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FORMULATION AND EVALUATION OF LIQUID CRYSTALS CONTAINING ACOTIAMIDE CAPSULE FOR ORAL DELIVERY

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Abstract:
The liquid crystals called as mesophase inter mediate between the crystalline solid state and therefore the amorphous liquid state. (Lagerwall, 2012) Liquid Crystals nano carriers are an intermediary state between the solid and liquid state. it's mostly named a mesomorphic state. (Imran, 2012) From reverse cubic phase colloidal particles are interior aqueous zones also afford certain benefits in technical applications compared by means of droplets of general oil-in-water emulsions The liquid could be a substance that which is thermodynamically situated in within the middle of the isotropic liquid and therefore the crystalline phase. They show flow properties sort of a liquid and at the identical time partly hold the order of a crystal. (Dierking, 2017) The liquid are often deliberated 1 / 4 states of matter following solid, liquid, and gas. Liquid-crystal phases, as their name suggests, be existent between the predictable crystal phase and therefore the liquid phase.

Keywords- Acotiamide, Poloxamer 407, Liquid crystal.

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1. INTRODUCTION:

1.1 Liquid Crystals

The liquid crystals are categorized into two main categories i.e. thermo tropic and lyotropic. These classes are further well-known into various phases reliant on the differences in their orientational or positional order under the effect of external issues like per temperature. They're shows in Figure 1. (Chandrasekhar, 1993; Jiang, et al., 2016)

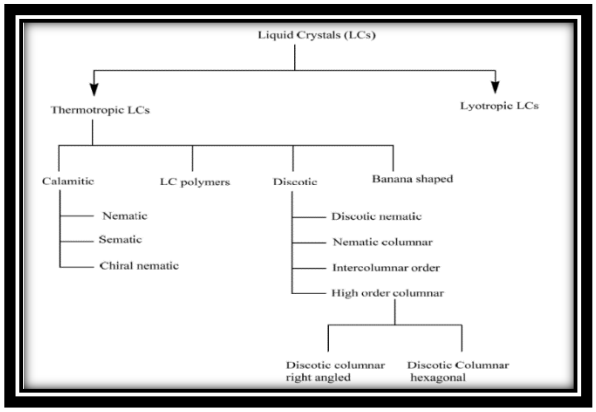

Fig-1 Types of liquid crystals (Jiang, 2016)

In appropriate conditions, the molecules of LCs show orientational way such all the axes line up and form a supposed hematic liquid. The molecules are still capable to transfer the globe over within the fluid, but their orientation remains the identical. It's the smallest amount well-arranged LC phase. On the conflicting, smectic (Sm) phase displays the orientational order but also positional. Within the smectic phase, the molecular cores of mass are organized in layers and therefore the drive is especially limited inside the layers. within the cholesteric LC phase, molecules express intermolecular forces that errand arrangement between molecules at a minor angle to at least one another.

1.1.1 Thermo tropic Liquid Crystals-

Thermotropic LCs are those which are extensively recognized thanks to their applicational influence within the laptop, flat screen televisions, and tablet displays, or mobile phones. of these applications depend upon the purpose that LCs reveal elastic behavior and might be addressed via electric or magnetic fields, which alter the orientation of the axis, and so the birefringence. Thermotropic LCs are further illustrious by their degree of order, show further stage of transitions inside the temperature regime of the liquid crystalline state. The kinds of Thermotropic LCs are shown in Figure 2. (Hina, 2016)

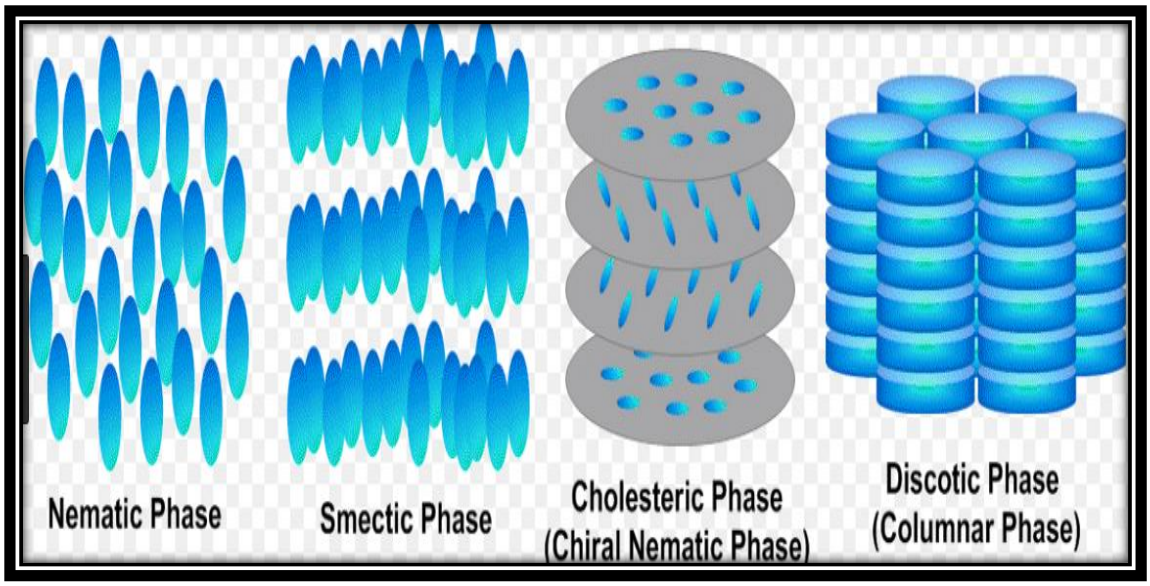

Fig- 2 Thermotropic Liquid Crystals and its types

1.1.2 Lyotropic Liquid Crystals (LLCs)

Lyotropic LCs has significance in medication conveyance application. Numerous amphiphilic molecules that have particular polar and non-polar units which might be ionic, non-ionic or cationic shows lyotropic LCs stage succession.

