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Project Title: FABRICATION, OPTIMIZATION AND CHARACTERIZATION OF ANTIPSYCHOTIC DRUG LOADED NANOSTRUCTURED CUBOSOMES AS IN SITU GEL FOR BRAIN TARGETING

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ABSTRACT

In India, every 3 out of 1000 individuals and 1% of world population suffer from schizophrenia. Lurasidone hydrochloride is BCS Class II drug with poor bioavailability of about 9-19% and lacking solubility to show quick response. Atypical antipsychotic agent that function as a dopamine D2 receptor antagonist; serotonin 5-HT2 receptor antagonist. It is used for the treatment of schizophrenia and bipolar disorder. The major drawback in drug treatmentwas crossing the blood brain barrier. Drug delivery to the brain is most challenging because ofthe presence of the blood brain barrier (BBB). Brain targeting through nasal cavity offers safeentry route of drug to brain by restricting BBB as the olfactory receptor cells are in direct contact with the CNS. It constitutes neuronal and extracellular pathways to delivery oftherapeutic agent, there is rapid onset of pharmacological action and higher bioavailability of lipophilic drugs. The reason for selecting intranasal *insitu* drug delivery is because of its largesurface area for drug absorption. The drug concentration increases with simultaneous reductionof dose and side effects. The major advantage is that it also avoids first pass metabolism and have rapid onset of action as compared to oral route. *insitu* delivery system favours the ease and convenience of administration as drops allowing accurate dosing. Cubosomes are nano-structures composed mainly of amphiphilic polar lipid. When this amphiphilic substance dissolved in water with concentration above the critical micelle concentration, it forms micellar aggregations. At higher concentrations, the formed micelles are forced to form cubic structure. Cubosomes may offer over other lipid-based systems, such as liposomes, are the potential to encapsulate a large drug-payload and for sustained release of the entrapped bioactive.

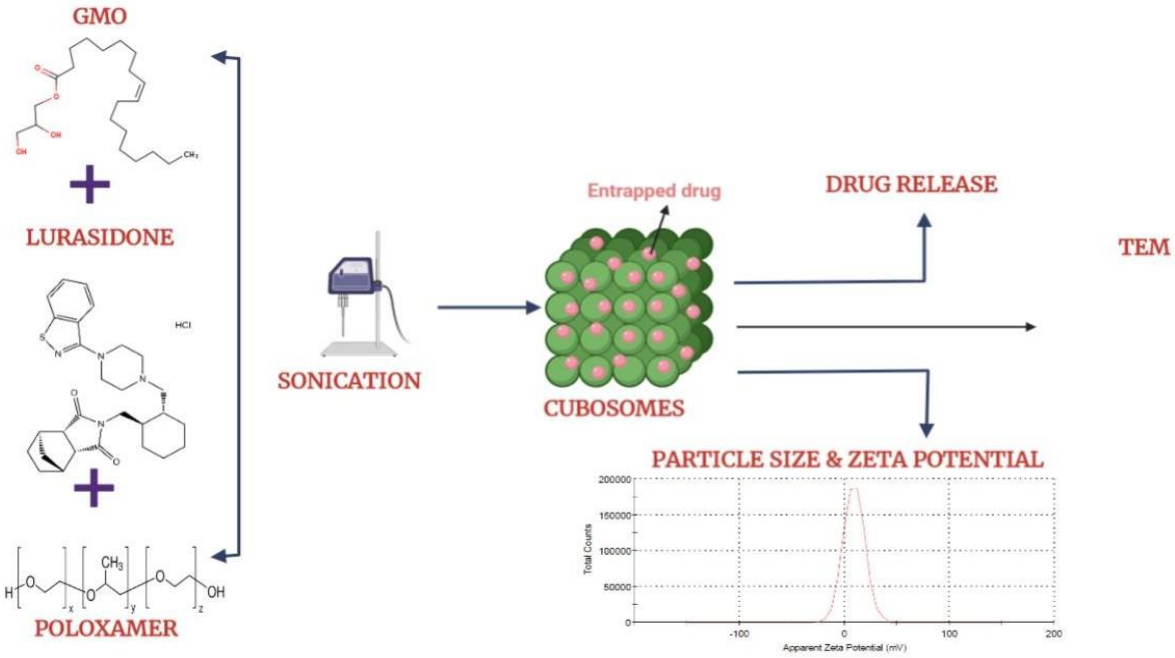


Figure 1: Graphical abstract

OBJECTIVE:

