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FORMULATION, OPTIMIZATION AND EVALUATION OF CELECOXIB LOADED CUBOSOME FOR TOPICAL DRUG DELIVERY SYSTEM

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ABSTRACT

The present study is mainly focused to formulate the celecoxib loaded cubosomes. Celecoxib is [NSAID] medication used to treatment of pain and inflammation, associate with rheumatoid arthritis, and several other inflammatory disorder. Celecoxib shows poor solubility and poor bioavailability, to overcome the problems, Celecoxib is formulated as cubosomal gel. Pre-formulation studies were carried out, in this FTIR study results indicate there is no chemical interaction between drug and excipients. Eight different formulations (F1- F8) were prepared by Top -Down technique. Among the eight formulations, formulation (F7) was optimized, because of its high Entrapment efficiency (88.2%) and maximum percentage of drug release (89.69%) at the end of 8hrs compared to other formulations. The average particle size of cubosomal gel (F7) was found to be 488.21nm. The SEM images represent cubosomal gel (F7) are cubic in shape. From the In- vitro kinetics studies Formulation (F7) fitted with Higuchi release kinetics. Stability studies indicate that the cubosomal gel (F7) were stable during stability studies. Celecoxib loaded cubosomal gel may improve the bioavailability and solubility, So The celecoxib is suitable for the formulation of cubosomal gel.

KEYWORDS: Celecoxib, cubosomes, GMO, Poloxamer 407, Top- Down technique, Bioavailability, solubility.

1. INTRODUCTION

1.1. NOVEL DRUG DELIVERY SYSTEM[2,14,16]

The aim of Novel Drug Delivery System is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration. The drug delivery system should deliver drug at a rate control by the necessarily of the body over a specified term of treatment.

A number of novel drug delivery system has emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structure is one such system, which can be predicted to prolong the existence of the drug in systemic circulation and reduce the toxicity if selective uptake can be achieved. Consequently a number of vesicular drug delivery system such as liposomes, niosomes, transfersomes and pharmacosomes were developed. Advances have since been made in the area of vesicular drug delivery, leading to the development of this system that allow drug targeting and sustained or controlled release of conventional drug medicines.

1.2. NANOTECHNOLOGY[5,17,24]

Nanotechnology can be defined as the science and engineering involved in the design, characterization, production and applications of structures, devices and systems by controlling shape and size at nanometer scale. Nanotechnology is providing solutions for several pharmaceutical drug delivery issues. For over 20 years, researchers have appreciated the potential benefits of, Nanotechnology in providing vast improvements in drug delivery and drug targeting. Improving delivery techniques that minimize toxicity, improve efficacy and offers great potential benefits to patients, and new markets for pharmaceutical and drug delivery companies.

1.3. TOPICAL DRUG DELIVERY[5,6,41]

Topical drug delivery system is defined as the application of a drug containing formulation to a skin or mucous membrane, to treat a specific cutaneous disorder (e.g. acne) or cutaneous manifestations of a generalized disease (e.g. psoriasis), with the intent of containing the pharmacological effect of the drug only to the surface or within the layers of skin or mucous membrane.

Figure 1: Shows the formulations of cubosomal gel.

1.3.1. TYPES OF TOPICAL DRUG DELIVERY SYSTEM

Includes two types of topical drug delivery system:

a) Internal - that are applied to the mucous membrane of eye (conjunctiva), ear, oropharyngeal cavity, nasal cavity, vagina or anorectal region for local activity.

b) External – that are spread or dispersed on the cutaneous surface covering the affected area

1.3.2 CLASSIFICATION – BASED ON PHYSICAL STATE

- SOLID – Powder, Aerosol, Plaster.
- LIQUID – Lotion, Liniment, Solution, Emulsion, Suspension, Aerosol.
- SEMISOLID- Ointment, Cream, Paste, Gel, Jelly, Suppository.

1.3.4 ADVANTAGES OF TOPICAL DRUG DELIVERY SYSTEM

- Avoidance of first pass metabolism
- Easy application
- Suitable for self-medication.
- Improved patient compliance
- Improved physiological and pharmacological response
- Avoidance of gastro- intestinal incompatibility.
- Drug can be delivered more selectively to a specific site.
- Relatively large area of application.

1.3.5 DISADVANTAGES OF TOPICAL DRUG DELIVERY SYSTEM

- Skin irritation / contact dermatitis due to drug and/or excipients
- Poor permeability of some drugs through the skin
- Possibility of allergic reactions
- Can be used only for those drugs which require low plasma concentration of action.
- Enzymes in epidermis may denature the drugs
- Drugs with larger particle size are difficult to get absorbed through the skin.

1.4. CUBOSOMAL DRUG DELIVERY SYSTEM[16,17,24]

Cubosomes are discrete, sub-micron, nanostructured particles of the bicontinuous cubic liquid crystalline phases. They consist of honeycombed structures separating two internal aqueous channels along with a large interfacial area. They contain similar microstructure as that of the parent with high surface area and their dispersions are less viscous than the parent cubic phases. In cubosomes, the cubic phases composed of two separate thermodynamically stable structure consisting of, continuous but non intersecting hydrophilic regions which are separated by a lipid bilayer. The structure of cubosome retains the stability and efficacy of activities like vitamins and proteins.

Cubosomes are thermodynamically stable, long lasting. By the addition of polymers, the colloidal dispersions of cubosomes can be stabilized. It also shows the potential for controlled delivery of drugs, in which diffusion is governed by the passage of the drug through the "regular" channel present in structure of the cubic phase. Cubosomes are liquid crystalline nanostructured particles with the same unique properties of the bulk cubic phase, although cubosome dispersions have much lower viscosity. Cubosome is a honey-combed structure separating two internal aqueous channels along with large interfacial areas.