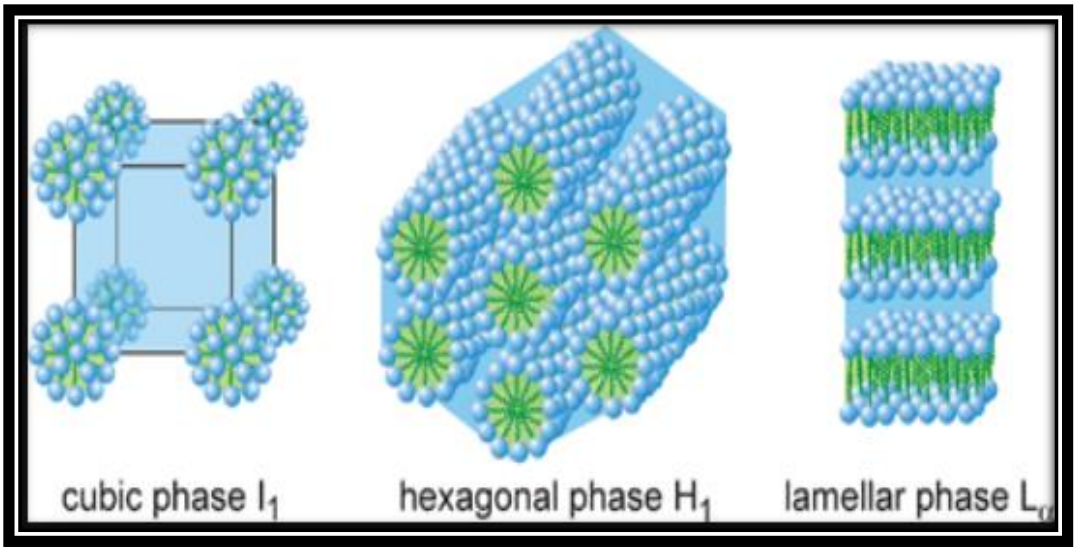

Fig-3 Lyotropic Liquid Crystals and its type

At the point when an amphiphile is broken down in the water, because of the polar head and nonpolar tail, the particles self-gather framing micelles, a comparable marvel is watched even to surfactants in cleanser arrangement. Numerous amphiphilic atoms show LLC stage successions dependent on the volume balance, hydrophilic and hydrophobic areas. At the point when the atoms self-gather, dissolvable particles fill the space around the mixes to give ease to the frame work. Structure of the particle relies upon the substance of the dissolvable. Micelles framed basically were amphiphilic monolayer in which totals are conveyed haphazardly in the dissolvable making an isotropic micellar arrangement. As the grouping of amphiphile transforms, it produces distinctive organized LLC. Molecules are haphazardly dispersed with no organization at low convergence of amphiphile. At the point when the fixation is marginally expanded, they will, in general, organize themselves into micelles or vesicles. At high fixation, get together ends up requested, a few structures to the hexagonal columnar stage, cubic or lamellar. In these paired frameworks, if the centralization of amphiphile is expanded past the lamellar stage, they will in general structure turn around hexagonal, switched cubic and invert micellar cubic stages. (Yang, 2016) Lyotropic LCs instead are detected when altering the concentration of a shape or property anisotropic dispersant in an isotropic solvent. A maximum, lyotropic phases are observed as a function of the amount of amphiphilic molecules in water or other solvents, as schematically shown in Figure 3. Below the critical micelle concentration (cmc), the amphiphiles are molecularly isolated in the solvent, at superior concentrations form micelles, which can be spherical, rod or disk–like type, depending on the molecular shape. At even greater concentrations, these micelles aggregate to well-ordered structures and can procedure hexagonal, cubic or lamellar phases, also of the inverse Nanomaterials type. The LLCs shows in Figure 3. (Yang, 2016)

1.1.3 Cubic phase

Cubic phase are discrete, sub-micron, nanostructured particles of the bicontinuous cubic fluid crystalline stage. Cubic LCs are nanoparticles which are self-assembled liquid crystalline particles of specific surfactants with an appropriate proportion of water with microstructure. Cubic liquid crystals are nanoparticles yet rather than the strong particles normally experienced, cubic LCs are self-amassed liquid crystalline particles with a solid like rheology that gives exceptional properties of practical interest. (Tileka, 2014) Most likely cubosomes are made out of polymers, lipids and surfactants with polar and non-polar parts consequently said as amphiphilic. The amphiphilic molecules are driven by the hydrophobic impact into polar dissolvable to imprudently distinguish and assemble into an LCs of nanometre scale. The cubic crystallographic symmetry and are optically isotropic, thick and strong as well. The cubic stage can break and shape colloidally as well as thermodynamically stable particulate scatterings. Cubic LCs have incredible significance in nano-drug. (Grace, 2015) The term 'cubic phase' is inferred by their structure since they have cubic crystals lattice, were called as cubic LCs. One of the main distributed cases of the term is found in a survey distributed, 'cubic' being a cubic are the nanoparticles of bicontinuous, lyotropic cubic stages,

involved bent lipid bilayers sorted out into a three-dimensional honeycomb (huge) like structures isolating two inside fluid channels and huge interfacial territory. Cubic stages are optically isotropic, exceptionally thick, and strong like (crystalline) with cubic crystallographic symmetry. (Spicer, 2004)

The structure of fluid monoglyceride cubic stages utilizing X-ray diffraction discovered cubosomes has constant areas of both hydrophobic and hydrophilic nature, which prompts an end that the cubic stage structures clarified with the idea of differential geometry and intermittent negligible surfaces. The basic highlights of cubic liquid stages were their interfacial area (~400m^2), the thickness of bilayers (3.5mm) and the breadth of pores (5mm). The Cubic phase and their composition shown in Figure 4. (Garg; Saraf *et al.*, 2007)