Primary Objective

- To formulate **lurasidone** hydrochloride loaded **cubosomes** to enhance the dissolution rate.
- To optimize the Nano carrier system for enhanced drug loading by adopting Quality byDesign (BD)
- To formulate thermosensitive *in situ* **gel** using nano cubosomes.
- To characterize the formulated nano cubosomes and *in situ* gel.
- To evaluate the toxicity of prepared nano cubosomes using **SH-Sy-5y Human Neuroblastoma cell lines**.
- To carry out the **short-term stability studies** as per ICH guidelines.

Secondary objective

- Based on the *invitro* dissolution and cytotoxicity data. The optimized formulation willbe subjected for *in vivo* pharmacokinetic study to confirm the enhanced drug uptake in brain

LITERATURE SURVEY

Nearly 150 published articles were reviewed for the purpose of study of experiment. The articles were relevant to the antipsychotic drugs, neurodegenerative disease, and drug deliverytechnology. Some important highlights are mentioned as follows:

AUTHOR	JOURNAL	TITLE	CONCLUSION
Mayuri Ahirrao *et al*	Drug Development and Industrial Pharmacy	*in vitro* and *in vivo* evaluation of cubosomal *in situ* nasal gel containing resveratrol for brain targeting	studied the delivery of resveratrol to the brain through the transnasal route by cubosomes. It showed significantly higher transnasal permeation and better distribution to brain, when compared to the drug solution (i.v.) and drug solution (oral).
Fatma Elzahraa Abdelrahman*et al*	International Journal of Pharmaceutics	Investigating the cubosomal ability for transnasal brain targeting: *in vitro* optimization, *ex vivo* permeation and *in vivo* biodistribution	concluded that cubosomal gel could be considered as a promising carrier for brain targeting of CNS acting drugs through the transnasalroute.
Radhakrishnan Nithya et al	Journal of Drug Delivery Science and Technology	Cubosomes of dapsone enhanced permeation across the skin	prepared Dapsone loaded cubosomes (DC) by ultrasonication. They concluded that DC is a good option to enhance permeation across the epidermal layers of the skin.
Hanisah Azhari et al	European Journal of Pharmaceutics and Biopharmaceutics	Stabilizing cubosomes with Tween 80 as a step towards targeting lipid nanocarriers to the blood–brain barrier	confirmed that Tween 80 can effectively stabilize phytantriol cubosomes, opening the possibility for future application in drug delivery across the BBB.

Haiqiao Wang et al	Journal of Colloid and Interface Science	Polymerization of cubosome and hexosome templates to produce complex microparticle shapes	stated that Cubic and hexagonal liquid crystalline particles are used as templates to polymerize various monomers to produce particles with unique micron-scale geometric shapes.

TOOLS/ HARDWARE/ SOFTWARE USED:

HARDWARE:

Several instruments are required for the formulation of cubosomes and characterizationof resulting product.

Table 2: Hardware used in study

S.NO.	EQUIPMENTS	MAKE /MODEL
FORMULATION		
1	Digital weighing balance	AX200 Shimadzu
2	Probe sonicator	VT- PROBE 250
CHARACTERIZATION		
3	UV-Visible Spectrophotometer	UV-1700 Pharmaspec, Shimadzu
4	Cooling centrifuge	C-30BL REMI
5	FTIR Spectrophotometer	4100 JASCO
6	Zeta sizer	MAL 1021384 Malvern Instruments
7	Brookfield viscometer	CAP 2000+
8	Transmission electron microscope	Tecnai sprit G2 FEI
9	Differential scanning calorimetry	DSC 60 Shimadzu

SOFTWARE:

- **Design-Expert®** Version 11

  It is a powerful tool employed for the design of experiment and optimization of cubosomes.