Cubosomes are Nano sized, more accurately nanostructure particles of a liquid crystalline phase having cubic crystallographic symmetry which is formed by the self assembly of surfactant like molecules. Methods for preparation of cubosomes are high-pressure homogenization, Probe Ultra sonication, Automated Cubosome Preparation; Some Special techniques for The Preparation of Cubosomes are Topdown Technique, Bottom-up Technique.

1.4.1 STRUCTURE OF CUBOSOMES

Cubosomes have honeycomb structures whose size range from 10-500 mm in diameter. They appear like dots, which are slightly spherical in structure. Each dot corresponding to the presence of pore containing aqueous cubic phase in lipid water system.

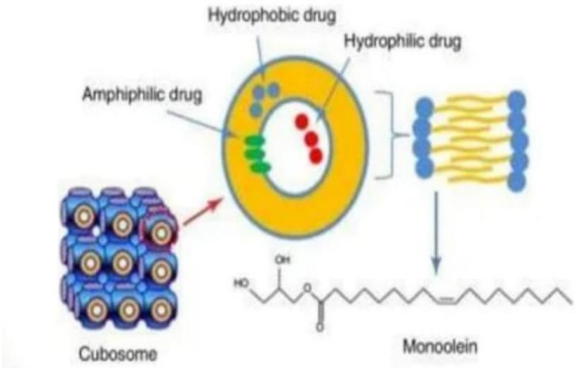


Figure 2: Structure of cubosomes.

1.4.2. Advantages of cubosomes

- Simple method of preparation
- Capability of encapsulating hydrophilic, hydrophobic and amphiphilic substance Excellent bio adhesive properties
- Skin permeation enhancement
- Controlled release and targeted release of bioactive agent.
- High drug loading efficiency due to their cubic crystalline structures and high internal surface area
- Thermodynamically stable
- Economic, non-toxic and biocompatible
- Composed of biodegradable lipid
- Protect the incorporated drug from physical and chemical degradation.

1.4.3. Disadvantages of cubosomes

- Low entrapment of water-soluble drugs due to presences of large amount of water inside the cubosomes
- Due to their high viscous nature large scale production is difficult.

1.5. Theories on cubic phase structure[43,27]

Cubosomes or bicontinuous cubic phase liquid crystals have several features that are intriguing as a generic medication delivery system. It is formed into bilayers inside the surfactant and wrapped into a three dimensional, periodic, and minimum surface, generating a densely packed structure. The material is an optically transparent, very viscous bicontinuous cubic liquid crystalline phase with a unique structure in the nanometer range. They are relatively easy to make, and the improved penetration power and emulsification properties of lipids allow them to encapsulate hydrophobic, hydrophilic, and amphiphilic compounds while ensuring the targeted and controlled release of bioactive compounds. The three macroscopic phases of the cubic structure that are often seen during cubosome synthesis are the precursor, bulk gel, and particle dispersion phases. A solid or semisolid material that produces the cubic phase in reaction to stimuli, including contacting a liquid, is designated the precursor state. The bulk gel-cubic phase, on the other hand, is rigid, isotropic, and can be expanded into cubosomes. Finally, the dispersion of the solid-like phase into smaller particles forms cubosomes.

a) Fontell & Drew theory: Cubic phases can be found in ternary systems of amphiphiles, oil and water, and various monoglycerides. Monoglycerides are polar lipids with low water solubility and aqueous phase behavior that is structurally similar to non-ionic surfactants. Lutton's results show that monoglycerides with hydrocarbon chain lengths between C-12 and C-22, particularly monoolein, have a bigger cubic phase area. Monoolein, also known as C-18 Monoglycerides, is an unsaturated fatty acid.

b) Gustafson et al. Theory: Cubosomes are single-crystal formations with unilamellar vesicles visible and distributed lamellar liquid-crystalline phase particles. The formation of larger vesicles is aided by increasing the polymer-to-monoolein ratio. Slow transport processes that form highly viscous crystalline structures and the high energy required for fragmentation result in mostly vesicles through ultrasonication of bulk cubic phases that are trace formed into cubosomes via membrane fusion over time. This metastability is one of the many characteristics of cubosomes systems (bulk cubic phase). Cubosomes are also colloidally stabilized by vesicles.

C) Schwarz, Jacob & Anderson theory: In non-ionic surfactant systems, cubic phases are frequently encountered wedged between lamellar and hexagonal liquid-crystalline phases. The mono olein water system is remarkable in that it has a cubic phase area with a wide range of composition and temperature. Surfactant packing concepts, on the other hand, are getting closer. Normally, monoolein has a hydrophilic head and a hydrophobic tail, resulting in reversed or inversed cubic phases, indicating polar medium phases. As a result, cubic phase structures can be represented using differential geometry and periodic minimum surfaces. The ideal way to characterize minimal surfaces is to compare them to soap films. Three types of minimum surfaces are investigated in cubic phases based on their curvatures. At high water levels, the monoolein-water system creates the D-surface, and at lower water levels, the G surface. The p-surface forms in the monoolein-water system, but only when a third component, such as caseins or amphiphilic molecules, is present. The block copolymer is incorporated. The existence of cubic phases can be determined using the Xray scattering technique. Cubosomes are visualized using transmission electron microscopy (TEM) and freeze fracture electron microscopy.

d) System forming theory: Cubosomes can form in binary and ternary systems if the cubic phase and the solvent have a significant miscibility gap. When poloxamer 407 is employed to prevent cubosome aggregation and flocculation, the cubosomes have good

colloidal stability. They can be encased in lamellar bilayer caps, which seal the cubic bilayer opening created by fragmentation and offer colloidal stability by preventing hydrocarbon chains from coming into contact with water. The colloidal stability of cubosomes coated with a solid crystalline bilayer is better, whereas lamellar liquid-crystalline coatings are rigid. In addition, sponge phase coatings as a cubosome stabilising coating have been proposed. Another molecule with a high potential for cubosome development is phytonadione.