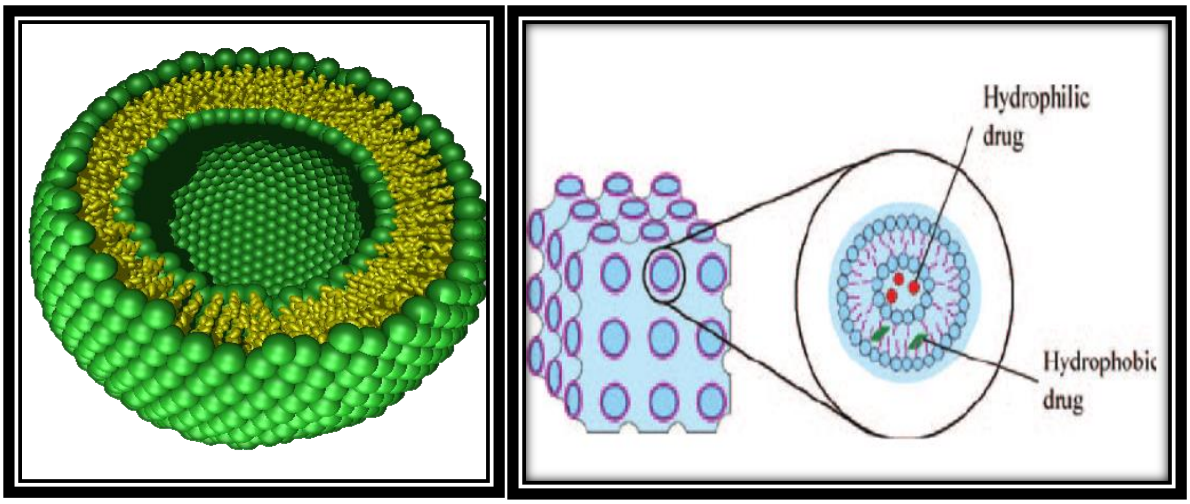

Fig-4 Cubic phase and their composition (Achouri, 2013**)**

2. NEED

Acotiamide has been reported to spice up meal-related signs of functional dyspepsia in clinical studies. Here, we observed the gastroprokinetic effects of acotiamide and its antiacetylcholinesterase action as a possible mechanism of action in conscious dogs. Acotiamide increased postprandial gastric motor activity in conscious dogs with chronically implanted force transducers and, furthermore, acotiamide improved clonidine-induced hypomotility and delayed gastric emptying. Acotiamide improved postprandial gastroduodenal motility was suppressed completely by pretreatment with atropine, a muscarinic receptor antagonist. In in vitro studies, acotiamide improved acetylcholine- but not carbachol-induced contractile replies of guinea pig [gastric antrum strips. Moreover, like itopride and neostigmine, acotiamide Prevent recombinant humanoid and canine stomach-derived acetylcholinesterase (AChE) activity in vitro. The way of the AChE inhibitory act of acotiamide was selective and reversible. The commercial dosage forms having the dose 90 mg twice daily, in line with the recent study of LCs reduce the frequency of dose and improve the patient's compliance. Hence sustain release LCs loaded with drug formulated. Sustained-release dosage forms are dosage forms designed to release (liberate) a drug at a predetermined rate so on carry on a relentless drug concentration for a selected period of some time with minimum side effects. Sustained release dosage form incorporates variety of advantages over conventional dosage form improved patient convenience thanks to less frequent dossing, reduction in fluctuation in steady-state level and thus better control of disease, maximum utilization of drug enabling a reduction within the whole amount of dose administered. Sustain release formulation are those which shows slow-release pattern. Slow dissolution and absorption of the drug, which might produce sustain action.

LCs formulation possessing high entrapment of drugs and avoid drug leakage during storage, so for its potential in improving solubility and oral bioavailability of poorly water-soluble drugs as compared to traditional approaches.

2.1 AIM

Formulation And Evaluation Of Liquid Crystals Containing Acotiamide Capsule For Oral Delivery.

2.2 OBJECTIVE

1) To optimize liquid cryatal formulation loaded with maximum drug.
1) To obtained free-flowing powder of LCs by Spray drying method.
3) To study the release kinetic of the optimized formulation.

3. PLAN OF WORK

1) Literature survey
2) Selection of drug and excipient
3) Confirmation of drug
   - Melting point
   - IR Spectroscopy
   - DSC
   - Solubility of drug
   - UV Method

4. DRUG PROFILE

Acotiamide.

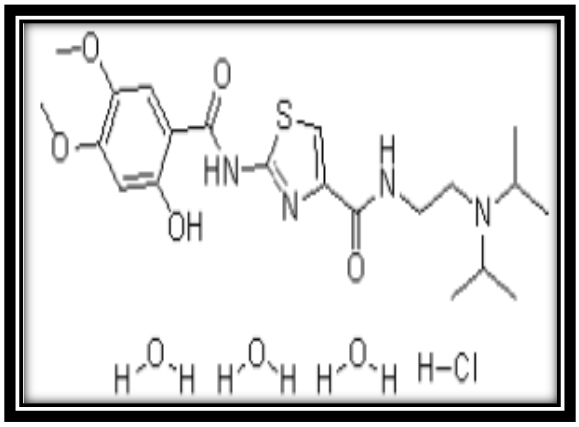

Fig.5: Chemical Strcture of Acotiamide.

Pharmacology

Acotiamide Hydrochloride is the hydrochloride salt form of acotiamide, a prokinetic agent with gastrointestinal (GI) motility-enhancing activity. Although the exact mechanism by which acotiamide exerts its effect has yet to be fully elucidated, this agent appears to inhibit acetylcholinesterase (AchE), an enzyme responsible for the breakdown of acetylcholine (Ach). Increased Ach concentrations lead to an
improvement of gastric emptying and GI motility and eventually to a reduction of dyspepsia symptoms.