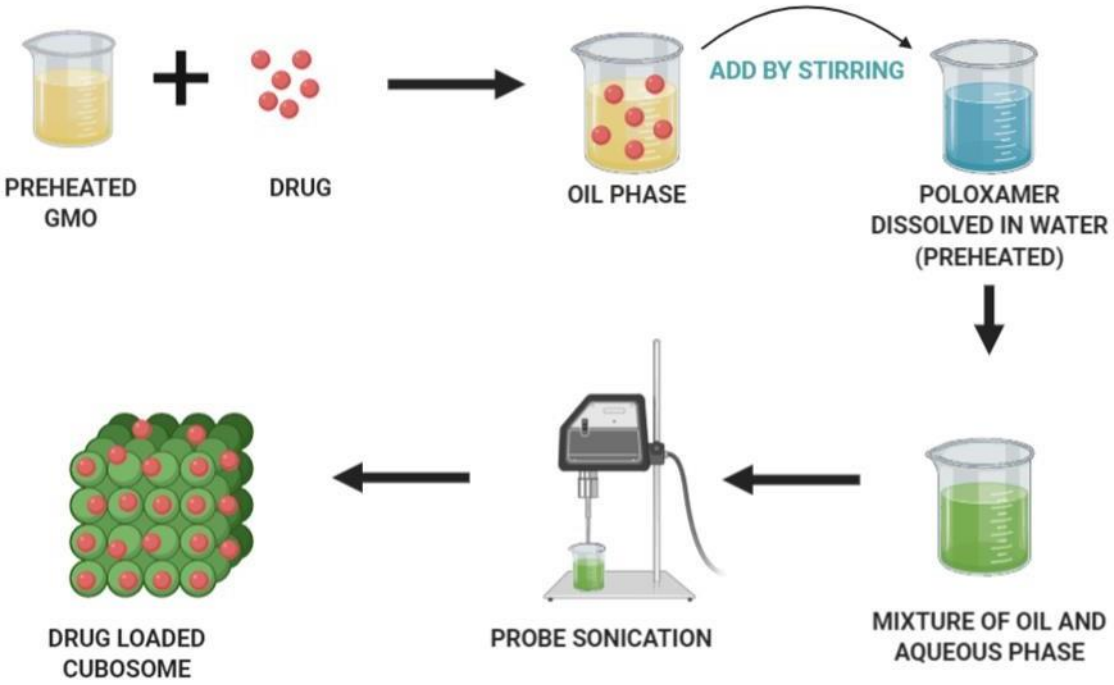
METHODOLOGY:

1. **Preformulation studies: FT-IR and DSC**

   To check compatibility of the drug and polymers.

2. **Formulation of lurasidone load nano cubosomes**

   - Cubosomes are prepared by high speed homogenization followed by probe



Sonicationmethod using the drug, glycerol monooleate and lutrol F127.

Evaluation of cubosomes:

- **Physiochemical properties:**

The Vesicle size, size distribution and zeta potential are determined by Dynamic Light Scattering system by Malvern Zeta sizer

- **Vesicle morphological analysis:**

The cubosome vesicle morphology can be visualized by Transmission electron microscopy (TEM), phase contrast microscopy, etc.

- **Entrapment efficiency:**

The entrapment efficiency is expressed as the percentage entrapment of the drug added.

Entrapment efficiency = (Amount entrapped / Total amount added) ×100

- **Drug content**

The cubosome formulation equivalent to 10mg of drug is taken and suitably diluted with appropriate solvent. The drug content was estimated by UV method as described earlier

- **In vitro drug release**

The *in-vitro* drug release is carried out by the dialysis bag technique using 0.1 N hydrochloric acid (HCl, pH 1.2) followed by phosphate buffer pH 6.8. Cubosomes equivalent to 20 mg is placed in the dialysis bag (12,000-14000 Da), tie at both ends and introduce into 50 mL of the diffusion medium at 37±1°C under magnetic stirring at 100 rpm. At predetermined time intervals aliquots are withdrawn and drug concentration is determined by UV spectroscopy at ʎmax. The kinetic analysis of the release data will be done by model fitting to different equations to characterize the release profile from cubosomes.

Formulation and characterization of cubosomal *in situ* gel

- The optimized cubosomes are re-dispersed into *in situ* gel to increase its physical stability; nasal residence time and patient feasibility.

Evaluation of Mucoadhesive Nasal Gel

- **Gelation Temperature:**

Visual inspection method is employed to determine gelation temperature of the prepared gels

- **Gelation Time:**

Gelation time is recorded when the magnetic bar stopped stirring and the test was triplicated.