1.6. Mechanism of drug release from cubosomes[10]

The drug release mechanism from cubosomes is based on the principle of drug diffusion, where the concentration gradient of the drug across the cubosomes is the driving force of the diffusion. Therefore, the drug release rate from cubosomes is generally coincidental with the Higuchi or Fick diffusion equation. There are many factors influencing the drug release rate, such as drug solubility, diffusion coefficient, partition coefficient, cubic liquid-crystalline geometry, pore size and distribution, interface curvature, temperature, pH, and ionic strength of the release medium. The release mechanism of several hydrophilic model drugs from the cubic and reversed hexagonal liquid crystalline was investigated. These studies indicated that diffusion is the predominant mechanism of drug release, and the drug release rate from cubic ones is faster than the hexagonal liquid crystalline. Furthermore, the in vivo drug release profiles of C-glucose from cubosomes and hexagonal phase were consistent with the in vitro release profiles, which indicated the nanostructure of cubosomes and the nature of lipid could be utilized to control the release rate of hydrophilic drugs. But it is difficult for the hydrophobic drug to escape from the cubosomes in vitro due to the affinity of the drug with the hydrophobic domain in the cubic phase. Hence, the release profiles of hydrophobic drug-loading cubosomes in distilled water media (pH 6.5) and digestion media (0.1 M Hydrochloric acid) were investigated and found that the drug release rate in the digestion media was drastically improved. Also, it is reported that the plasma concentration of Silymarin in vivo showed an increased drug release rate from cubosome formulation as compared to Legalon®, a commercial capsule formulation.

1.7. Method of preparation[9,42,10]

1. Top-down approach
2. Bottom-up approach
3. Heat treatment
4. Spray drying

1. Top-down approach

It is the most widely used in research area, where by bulk cubic phase is first produced and then dispersed by high energy processing in to Cubosomes nanoparticles. Bulk cubic phase is resembling a clear rigid gel formed by water swollen crossed linked polymer chains, whereas cubic phases are like liquid crystalline structure. The cubic phases exhibits a yield stress that increases with increasing amount of bilayer forming surfactant and oils.

Based on most existing studies comparison of dispersion produced by sonication and high pressure homogenization suggests the formation of complex dispersions containing vesicles and Cubosomes with time dependent ratios of each particle type. Coarse Cubosomes on micron scale possess the same Dsurface structure as their originating bulk cubic phase, but after homogenization, the P-surface dominates because of added polymers. The extreme viscous bulk phase is prepared by mixing structure-forming lipids with stabilizers, then the resultant is dispersed into aqueous solution through the input of high energy (such as high-pressure homogenization [HPH], sonication or shearing) to form LLC nanoparticles. At present, HPH is the most extensively used technique in the preparation of LLC nanoparticles (cubosomes).

2. Bottom-up approach

In this Cubosomes are allowed to form or crystallize from precursors. Almgren et., al. discuss the formation of Cubosomes by dispersing L2 or inverse micellar phase droplets in water at 80ºC, and allow them to slowly cool, gradually droplets get crystallizes to Cubosomes. This is more robust in large scale production of Cubosomes. Spicer et.al developed Cubosomes at room temperature is by diluting monoolein-ethanol solution with aqueous poloxamer 407 solution. The Cubosomes are spontaneously formed by emulsification.

The key factor in the bottom-up approach is hydrotrope, which can dissolve water- insoluble lipids to create liquid precursors and prevent the formation of liquid crystals at high concentration. Compared with the top-down approach, this dilution-based approach can produce Cubosomes without laborious fragmentation. In other words, it needs less energy input. Moreover, this approach is far more efficient at generating small particles. The reason for this might relate to the forming mechanism of Cubosomes. The dilution-based approach can be regarded as a process of small particles forming big particles through aggregation, which is analogous to the use of precipitation processes to produce nanoparticles, whereas the top-down approach is more analogous to the attrition of big particles. In addition, Cubosomes prepared through dilution show long-term stability, which might be attributed to the homo disperse stabilizers onto the surface of Cubosomes. Indeed, the use of hydrotrope can simplify the preparation process and produce Cubosomes possessing similar or even better properties than those fabricated by the top-down approach.

3. Heat treatment

In this case, heat treatment can be regarded as a good approach. Note that in the strictest sense, heat treatment is not an integrated process for the manufacture of Cubosomes because it only promotes the transformation from non-cubic vesicles to well-ordered cubic particles.

The dispersed particles, therefore, can be produced by a simple processing scheme comprising a homogenization and heat-treatment step. From the reported studies, heat treatment could cause a decrease in the small particle size fraction that corresponded to vesicles and form more cubic phases with narrow particle distribution and good colloidal stability. Taking the whole process of preparation into account, it is obvious that the transition takes place during the procedure of heat treatment. The reason for transition could be speculated as an elevated temperature giving rise to a reduction in solubility and stability. When the temperature was below cloud point, the surfactant had a high solubility and thus the particles could exist stably and the phenomenon of fusion was hardly observed. Once reaching cloud point, the solubility of surfactant decreased notably and a notable fast fusion among vesicles would occur.

