The most satisfactory formulation released drug for 24hours. SEM studies of the most satisfactory formulation showed that the microspheres were spherical and porous in nature. The data obtained from in vitro release showed highest correlation with Higuchi model and the drug release was found to be diffusion controlled.the physico-chemical parameters and in vitro drug release profile requirements for an oral controlled release microsphere formulation of leviteracetam, in addition to masking the bitter taste and faint odor of the drug.

- **Hasan H. Sonmezturk *et al*** [43] Levetiracetam Extended Release as Adjuvant Therapy for the Control of Partial-onset Seizures. The precise mechanism of action is unknown. Animal studies showed binding to synaptic vesicle protein SV2A, thought to be involved in modulating synaptic neurotransmitter release. LEV-IR is proven effective as adjunctive therapy for partial-onset seizures, It was shown to be equivalent to carbam azepine as first-line treatment for partial-onset seizures. The extended release formulation added advantages such as better tolerance and increased compliance. LEV-XR is demonstrated to be safe and effective add-on treatment for partial onset seizures in adult patients.

- **Sakthivel *et al*[44]** Formulation and *Invitro* Evalution of Niosomes contaninng Oxacarbazepine. Niosomes are the novel vesicular drug delivery system by which we can achieve the constant plasma drug concentration for the extended period of time. Oxcarbazepine niosomes were prepared by thin film hydration method using span60 in order to achieve prolonged circulation time and sustained release. From this study it was observed that the formulation F-II showed satisfactory particle size 230-275nm, entrapment efficiency 58.87% and *in vitro* release 78.08% for the period of 16 hours. Thus the niosomal formulation could be a promising delivery system for Oxcarbazepine with improved anticonvulsant activity, stability and sustained drug release profile. Niosomes containing Oxcarbazepine were formulated using different surfactants such as span 40, span 60 and span 80 and evaluated for various parameters. Thus the prepared noisome could be promising delivery system for Oxcarbazepine with sustained drug release profiles.

- **Tim De Smedt *et al*[45]** Levetiracetam: Part II, the Clinical Profile of a Novel Anticonvulsant Drug. The objective of this article was to review and summarize the available reports on the profile of the novel anticonvulsant drug levetiracetam (LEV) in a clinical setting. This article is devoted to the clinical pharmacology and clinical trials of LEV investigating its efficacy and safety as add-on therapy or monotherapy for various seizure types. LEV is shown to be a safe, broad-spectrum anticonvulsant drug with highly beneficial pharmacokinetic properties, a favorable long-term retention rate, and a high responder rate, indicating that LEV is an efficient therapeutic option for the treatment of several types of epilepsy. In a clinical setting, LEV has proven to be a well tolerated and

efficacious ACD against a broad range of seizure types. a broad range of seizure types. In conclusion, LEV is a safe and unique addition to ACDs used in clinical practice.

- **S. Poongothai *et al*[46]** A Sensitive Dissolution Test Method for the Development and Validation of Levetiracetam Tablets by Reverse Phase-HPLC Technique. This study describes the development and validation of dissolution tests for levetiracetam tablets using a reverse phase high performance liquid chromatography method. Optimal conditions to carry out the dissolution tests were 900 mL of purified water as dissolution medium, paddle at 50 rotations per minute (rpm) stirring speed for tablets and detection was carried out at 217 nm. The obtained results provided adequate dissolution profiles. The HPLC method was validated to meet requirements for a global regulatory filing and also to quantify levetiracetam tablets from the dissolution tests.

- **V. Pola Chandu *et al*[47]** Niosomes: A Novel Drug Delivery System. Niosome are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or their lipids. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. Niosomes can entrap both hydrophilic and lipophilic drugs and can prolong the circulation of the entrapped drug in body. This review article focuses on the advantages, Disadvantages, preparation methods, factors affecting, characterizations, invitro methods, drug release kinetics, and applications of noisome.

- **Kandikonda Saikrishna *et al*.**[48] New Rp-Hplc Method For The Determination of Levetiracetam In Bulk And Prepared Tablets. A simple Reverse Phase HPLC method was developed for the determination of LEVETIRACETAM (LCM) present in bulk and prepared tablets. An ODS C18 (250cm ´4.6 mm) column from Shimadzu in gradient mode, with mobile phase acetonitrile: KH2PO4 buffer [0.05M] (50:50) was used. The proposed method is precise, accurate, reproducible and rapid for the determination of Levetiracetam in bulk and their pharmaceutical dosage forms.

AIM AND OBJECTIVE

AIM:

- To formulate and evaluate Levetiracetam niosomes and compare its anti-convulsant activity with the pure drug.

OBJECTIVE:

- To prepare and evaluate Levetiracetam niosomes .
- The need for present study is to encapsulate the drug in the niosomes vesicles for effective central nervous system drug delivery for the prolonged period of time.
- To compare the anti convulsant activity of Levetiracetam niosomes with a Levetiracetam pure drug.
- To formulate Levetiracetam in niosomal drug delivery there by dose can be minimised and also to achieve sustained release for a prolonged period of time.
- To carry out the stability studies on the optimised formulation of niosomes as per ICH guidelines.

PLAN OF WORK

- **Pre-formulation studies**
    - ✓ Solubility: To study the solubility behaviour of the drug with different solvent.
    - ✓ FTIR: Identification of levetiracetam in pure drug by FTIR technique.
    - ✓ DSC: To observe the compatibility of the product and excipients used in the formulations.
- Optimization of the formula**:**
    - ✓ Optimization of excipients (surfactants).
    - ✓ Optimization of rotating speed.
    - ✓ Optimization of temperature.
- To formulate the levetiracetam niosomes with different surfactants by liquid hydration technique using Rotator Flash Evaporator.
- To characterize the formulated niosomes by following methods.
    - ✓ Surface Morphology.
    - ✓ Particle size distribution of the prepared niosomes.
    - ✓ Determination of entrapment efficiency.
- To test the stability of the formulated niosomes.
- To test the sterility of the formulated niosomes.
- To examine In-vitro diffusion study of the niosomes prepared with different in the surfactants and to compare the drug release profile.
- To test the anti-convulsant activity of the formulated niosome with the formulation having best drug release.
- The animals are to be weighed numbered. They should be divided in to 4 groups each consisting of 4-6 mice. one group will be used as control and the other group for treatment.
- The animal should be hold properly, the corneal electrode will be placed on the cornea and prescribed current will be applied.

➢ The different stages of convulsion are to be noted (i.e).

- ✓ Tonic flexation
- ✓ Tonic extension
- ✓ Clonic convulsion

- ✓ Stupor
- ✓ Recovery (or) death.

- The time spent by the animal in each phase of convulsion is to be noted.
- The experiment will be repeated with other animal of control group.
- Drug sample will be injected intraperitonially to a group of mice and after 30 min, the animal will be subjected to electro convulsion as described in step-2.
- The reduction in abolism of tonic extension of MES convulsion will be noted.

MATERIALS AND METHODOLOGY

Table No 5: List of Materials used

S.NO.	MATERIALS USED	MANUFACTURER
1.	Levetiracetam	Lupin
2.	Cholesterol	Qualigens Fine Chemicals, Mumbai
3.	Span 40	Kemphasol
4.	Span 60	Kemphasol
5.	Chloroform	Ranbaxy
6.	Diethylether	Qualigens Fine Chemicals, Mumbai
7.	Buffer (pH 7.4)	Laboratory preparation
8.	Mice	Seiss Albino mice (25-30)

Table No 6: List of Equipments used

S.NO.	EQUIPMENTS	MANUFACTURER
1	Rotary Flash Evaporator	Equitron Roteva,Medica Instrument Mfg-Co, Mumbai
2	Ultra Sonicator	Vibronics
3	UV-Double Beam Spectrophotometer	UV-Pharmaspec 1700, Shimadzu
4	Electronic Balance	Sartorius
5	pH Meter	Elico LI 120
6	Magnetic stirrer	Remi- 1MLH
7	Environment stability testing chamber	"HECO". Environment chamber
8	FTIR	Perkin Elmer
9	Centrifuge	Remi
10	Scanning Electronic Microscope	JEOL, JSM -6701 F, Japan
11	Particle size Analyzer	Microtrac – Bluewave US
12	Anti convulsometer	Kmch Laboratory

METHODOLOGY

PREFORMULATION STUDY

Preformulation study is one of the important prerequisites in development of any drug delivery system. Thus, a preformulation study was carried out to check the compatibility between drug and various excipients and development of analytical method of drug.

DRUG EXCIPIENT COMPATIBILITY STUDIES

FT-IR spectra matching approach was used for detection of any possible chemical interaction between drug and excipients. A physical mixture (1:1:1) of drug,cholesterol and surfactants (span40,span60) was prepared and mixed with the suitable quantity of potassium bromide. About 100mg of mixture was compressed to form a transparent pellet using a hydraulic press at 6 tons pressure. It was scanned from 4000 to 400 cm^{-1} in FT-IR spectrometer. The IR spectrum of the physical mixture was compared with those of pure drug and excipients and matching was done to detect any appearance or disappearance of peaks. This study was done by using SHIMADZU FT/IR spectrum.

DIFFERENTIAL SCANNING CALORIMETER

The DSC curves of levetiracetam, Cholesterol, and surfactant (span 40,span 60) Physical mixture of Levetiracetam were obtained using differential scanning calorimeter (DSC Q20 V24.2 Build 107) increasing heating rate at 10˚ C/min and heated over a temperature range of 50˚ C to 250˚ C in an atmosphere of nitrogen (20ml/min). Accurately twelve mg of sample was taken in a hermetically sealed, flat bottom aluminum sealed pan and placed at sample stage and thermograms were recorded.

PREPARATION OF STANDARD CURVE FOR LEVETIRACETAM NIOSOMES

Preparation of phosphate buffer pH 7.4

Dissolve 125 ml of 0.2M potassium dihydrogen phosphate and 195.5 ml 0.2M sodium hydroxide in 500 ml of distilled water.

Determination of λmax of Levetiracetam

50 mg of accurately weighed drug was dissolved in the phosphate buffer pH 7.4 which upon suitable dilution, analyzed in UV spectrophotometer between 200 to 400 nm. The point of absorption maximum obtained in the graph was considered as the λ_{max} of the pure drug.

Preparation of standard curve

- 100mg of Levetiracetam pure drug was dissolved in 100 ml of phosphate buffer pH 7.4 (solution A).
- From the solution A, 2.5 ml was pipetted out and made up to 25 ml with phosphate buffer pH 7.4 (solution B).
- From the solution B, serial dilutions were made to produce 0.5, 1, 1.5, 2, 2.5, 3, and 5µg/ml concentrations.
- These samples were analysed spectrophotometrically at 220 nm usin phosphate buffer pH 7.4 as blank.

FORMULATION DEVELOPMENT OF NIOSOMES CONTAINING LEVETIRACETAM

Levetiracetam niosomes were prepared by Thin film hydration technique by handshaking method.

According to this method, accurately weighed quantity of cholesterol and non-ionic surfactant were dissolved in 5ml of chloroform and poured into a round bottom flask. The flask was rotated at 1.5 cm above a water bath at $60^oC\pm 2^0c$ under reduced pressure, until all the organic phase evaporated and a thin layer was formed on the wall of a round bottom flask. Then accurately weighed quantity of drug was dissolved in 5ml of phosphate buffer. The dried non- ionic surfactant and cholesterol film was subsequently hydrated with this drug solution and the mixture was rotated by immersing in a water bath at $60^oC\pm 2^0c$ for 1 hour until a good dispersion of mixture was obtained. The niosomes vesicles containing were subsequently formed. The suspension was then sonicated to form unilamellar vesicles.

Preparation of Niosomes

Hand shaking method (Thin film hydration technique)[4]: The mixture of vesicle forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20^{o}c) using rotator evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60^{o}c with gentle agitation. This process forms typical multilamellar Niosomes.

Thermosensitive niosomes can be prepared by evaporating the organic solvents at 60^{o}c and leaving a thin film of lipid on the wall of rotary flash evaporator. The aqueous phase containing drug is then slowly added with intermittent shaking of flask at room temperature followed by sonication.

Table No 7: Formulation Chart of Niosomes Containing Levetiracetam

Formulation code	**FI**	**FII**
Amount of Levetiracetam	5mg	5mg
Cholesterol	5mg	5mg
Span40	5mg	-
Span 60	-	5mg
Chloroform	5ml	5ml
Phosphate Buffer	5ml	5ml
Cholesterol surfactant ratio	1:1	1:1

Table 8: Formulation code

S. No	Formulation code	Type Of Surfactant Used
1.	FI	Span 40
2.	FII	Span 60

EVALUATION OF LEVETIRACETAM NIOSOMES

Optical microscopy[29]

Optical microscopy shows niosomes under magnification of 40X.

Transmission Electron microscopic observation[72]

The niosomal dispersion was spread on the glass slide using a glass rod. Formation of multilamellar vesicles was confirmed by examining the niosomal suspension under an transmission electron microscope with the magnification power of 71 lacks Sx.

Vesicle shape[42]

The vesicle shape of best formulation F2 was determined by using scanning electron microscopy.

Entrapment efficiency[41]

An1ml of the sample is taken and centrifuged at 13000 RPM at 4^0c for 60 minutes using Eppendorf centrifuge. Supernatant was separated without disturbing the sediment layer using micropipette. Then the supernatant layer (free drug) was diluted using PBS pH 7.4 and analysed using UV spectrophotometer at 220 nm.

Percent drug entrapment = amount of drug entrapped/initial amount of drug*100

In vitro diffusion study of Levetiracetam niosomes[71]:

The *in vitro* release of niosomes was studied by using simple diffusion cell apparatus. The diffusion cell apparatus consists of a glass tube with an inner diameter of 2.5 cm, open at both ends, one end of the tube is tied with cellophane membrane. The

cellophane membrane soaked in glycerin for 6-8 hours. It was clamped carefully between donor and receptor compartment. Niosomes equivalent to 5mg of Levetiracetam was taken in a dialysis tube and placed in 200ml of Phosphate buffer 7.4. The medium was stirred by using the magnetic stirrer and the temperature was maintained at 37 ± 2^0C. Periodically, 5 ml of samples were withdrawn and after each withdrawal same volume of medium was replaced. Then the samples were assayed spectrophotometrically at 220 nm using phosphate buffer as blank.

RELEASE KINETICS [70]

The results of *in vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows.

1. Log cumulative percent drug remaining versus time (first order kinetic model)
2. Cumulative percent drug release versus time (zero order kinetic model)
3. Cumulative percent drug release versus square root of time (Higuchi's model)

Drug release kinetics- model fitting of the diffusion data

It is necessary to ensure that drug diffusion occurs in an appropriate manner. Drug diffusion from solid dosage forms has been described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time, t or Q = f (t). Some analytical definitions of the Q (t) function are commonly used such as zero order, first order, Higuchi, models. Other release parameters, such as diffusion time ($t_{x\%}$), diffusion efficacy (ED), difference factor *(f_1),* similarity factor *(f_2)* can be used to characterize drug diffusion / release profile.

Zero order kinetics

A zero-order release would be predicted by the following equation.

$$\mathbf{A_t = A_o - K_o t} \qquad \text{eq…….. (1)}$$

Where,

A_t = Drug release at time" t"

A_o = Initial drug concentration

K_o = Zero-order rate constant (hr)

When the data is plotted as cumulative percent drug release versus time if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to k_o.

Use: The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a prolonged pharmacological action.

First order kinetics

A first order release would be predicted by the following equation.

$$\text{Log } C = \text{Log } Co - K_t / 2.303 \qquad \text{eq}\ldots\ldots (2)$$

Where

C = Amount of drug remained at time" t"

C_o = Initial amount of drug

K_t = First-order rate constant

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line indicating the release follows first-order kinetics, the constant k can be obtained by multiplying 2.303 with slope values

Use: The pharmaceutical dosage forms containing water-soluble drugs in porous matrices, follows this type of diffusion profile. The release of the drug is proportional to the amount of drug remaining in its interior so that the amount of drug release by unit of time diminishes.

Higuchi model

Drug release from the matrix devices by diffusion has been described by following higuchi's classical diffusion equation.

$$\mathbf{Q = [DE/ \tau(2A- EC_s) C_{st}]} \qquad \text{eq} \quad (3)$$

Where,

Q = Amount of drug release at time" t"

D = Diffusion coefficient of the drug in the matrix

A = Total amount of drug in unit volume of matrix

C_s = The solubility of the drug in the matrix

E = Porosity of the matrix

T = Time in hrs at which q is the amount of drug is release

Equation 3 may be simplified if one assumes that D, Cs and A are constant. Then equation-3 becomes

$$\mathbf{Q = K_t ½} \qquad \text{eq} \quad (4)$$

When the data is plotted according to equation-4 i.e. cumulative drug release versus Square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to k.

Use: The relation can be used to describe the drug diffusion from several types of modified release pharmaceutical dosage forms, as in case of some water soluble drugs.

STABILITY STUDIES[41-42]

Pharmaceutical preparations or products often may exhibit physical or chemical reactions and that may end in instability. Due to such instability, the preparation gets deteriorated. This deterioration may lead to

(a) Reduction in the activity of a preparation.
(b) Formation of toxic products.
(c) An inelegant product.

Apart from these effects, microbial contamination also may cause deterioration of the product. Ultimately, the product becomes unacceptable. Hence it is necessary to perform stability testing to find out the extent of deterioration or degradation and to ensure the degradation has not exceeded an acceptable level assuring,

1) The safety of the patient and
2) The activity of the product.

So, the formulated niosomes were subjected for stability studies for a period of three months. The formulated niosomes were divided into 3 portions. First portion was

kept at refrigeration ($4^0C \pm 1^0C$) temperature. Second portion was kept at room temperature. Third portion was kept at $40^0C \pm 2^0C$, 70% ±5 %. Optimized niosomal formulation was selected for stability studies of vesicles.

STERILITY TEST[69]

- ❖ 0.2ml of given sample of 10% concentration of medium is used and inoculated into three tubes, i.e 3 blanks, 3 possitive controls and 3 negative controls.
- ❖ Nutrient media used to check the presence of aerobic and anerobic organisms.
- ❖ Sabourands agar medium is used to check fungi kept at 37°C.
- ❖ Incubate and observe the growth after 24 hours.
- ❖ All the media are adjusted to P^H (7.2-7.6) and sterilized by autoclaving at 121°C for 20 minutes. Sterility of these media should also be tested.
- ❖ All above tests are conducted and observed for 7 days.

ANTICONVULSANT ACTIVITY[68]

- The animals are to be weighed numbered. They should be divided in to 4 groups each consisting of 4-6 mice. one group will be used as control and the other group for treatment.
- The animal should be hold properly, the corneal electrode will be placed on the cornea and prescribed current will be applied.
- The different stages of convulsion are to be noted (i.e).
    i. Tonic flexation
    ii. Tonic extension
    iii. Clonic convulsion
    iv. Stupor
    v. Recovery (or) death.
- The time spent by the animal in each phase of convulsion is to be noted.
- The experiment will be repeated with other animal of control group.
- Drug sample will be injected intraperitonially to a group of mice and after 30 min, the animal will be subjected to electroconvulsion as described in step-2.