5. EXCEPIENT

5.1 Poloxamer 407

5.1.1 Synonym: Pluronic, Lutrol,Polyethylene-propylene glycol copolymer.

Structure:

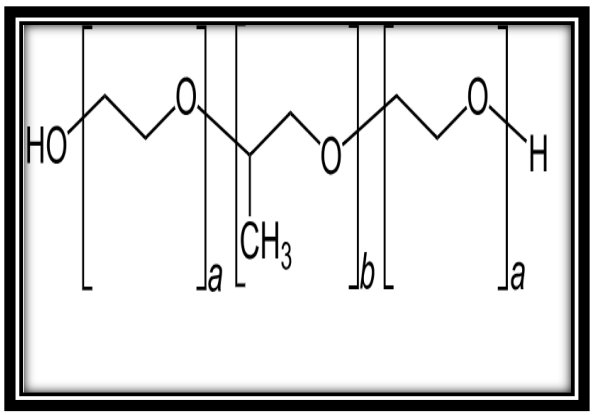

Fig-6 Structure of Poloxamer407

5.1.2 Melting point: 53-57

5.1.3 Pharmaceutical Application: Poloxomer 407 are the non-ionic polyoxyethylene-polyoxypropylene copolymers used primarily in pharmaceutical formulations as emulsifying or solubilizing agents the polyoxyethylene segment is hydrophilic while the polyoxypropylene is hydrophobic. Poloxomers 407 are used as emulsifying agents in intravenous fat emulsions, and as solubilizing agents to require care of the clarity of elixirs and syrups. Poloxomers 407 may additionally be used as wetting agents; in ointments, suppositories bases, and gels; and as tablet binders and coating.

5.2 Oleic acid

5.2.1 Synonyms

Acidumoleicum, crodolene, crossesential 094, eleic acid, emersol, glycon, oleinic acid, priolene.

5.2.2 Structure:

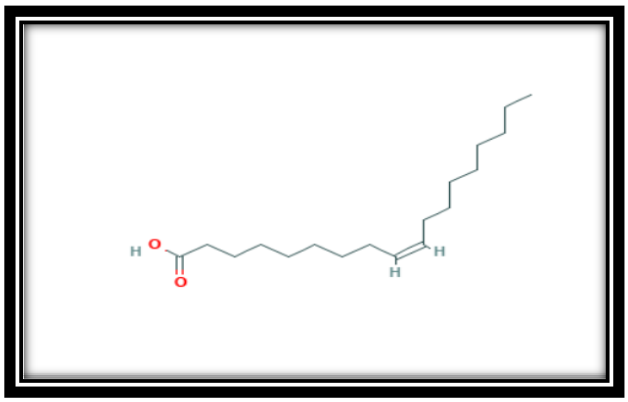

Fig-7 Structure of oleic acid

5.2.3 Nonproprietary name: Oleic acid

5.2.4 Chemical name: Z-(9)-octadecenoic acid

5.2.5 Molecular formula: C18 H34 O2

5.2.6 Description:

A yellowness colour and oily liquid with a characteristic's odour. monounsaturated carboxylic acid consists of Z-(9)-octadecenoic acid having

formula C18 H34 O2. it's visiting contain suitable antioxidant, to help prevent oxidation.

5.2.7 Functional category: Emulsifying agent and skin permeate.

5.2.8 Application:

Oleic acid utilized within the emulsifying agent in food and a topical formulation. It also used as permeation enhancer within the transdermal formulation to spice up the bioavailability of the poorly water-soluble drug in tablet formulation partially of the vehicle in an exceedingly soft gelatin capsule in topical microemulsion formulation in oral self-emulsifying drug delivery system in oral mucoadhesive patches and in metered dose inhaler. monounsaturated carboxylic acid was shown to an important consider oral hypoglycemic effect to supply by multiple emulsions containing insulin intended for essential drug delivery system.

6. EXPERIMENTAL WORKS

6.1 Confirmation of Drug

Confirmation of drug was carried out by using following method such as melting point determination, UV spectroscopy, infrared spectroscopy, and differential scanning calorimetry (DSC) and compared with standard. (Spicer, 2001; Wallentin, *et al*., 2009)

1. Melting Point Determination

Melting point determination is prime requirement for the confirmation of drug. In this method, drug whose analysis to be carried out was filled into capillary tube and tied to the thermometer in such a way that it remains dipped in liquid paraffin bath. The temperature range at which the drug starts melting and complete melting was noted, this method is applicable for the "Capillary melting point determination" and also confirming the drug by DSC method. (Spicer, 2001; Wallentin, *et al*., 2009)

2. UV Spectrophotometer

Accurately weighed 10 mg of Acotiamide was dissolved in 100 mL of ethanol to obtain working standard solution of 100µg/ml. The above solution was scanned in the range 400-200nm. The λMax of drug was confirmed with reported value in the literature.

3. Infrared Spectroscopy

IR spectrum of medication was measured in the solid state as potassium bromide (KBr) mix. The pure Acotiamide was previously ground and mixed thoroughly with KBr, an infrared transparent matrix at 1:100 (sample: KBr) ratio.

The KBr pellet was equipped by applying 10-12 metric ton of pressure in a motor-powered pellet press (Kimaya engineers, India). The pellet was then scanned over a wave range of 4000 – 400 cm^{-1} and spectra was obtained by using a FTIR spectrometer-430 Shimadzu 8400S, Japan. (Musallam, 2015; Thomas, *et al*., 2016)

4 DSC Study

The thermal behavior of pure drug was determined by using, Differential Scanning Calorimetry.

Thermogram for Acotiamide was obtained using DSC (Mettler DSC1 star system, Mettler-Toledo, Switzerland). The drug was sealed in perforated aluminum pan and heated at constant rate of 10°C/min over the temperature ranges of 40-300°C.