4. Spray drying

Another method of cubosome preparation is the spray drying process. Spray-dried encapsulated particles are made from an emulsion of liquid droplets or dispersions of solid particles in concentrated water-polymer solutions. Both phases are sprayed through a curated nozzle, creating suspension droplets to collide with a dry, hot airflow. Excess water quickly evaporates, leaving dry powder particles made composed of the dispersed phase surrounded by an encasing of the previously dissolved polymer. The spray-drying process is easy to scale up and is now frequently utilized in consumer goods such as detergents and meals. Furthermore, the method makes it simple to preload actives into cubosomes before drying. Finally, the polymer coating on the powder gives the hydrated cubosomes surface properties, which can be changed by identifying the perfect encapsulating polymer. The liquid feed can be changed to alter the resulting powder's properties. For the production of starch- coated cubosomes powder precursors, high shear treatment of monoolein in aqueous starch solution produces a coarse cubosomes dispersion that is then pushed through a nozzle and dried.

1.8. Applications of cubosomes [23,30]

- Cubosomes are used in cancer therapy.
- Cubosomal dispersion that containing monoglyceride used topically for mucosal or percutaneous application.
- Cubosomal technology is used in development of synthetic vernix which is a white substance that coats infants in late gestation for premature infants who born without it.
- Cubosomes are also been used in the treatment of fungal infections.
- Cubosomes are been used as a agent for delivery of vaccines.
- Controlled release of solubilized actives is the most popular application of cubosomes.
- Biodegradable by simple enzymes.
- Monoglycerides has microbiocidal properties therefore used in the treatment of sexually transmitted diseases by both bacteria and viruses.
- Cubosomes are recently used in cosmetics, skin care, antiperspirant and hair care

1.9 SKIN

Skin consists of three main parts:

1. Epidermis - outer layer
2. Dermis - beneath the epidermis
3. Hypodermis - inner layer

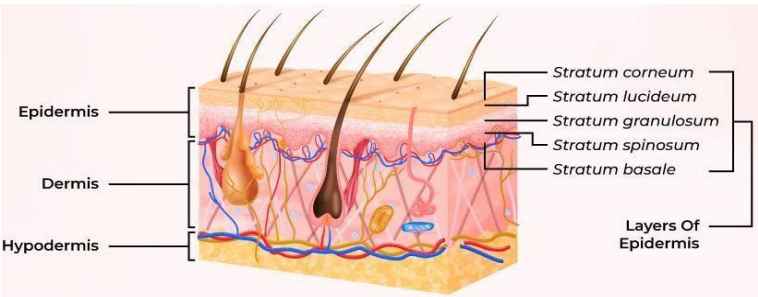


Figure 3: Shows the layers of skin.

1. The epidermis

The epidermis (outer layer) contains no blood vessels and is divided into five layers. Cells move from the base of the epidermis up to the surface, changing shape and structure as they go. The epidermis is made up of stratified squamous epithelium or hardened cells which play a role in the skin's protective function. This is referred to as the stratum corneum. Epidermal cells line the hair follicles, sebaceous glands and sweat glands.

2. The dermis

The main function of the dermis is to provide physical support and nutrients to the epidermis. The two layers identified within the dermis are the papillary layer and the reticular layer. Key substances found in the dermis include elastin, fibrillin and collagen (which helps give support and protection), all of which will decrease with age. The dermis also contains nerve endings, sweat glands, sebaceous gland, hair follicles and blood vessels. The papillary dermis contains smaller blood vessels which supply oxygen, elastic fibers and nutrients to the lower epidermis.

3. The hypodermis

The skin is supported by a layer of fatty tissue. The fatty area helps to act as a cushion to protect the body and is also important for insulation.

1.9. PENETRATION OF DRUG THROUGH HUMAN SKIN

The skin is the largest organ of the body, with a total area of about 20 square feet. The skin protects us from microbes and the elements, helps regulate body temperature, and permits the sensations of touch, heat and cold.

- Waterproof barrier and creates our skin tone.
- The dermis, beneath the epidermis, contain tough connective tissue, hair follicles, sweat glands.
- The deeper subcutaneous tissue (hypodermis) is made of fat and connective tissue.

1.9.2 PENETRATION PATHWAYS

There are three penetration pathways available for topically applied drugs.

- ❖ Intercellular
- ❖ Intracellular
- ❖ Follicular

1. *Intercellular*

It is defined as the transport of drugs through junction between the epithelial cells.

2. *Intracellular*

It is defined as passage of drugs across epithelial cells.

3. *Follicular*

Here the hair follicle act as a pathway for penetration of topically applied drugs.

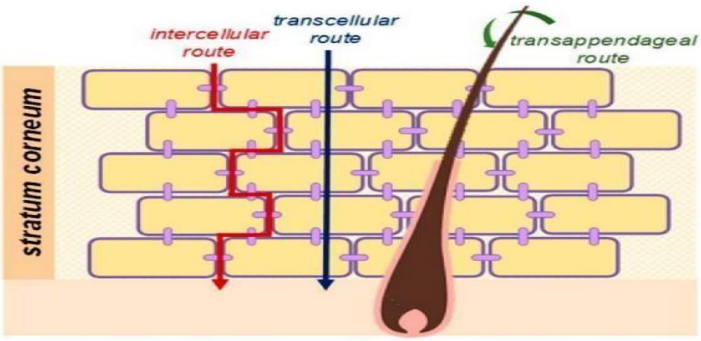


Figure 4: Shows the route of drug penetration through the skin.