- The reduction in abolism of tonic extension of MES convulsion will be noted.

Aimals : Seiss Albino mice (25-30)

Gender : Male/Female

Number to be used : 24

Duration : 1 month

Table 9: Grouping of Animals

Group	Treatment : Dose and Route of Administration	No of Animals (mice)
1	Control (only vehicle)	6
2	Only MES induced	6
3	MES with standard (Levetiracetam plain)	6
4	MES of Levetiracetam niosomes	6

RESULTS & DISCUSSION

Table 10: Solubility Studies of Levetiracetam

SOLVENTS	SOLUBILITY
Water	Highly soluble in water
Diethyl ether	Highly soluble in water
Chloroform	Freely soluble in water
Methanol	Freely soluble in water
Ethanol	soluble in water
Acetonitrile	Sparingly soluble in acetonitrile

DRUG EXCIPIENT COMPATIBILITY STUDIES

Figure 3: FT-IR Spectrum of Levetiracetam pure drug

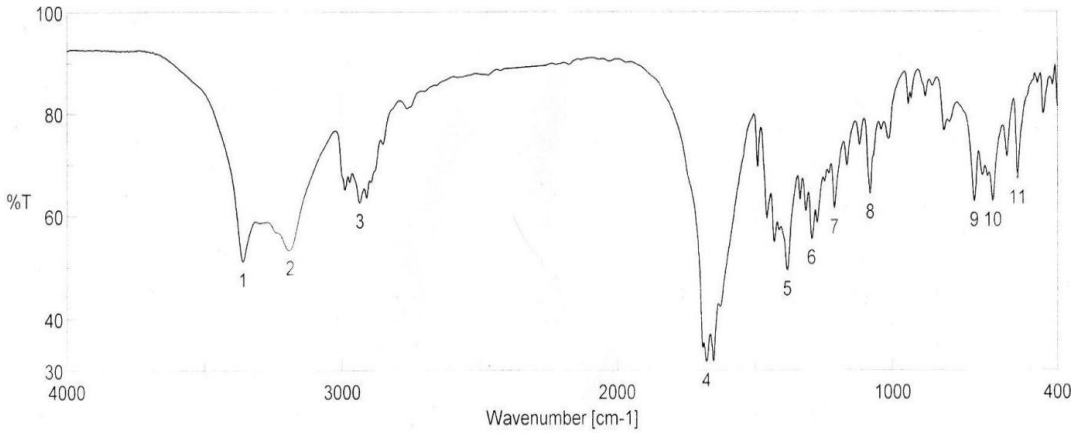


Table No 11: FT-IR Spectrum of Levetiracetam pure drug

Peak	**Group**
3360	Aliphatic primary amine
3187,2937	CH Stretching
1675	O=C-NH_2
703.89,636	Out of plane N-H Wagging

Figure 4: FT-IR Spectrum of Cholesterol

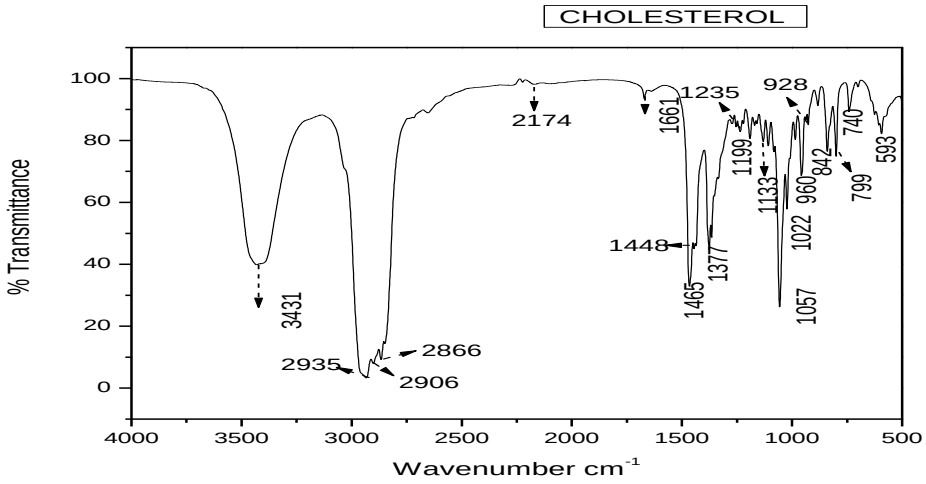


Figure 5: FT-IR Spectrum of Span 40

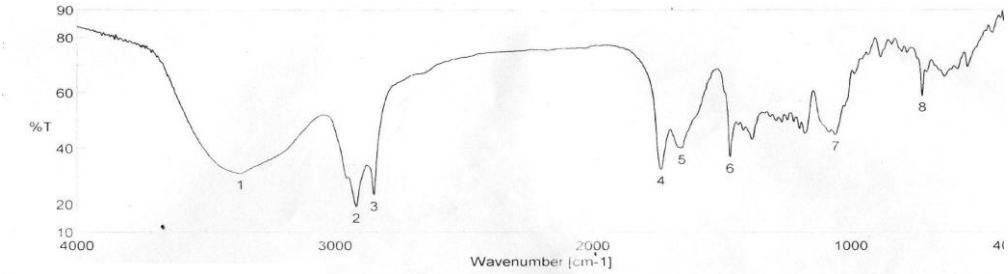


Table No 12: FT-IR Spectrum of Span 40

Peak Tr	Group
3366	Aliphatic primary amine
2917,2849	CH Stretching vibration
1735	O=C Stretching vibration
1654	O=C-NH_2 Group

Figure 6: FT-IR Spectrum of Span 60

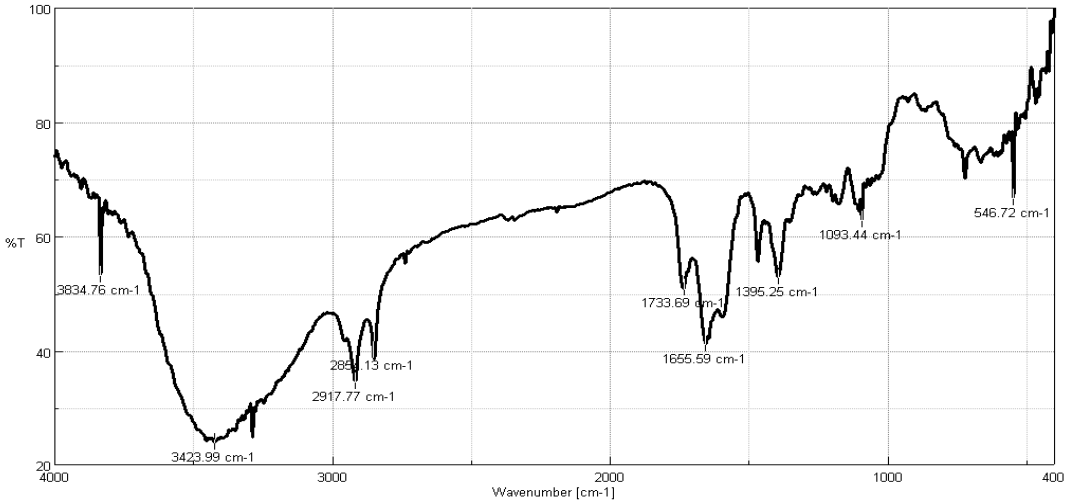


Figure 7: FT-IR Spectrum of Drug+ Cholesterol+Span40

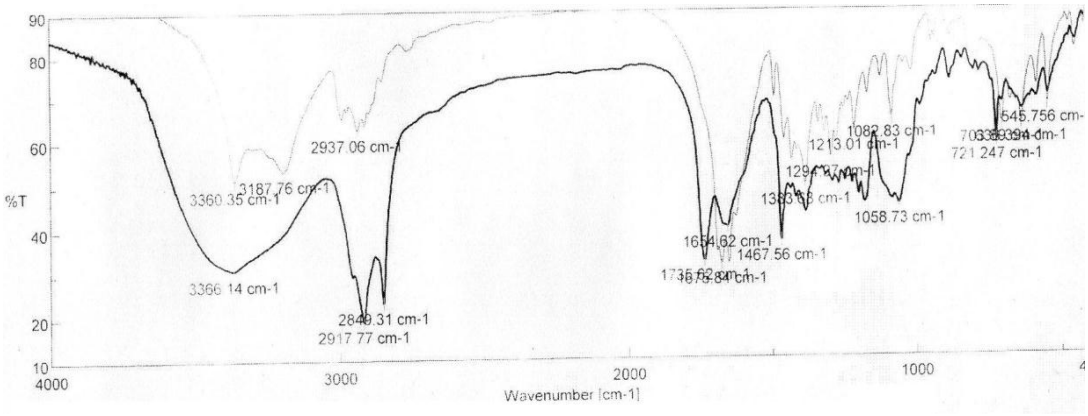
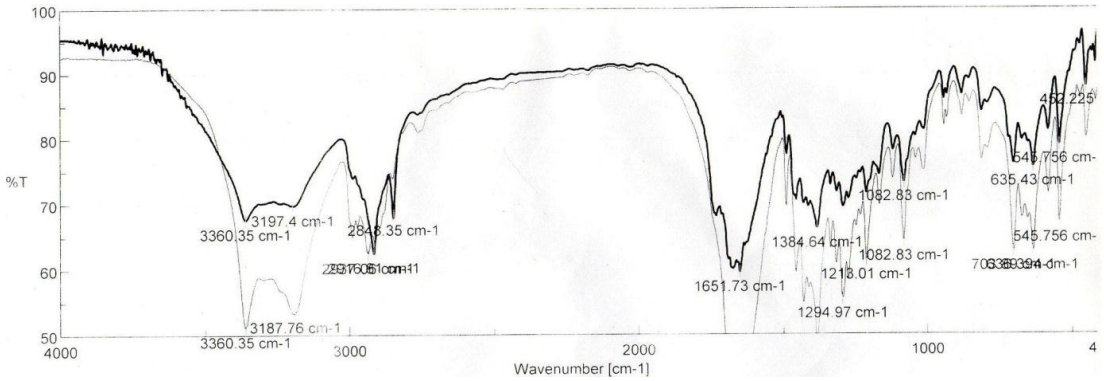


Table No 13: FT-IR Spectrum of Drug+ Cholesterol+Span40

Peak	Group
3360	Aliphatic primary amine
3187,2937	CH Stretching
1675	$O=C-NH_2$
703.89,636	Out of plane N-H Wagging

Figure 8: FT-IR Spectrum of Drug+ Cholesterol+Span60



DIFFERENTIAL SCANNING CALORIMETRY STUDIES

DSC thermogram of Levetiracetam and mixture's are depicted in **(Figure 9, 10, 11, 12, 13, 14)**, respectively. The thermogram of pure drug, cholesterol, span40, span60 exhibited a sharp endothermic peak at 192.53˚ C, 140.76 ºC, 53.14 ºC, 57.84 ºC. Corresponding to its melting point respectively,while the mixture's exhibited a broad endothermic peak at 51.96˚C, 56.80ºC. The DSC thermogram of cholesterol , span40, span60 and levetiracetam mixture showed identical peaks corresponding to pure drug indicated the absence of well defined chemical interaction between the drug and the excipients.