6.2 Standard calibration curve of Acotiamide in 1.2, 6.8 and 7.4 pH buffer

a) Standard calibration curve in Ethanol

Accurately weighed 10 mg of Acotiamide was dissolved in 100 mL of ethanol to obtain working standard solution of 100 µg/ml. Aliquots of 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml up to 3 ml from the stock solution representing 5, 10, 15, 20, 25, up to 30 µg/ml of drug were transferred to 10 ml volumetric flask and the volume was adjusted up to mark with ethanol. Absorbances of the above solutions were taken at 256nm against the blank solution that is ethanol without drug. A graph of absorbance vs. concentration was plotted.

b) Standard calibration curve in pH 1.2 buffer

Accurately weighed 10 mg of Acotiamide was dissolved in 100 mL of pH 1.2 buffer solution to obtain working standard solution of 100 µg/ml. Aliquots of 0.5, 1, 1.5, 2, 2.5 up to 3 mL from the stock solution representing 5, 10, 15, 20, 25 up to 30 µg/mL of drug were transferred to 10 mL volumetric flask and the volume was adjusted to mark with same blank solution. Absorbance's of the above solutions were taken at 256nm against the blank. A graph of absorbance vs. concentration was plotted. (Zhang, 2010; Musallam, 2015; Lampis, *et al*., 2018)

c) Standard calibration curve in pH 6.8 Phosphate Buffer

Accurately weighed 10 mg of Acotiamide was dissolved in 100 mL of pH 6.8 buffer to obtained working standard of 100 µg/ml. Aliquots of 0.5 ml, 1, 1.5, 2, 2.5 ml up to 3 ml from the stock solution representing 5, 10, 15, 20, 25, up to 30 µg/ml of drug were transferred to 10 ml volumetric flask and the volume was adjusted up to mark with pH 6.8 buffer. Absorbances of the above solution were taken at 256nm against the blank solution prepared in the same manner without adding the drug. (Zhang, 2010; Musallam, 2015; Lampis, *et al*., 2018)

d) Standard calibration curve in pH 7.4 Phosphate Buffer

Accurately weighed 10 mg of Acotiamide was dissolved in 100 mL of pH 7.4 buffer to obtained working standard of 100 µg/ml. Aliquots of 0.5, 1, 1.5, 2, 2.5 ml, up to 3 ml from the stock solution representing 5, 10, 15, 20, 25, to 30 µg/ml of drug were transferred to 10 ml volumetric flask and the volume was adjusted up to mark with pH 7.4 buffer. Absorbances of the above solution were taken at 256nm against the blank solution prepared in the same manner without adding the drug. (Zhang, 2010; Musallam, 2015; Lampis, *et al*., 2018)

6.3 Solubility Study of drug

a) Solubility study of Acotiamide in various lipids

The solubility of Acotiamide in oleic acid, GMS, GMO and labrafac. Excess amounts of T were placed in the oleic acid, GMS, GMO and labrafac, the contents were gently shaken for 24 hrs at room temperature in to mechanical shaker (Remi mechanical shaker, Mumbai). The saturated drug solutions were filtered through whatman filter paper and then assayed spectrophotometrically (UV-1700, UV-visible spectrophotometer, Shimadzu, Japan) at 256nm after appropriate dilutions. (Winter, 2015)

b) Solubility study of Acotiamide in ethanol, water and various buffers

The solubility of Acotiamide in ethanol, water and various media with varying pH was studied. Excess amounts of T were placed in the ethanol, acidic buffer (pH 1.2), phosphate buffer (pH 6.8) and alkaline buffer (pH 7.4) and water the contents were gently shaken for 24 hrs at room temperature in to mechanical shaker (Remi mechanical shaker, Mumbai). The saturated drug solutions were filtered through whatman filter paper and then assayed spectrophotometrically (UV-1700, UV-visible spectrophotometer, Shimadzu, Japan) at 256nm after appropriate dilutions. (Winter, 2015)

6.4 Drug Polymer Interaction Study:

The drug-excipients interaction study was carried out by using FTIR & DSC.

1. FTIR spectroscopy study.

IR spectroscopy was used to determine the molecular interaction between polymer and drug. The all physical mixtures and drug sample were mixed with dried KBr in ratio 1:100. Then minor fraction of mixture was compressed on automatic IR press (Kimaya Engg. Thane, India) pressure 10 tones to form transparent pellet. Then the IR spectrum of pellet was taken on FTIR spectrophotometer (Musallam, 2015; Lampis, *et al*., 2018)

2. DSC study

Plain drug, physical mixtures of drug and polymers were filled in the prewashed, dried ampoules and sealed. The sealed ampoules were stored at 37 ± 0.5ºC for 28 days in stability chamber. At the end of 28 days ampoules were removed from stability chamber and subjected for interaction study. Drug polymer interaction study was carried out by using DSC. In this study thermogram of pure drug, mixtures of drug: Acotiamide, Acotiamide and oleic acid were done at a scanrate of 10°C/min over the temperature range of 40°-300°C. (Nylander, 2013)

6.5 Formulation and Development

A) Magnetic stirring method

In the magnetic stirring method, a pre-emulsion was obtained under stirring by adding liquid lipid to a mixture of surfactants and water. A magnetic stirring method the pre-emulsion is obtained which lead to droplet breakage due to the continuous stirring and subsequent formation of oil in water (o/w) nanoemulsion which immediately cooled down to room temperature and take a side for two days at room temperature for making a gel-like appearance and to generate T-LCs. particles size and PDI of scattering formulation of liquid crystals are less according to reported technique. In this method principles based on the impact or attrition due to obtained particles size in nano size. This process shows in Figure 11. (Ola, 2018; Thomas, *et al*., 2017)