2. MATERIALS AND METHODS

Table No. 1: List of raw material and name of their suppliers.

S.NO	NAME OF THE MATERIAL	NAME OF THE SUPPLIER
1.	Celecoxib	Strides pharma science.Ltd
2.	GMO	Mohini Oraganics pvt.Ltd
3.	Poloxamer 407	Strides pharma science.Ltd
4.	Carbopol 934	Loba Chemie Pvt.Ltd, Mumbai.
5.	Methyl paraben	Loba Chemie Pvt.Ltd, Mumbai.

7. EXPERIMENTAL WORK

PRE-FORMULATION STUDIES

Pre-formulation testing was an investigation of physical and chemical properties of a drug substance alone. It was the first step in rational development of dosage form.

7.1. IDENTIFICATION OF DRUG

A] Identification of drug by FTIR Spectroscopy [7,32]

FTIR study was carried out to identity the drug. Infrared spectrum of celecoxib was determined on Fourier transform Infrared Spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was performed by using FTIR spectrophotometer. Absorption maximum in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum.

B] Determination of Melting Point [3]

Small amount of drug was loaded in a capillary tube where one of capillary tube was closed and kept in the melting point apparatus and temperature was noted when drug melts.

7.1.1 *Physicochemical parameters*

a) Organoleptic properties [8,25]

The colour, odour and nature of the drug were recorded using descriptive terminology.

b) Solubility study [3,4]

It is important to about solubility characteristic of a drug in aqueous system, since they must possess some limited aqueous solubility to elicit a therapeutic response. The solubility of drug was recorded by using various descriptive terminology specified in indian pharmacopoeia, 2007. the solubility study was shown in table.

Table 3: Values for estimating drug solubility based upon USP definition.

Descriptive Term	Appropriate Volume of solvent in milliliters per gram of solute
Very soluble	Less than 1 part solvent needed to dissolve 1 part solute
Freely soluble	From 1 to 10 parts solvent needed to dissolve 1 part solute
soluble	From 10 to 30 parts solvent needed to dissolve 1 part solute
Sparingly soluble	From 30 to 100 parts solvent needed to dissolve 1 part solute
Slightly soluble	From 100 to 1000 parts solvent needed to dissolve 1 part solute
Very slightly soluble	From 1000 TO 10000 parts solvent needed to dissolve 1 part solute
Practically insoluble	More than 10000 parts solvent needed to dissolve 1 part solute

8.2 Drug Excipients Compatibility Study

The optimization of a formulation can be done only after a through investigation of its physical and chemical properties of the drug and excipients. The drug and the polymer must be compatible for a successful formulation.

8.3 *Determination of λ max [Methanol]*[29]

Stock solution of celecoxib was prepared using methanol. Absorbance of Celecoxib was scanned by UV-Visible spectrophotometer from wavelength of 400-200nm. From the standard stock solution, all the required concentrations were prepared using methanol. The spectrum shows maximum absorbance at 252nm, was selected as the wavelength (λ max) and utilized for analysis, in the present investigation. Standard plot was drawn using the data obtained.

8.3.1 *Procedure for determination of λ max using methanol*

10 mg of celecoxib was weighed accurately and transferred into 100ml(100mcg/ml) volumetric flask and dissolved in methanol. After dissolution the volume was made up to the mark with methanol. Further dilution was made by Pipetting 10ml into 100 ml (10 mcg /ml) volumetric flask to acquire solution made up with pbs 7.4. The absorption maximum of the standard solution was scanned between 200-400 nm region on uv spectroscopy.

8.4 *Procedure for determination of λ max (phosphate buffer 7.4)*[38]

To establish Celecoxib standard curve, a solution in stock was come about in 10 mg of Celecoxib powder, dissolved in ethanol (50 ml) through the stirring of multiple minutes and accomplishing 100 ml(100mcg/ml)volume in ethanol further diluted in phosphate buffer contains (pH 7.4). Further dilution was made by pipetting 10ml into 100ml(10mcg/ml) volumetric flask to acquire solution made up with phosphate buffer 7.4. The absorption maximum of the standard solution was scanned between 200-400 nm region on uv spectroscopy.

8.4.1 *Preparation of phosphate buffer 7.4*

1.083g of sodium dihydrogen ortho phosphate and 3.258g of disodium hydrogen phosphate is dissolved with 100 ml of distilled water, stirred continuously until its completely dissolved. After dissolved make up with 200 ml of distilled water.

8.5. Standard curve of celecoxib in methanol

10 mg of celecoxib was weighed accurately and transferred into 100ml(100mcg/ml) volumetric flask and dissolved in methanol. After dissolution the volume was made up to the mark with methanol. Further dilution was made by Pipetting 10ml into 100 ml (10 mcg /ml)volumetric flask to acquire solution made up with methanol. Further dilution was made by pipetting 2ml,4ml,6ml,8ml and 10ml into 10mlvolumetric flask acquire solution made up with pbs 7.4. The absorbance measurement of these solution were carried out against methanol as blank at 252 nm.A celecoxib was plotted.