Figure 9: DSC of Levetiracetam pure drug

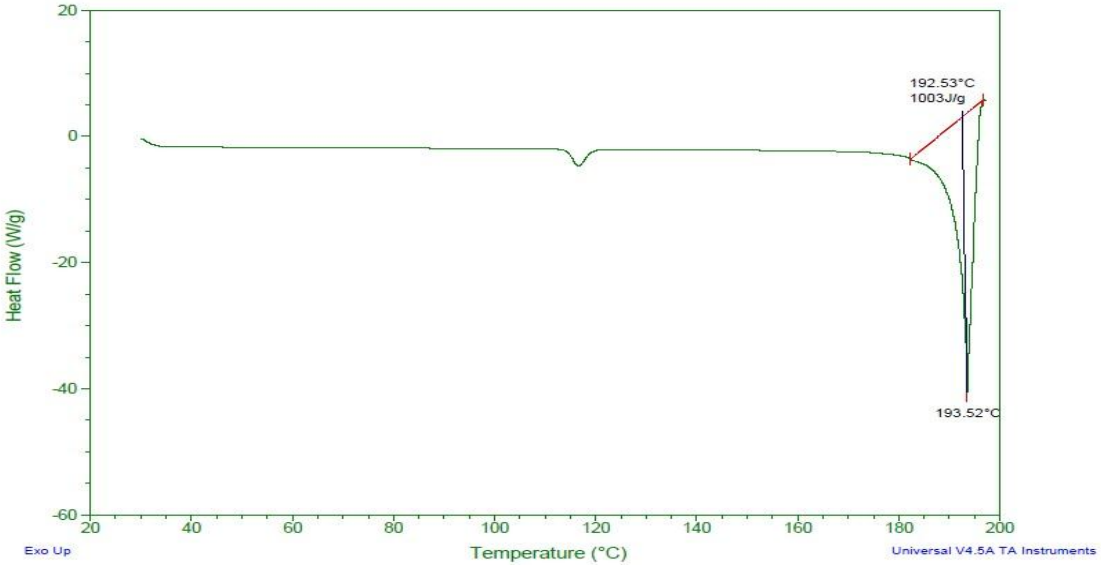


Figure 10: DSC of Cholesterol

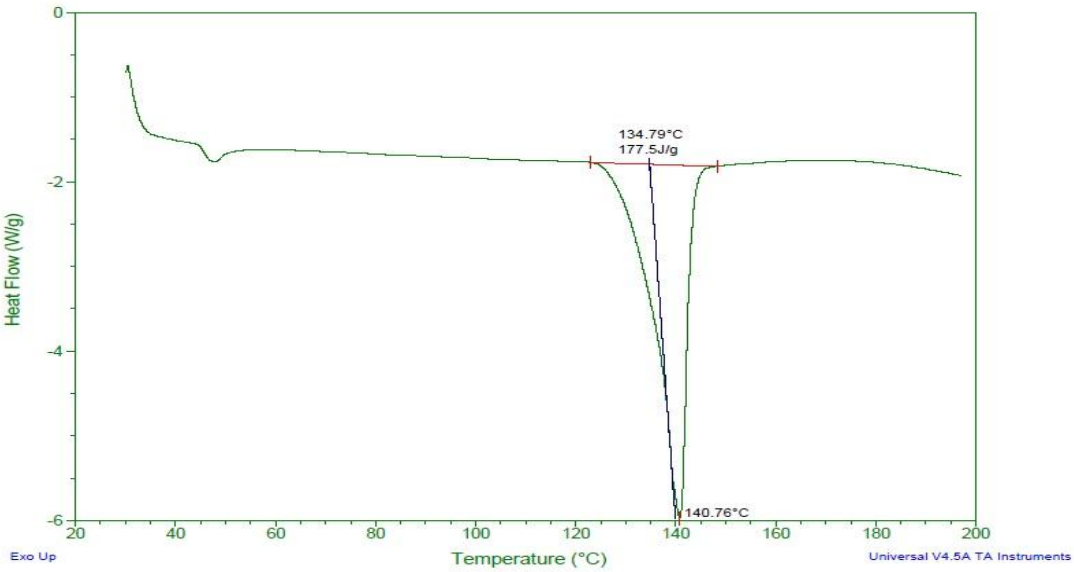


Figure 11: DSC of Span 40

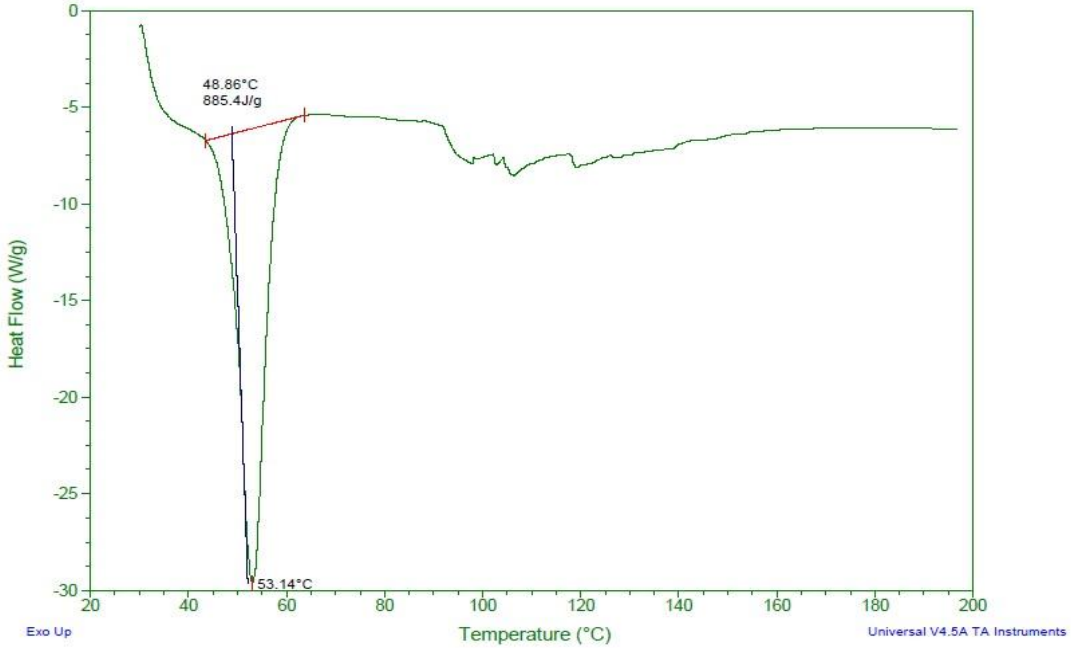


Figure 12: DSC of Span 60

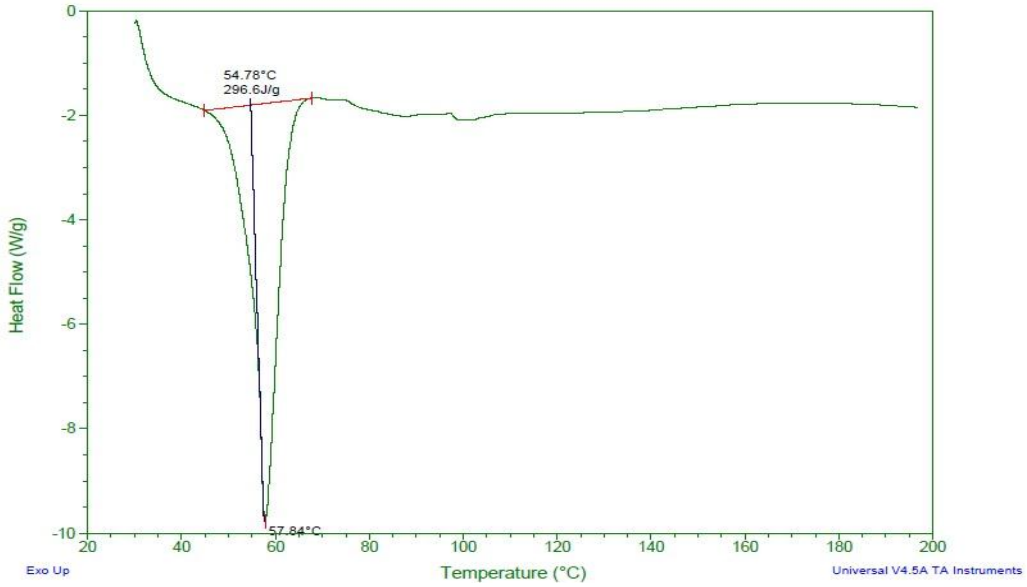


Figure 13: DSC of Drug+ Cholesterol+Span40

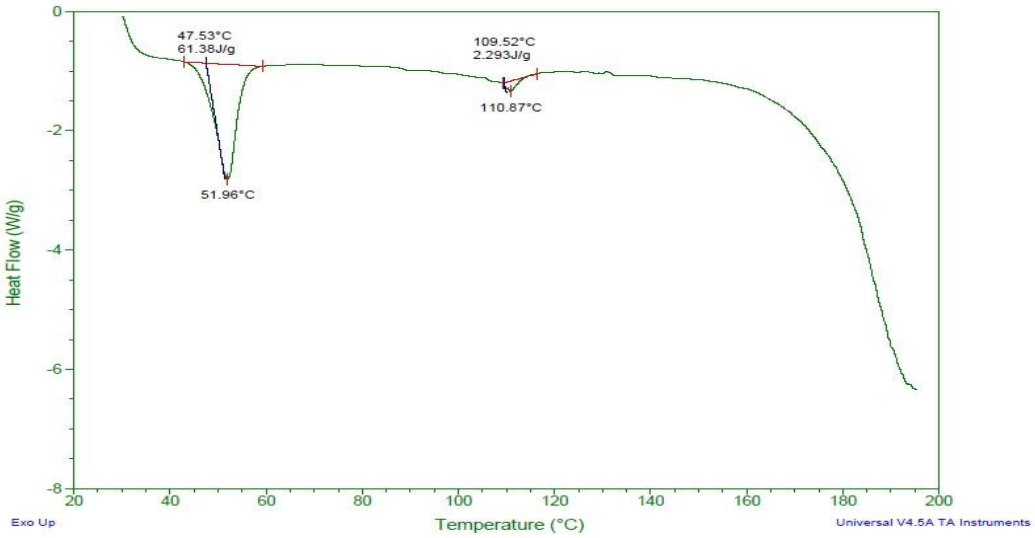


Figure 14: DSC of Drug+ Cholesterol+Span60

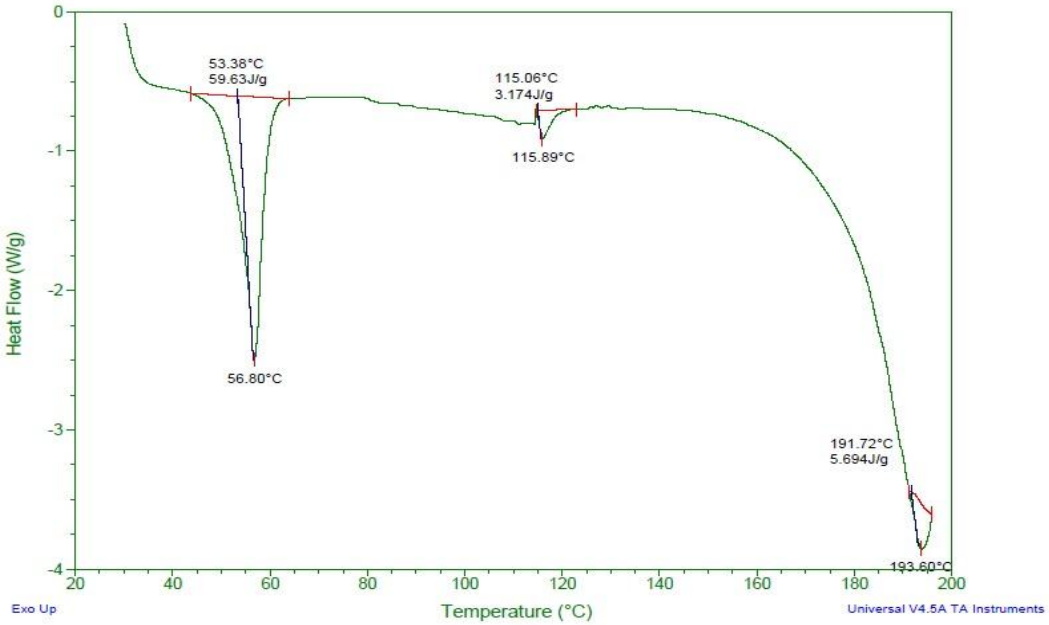


Table 14: DSC Observed for the Drug and excipients

S.NO	SAMPLE	MELTING POINT
1	Levetiracetam	192.53^{o}C
2	Cholesterol	140.76^{o}C
3	Span 40	53.14^{o}C
4	Span 60	57.84^{o}C
5	Drug+Cholesterol+Span 40	51.96^{o}C
6	Drug+Cholesterol+Span 60	56.80^{o}C

CALIBRATION CURVE OF LEVETIRACETAM

A calibration curve for Levetiracetam was constructed in pH 7.4 phosphate buffer, wave length at 220nm using UV Spectrophotometer. The linearity of calibration curve was found to be in the range of 5-30µg/ml. A regression coefficient value of 0.992 was noticed for Levetiracetam.

Figure 15: Standard calibration curve of Levetiracetam

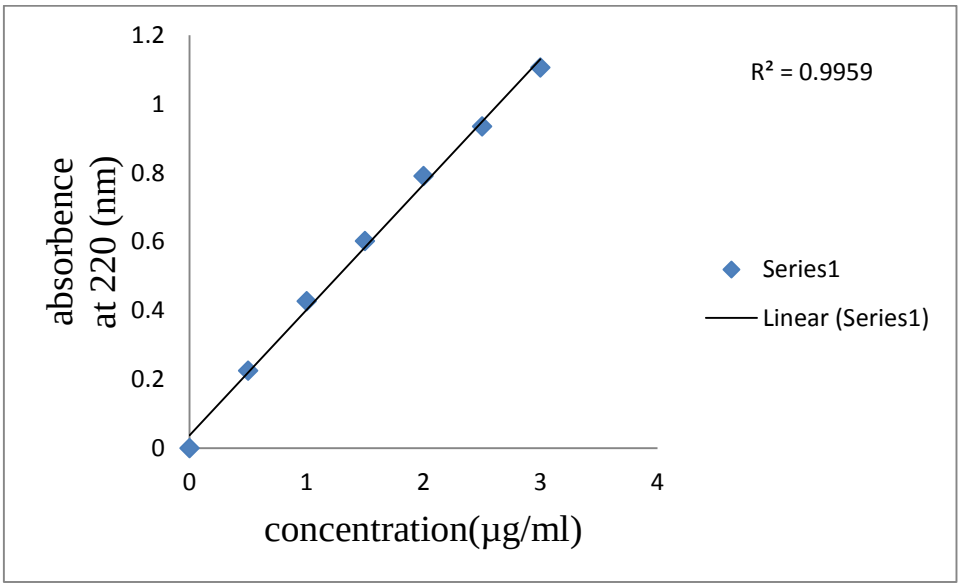


Table No 15: Calibration curve of Levetiracetam

Concentration(µg/ml)	Absorbance at 220nm
0.5	0.225
1	0.427
1.5	0.602
2	0.791
2.5	0.935
3	1.106

Table 16: Optimization of temperature during niosomes preparation

S.NO.	TEMPERATURES	OBSERVATION
1.	Below 60^0 C	Irregular Shaped vesicles were formed.
2.	60^0 C± 2^0 C	Spherical vesicles were formed.
3.	Above 80^0 C	Less and irregular vesicles were formed.

Table 17: Optimization of Rotating speed

S.NO	RPM	OBSERVATION
1.	Below 80	Clumped vesicles were formed.
2.	100 ± 2	Spherical vesicles were formed.
3.	Above 100	Irregular shaped vesicles were formed.

Table 18: Optimization of temperature during Ultrasonication

S.NO.	TEMPERATURES	OBSERVATION
1.	Below 10^0 C	Small sized vesicles were formed.
2.	10-20^0 C	Numerous vesicles of moderate size.
3.	Room temperature	Large sized vesicles.

Table 19: Optimization of Ultrasonication time

S.NO.	TIME	OBSERVATION
1.	10 Mins	Incomplete and large sized vesicles were formed.
2.	15 Mins	Spherical vesicles were formed.
3.	30 Mins	Broken vesicles were formed.

Table 20: Selection of Surfactant

S.NO.	SURFACTANT	OBSERVATION
1.	Span 40	Less vesicles were formed.
2.	Span 60	Good number of vesicles were formed.

Formulation development of Niosomes containing Levetitracetam

Various formulations of Niosomes were developed for Levetiracetam by Hand shaking method. The formulation of Levetiracetam niosomes was also prepared by thin film hydration technique. Before the formulation of Niosomes were subjected to internal morphology, vesicle shape are given in Figure:-17-18 and entrapment efficiency values are given in Table No: 22.

EVALUATION OF PHYSICO CHEMICAL PARAMETERS

Optical microscopy

Optical microscopy observed niosomes under magnification of 40X of F1 and F2.The scale is 50 µm.

FORMULATION 1 **FORMULATION 2**

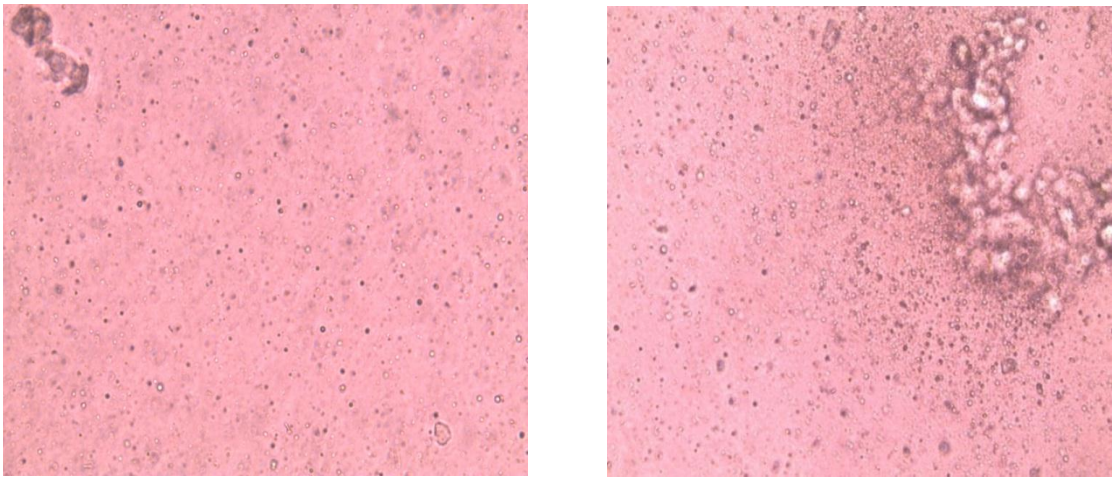


Fig 16: Optical microscopy A) Formulation 1 B) Formulation 2

Transmission Electron microscopic observation

Formation of multilamellar vesicles was confirmed by examining the niosomal suspension under an transmission electron microscope with the magnification power of 71 lacks Sx and observe the internal morphology.

FORMULATION 1 **FORMULATION 2**

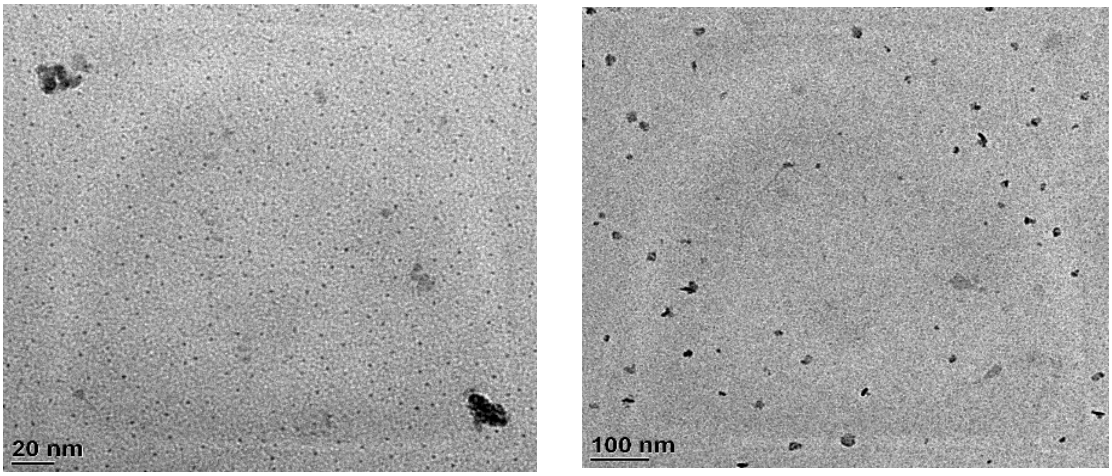


Fig 17: Transmission Electron microscopic observation

Vesicle shape by SEM Technique

The formulated niosomal vesicles were confirmed by scanning electron microscopy in the. It was found to be spherical in shape with smooth surface.

FORMULATION 1 **FORMULATION 2**

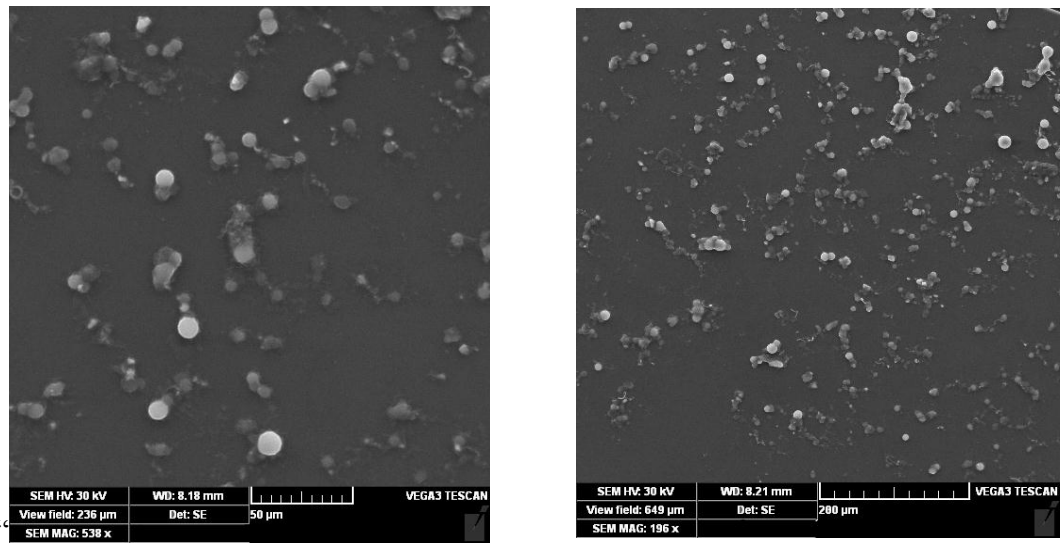


Fig 18: Scanning electron micrographs A) Formulation 1 B) Formulation 2

Table 21: Vesicle diameter of Niosomes

S.No	Type of formulation	Size (µm)
1	F1	50
2	F2	200

Drug Entrapment Efficiency

The quantity of the drug entrapped in the niosomes is very essential to know before studying the behaviours of the entrapped drug in physical or biological system. The process and formulation variables (cholesterol and surfactant) were altered and optimized to obtain the niosomes with maximum drug entrapment. Two formulations FI and FII were subjected to percentage drug entrapment.

The entrapment efficiency of drug in FII containing span 60 was found to be 68% which showed maximum percent drug entrapment where as those containing span 40 was found to encapsulate 55%. This showed that span 60 is the more suitable surfactant along with cholesterol for enhancing maximum entrapment for the drug Levetiracetam.

Further, the percent drug entrapment, is increased by decreasing the sonication time. Therefore, the sonication time was optimized to 15 minutes and further reduction in the size by increasing sonication time was not attempted.

Table 22: Percentage Drug Entrapment of the Formulated Niosomes

Formulation code	% Drug entrapment
FI(Span 40)	55%
FII(Span 60)	68%

***In vitro* Drug release**

The formulated niosomes were subjected to *in vitro* drug release using 0.1 M in a tubing. The amount of Levetiracetam diffused was estimated spectrophotometrically at 220 nm. FI showed 57.49% of drug release within 24 hours. FII showed 72.37% of drug release within 24 hours. These results showed that Levetiracetam noisome has sustained release up to 24 hours. This is because the drug is released slowly for a prolonged period of time from Levetiracetam niosomes. Also, therefore FII is selected for further studies like stability studies and pharmacological study.

Table 23: *In Vitro* Drug Release of FI

Time (hr)	Percentage drug diffused
30	14.85 ± 0.48
1	17.23 ± 0.54
2	20.29 ± 0.46
3	22.46 ± 0.42
4	23.57 ± 0.57
5	30.40 ± 0.69
6	34.42 ± 0.75
7	37.02 ± 0.42
8	41.75 ± 0.34
9	45.14 ± 0.49
10	47.36 ± 0.53
11	50.31 ± 0.67
12	55.89 ± 0.78
13	57.70 ± 0.83
14	59.97 ± 0.25
15	62.24 ± 0.13
16	66.13 ± 0.18
17	70.25 ± 0.33
18	71.68 ± 0.39
19	73.21 ± 0.21
20	77.94 ± 0.17
21	81.28 ± 0.45
22	86.17 ± 0.72
23	88.77 ± 0.28
24	90.52 ± 0.19

All the results were mean ± S.D (n= 3).

Table 24: *In Vitro* Drug Release of F II

Time (hr)	Percentage drug diffused
30	15.43 ± 0.35
1	17.60 ± 0.26
2	21.03 ± 0.57
3	22.88 ± 0.51
4	24.15 ± 0.46
5	31.09 ± 0.11
6	34.90 ± 0.39
7	37.49 ± 0.41
8	42.33 ± 0.49
9	45.51 ± 0.13
10	47.57 ± 0.48
11	50.78 ± 0.37
12	56.31 ± 0.08
13	58.12 ± 0.21
14	60.55 ± 0.12
15	63.08 ± 0.28
16	66.71 ± 0.69
17	70.72 ± 0.47
18	72.37 ± 0.13
19	73.90 ± 0.55
20	78.89 ± 0.17
21	82.38 ± 0.66
22	86.18 ± 0.53
23	90.40 ± 0.10
24	94.46 ± 0.37

All the results were mean ± S.D (n = 3)**.**

Release kinetics

In-vitro release data obtained for the formulations was subjected to kinetic analysis. The cumulative percentage drug release data obtained were fitted to zero order, first order, Higuchi's square root of time and equation to understand the mechanism of drug release from the Levetiracetam niosomal formulation. The diagrams were shown in Figure: 20-28. Higuchi model explains the diffusion controlled release mechanism. The slopes and the regression coefficient of determinations (R^2) were listed in Table No: 25. The coefficient of determination indicated that the release data was best fitted with Higuchi's kinetics.