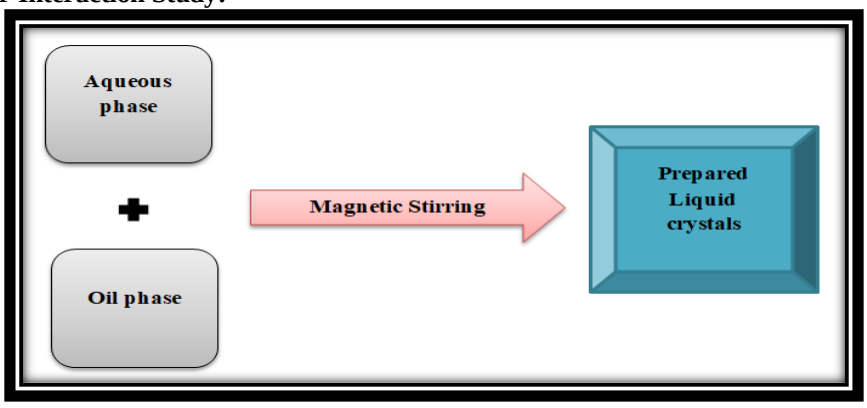

Fig-8 Preparation of LCs by magnetic stirring method

B) Composition of process optimization

In the process used drug, lipid, surfactant and aqueous phase. This composition is gives the LCs formulation the list of excipients is selected according to the above study that is, lipid screening and solubility of drug and excipients are given in Table 5.

Table-1 Composition of process optimization

Sr. No	Content
1	**Acotiamide**
2	**Poloxamer 407**
3	**Oleic Acid**
4	**Aqueous phase**

C) Process of preliminary trials for set the different ratio

For the preparation of oleic acid-based liquid crystals containing Acotiamide, ingredients were taken according to Table 6. Acotiamide was firstly dissolved in 2-5ml of ethanol for the 10ml batch. P407 used different ratio and oleic acid used as different ratio 1:9 to 9:1 (w/w), a pre-emulsion was prepared under stirring by adding liquid lipid to a mixture of surfactants and water. A magnetic stirring method the pre-emulsion is obtained which lead to droplet breakage due to the continuous stirring particles size and PDI of scattering formulation of liquid crystals are less according to reported technique. In this method principles based on the impact or attrition due to obtained particles size in nano size. After which added and stirred continuously until total dissolution. The required amount of water was gradually added to the melted mixture with a mechanical stirrer for 30 minutes followed by sonication (PCI Analysis, Mumbai) for 20 minutes panda, Italy) at 2900 rpm to obtain a dispersion of the LCs. (Collet, 2014) and subsequent formation of oil in water (o/w) nanoemulsion which immediately cooled down to room temperature and take a side for two days at room temperature for making a gel-like appearance and to generate T-LCs. By this way obtained the fixed ratio and this ratio used in to the Central composite design, for the set the higher and lower concentration of the polaxamer407 and oleic acid and then gives the design of batches.

Table-2 Process of preliminary trials for set the different ratio

Sr. No	Oleic acid(ml)	Poloxamer 407 (gm)	Water (ml)
1	1	0.3	9
2	2	0.3	8
3	3	0.3	7
4	4	0.2	6
5	5	0.2	5
6	6	0.2	4
7	7	0.1	3
8	8	0.1	2
9	9	0.1	1

6.6 Development and Optimization Acotiamide loaded LCs

Following Table 8 is the central composite design with the content of concentration of lipid (X_1), and Surfactant (X_2) as independent variables or MPS and EE as responses. The results of the experimental design were analyzing using design expert software. Each response coefficient was studied for its statistical significance at 95 % confidence level. The *P* value of 'probe >*F*' less the 0.05 indicates that model terms are significant and greater than 0.05 indicates that model terms as insignificant and should be remove from analysis to generate the reduced model. All independent variables, their levels along with actual and coded values of these variables are shown in Table 7. Whereas MPS of LCs (Y_1) and (EE %) drug entrapment efficiency (Y_2) were selected as response parameter as the dependent variables. Using this design, best model among the linear can choosen, central composite interaction model and quadratic model due to the analysis of variance (ANOVA) *F*-value.

Table-3 The coded and actual values of the variables used in the Central composite design of Acotiamide –LCs

Independent variables	**Levels**	
	Low (−1)	**High (+1)**
A: Amount of Poloxamer 407	1	3
B: Amount of oleic acid	8	12
Dependent variables	**Constraints**	
Y1: Particle size)nm)	Minimum	
Y2: Encapsulation efficiency (%)	Maximum	

Design-Expert software was employed for statistical analysis and graph plotting. The effect of independent variables on the responses was calculated by ANOVA through Fisher's test. The *P*-value of less than 0.05 was considered to be statistically significant. Evaluation of multiple correlation coefficients (R^2) and adjusted R^2 were employed for the best suitability of the model. Contour and three-dimensional surface plots were used to reveal the relationship and interaction between the coded variables and the responses. For optimization, selection of the MPS is in its minimum, entrapment efficiency (%) at maximum levels and ZP should be in range.
(Mulhan, 1998; Araujo, 2011; Chalikwar, *et al*., 2012)

On the basis of trial batches mention in the Table 6. The ratio of lipid and surfactant is selected, with the help of lower and higher ratio of excipients used to obtained the batches of factorial design and the batches shown in Table 8. The selection of the dependent variable Y1 are recognized increased the solubility by minimum size and Y2 are maximum drug contain by maximum EE%.

Table-4 Formulations of liquid crystals loaded with A by central composite designs

Code (Run)	**Drug (mg)**	**Lipid Oleic acid (ml) (A)**	**Surfactant Polaxamer407 (mg) (B)**	**Water (ml)**
1	90	10	0.59	90
2	90	10	2.00	90
3	90	12	1.00	88
4	90	8	1.00	92
5	90	10	3.41	90
6	90	12	3.00	88
7	90	8	3.00	92
8	90	7.17	2.00	93
9	90	12.83	2.00	88

6.7 Hard gelatin capsule as a finished product
Acotiamide after preparation of A-LCs enhanced solubility as well as the minimized the dose frequency. The dose of initiate Acotiamide treatment with a 180 mg loading dose. Administer 90 mg twice daily during the first year. After one year administers 60 mg twice daily. According to recent studies selected 90 mg dose. So the size of the capsule was selected with 130 mg capacity. The LCs Powder was obtained in which 92.89 mg powder of LCs contain 90 mg of drug, that's why (capsule size 4) 130 mg capacity of the capsule was selected. In %EE the 2 mg of formulation captained the 1.93 mg of entrapped drug, so the 90 mg of drug present in 92.89 mg of powdered formulation was calculated. (Moursy, 2003; Hasnsen 2005; Tilekar, *et al*., 2015)