8.6. Standard curve of celecoxib in phosphate buffer 7.4

To establish Celecoxib standard curve, a solution in stock was come about in 10 mg of Celecoxib powder, dissolved in ethanol (50 ml) through the stirring of multiple minutes and accomplishing 100 ml(100mcg/ml)volume in ethanol further diluted in phosphate buffer contains (pH 7.4). Further dilution was made by pipetting 10ml into 100ml(10mcg/ml) volumetric flask to acquire solution made up with phosphate buffer 7.4. Further dilution was made by pipetting 1ml, 2ml, 3ml, 4ml and 5ml into 10mlvolumetric flask acquire solution made up with pbs 7.4. The absorbance measurement of these solution were carried out against pbs 7.4 as blank at 252 nm.A celecoxib was plotted

8.7. Optimization of celecoxib Loaded cubosomal dispersion by Factorial design[15,21]

A Factorial Design was developed to statistically optimize the formulation factors and evaluate the main effects, interaction effects and linear effects on the independent factors. It was 3 factors, 2 levels Factorial Design was used to explore linear response surfaces with Design Expert (Version 13), and a matrix comprising 3 factors, 2 level and 8 runs is selected for the optimization study. The experimental design is summarized in Table 4.

8.8. Validation and data analysis

Statistical validation of the polynominal equation and ANOVA was calculated using Design Expert Software. The resultant experimental values of the responses were quantitatively compared with the predicted values to calculate the prediction error. Factorial Design was used for the optimization of celecoxib loaded cubosomal dispersion formulation. The drug concentration, Surfactant Concentration and Lipid Concentration were

the three factors (independent variables) studied. The responses (dependent variables) studied were Percentage Entrapment efficiency and Percentage Drug release.

Table 4: Summary of Experimental design.

Independent variable	Units	Level	
		Low (-1)	High (+1)
X1 = Stirring speed	RPM	500	1000
X2 = Concentration of lipid	Gram	2.5	1.5
X3 = Concentration of surfactant	Gram	0.3	0.2
Dependent variable	**Units**	**Constraints**	
R1 = Entrapment Efficiency	%	Maximize	
R2 = Drug Release	%	Maximize	

8. METHOD OF PREPARATION AND EVALUATION OF CELECOXIB LOADED CUBOSOMES[28,26]

Cubosomal dispersions of celecoxib were prepared by top-down technique. Accurately weighted quantity of Glyceryl monooleate (GMO) and poloxamer 407 polymer mixed and melted in a water bath at 60^0C, to this mixture add celecoxib drug and stir until completely dissolved, then to this solution add drop by drop preheated (up to 70^0C) distilled water of suitable quantity by continuous stirring for 2 hours, This whole system is taken into subjected for homogenization at 1500rpm for 1 minute under at room temperature. Thus formed liquid dispersion of cubosomes was kept at a room temperature, avoids direct sunlight and which will used for further study. The formulation design was given table.

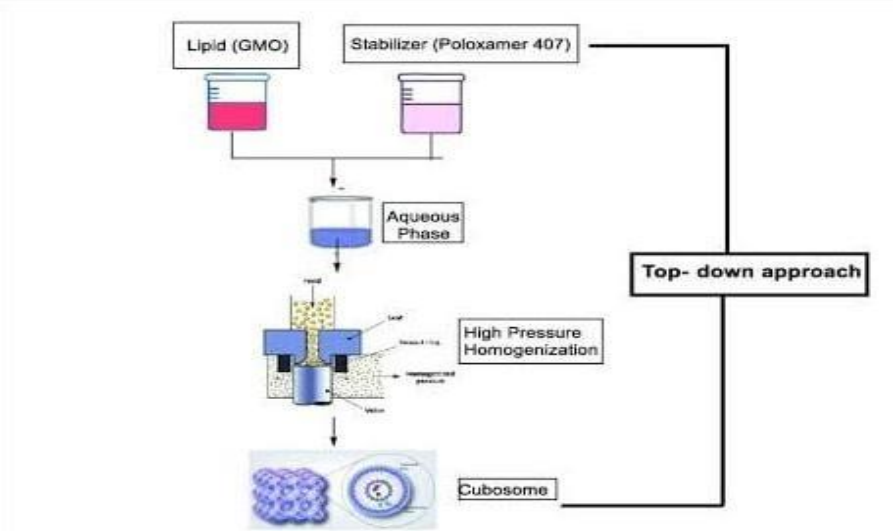


Figure 6: Formulation of the cubosomal dispersion by top-down technique.

Table No. 5: Formulation chart of celecoxib cubosomal dispersion by using optimisation technique.

FORMULATION	POLOXAMER 407(gm)	GMO (gm)	CELECOXIB (mg)	DISTILLED WATER (ml)
F1	0.3	2.5	100	50
F2	0.3	2.0	100	50
F3	0.3	1.5	100	50
F4	0.25	2.5	100	50
F5	0.25	2.0	100	50
F6	0.25	1.5	100	50
F7	0.2	2.5	100	50
F8	0.2	1.5	100	50

Figure 7: preparation of cubosomal dispersion.

8.1. Evaluation of cubosomal dispersions A] Optical Microscopy[34]

The cubosomal dispersions prepared were observed under binocular compound microscope at 10X and 40X magnification for studying the shape and surface morphology.

B] Particle Size and Polydispersity Index[44]

Particle size (z-average diameter) and polydispersity index (as a measure of particle size distribution) of celecoxib loaded cubosomal dispersion is performed by dynamic light scattering also known as photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000 Nano S (Malvern instruments, UK) at 25°C. Prior to measurements all samples were diluted using ultra–purified water to yield a suitable scattering intensity. The diluted cubosomal dispersion was poured into disposable sizing cuvette which is then placed in the cuvette holder of the instrument and analyzed. Air bubbles were removed from the capillary before measurement. Monodisperse samples have a lower PDI value, whereas higher PDI value indicates a wider particle size distribution and the polydisperse nature of the sample can be calculated by following equation:

$$PDI = d/d\ avg$$

Where,
d is the width of distribution denoted by SD,
d avg is the average particle size denoted by MV(nm) in particle size data sheet.