Kinetic modelling and release mechanisms

Figure 19: Zero order plot of F1formulations

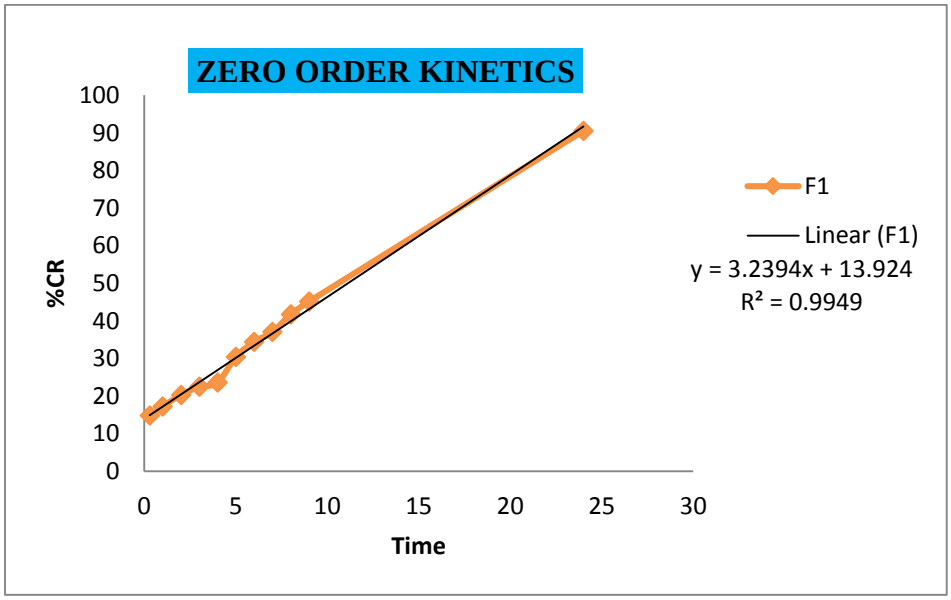


Figure 20: First order plot of F1 formulations

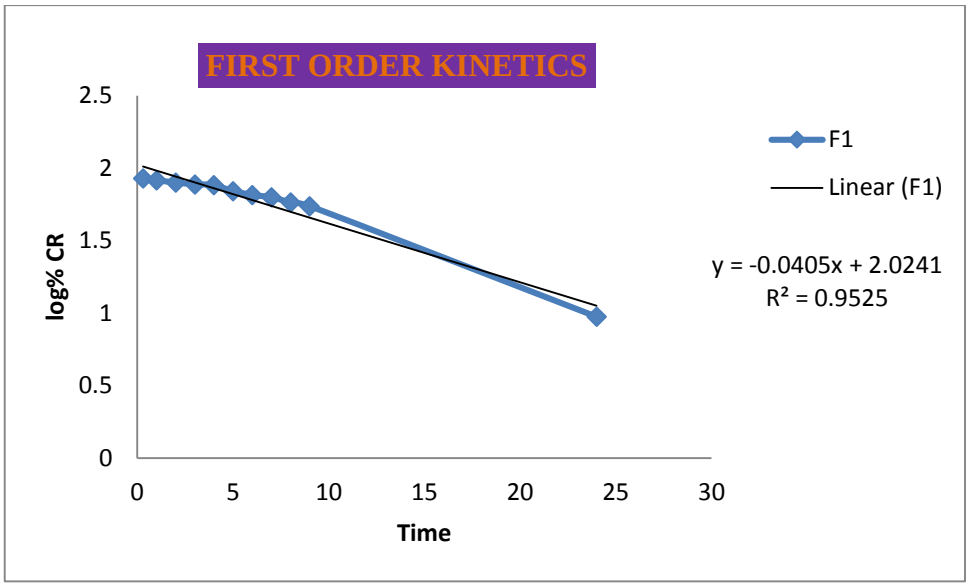


Figure 21: Higuchi plot of F1 formulations

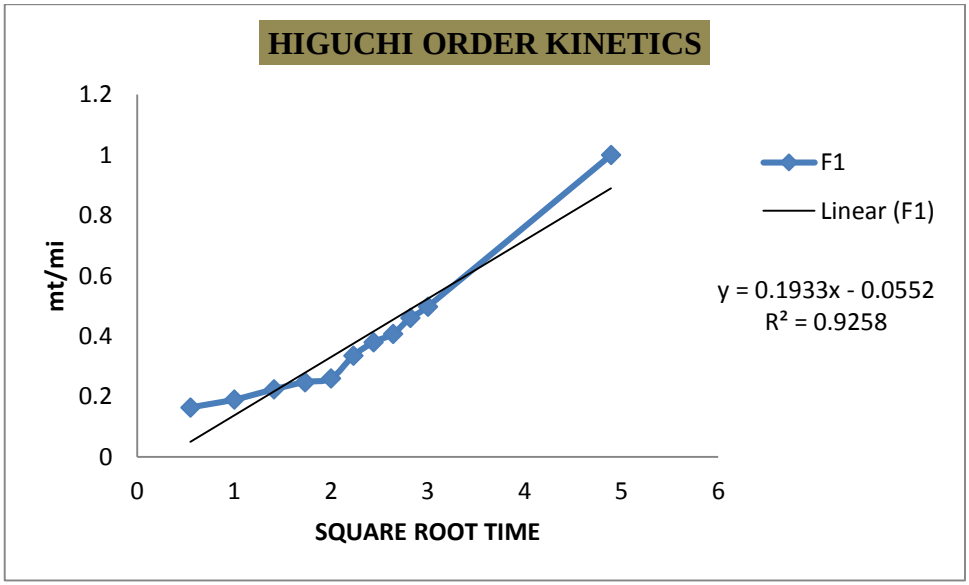


Figure 22: Zero order plot of F2 formulations

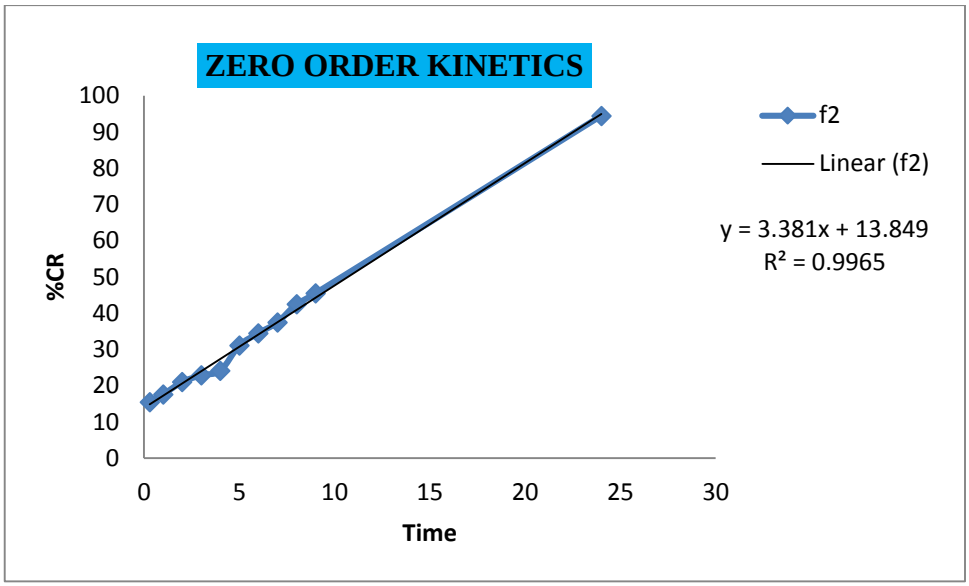


Figure 23: First order plot of F2 formulations

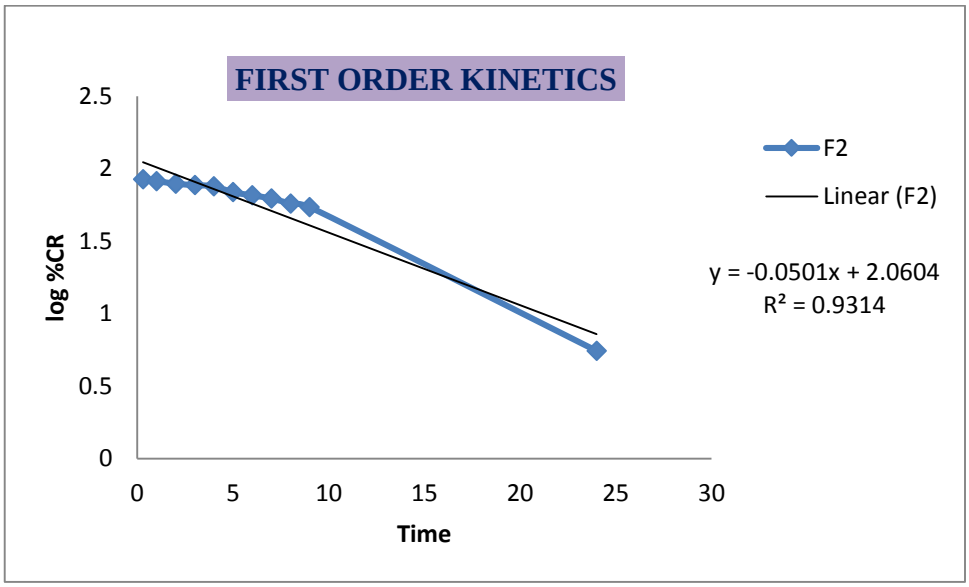
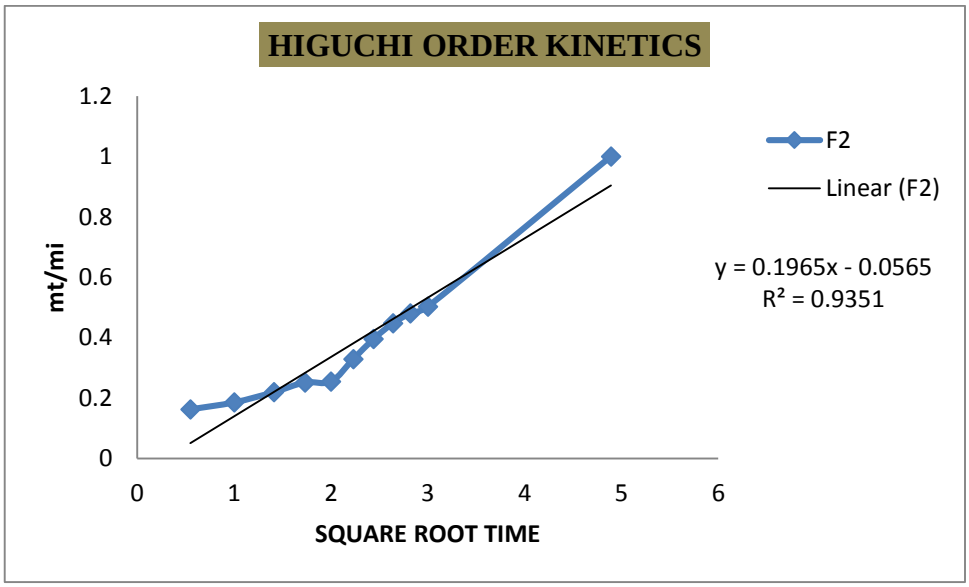


Figure 24: Higuchi plot of F2formulations



Kinetic Modeling Data

Table No 25: Kinetic Modelling Data

Formulation	Zero order	First order	Higuchi model
	r^2	r^2	r^2
F2	0.996	0.931	0.935

STBILITY STUDIES

The result of accelerated stability studies, carried out according to ICH guidelines, The stability studies were performed for FII containing span 60. The formulation was divided into three portions and stored at three different temperatures (i.e.) refrigeration temperature ($4^0C\pm 1^0C$), room temperature and at $40^0C\pm2^0C$ and RH 70% ±5% for a period of three months. Drug leakage from the niosomal formulation were analysed in terms of percent drug retained at the end of every month.

Storage under refrigerated condition showed greater stability with 94.85% of drug content at the end of three months, whereas storage under room temperature and

at 40^0C ± 2^0C / R-H 70 % ± 5% showed drug content of 92.83% and 85.41% at the end of three months.

Table 26: Stability Studies

Temperature	Amount of drug retained (%) after months			
	Initial	**I**	**II**	**III**
Refrigeration (4^0C±1^0C	100	97.42	96.22	94.85
Room Temperature	100	95.68	94.77	92.83
40^0C±2^0C RH-70% ± 5%	100	89.79	87.73	85.41

STERILITY TEST

- Sterility test was done by I.P.1996.
- After 7 days of observation, all the tubes containing sample was found to be sterile.

ANTICONVULSANT ACTIVITY

Table 27: Different stages of convulsion studies

Groups	Duration (sec)				
	Flexion	**Extension**	**Convulsion**	**Stupor**	**Death**
Control	1.52, 3.56, 3.75, 2.84, 3.16, 4.51	21.46, 34.61, 23.25, 18.56, 15.92, 20.59	8.91, 9.14, 14.63, 11.50, 9.12, 8.76	156,121, 186, 0, 155, 0	2/6
Standard	4.19, 2.11, 5.13, 2.62, 3.14, 2.19	2.81, 1.96, 3.91,2.46, 1.08, 0	4.36, 4.59, 8.83, 0, 0, 3.61	111, 123, 125, 129, 108, 105	0/6
Formulation	1.08, 1.28, 5.13, 0.88, 1.87, 1.71	0.68, 1.63, 0, 1.6, 0, 0	4.6, 5.87, 0, 1.38, 4.44, 6.21	77, 69, 91, 86, 81, 94	0/6

Table 28: Deviations obtained from convulsion studies

Groups	Duration (sec)				
	Flexion	**Extension**	**Convulsion**	**Stupor**	**Death**
Control	3.223333 ± 0.4122513	22.39833 ± 2.650172	10.34333 ± 0.9525288	103.000 ± 33.63728	2/6
Standard	2.018333 ± 0.3203167	0.7066667 ± 0.3590605**	3.565 ± 1.35183**	108.500 ± 6.716894	0/6
Formulation	2.701667 ± 0.6589305	1.981667 ± 0.562645**	3.750 ± 1.023458**	92.000 ± 8.449852	0/6

Values are expressed as mean ± SEM (n=6); *p<0.05, **p<0.01 vs Control (Oneway ANOVA followed by Dunnett's test)

From the above tabular column it was concluded that formulation (F_2) showed better anticonvulsant activity than the standard in lesser time.

SUMMARY

The present study was aimed to prepare niosomes containing drug levetiracetam. It belongs to the class of drugs called anticonvulsant drug. They work by maximum stimulation with electrical current or different chemoconvulsants and showed only minimal activity in submaximal stimulation. Hence, the present work was made to formulate and evaluate the levetiracetam niosomes and compared its anti-convulsant activity with the pure drug.

- FT-IR of pure drug and drug excipients mixture revealed no chemical interaction. Hence they were compatible.
- UV Spectrophotometric method was developed for the determination of levetiracetam in 7.4 pH phosphate buffer at 220nm. A regression coefficient value was found to be 0.99 for levetiracetam.
- Two formulations of levetiracetam niosomes were prepared by hand shaking method.
- Formulation of multilamellar Niosomes vesicles were confirmed by examing the noisomal suspension under an TEM and observed the Internal Morphology
- Various parameters like vesicle shape and entrapment efficiency were done for niosomes. Vesicle shape was analysed by SEM technique. The vesicles were found to be smooth and spherical in shape. Entrapment efficiency was noticed for F2 with 68%.
- Niosomes were subjected to *in-vitro* diffusion studies. It reveals that F2 formulation has higher release with 94.46%.
- The formulations were subjected to release kinetics and the best formulation was found to be F2 and its follows zero order kinetics.
- Anticonvulsant activity of Niosomal formulations and the plain levetiracetam were conducted on 24 mice. It was found that niosomal formulation (F2) shows better activity than plain levetiracetam.
- Stability studies were carried out at $4^{o}C \pm 1^{o}C$, $40^{o}C \pm 2^{o}C$ RH-70% ± 5% and room temperature for 3 months. There were no significant changes in entrapment efficiency and *in-vitro* drug diffusion profile.
- Sterility tests are performed for the optimized niosomal formulations, after 7 days observations all the tubes containing samples were found to be sterile.

CONCLUSION

Levetiracetam niosomal formulation was prepared by hand shaking method and it was evaluated for its entrapment efficiency, *in-vitro* drug diffusion profile, sterility tests and stability studies. The optimized formulation F2 and levetiracetam plain were evaluated for it's anti-convulsant activity using flexion, extension, convulsion, stupor, recovery and death. The effects produced by the formulation (F2) in the mice were comparable with that of the *in-vitro* drug diffusion profile. Hence, the present study concluded that the anti-convulsant activity of levetiracetam formulation (F2) shows better activity than the levetiracetam plain.

FORMULATION & EVALUATION OF LEVETIRACETAM NIOSOMES FOR IMPROVED ANTI-CONVULSANT ACTIVITY

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CONTENT

- Introduction
- Aim and Objective
- Literature Review
- Methodology
- Formulation and Development
- Results and Discussion
- Summary
- Conclusion
- References

INTRODUCTION

TARGETED DRUG DELIVERY SYSTEM

DEFINITION:-

Drug Targeting can be defined as the ability to direct a therapeutic agent specifically to desired site of action with little (or) no interaction with non targeting tissue.

NIOSOMES

- Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic non-ionic surfactants,with (or) without incorporation of cholestrol (or) other lipids.

- They are vesicular systems similar to liposomes that can be used as carriers of hydrophilic and lipophilic drugs.

- It is less toxic and improves the therapeutic index of drug by restricting its action to target cells.

- Niosomes are novel drug delivery system,in which the medication is encapsulated in vesicle.

- The niosomes are very small, and microscopic in size. Their size lies in the nanometric scale.

- Niosomes are uni-lamellar (or) multi-lamellar vesicles. The vescile is composed of a bilayer of non-ionic surface active agents and hence the name niosomes.

AIM AND OBJECTIVE

AIM:

- To formulate and evaluate Levetiracetam niosomes and compare its anti-convulsant activity with the pure drug.

OBJECTIVE:

- To prepare and evaluate Levetiracetam niosomes .
- The need for present study is to encapsulate the drug in the niosomes vesicles for effective central nervous system drug delivery for the prolonged period of time.
- To compare the anti convulsant activity of Levetiracetam niosomes with a Levetiracetam pure drug.
- To formulate Levetiracetam in niosomal drug delivery there by dose can be minimised and also to achieve sustained release for a prolonged period of time.

PLAN OF WORK

1. Pre-formulation studies
    1. Solubility : To study the solubility behaviour of the drug with different solvents.
    2. FTIR: Identification of Levetiracetam pure drug by FTIR technique.
    3. DSC : To observe the compatibility of the product and excipients used in the formulations.
2. Optimization of the formula
    1. Optimization of excipients (surfactants)
    2. Optimization of rotating speed
    3. Optimization of temperature
3. To formulate the Levetiracetam niosomes with different surfactants by liquid hydration technique using Rotory Flash Evaporator

4. To characterize the formulated niosomes by following methods:
    1. Surface Morphology.
    2. Particle size distribution of the prepared niosomes.
    3. Determination of entrapment efficiency.
5. To test the stability of the formulated niosomes.
6. To test the sterility of the formulated niosomes.
7. To examine the *In vitro* diffusion study of the niosomes prepared with different surfactants and to compare their drug release profile.
8. To Test the anti-convulsant activity of the formulated niosomes with the formulation having best drug release.