6.8 Evaluation of filled capsules
The capsules evaluated for weight variation, drug content uniformity.
1. Weight variation test
Filling the 20 capsules in equal amoun with powder formulation, each capsule is cantained 92.89 mg powder formulation weighed individually and the average weight was determined. Then percentage

deviation from the average weight was calculated. (Hossain, 2013)

2. Drug content uniformity

For the drug content uniformity test 10mg of liquid crystals, the powders was weighted and dry LCs to a fine powder, and a quantity of powder equivalent to 100 mg of the formulation was dissolved in 100 mL ethanol and the liquid was filtered using whatman filter paper and diluted up to 50mg/ml. The A-LCs content was determined by measuring the absorbance at 256nm using UV spectrophotometer, after appropriate dilution with ethanol. (Hundekar, 2014)

3. *In vitro* dissolution study

The drug release study was carried out using a dissolution study apparatus (USP Apparatus I, basket type). The dissolution medium was having pH 1.2. The dissolution experiments were conducted at 37 ± 0.5°C at a basket rate of 50 rpm for 12 hours. Sampling was performed with interval of 0.5,1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hours (0.1N HCl). Detection of Acotiamide concentration in samples was identifying by using UV- spectrophotometer at 256nm.

The drug release study was carried out using a dissolution study apparatus (USP Apparatus I, basket type). The dissolution medium was having pH 6.8. The dissolution experiments were conducted at 37 ± 0.5°C at a basket rate of 50 rpm for 12 hours. Sampling was performed with interval of 0.5, 1,1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hours (0.1N HCl). Detection of Acotiamide concentration in samples was identifying by using UV- spectrophotometer at 256nm. (Thomas, 2016; Pai, *et al.*, 2017)

6.9 Stability studies of A-LCs

The LCs powder sample of final optimized formulation was utilized for carrying out accelerated stability. Drug products intended for storage in a refrigerator for long term stability at 5°C ± 3°C and accelerated stability at 25°C ± 2°C/60% RH ± 5% RH for 3 Months. Accelerated stability study was performed with the principal aim to assess the stability of LCs at 25°C ± 2°C/60% RH ± 5% RH with respect to particle size, PDI and EE. (Kalam, 2010; Nylander, 2013; Ola, *et al*., 2018)

7. RESULT AND DISCUSSION:

7.1 Confirmation of Drug

7.1.1 UV Spectroscopy

Acotiamide solution was scanned at 400 nm to 200 nm, the maxima were observed at 256nm shown in Figure 9. The reported pure Acotiamide peak was observed at 257nm. This was nearly same to the refrance peak so, confirmed with reported UV Acotiamide.

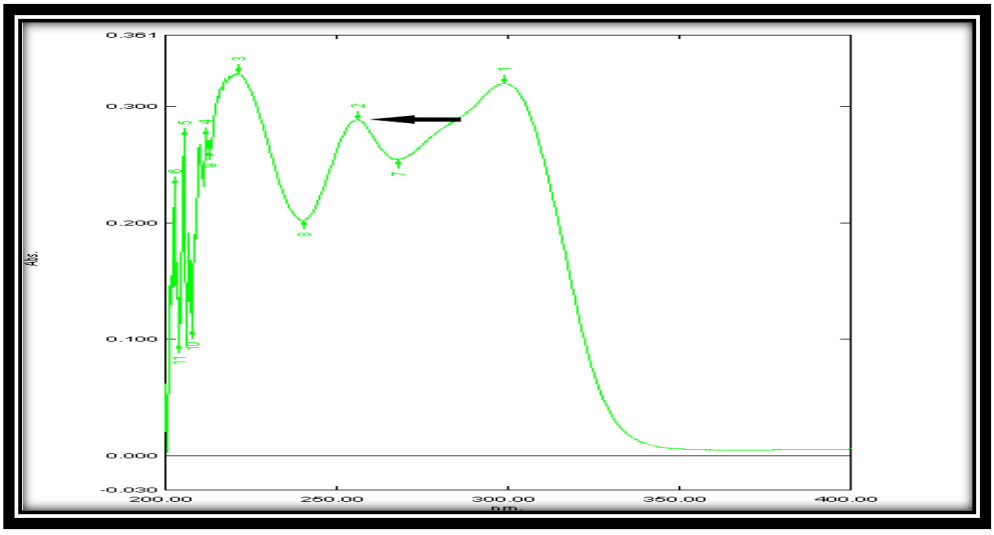

Fig-9 UV Spectra of Acotiamide Pure drug

7.1.2 Infrared Spectrum

IR spectrum of the drug was Determined in the solid state as potassium bromide dispersion. The IR spectrum of Acotiamide presented in Figure 10 and 11,

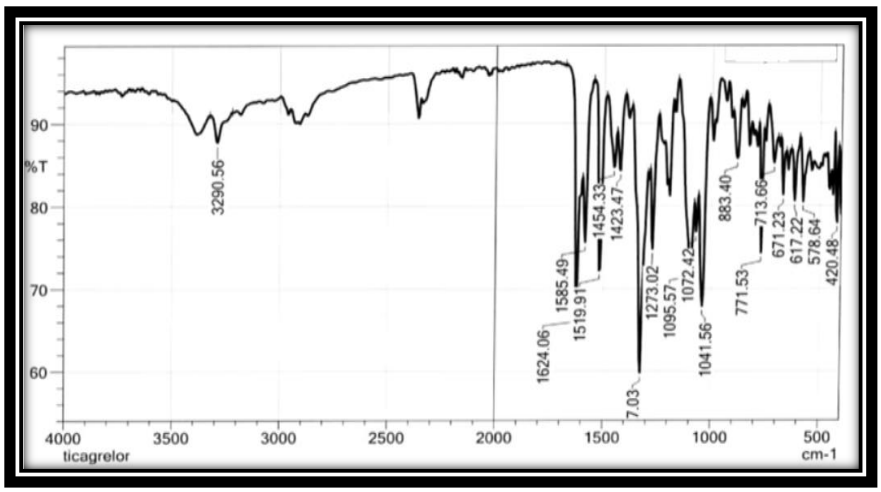

Fig-10 IR Spectra of Pure Acotiamide

Table-5 Peak and chemical group present in the IR spectrum of Acotiamide.