Table No. 6: Polydispersity Index.

Polydispersity Index	Type of dispersion
0 – 0.05	Monodisperse stand
0.05 – 0.08	Nearly monodisperse
0.08 – 0.7	Mid range polydisperse
> 0.7	Very polydisperse

C] SEM[22,19]

The prepared samples of cubosomes are coated with a gold film under vaccum for 2min. The specimens are transferred to an ISI ABT SX-40A scanning electron microscope and digital images captured.

D] Determination of percentage drug content[44]

5 ml of dispersion was taken and was further diluted with pH 7.4 phosphate buffer saline and the samples were analyzed spectrophotometrically at 252nm.

E] Determination of percentage entrapment efficiency[44]

The %EE of the vesicles was determined using centrifugation technique. The vesicular dispersion was centrifuged for 20 min. Supernatant containing unentrapped drug was withdrawn and measured UV spectrophotometrically at 252nm against phosphate buffer saline pH 7.4. The amount of drug entrapped in cubosomes was determined by.

$$\%\ .E.E\ \frac{T-C}{T}\times 100$$

Where, T=Total amount of drug calculated in both supernatant and sediment. C=Drug in supernatant.

F] *In Vitro* drug release[18]

In vitro drug release was measured using Franz diffusion cell. 4mg celecoxib containing cubosomal dispersion was placed on one side of cellophane membrane in a vertical franz diffusion cell. Other side of membrane was in contact with the dissolution medium phosphate buffer saline of pH 7.4. Entire dissolution assembly was placed on a magnetic stirrer at temperature of 37°C. Aliquots of dissolution medium was withdrawn at different time intervals for 8hr. Drug concentration in the dissolution medium were determined by UV spectrophotometry at 252nm.

8.2. Preparation of cubosomal gel[11,37]

The cubosomal gel was obtained by addition of weighted amount of carbomer (1% w/w) in distilled water and kept for half day forgetting to swell of carbomer and then add triethanolamine drop by drop up to pH 7. Propylene glycol is added to adjust the consistency. The obtained gel was then diluted with an appropriate amount of cubosomes dispersion in the ratio between the dispersion and the gel was 2:1 w/w.

9.2.1. Evaluation of cubosomal gel[40]

A] Appearance[1]

About 1 week after preparation, the gel were visually

assessed for optical appearance (e.g., colour, turbidity, homogeneity, presence of macroscopic particles)

B] PH[37]

PH of all formulations is determined by using digital pH meter by immersing the electrode in gel formulation and pH was measured.

C] Drug content[12]

1g of the prepared gel[7] was mixed with 100ml of methanol. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and analyzed using UV.

D] Ex- vivo skin permeation study[6]

In vitro skin permeation studies were performed using goat ear skin. The superficial skin was collected from the back of goat ear and the hair on the skin was removed. Skin was then mounted in a modified Franz diffusion cell, which is kept at 37ºC. Weighed quantity of cubosomal gel[7] was then spreaded on the stratum corneum side of skin (donor compartment) and dermis side was facing receptor compartment. Receptor compartment contains 30 ml of pH 7.4 phosphate buffer and after everyone hour 1 ml of sample was taken and replaced with the same volume of phosphate buffer. After 6 hours sampling, absorbance was measured at 252 nm against blank of pH 7.4 phosphate buffer by UV spectrophotometer. And the percentage drug permeated was calculated.

E] Release kinetics[13]

To study the *in vitro* release kinetics of the optimized formulation, data obtained from dissolution study were plotted in various kinetics models. Different kinetic models such as zero order (cumulative amount of drug released vs. time), first order (log cumulative percentage of drug remaining vs. time), Higuchi model (cumulative percentage of drug released vs. square root of time), Korsmeyer- Peppas model (Log Cumulative percent drug release versus log time) and Hixson Crowell model(cube root of log cumulative percentage of drug remaining vs. log time) were applied to interpret the drug release kinetics from the formulations. Based on the highest regression values for correlation coefficients for formulations, the best fit model was decided.

Zero order equation

The zero order release can be obtained by plotting cumulative % percentage drug released vs Time (hr)

It is used to describe the drug dissolution of several types of modified release Pharmaceutical dosage forms, as in the case of some transdermal systems, as well as tablets with low soluble drugs in coated forms, osmotic systems, etc.

First order reaction

The graph was plotted with log % cumulative drug remaining vs. time (h) Log

$$C = \text{Log } C_0 - Kt / 2.303$$

Where,

C_0 = initial concentration of drug,

K = First order, t = time (hr)

The first order equation describes the release from systems where the dissolution rate is dependent upon the concentration of the dissolving species.

The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release the drugs in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminishes.

Higuchi kinetics

It was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then sustained to different geometrics and porous systems. This model is based on the hypothesis that

A] Initial drug concentration in the is much higher than drug solubility.

- Drug diffusion takes place only in one dimension (edge effect must be negligible).
- Drug particles are much smaller than system thickness.
- Swelling and dissolution are negligible.
- Drug diffusivity is constant and Perfect sink conditions are always attained in the release environment.

The graph was plotted with % cumulative drug release vs. square root of time.

$$Q = Kt^{1/2}$$

Where,

K = constant reflecting design variable system (differential rate constant) t = time (hr)

The drug release rate is inversely proportional to the square root of time

Application

This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and tablets with water soluble drugs.