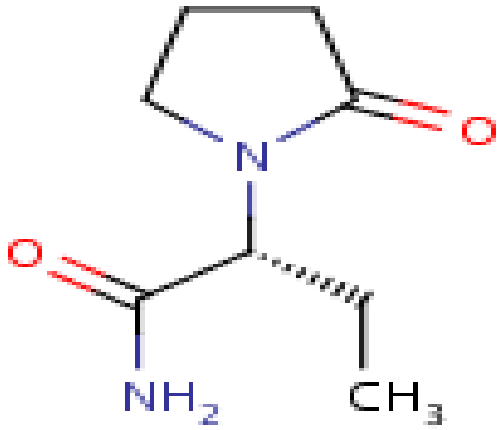
LITERATURE REVIEW

S. No	Author's Name	Journal Name	Objective	Conclusion
1	Raja K. *et al*	International journal Pharm Tech.	Formulated and evaluated the Maltodextrin based Proniosomal Drug Delivery System containing Anti-diabetic (Glipizide) drug.	It was concluded that the formulation developed using the desirability approach produced high drug encapsulation efficiency and sustained anti-diabetic activity in rats following oral administration.
2	Rajesh Z. Mujoriya* kishordhamonder *et al*	Int J App Pharm	Niosomes drug delivery system.	It was concluded that Niosomes are thoughts to be better candidate's drug delivery as compared to liposomes.

Sl No	Author's Name	Journal Name	Objective	Conclusion
3	Anchal Sankhyan and Pravin Pawar	Journal of Applied Pharmaceutical Science.	Recent Trends in Niosome as Vesicular Drug Delivery System.	It was concluded that Niosomes have evolved for treatment of many dreadful diseases efficiently with reduced side effects and better patient compliance.
4	Sakthivel *et al*	Int J Pharm Pharm Sci.	Formulation and *Invitro* Evalution of Niosomes contaninng Oxacarbazepine.	Thus It was concluded that Thus the prepared noisome could be promising delivery system for Oxcarbazepine with sustained drug release profiles.

DRUG PROFILE

- **Name : Levetiracetam**
- **Brand Name : Keppra**
- Levetiracetam is an anticonvulsant medication used to treat epilepsy.
- **Structure Levetiracetam :**



- **IUPAC Name : (2R)-2-(2-oxopyrrolidin-1-yl)butanamide**

- **Solubilty :** Very soluble in water, Freely soluble in Chloroform, Freely soluble in Methanol, Soluble in Ethanol, Sparingly soluble in acetonitrile.
- **Categoties :** Anticonvulsants

  Nootropic Agents

Pharmacokinetic profile of Levetiracetam

Pharmacokinetic Characters	Levetiracetam
bioavailability (%)	~100%
protein binding (%)	<10%
Volume of distribution(L/Kg)	Not Available
% metabolized	24%
Elimination $T^{1/2}$(hr)	6-8hrs

MATERIALS AND METHODOLOGY

List of Materials used

S.NO.	MATERIALS USED	MANUFACTURER
1.	Levetiracetam	Lupin
2.	Cholesterol	Qualigens Fine Chemicals, Mumbai
3.	Span 40	Kemphasol
4.	Span 60	Kemphasol
5.	Chloroform	Ranbaxy
6.	Diethylether	Qualigens Fine Chemicals, Mumbai
7.	Buffer (pH 7.4)	Laboratory preparation
8.	Mice	Seiss Albino mice (25-30)

List of Equipments used

S.NO.	EQUIPMENTS	MANUFACTURER
1.	Rotary Flash Evaporator	Equitron Roteva,Medica Instrument Mfg-Co, Mumbai
2.	Ultra Sonicator	Vibronics
3.	UV-Double Beam Spectrophotometer	UV-Pharmaspec 1700, Shimadzu
4.	Electronic Balance	Sartorius
5.	pH Meter	Elico LI 120
6.	Magnetic stirrer	Remi- 1MLH
7.	Environment stability testing chamber	“HECO”. Environment chamber
8.	FTIR	Perkin Elmer
9.	Centrifuge	Remi
10.	Scanning Electronic Microscope	JEOL, JSM -6701 F, Japan
11.	Particle size Analyzer	Microtrac – Bluewave US
12.	Anti convulsometer	Kmch Laboratory

- **Compatibility studies:**

  The compatibility studies of drug with excipients were done using FT-IR spectroscopy. The scanning range was between 400- 4000cm^{-1}

- **Determination of λmax of Levetiracetam:**

  50 mg of accurately weighed drug was dissolved in the phosphate buffer pH 7.4 which upon suitable dilution, analyzed in UV spectrophotometer between 200 to 400 nm. The point of absorption maximum obtained in the graph was considered as the l_{max} of the pure drug.

- **Preparation of Niosomes**
- **Hand shaking method (Thin film hydration technique):** The mixture of vesicle forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20^{o}c) using rotator evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60^{o}c with gentle agitation. This process forms typical multilamellar Niosomes.
- Thermosensitive niosomes can be prepared by evaporating the organic solvents at 60^{o}c and leaving a thin film of lipid on the wall of rotary flash evaporator. The aqueous phase containing drug is then slowly added with intermittent shaking of flask at room temperature followed by sonication.

FORMULATION

Formulation code	FI	FII
Amount of Levetiracetam	5mg	5mg
Cholesterol	5mg	5mg
Span40	5mg	-
Span 60	-	5mg
Chloroform	5ml	5ml
Phosphate Buffer	5ml	5ml
Cholesterol surfactant ratio	1:1	1:1

EVALUATION OF LEVETIRACETAM NIOSOMES

- **Entrapment efficiency**

  An1ml of the sample is taken and centrifuged at 13000 RPM at 4^0c for 60 minutes using Eppendorf centrifuge. Supernatant was separated without disturbing the sediment layer using micropipette. Then the supernatant layer (free drug) was diluted using PBS pH 7.4 and analysed using UV spectrophotometer at 220 nm.

- **Percent drug entrapment = amount of drug entrapped/initial amount of drug*100**

- **Transmission Electron microscopic observation**

  The niosomal dispersion was spread on the glass slide using a glass rod. Formation of multilamellar vesicles was confirmed by examining the niosomal suspension under an transmission electron microscope with the magnification power of 71 lacks Sx.

- **Vesicle shape**

  The vesicle shape of best formulation F2 was determined by using scanning electron microscopy.
- **In-vitro diffusion studies**
- The in-vitro diffusion of the drug through cellophane membrane was performed.
- The cellophane membrane soaked in glycerin for 6-8 hours.
- Franz diffusion cell contains donor compartment and receiver compartment
- Formulation was applied through donor compartment on the dialysis membrane
- Reservoir compartment was filled with phosphate buffer.
- The study was carried at 37ºc at 100 rpm for 24hrs. samples were withdrawn for every 1hr and absorbance measured at 220nm.

- **Release kinetics**

  The results of *in vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows.

  - Log cumulative percent drug remaining versus time (first order kinetic model)
  - Cumulative percent drug release versus square root of time (Higuchi's model)
  - Cumulative percent drug release versus time (zero order kinetic model)

Stability studies

- Stability testing of drug products begins as a part of drug discovery and ends with the commercial product

- To assess the drug and formulation stability, stability studies were done. The stability studies were carried out for the most satisfactory formulation.
- The most satisfactory formulation was sealed in a container and kept at 4 ± 2°C and at Room temperaturefor 90 days.

At the end 90days, the sample was analyzed for the entrapment efficiency percentage, and *in vitro* diffusion study.

ANTICONVULSANT ACTIVITY

Animals : Swiss Albino mice (25-30g)

Gender : Female

Number to be used : 24

Duration : 1 month

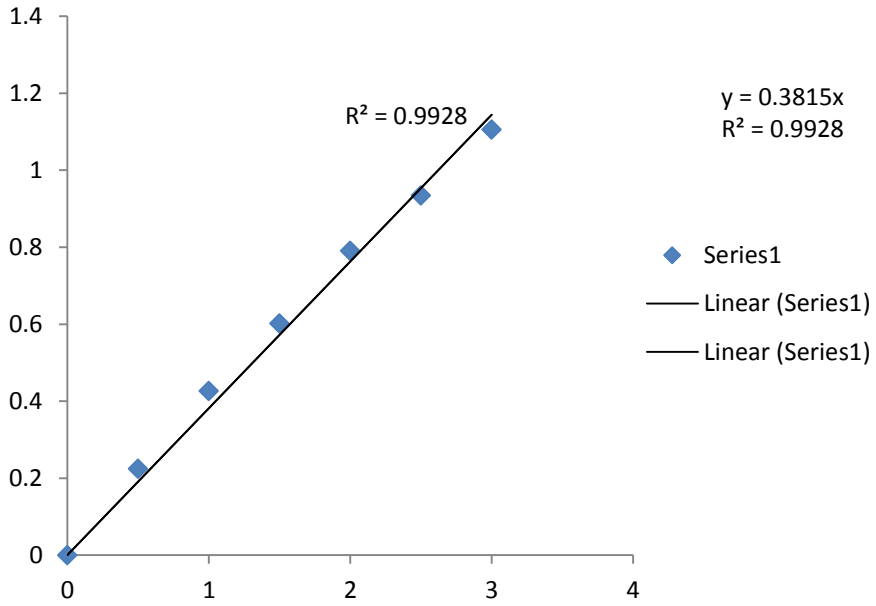
GROUPING OF ANIMALS:

Group	TREATMENT : DOSE AND ROUTE OF ADMINISTRATION	NO OF ANIMALS (mice)
1.	Control (only vehicle)	6
2.	Only MES induced	6
3.	MES with Standard (Levetiracetam plain)	6
4.	MES of Levetiracetam niosomes	6

RESULTS AND DISCUSSION

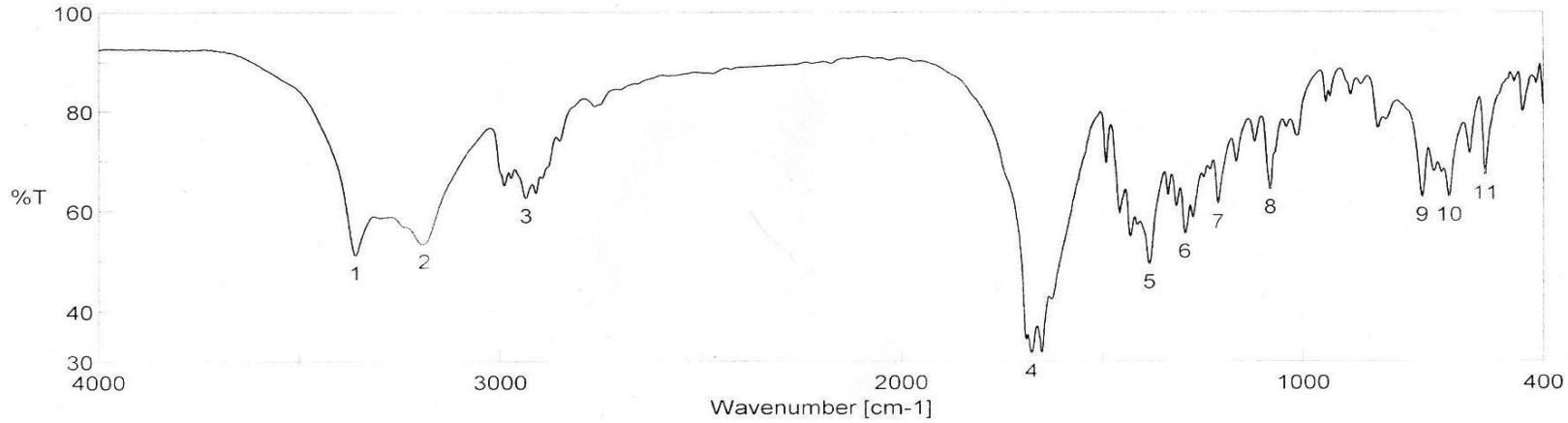
Determination of calibration curve:
Calibration curve of Levetiracetam at 220nm

Concentration (µg/ml)	Absorbance
0.5	0.225
1.5	0.427
2	0.602
2.5	0.935
3	1.106



Compatibility studies

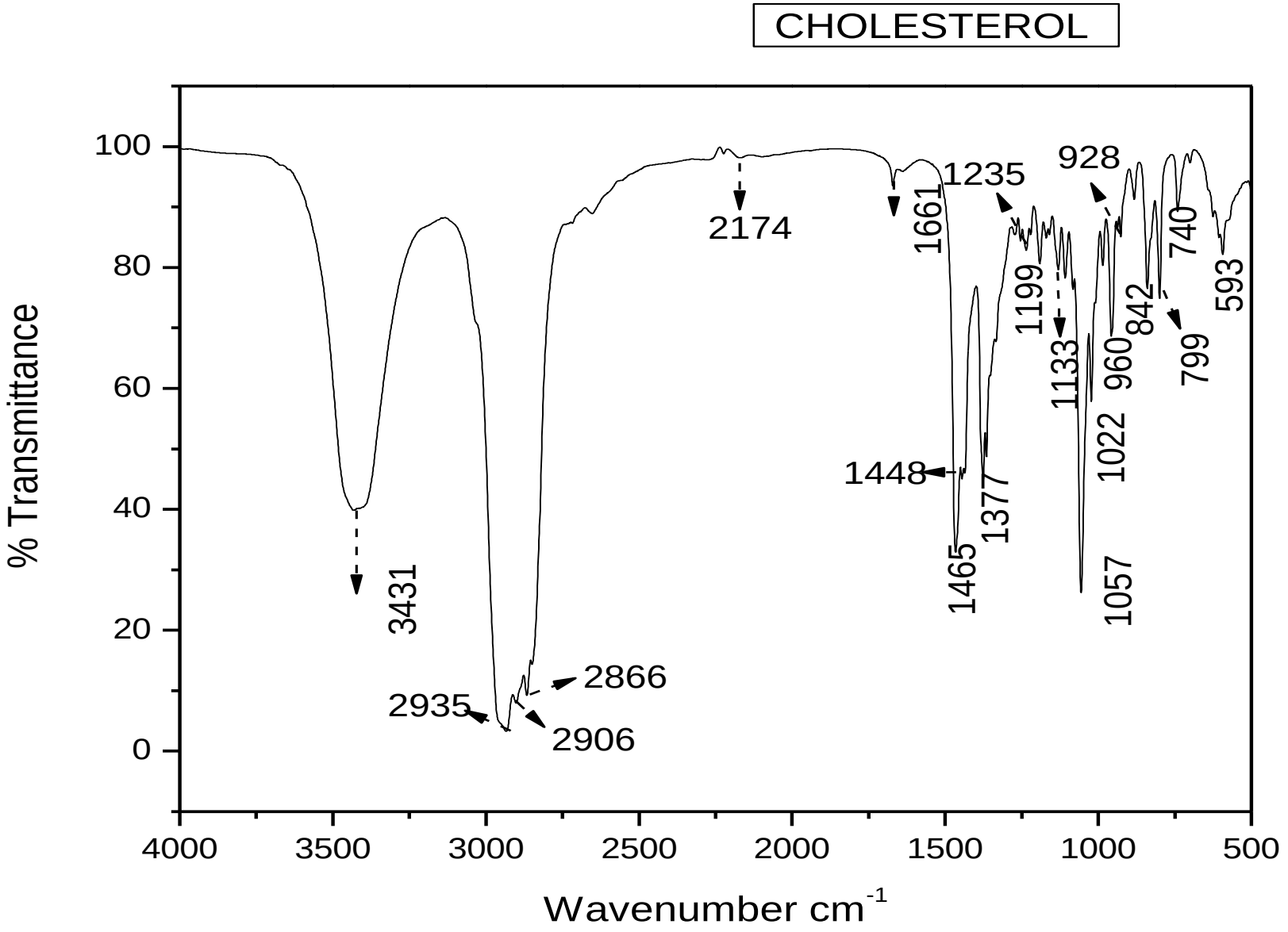
IR spectrum of levetiracetam



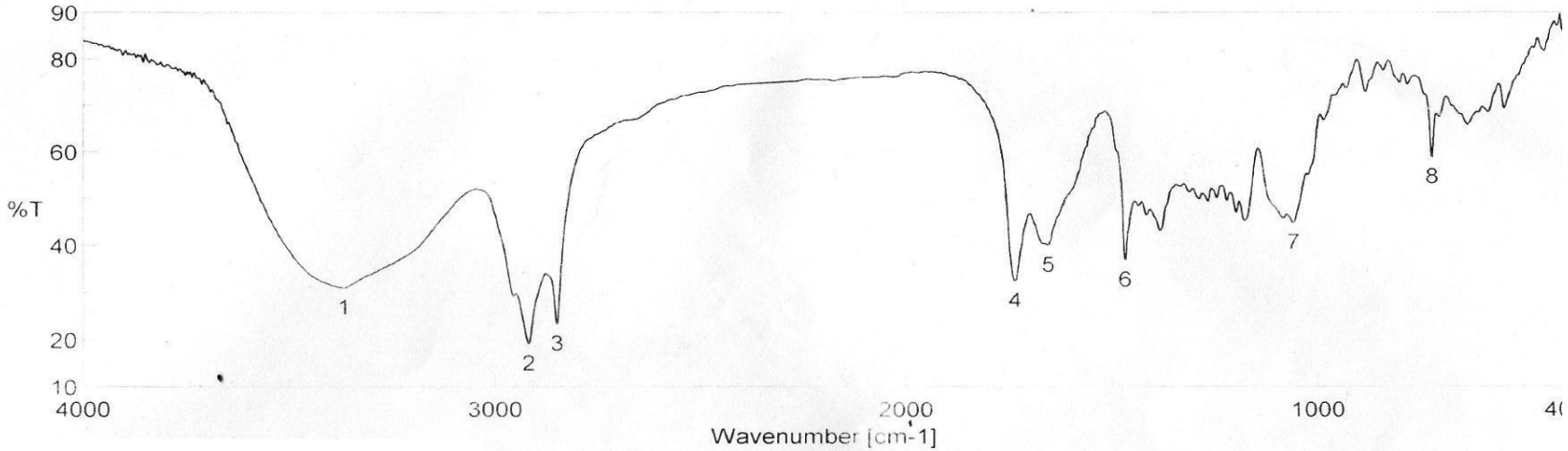
Division Pharm analysis
Company KMCH

[Result of Peak Picking]