- **Viscosity:**

The rheological studies were carried out using Brookfield viscometer

- ***In-vitro* drug release.**

The *In-vitro* release studies of the formulated in situ gel are carried out using Franz diffusion cell. The pre-treated dialysis membrane is mounted in between reservoir compartment and donor compartment with help of clam and the dose equivalent amount of gel is placed in donor compartment

- ***in vitro* cytotoxicity study:**

To find the cytotoxicity of the prepared nano cubosomes a twice-sub cloned cell line derived from the SH-Sy-5y Human Neuroblastoma cell lines is used. It serves as a model for neurodegenerative disorders, since the cells can be converted to various types of functional neurons by the addition of specific compounds. Being similar to blood brain barrier, cell line study is planned to be carried out.

PROGRESS OF WORK:

- Collection of data
- Literature review
- Procurement of raw materials
- Preformulation studies:

  From the results of preformulation studies we observe that there was **no interaction** between drug and excipients.
- Formulation of cubosomes
- Particle size analysis:

  We have noticed that expected particle size was achieved which ranges between 200-250MM

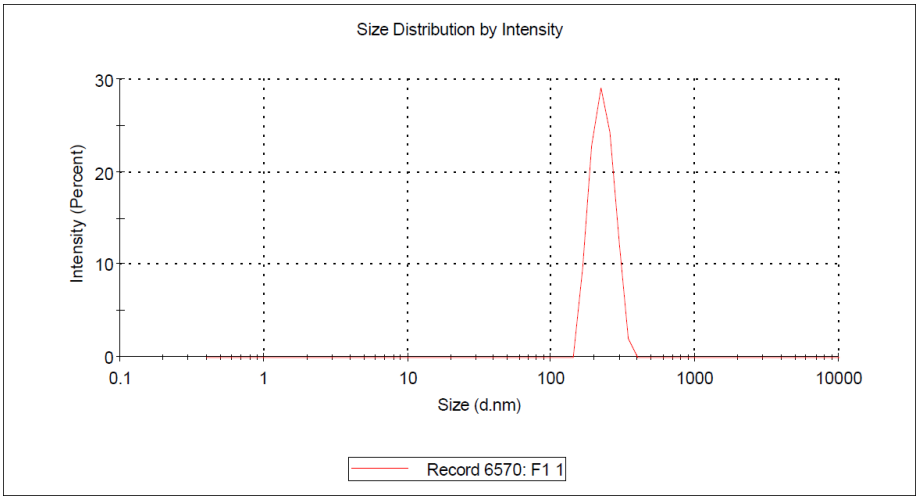


Figure 3: Particle size distribution

EXPECTED OUTCOME:

- **Produce lipophilicity:**

  It enables easier transmission of drug across Blood Brain Barrier.

- **Decreased particle size:**

  It results in increased surface area and enhanced absorption through EPR effect

- **Promote controlled release:**

  Controlled release maintains optimum drug concentration and leads to dose reduction Delivery drug to the brain by crossing BBB.

  Higher drug concentration is delivered to the targeted site by surpassing the complexnetwork of BBB.

- **Effective treatment of psychiatric diseases:**

  Improved patient compliance and better therapeutic effect

CONCLUSION:

Cubosomes can be considered as a **potential carrier** in the treatment of psychiatric diseases. With the increased bioavailability, dosing can be considerably reduced and enable patient compliance. Hence, we conclude that cubosomes can play an enormous role in drug delivery to target neurodegenerative diseases.

BASE PAPER:

Mayuri Ahirrao & Shilpa Shrotriya (2017): In vitro and in vivo evaluation of cubosomal *in situ* nasal gel containing resveratrol for brain targeting, Drug Development and Industrial Pharmacy, DOI: 10.1080/03639045.2017.1338721

Ahirrao *et al*, studied the delivery of resveratrol to the brain through the transnasal routeby cubosomes. It showed significantly higher transnasal permeation and better distribution to brain, when compared to the drug solution (i.n.) and drug solution (oral). Finally, they concluded that cubosomal gel could be considered as a promising carrier for brain targeting of Resveratrol through transnasal route.

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