Standard Peak (cm^{-1})	Observed peak	Interpretation of chemical group
1450-1375	1454.33	C-H(-CH3bend)
3400-3200	3290.56	-OH
1300-1000	1095.57	-CO
1350-1000	1041.56	-CN(Amines)
1680-1600	1624.06	C=C
1690-1640	No Reading	C=N(imines and oximes)
1640-1550	1585.49	N-H(Bend)
1400-1000	1327.03	C-F
1350-1140	1273.02	S-O(sulfon)

7.1.3 DSC of Pure Drug

Table-6 DSC of Pure Drug

Sr no.	Parameter	Values Obtained (°C)
1	Onset Point	138.80
2	Peak Point	141.03
3	End Point	143.44

7.2 Standard calibration curve of Acotiamide in 6.8 pH phosphate buffer

Graph of absorbance Vs concentration was plotted and found to be linear over the range of 0.5 to 3µg/ml indicating its compliance with Beer's and Lambert's law. Results are shown in Table 12 7 and Figure 11

Table-7 Standard calibration curve of Acotiamide in 6.8 pH phosphate buffer

Sr.no	Concentration (µg/ml)	Absorbance (nm)
1	0.5	0.107
2	1	0.15
3	1.5	0.174
4	2	0.221
5	2.5	0.244
6	3	0.273

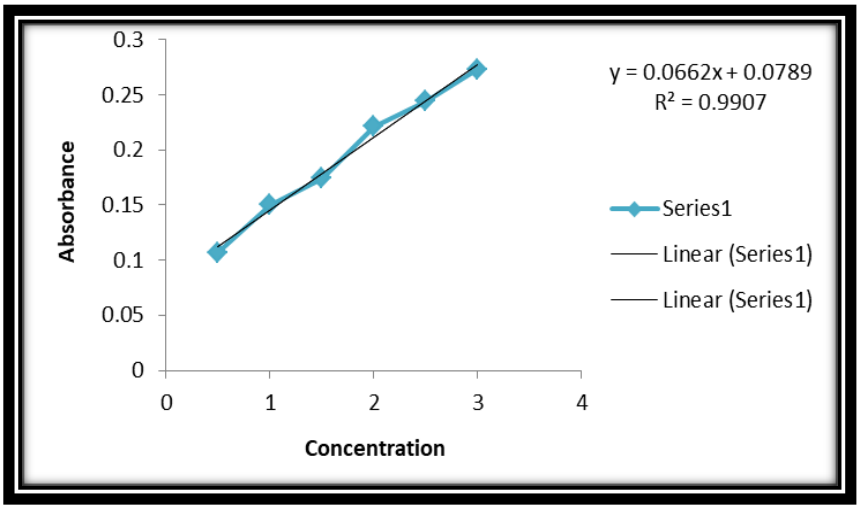

Fig-11 Standard calibration curve of Acotiamide in 6.8 pH buffer

Table-8 Result of Preformulation study of capsules

PS (nm)	PDI	Zeta Potential (mV)	Angle of repose (°)	Bulk Density	Tapped Density	Hausner´s ratio	EE %	Solubility (mg/ml) (in buffers)
301	0.51	-23	26.99	0.384	0.4081	1.06	89	pH1.2=0.0054 PH6.8=0.001 PH7.4=0.00216

7.3 Stability of Optimized A-LCs:

For formulation to see the market, it should be stable during the self-life (storage and transports. In general, a shelf life of at least one year is minimum prerequisite criterion for a commercial product, to be pharmaceutically acceptable with high drug retention capacity and particle size should be maintained during storage time.

Table-9 Stability study of lyophilized LCs loaded A in terms of PS, PDI and zeta potential studied.

Stability Parameter		Test period		
	0 month	1 month	2 months	3 months
Particle size(nm)	301 ± 3	301 ± 10	301±11	301. 2 ± 12
PDI	0.5 ± 0.04	0.580 ± 0.08	0.580 ±0.09	0.581 ± 0.09
Zeta potential(mV)	-23 ± 2.6	-23.1 ± 1.4	- 23.3 ± 1.6	-23.4 ± 1.2

8. CONCLUSION:

The objective of the present work was to formulation, development, and evaluation, of liquid crystals formulation containing Acotiamide for oral delivery, in a sustain release.

The compatibility of the drug, excipients was determined by DSC and IR spectroscopy. A result shows that the drugs are compatible with excipients.

The structure and particle size of formulation characterized by the SEM and crystalline nature of the drug characterized by X-ray diffraction. Oleic acid-based LCs provided significant in the sustained drug delivery which helps to reduce the frequency of dose and improve bioavailability. In-vitro dissolution study confirmed the sustain release profile.

LCs are performed under the stability study and the shows the stable nature in their particle size and PDI. The drug having a high dose of frequency and having poor dissolution rate from its oral solid dosage forms. Liquid crystal formulations have lesser drug particles size which help to speed up dissolution by enlarging the effective surface area. According to the Ostward–Freundlich, and Noyes–Whitney equation, the dissolution rate of a drug can be increased by reducing the particle size to increase the interfacial surface area. The main objective of the research is to improve the dissolution and solubility of this poorly water-soluble drug, to increase its oral bioavailability. Also by delivering in sustain manner the patient compliance increased.

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