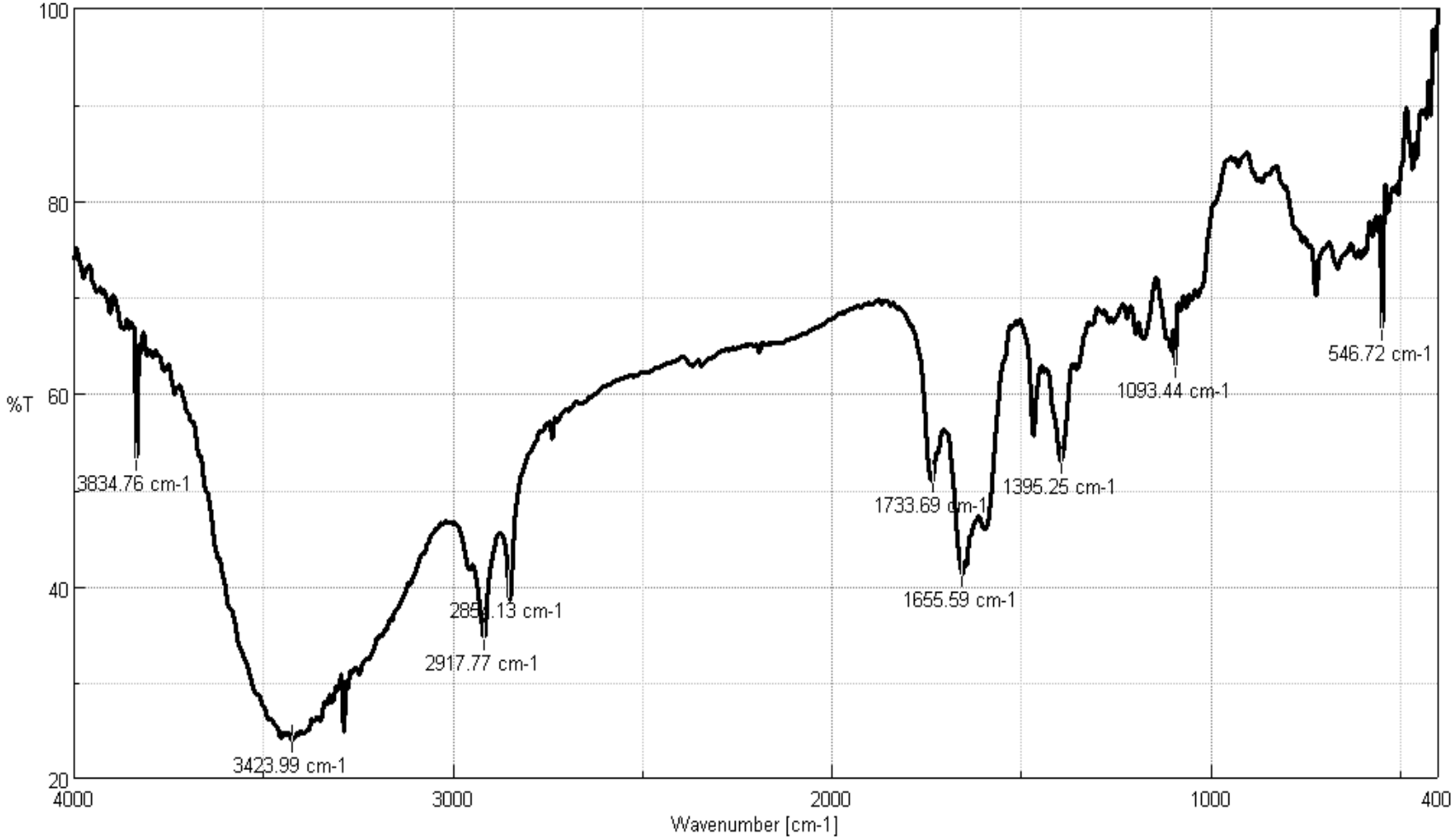
No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3360.35	51.113	2	3187.76	53.4139	3	2937.06	62.4532
4	1675.84	31.6137	5	1383.68	49.291	6	1294.97	55.396
7	1213.01	61.289	8	1082.83	64.1972	9	703.89	62.6492
10	636.394	62.6643	11	545.756	66.9517			


CHOLESTEROL
% Transmittance
100
80
60
40
20
0
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1
3431
2935
2866
2906
2174
1661
1235
928
740
593
1199
842
1133
960
799
1448
1377
1022
1465
1057

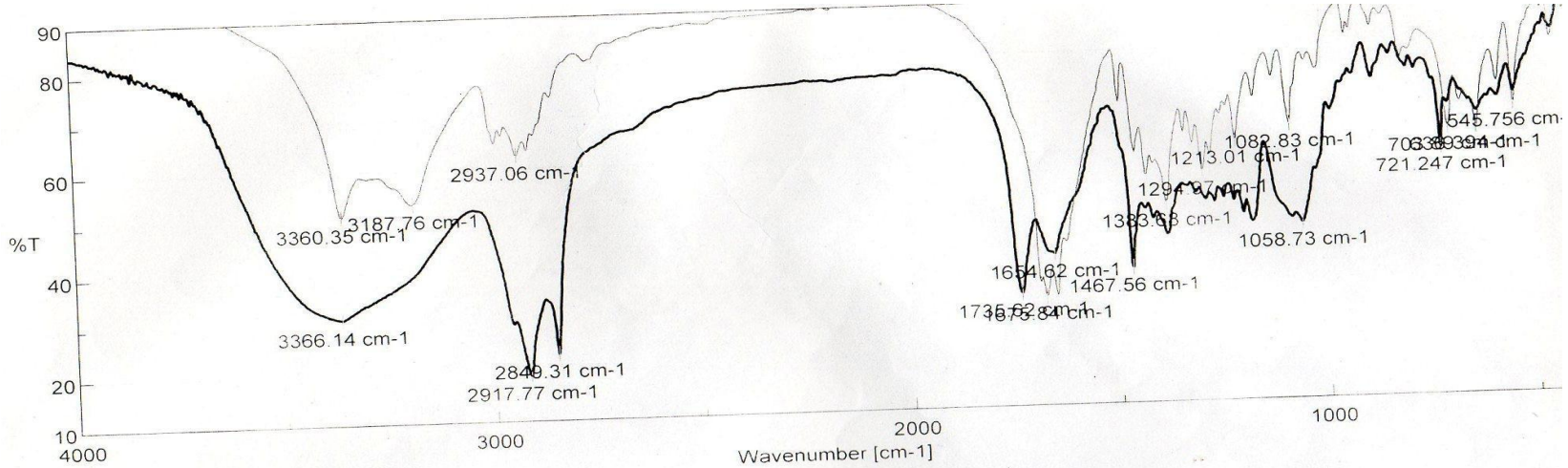
IR SPECTRUM OF SPAN 40



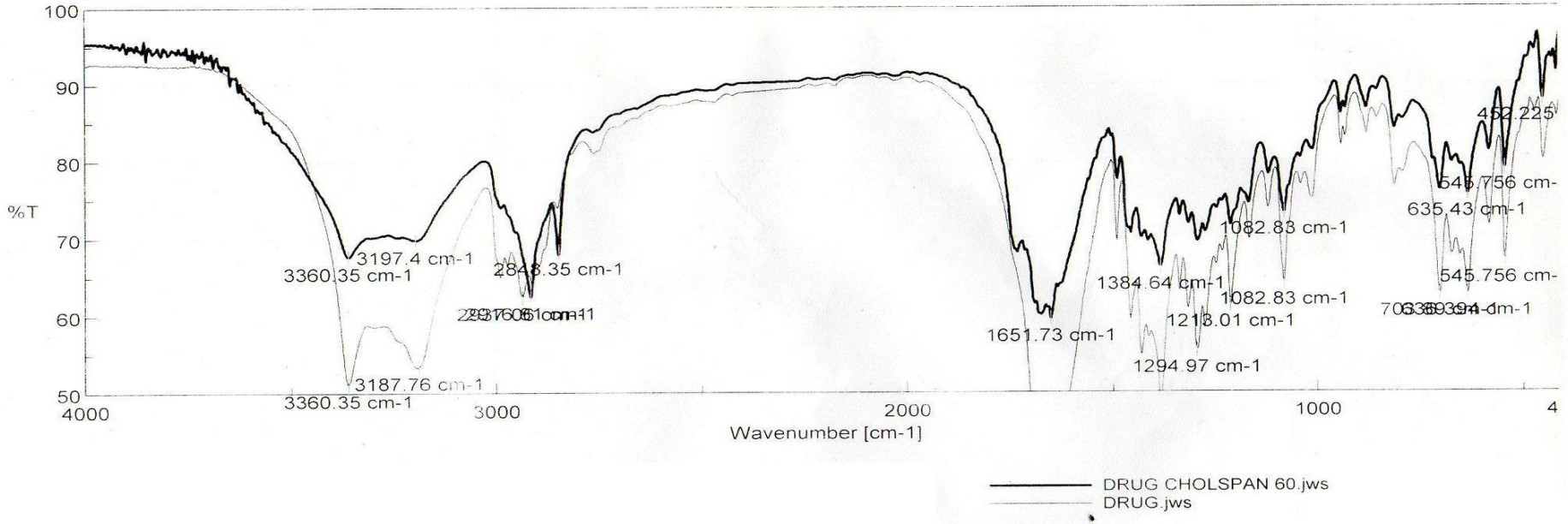
IR SPCTRA OF SPAN 60



IR SPECTRA OF DRUG+CHOL+SPAN40

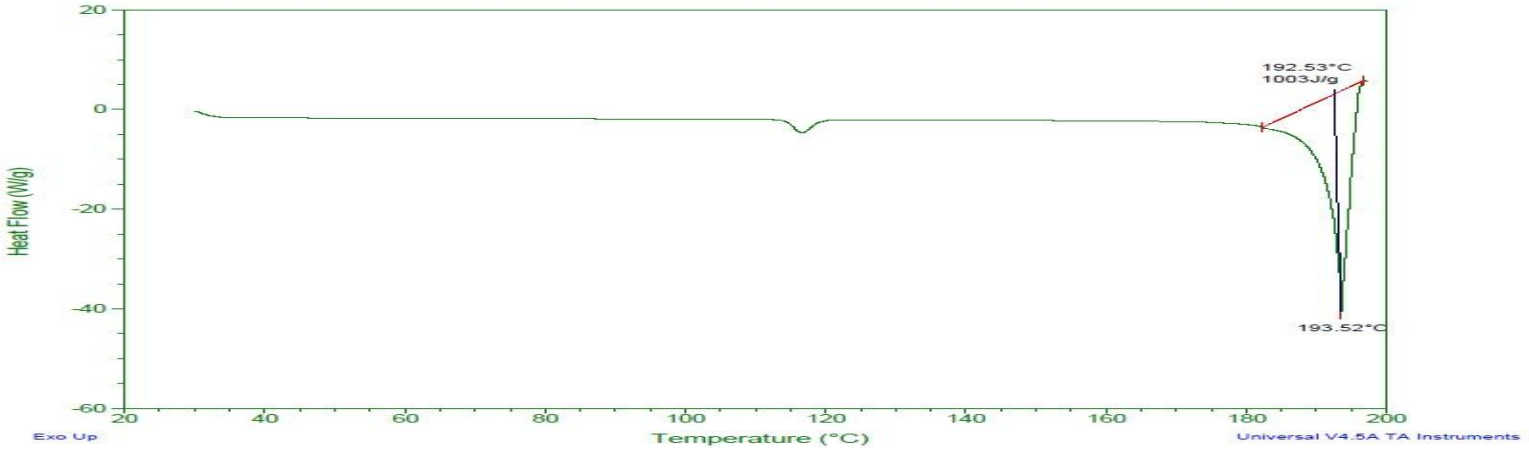


IR SPECTRA OF DRUG+CHOL+SPAN60

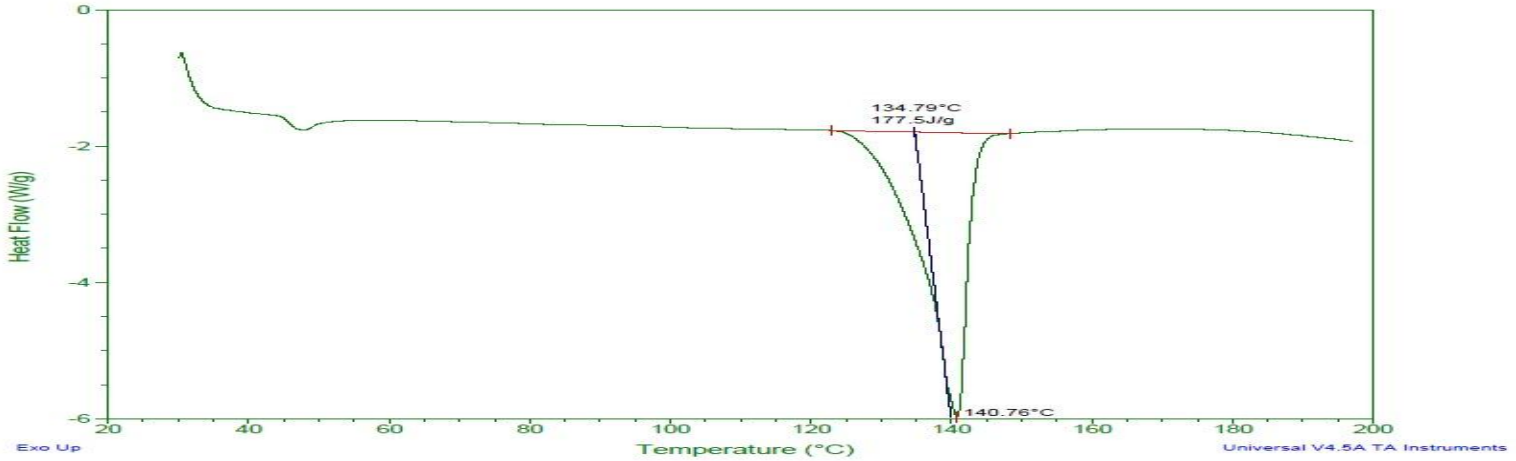


DIFFERENTIAL SCANNING CALORIMETRY

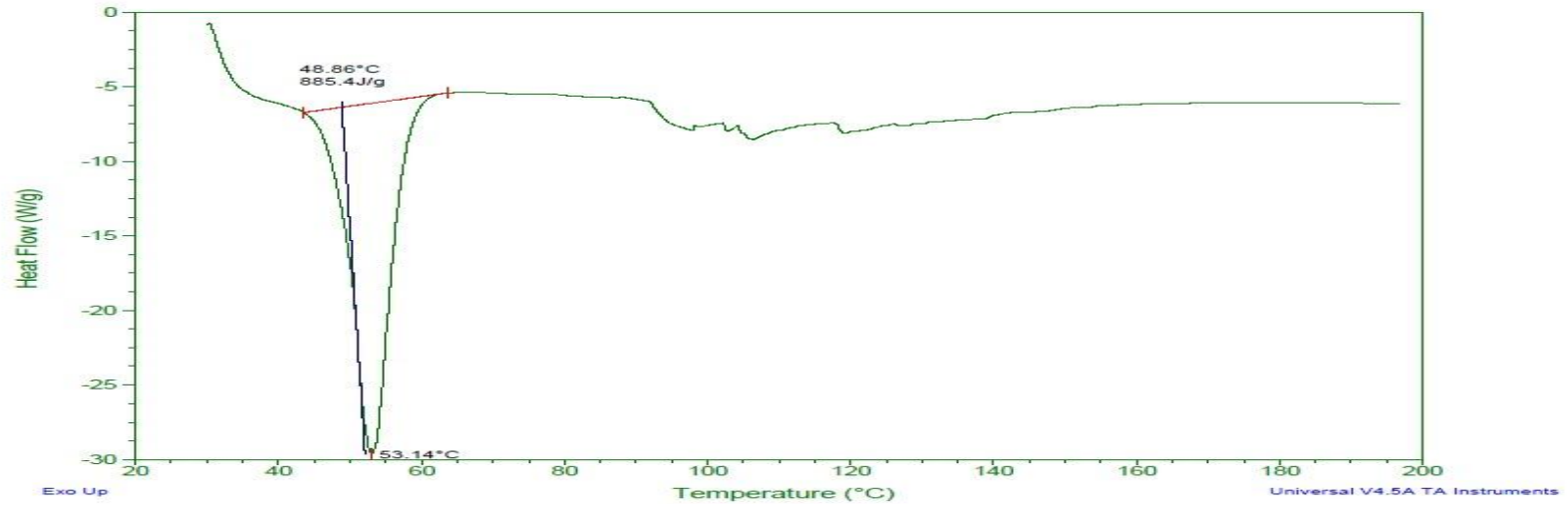
DSCof Levetiracetam pure drug



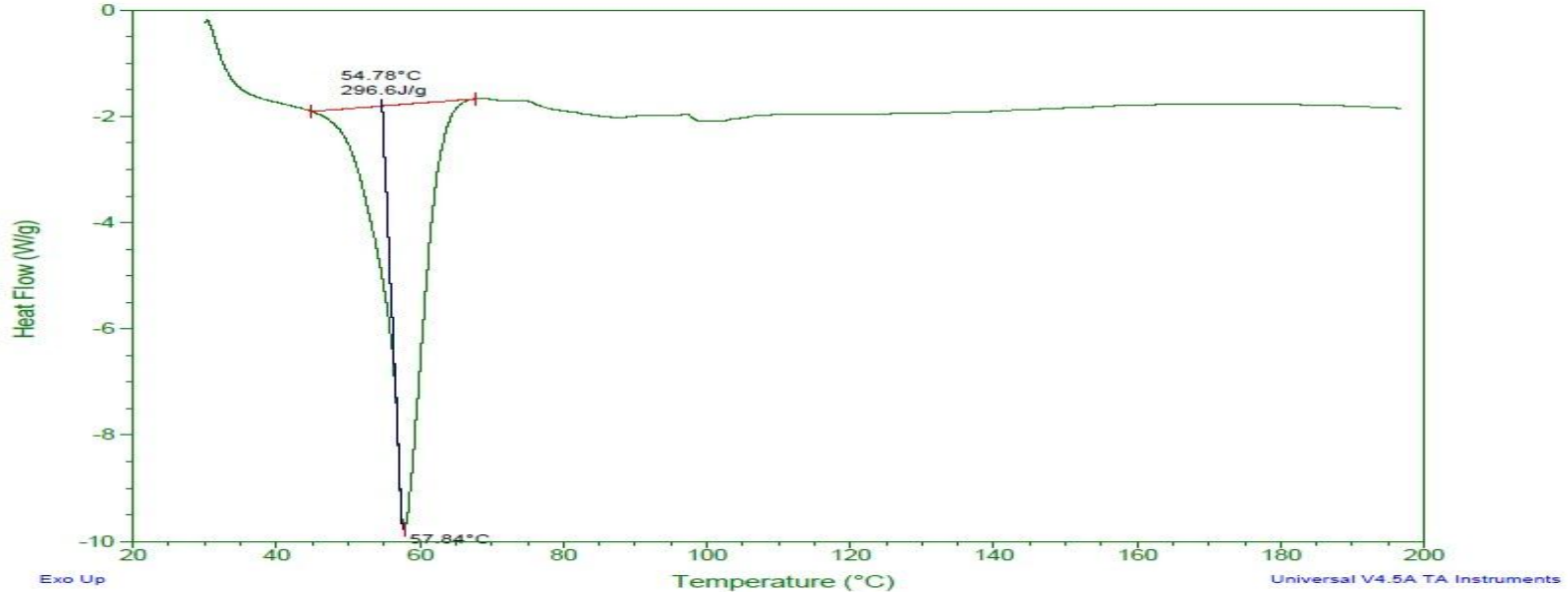
DSC of Cholesterol



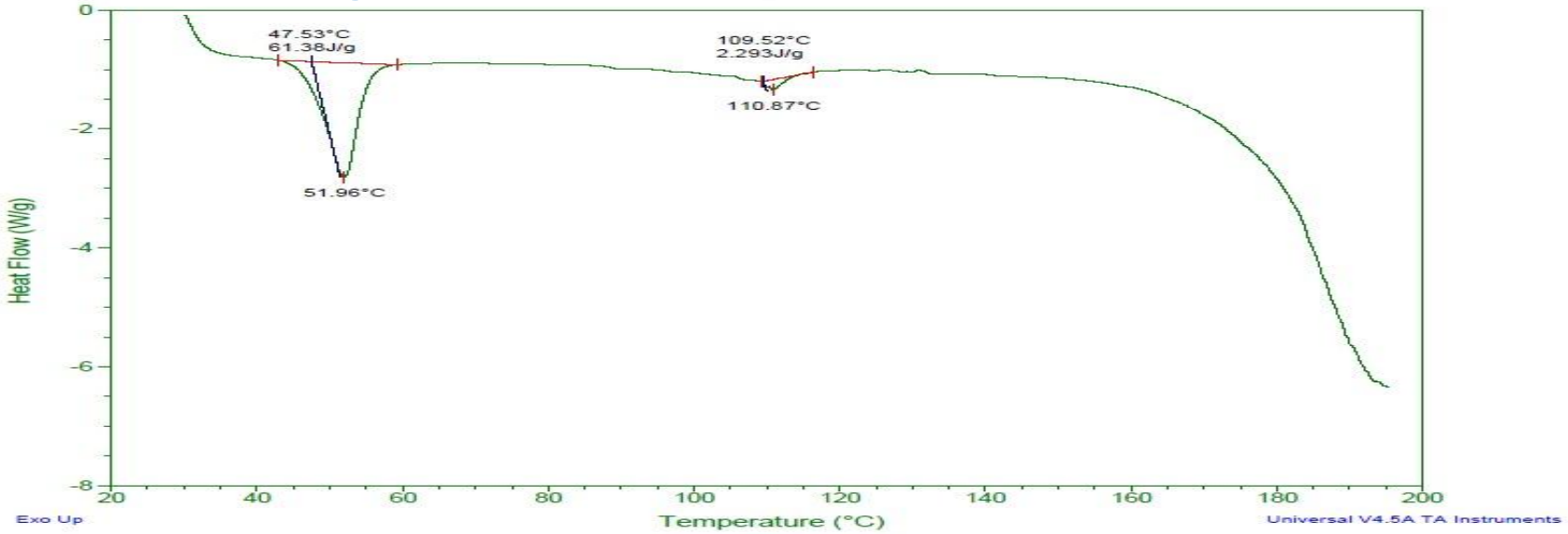
DSC of Span 40



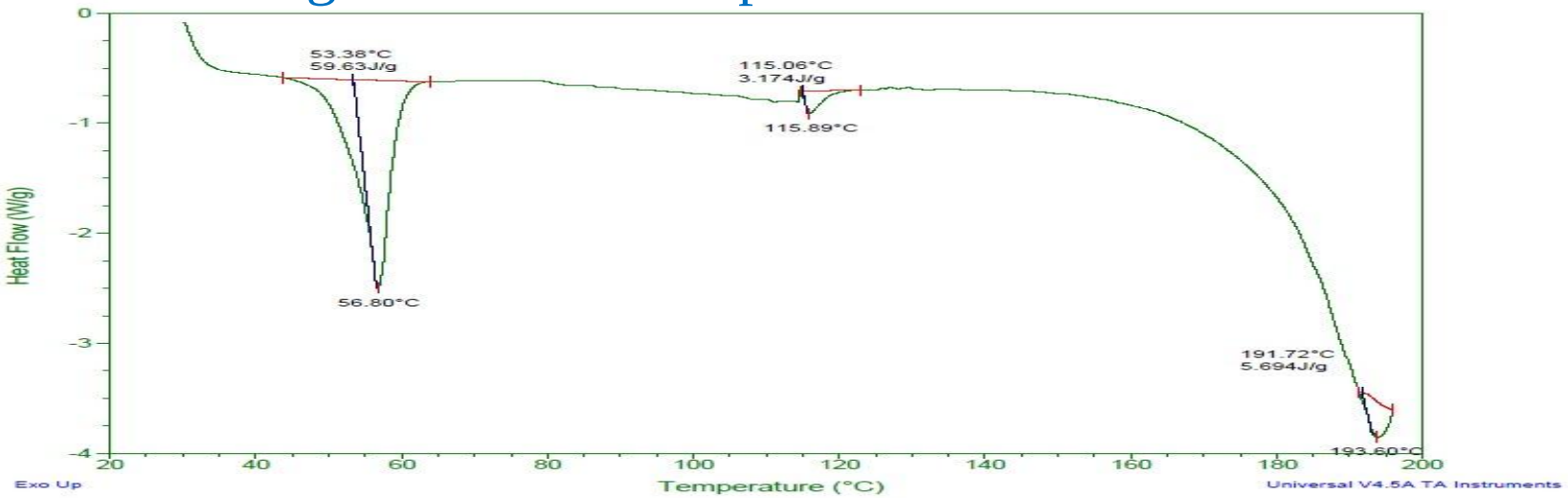
DSC of Span 60



DSC of Drug+ Cholesterol+Span40



DSCof Drug+ Cholesterol+Span60

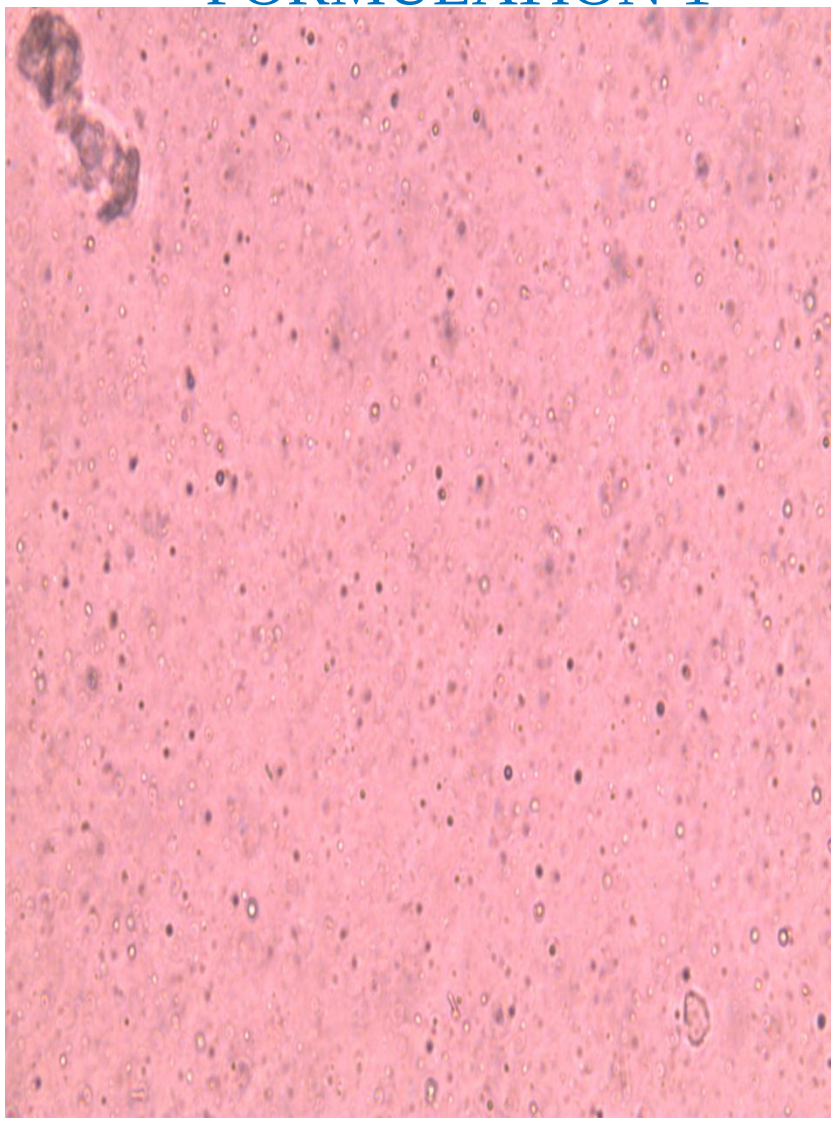


DSC Observed for the Drug and excipients

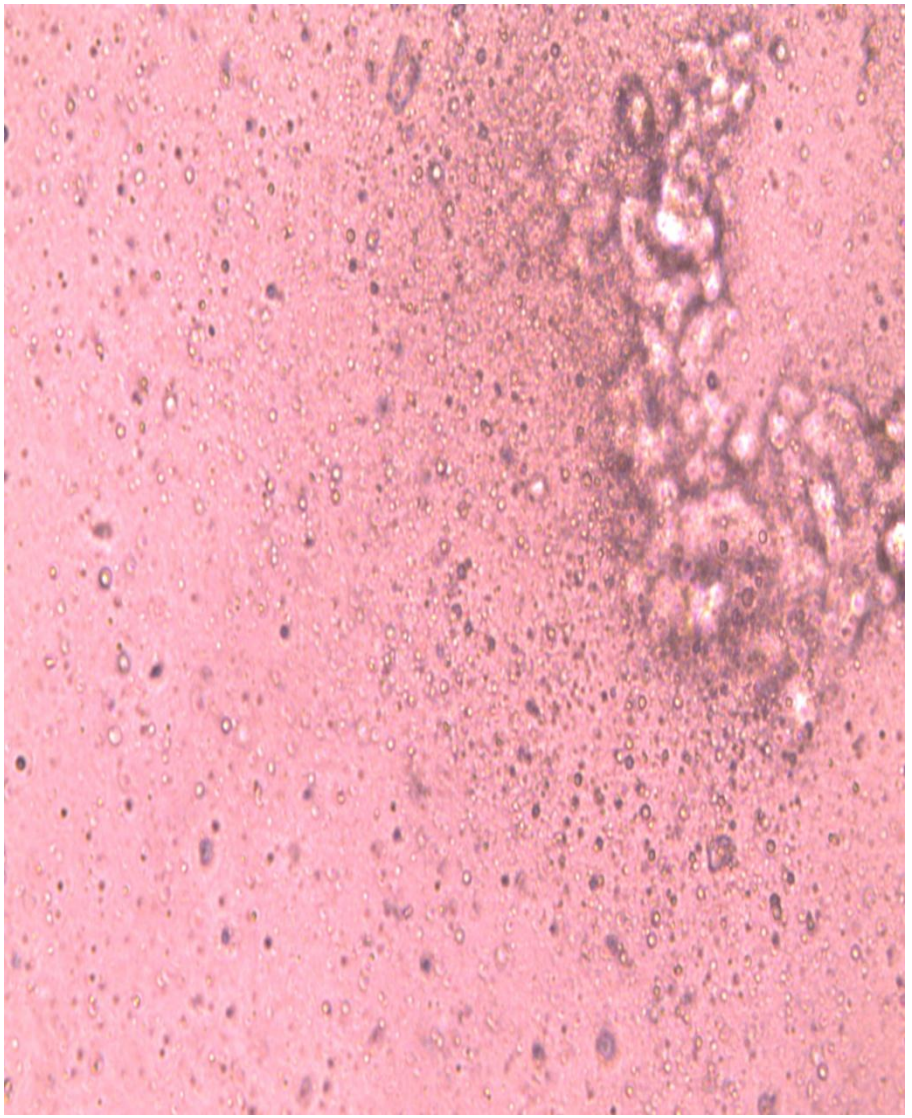
S.NO	SAMPLE	MELTING POINT
1	Levetiracetam	192.53^{o}C
2	Cholesterol	140.76^{o}C
3	Span 40	53.14^{o}C
4	Span 60	57.84^{o}C
5	Drug+Cholesterol+Span 40	51.96^{o}C
6	Drug+Cholesterol+Span 60	56.80^{o}C

OPTICAL MICROSCOPY (40X)

FORMULATION 1



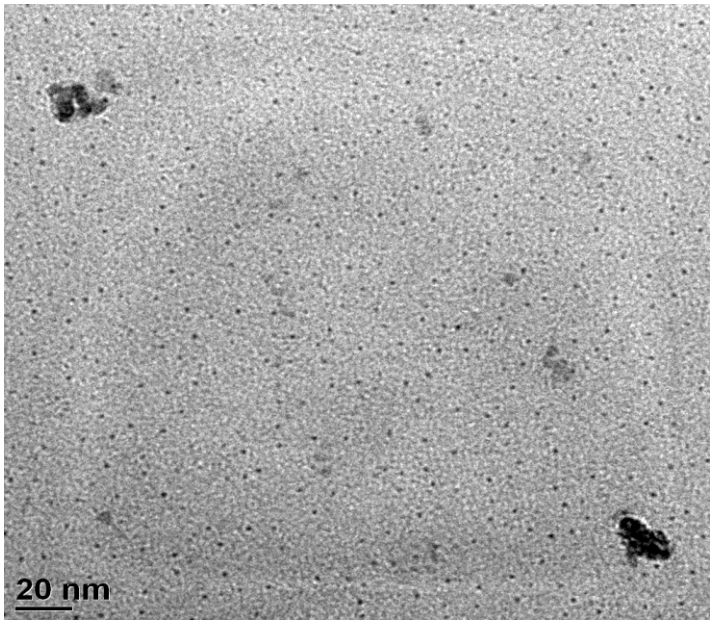
FORMULATION 2



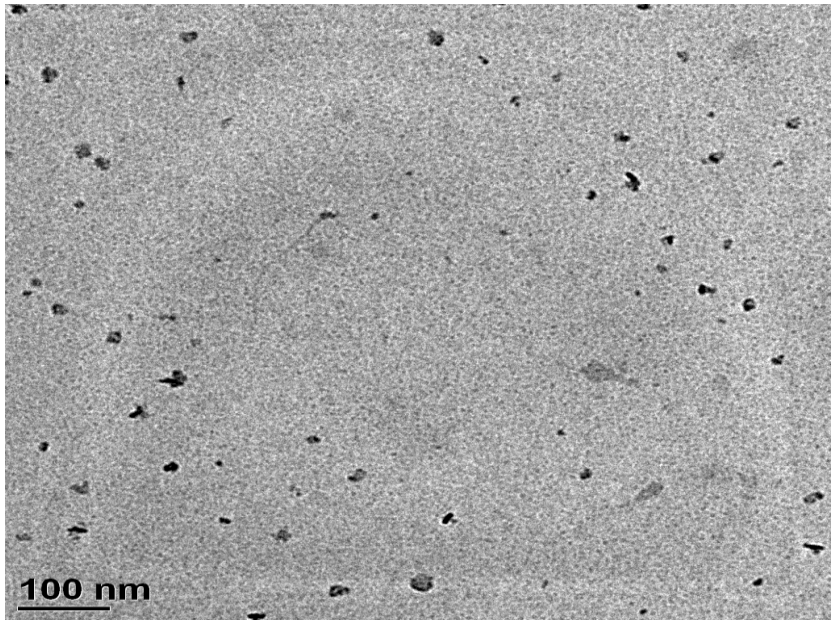
- **Transmission Electron microscopic observation:**

Formation of multilamellar vesicles was confirmed by examining the niosomal suspension under an transmission electron microscope with the magnification power of 71 lacks Sx and observe the internal morphology.

Formulation 1

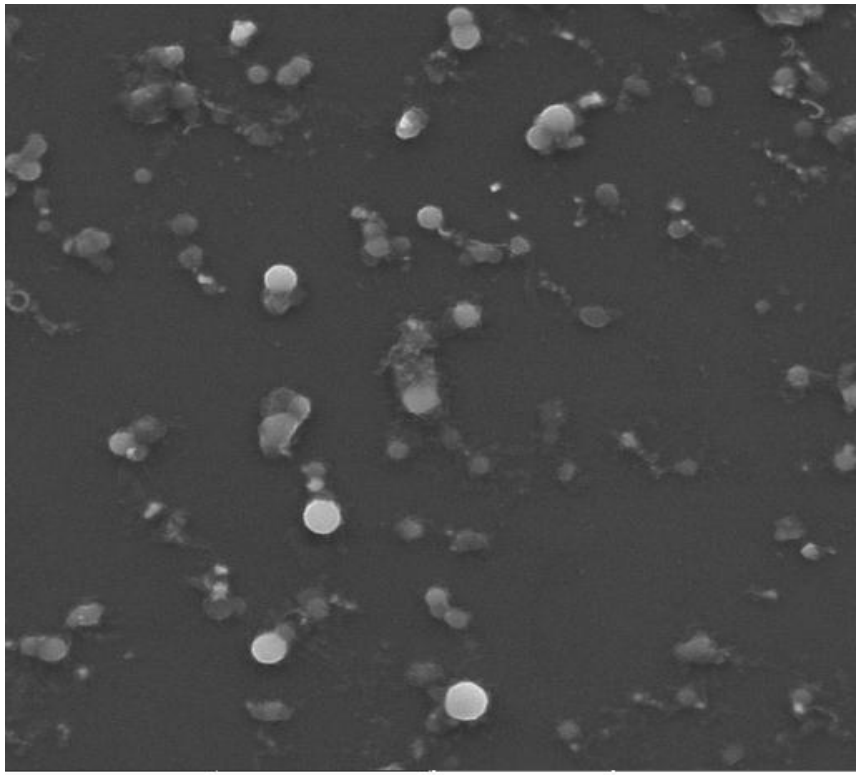


Formulation 2



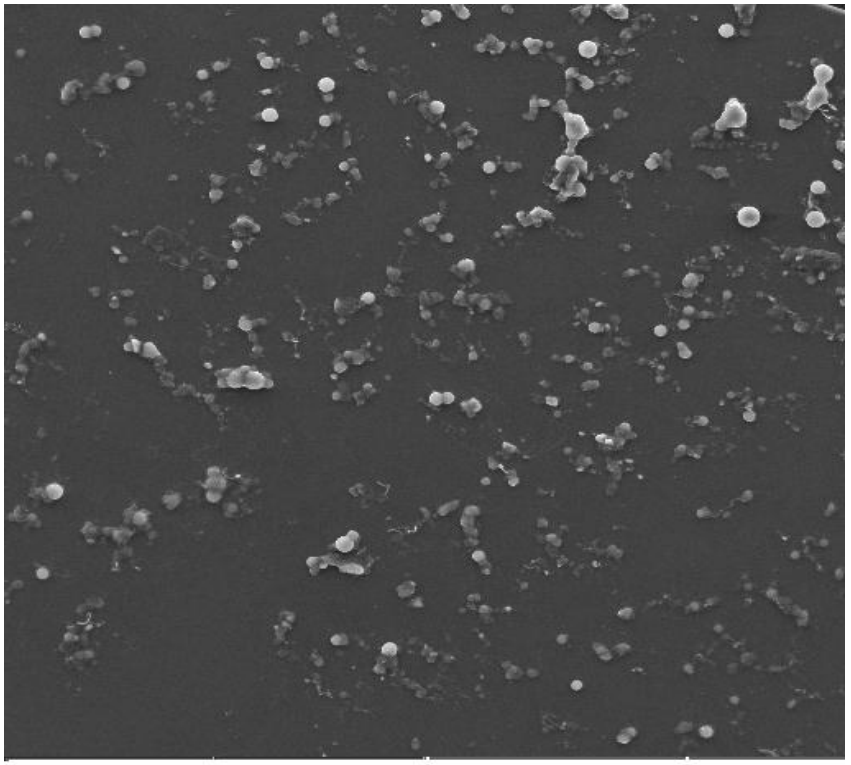
SCANNING ELECTRON MICROSCOPY(538x,196x)

FORMULATION 1



SPAN 40: 50µm

FORMULATION 2



SPAN 60: 200µm

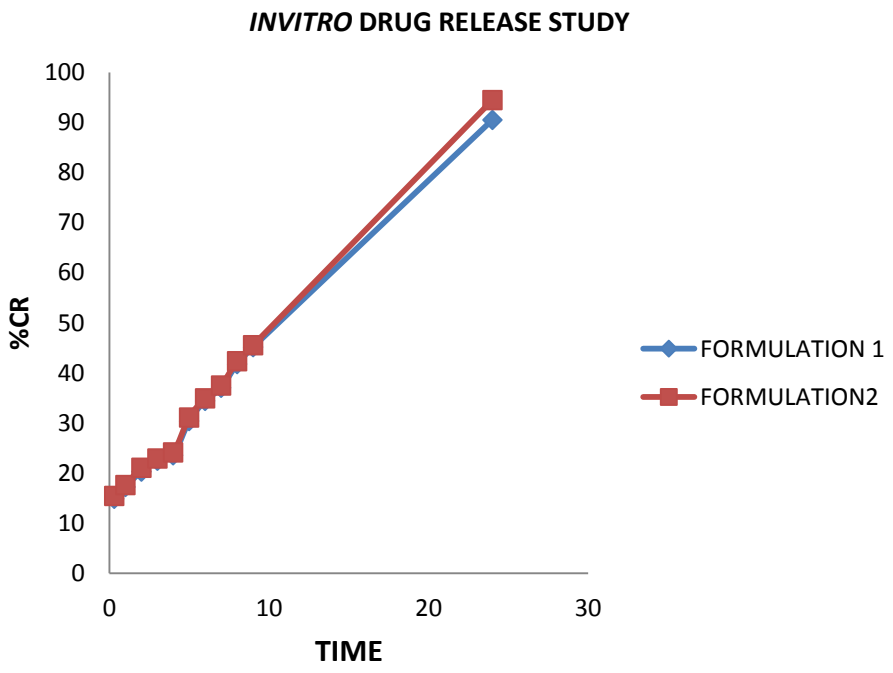
Entrapment efficiency

Percentage Drug Entrapment of the Formulated Niosomes

S.No	Formulation code	% Drug entrapment
1	FI(Span 40)	55%
2	FII(Span 60)	68%

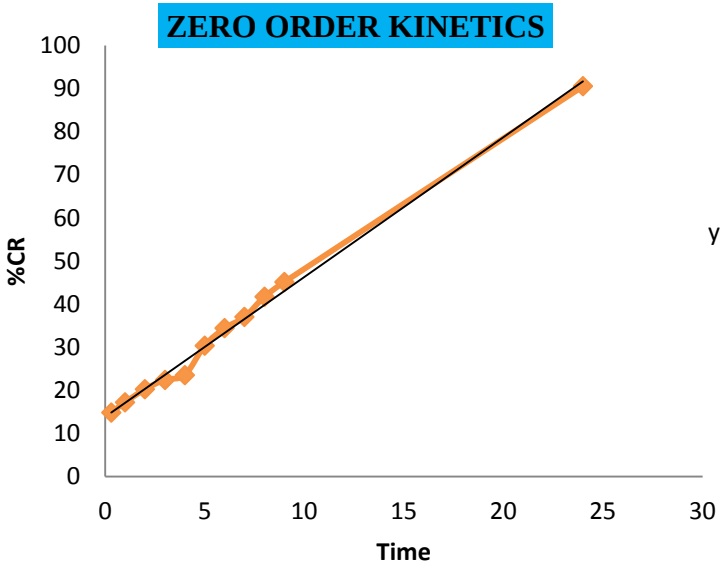
IN VITRO RELEASE STUDIES

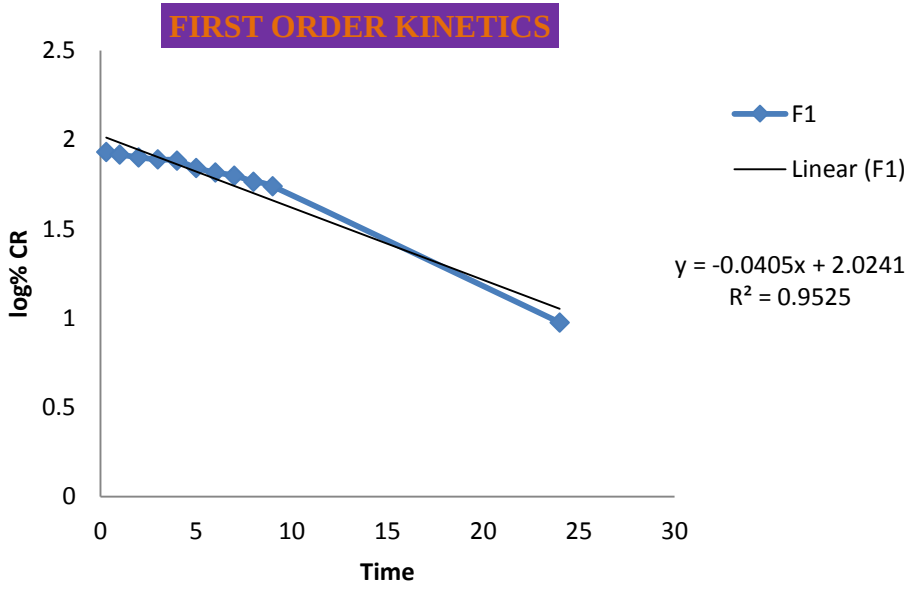
S.No	% CR(F 1)	% CR (F2)
0.3	14.85	15.43
1	17.23	17.60
2	20.29	21.03
3	22.46	22.88
4	23.57	24.15
5	30.40	31.09
6	34.42	34.90
7	37.02	37.49
8	41.75	42.33
9	45.14	45.51
24	90.52	94.46



RELEASE KINETICS

FORMULATION 1

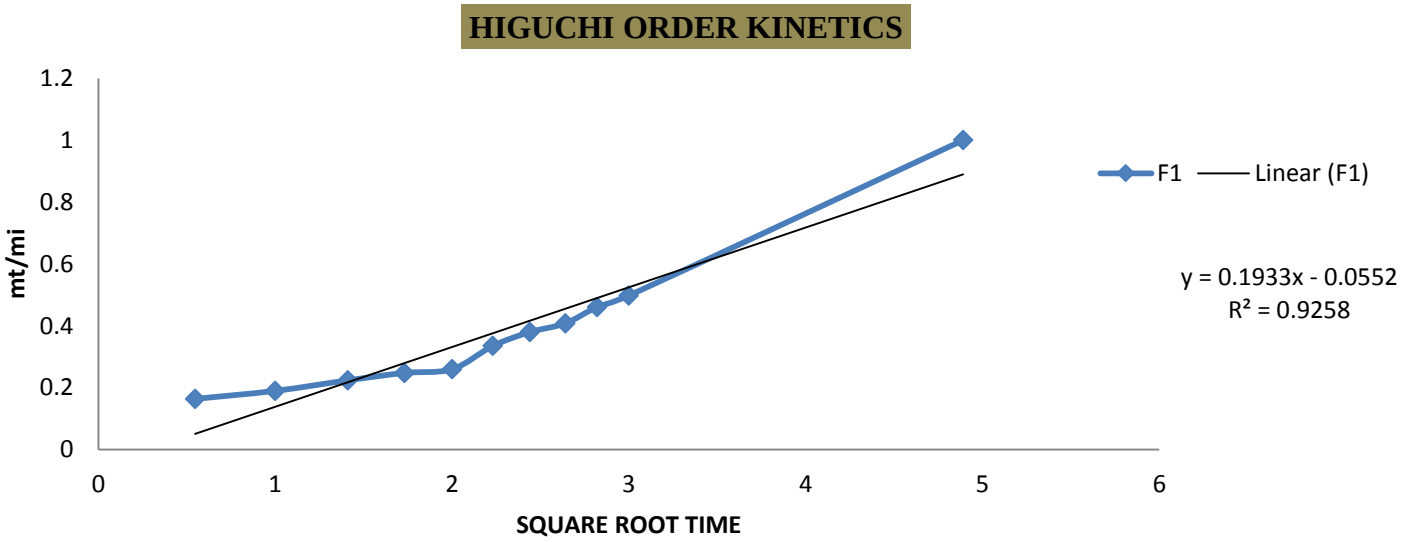




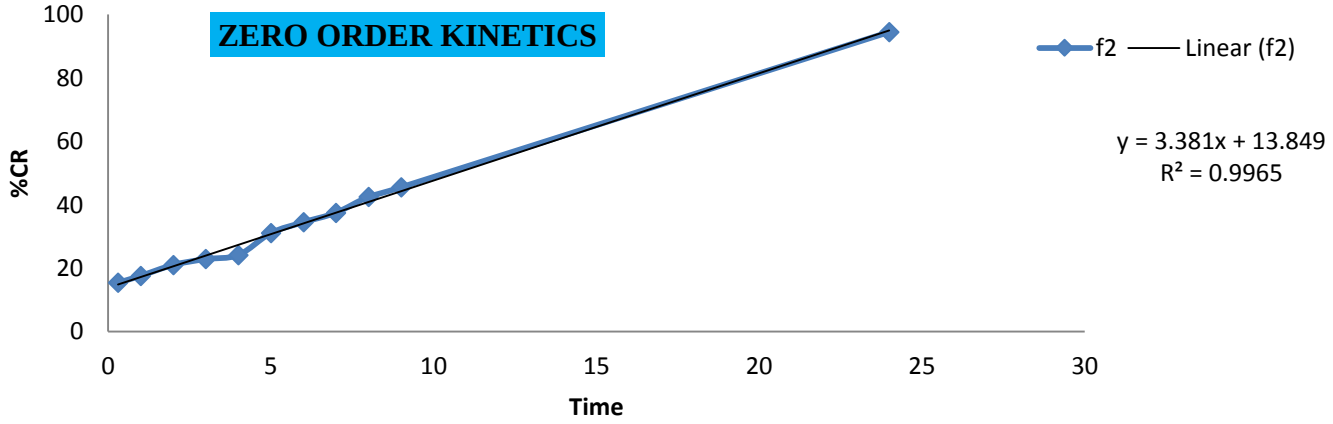
Zero order kinetics

First order kinetics

Higuchi order kinetics

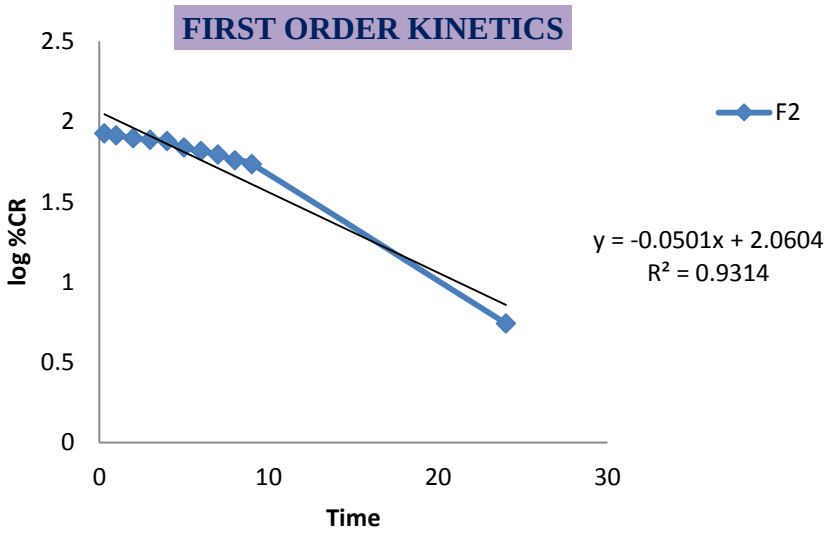


FORMULATION 2

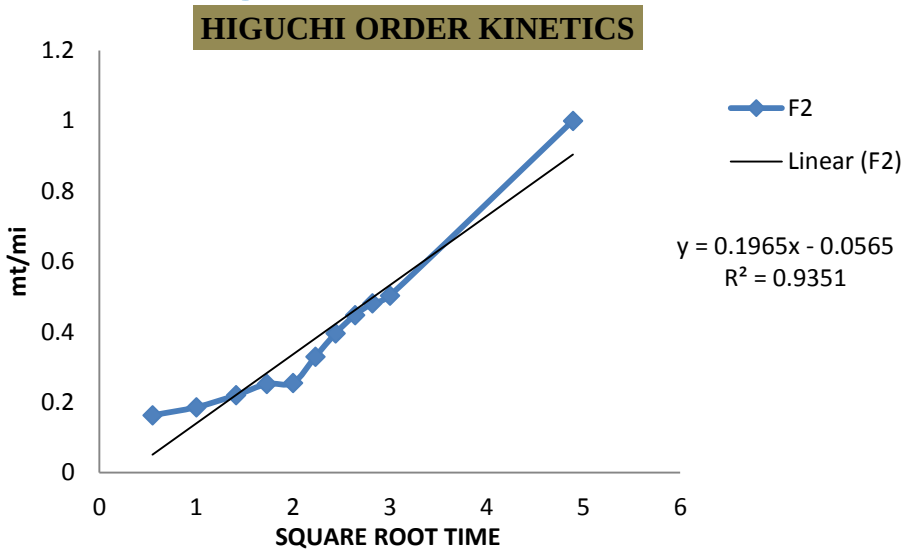


Zero order kinetics

First order kinetics



Higuchi order kinetics



Kinetic modeling data

S.No	Kinetics model	r^2
1	Zero order	0.996
2	First order	0.931
3	Higuchi model	0.935

Stability studies:

Storage under refrigerated condition showed greater stability with 94.85% of drug content at the end of three months, whereas storage under room temperature and at $40^0C \pm 2^0C$ / R-H 70 % ± 5% showed drug content of 92.83% and 85.41% at the end of three months.

Temperature	Amount of drug retained (%) after month			
	Initial	I	II	III
Refrigeration ($4^0C \pm 1^0C$	100	97.42	96.22	94.85
Room Temperature	100	95.68	94.77	92.83
$40^0C \pm 2^0C$ RH-70% ± 5%	100	89.79	87.73	85.41

Sterility Test:

- Sterility test was done by I.P.1996.
- After 7 days of observation, all the tubes containing sample was found to be sterile.

ANTICONVULSANT ACTIVITY:

Different stages of convulsion studies

Groups	Duration (sec)				
	Flexion	Extension	Convulsion	Stupor	Death
Control	1.52, 3.56, 3.75, 2.84, 3.16, 4.51	21.46, 34.61, 23.25, 18.56, 15.92, 20.59	8.91, 9.14, 14.63, 11.50, 9.12, 8.76	156,121, 186, 0, 155, 0	2/6
Standard	4.19, 2.11, 5.13, 2.62, 3.14, 2.19	2.81, 1.96, 3.91,2.46, 1.08, 0	4.36, 4.59, 8.83, 0, 0, 3.61	111, 123, 125, 129, 108, 105	0/6
Formulation	1.08, 1.28, 5.13, 0.88, 1.87, 1.71	0.68, 1.63, 0, 1.6, 0, 0	4.6, 5.87, 0, 1.38, 4.44, 6.21	77, 69, 91, 86, 81, 94	0/6

Deviations obtained from convulsion studies

Groups	Duration (sec)				
	Flexion	Extension	Convulsion	Stupor	Death
Control	3.223333 ± 0.4122513	22.39833 ± 2.650172	10.34333 ± 0.9525288	103.000 ± 33.63728	2/6
Standard	2.018333 ± 0.3203167	0.7066667 ± 0.3590605**	3.565 ± 1.35183**	108.500 ± 6.716894	0/6
Formulation	2.701667 ± 0.6589305	1.981667 ± 0.562645**	3.750 ± 1.023458**	92.000 ± 8.449852	0/6

From the above tabular coloum it is concluded that formulation(f_2) shows better anticonvulsant activity than the standard in lesser time.

SUMMARY

The present study was aimed to prepare niosomes containing drug levetiracetam. It belongs to the class of drugs called anticonvulsant drug. These drugs are used for the epilepsy. They work by maximum stimulation with electrical current or different chemoconvulsants and showed only minimal activity in submaximal stimulation. Hence the present work was made to formulate and evaluate the niosomes of levetiracetam by intraperitoneal route of drug and the anticonvulsant activity is to conduct on mice to find the best formulation.

- FT-IR of pure drug and drug excipients mixture revealed no chemical interaction. Hence they were compatible.
- UV Spectrophotometric method was developed for the determination of levetiracetam in 7.4 pH phosphate buffer at 220nm. A regression coefficient value was found to be 0.99 for levetiracetam.

- Two formulations of levetiracetam niosomes were prepared by hand shaking method.
- Formulation of multilamellar niosomes vesicle were confirmed by examing the niosomal suspension under an TEM and observed the internal morphology.
- Various parameters like vesicle shape and entrapment efficiency were done for niosomes. Vesicle shape was analysed by SEM technique. The vesicles were found to be smooth and spherical in shape. Entrapment efficiency was noticed for F2 with 68%.
- Niosomes were subjected to *in-vitro* diffusion studies. It reveals that F2 formulation has higher release with 94.46%.
- The formulations were subjected to release kinetics and the best formulation was found to be F2 and its follows zero order kinetics.

- Anticonvulsant activity of Niosomal formulations and the plain levetiracetam were conducted on 24 mice. It was found that niosomal formulation (F2) shows better activity than plain levetiracetam.
- Stability studies were carried out at $4^{o}C \pm 1^{o}C$, $40^{o}C \pm 2^{o}C$ RH-70% ± 5% and room temperature for 3 months. There were no significant changes in entrapment efficiency and *in-vitro* drug diffusion profile.
- Sterility tests are performed for the niosomal formulations, after 7 days observations all the tubes containing samples was found to be sterile.

CONCLUSION

- Levetiracetam niosomal formulation was prepared by hand shaking method and it was evaluated for its entrapment efficiency, *in-vitro* drug diffusion profile, sterility tests and stability studies. The optimized formulation F2 and levetiracetam plain were evaluated for it's anti-convulsant activity using flexion, extension, convulsion, stupor, recovery and death. The effects produced by the formulation (F2) in the mice were comparable with that of the *in-vitro* drug diffusion profile. Hence, the present study concluded that the anti-convulsant activity of levetiracetam formulation (F2) shows better activity than the plain levetiracetam.

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THANK YOU

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