Hixson and Crowell erosion equation

To evaluate the drug release with changes in the surface area and the diameter of the particles, the data were plotted using the Hixson and Crowell rate equation. The graph was plotted by cube root of % drug remaining vs. time in hr.

$$Q0^{1/3} - Qt\, t^{1/3} = HKCHC$$

Where,

Q0 = Initial amount of drug

Qt = Amount of drug released in time t

K_{HC} = Rate constant for Hixson Crowell equation

Korsmeyer- peppas equation

Korsmeyer et al., (1983) derived a simple relationship which described drug release from a polymeric system equation. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model.

To evaluate the mechanism of drug release, t was further plotted in korsmeyer- peppas equation as log cumulative % of drug released vs log time

$$Mt/M\alpha = Kt^n$$

Where,

Mt / Mα = Fraction of rug released at time t

T = Release time

K = kinetics constant (instructing structural and geometric characteristic of the formulation)

N = Diffusional exponent indicative of the mechanism of drug release.

E] Stability studies[33]

Accelerated stability studies for optimized gel formulation (F7) were conducted as per ICH guidelines at 40°C ± 2°C/75% ± 5% RH at sampling intervals of 0, 15, 30, and 60 days respectively. The drug content, pH and drug release are determined periodically.

$$C = K_0 t$$

9. RESULT AND DISCUSSION

10.1 PREFORMULATION STUDY

Preformulation testing was an investigation of physical and chemical properties of a drug substance alone. It was the first step in rational development of dosage form.

10.2. IDENTIFICATION OF DRUG

A) FTIR Spectroscopic studies

FTIR spectroscopy gives the possible information about the interaction between the drug and polymers. The results are as follows,

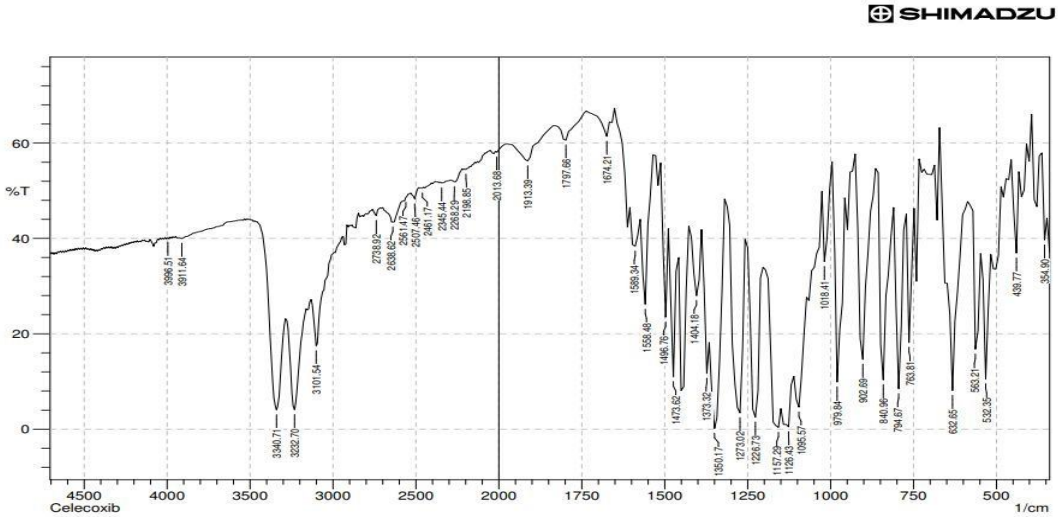


Fig. No. 8: FTIR Spectrum of celecoxib drug.

Table No.7: FTIR spectral interpretation of celecoxib.

Wave number (cm^{-1})	Types of vibrations
794.67 (cm^{-1})	Aromatic CH Stretching
1226.73 (cm^{-1})	S=O Stretching
1558.48 (cm^{-1})	N-H Stretching
3232.70 (cm^{-1})	NH_2Stretching

Interpretation of FTIR Spectrum

Major functional groups like NH_2 stretching, aromatic CH stretching, and S=O Stretching (sulfonamide group) were present in celecoxib showed characteristic peaks in FTIR spectrum. The major peaks were identical group of celecoxib. Hence, the sample was confirmed as celecoxib.

B) By melting point

Melting point values of celecoxib drug was found to be in range of 160 to 164^{0}C, which was represent in below table 8. the observed melting point for celecoxib was 161^{O}C, which meets the specification limit.

Table 8: Melting Point of Celecoxib.

S.No	MELTING POINT	AVERAGE
1	162 º C	162 º C
2	162 º C	
3	161 º C	

10.3 PHYSICOCHEMICAL PARAMETERS

A] Organoleptic propeties

Table 9: Organoleptic properties of celecoxib.

Property	Specification	observation
Color	white or almost white	White color
Nature	Amorphous or crystalline powder	Amorphous powder
Odour	NA	odourless

B] Solubility studies of celecoxib

Solubility of celecoxib pure drug in different solvents was represented in table 10.

Table No. 10: Solubility of celecoxib pure drug in various media.

S.NO	SOLVENT	INFERENCE
1	Water	practically insoluble
2	Methanol	freely soluble
3	Ethanol	soluble
4	0.2N NAOH	freely soluble
5	Buffer phosphate 6.8	sparingly soluble
6	Buffer phosphate 7.4	sparingly soluble

Inference

The solubility of the drug at methanol was significantly higher than that of distilled water. Pure drug of celecoxib in distilled water was found to be insoluble.

10.4. Physical compatibility study

Table No.11.physical compatibility study of drugs and excipients.

S.No	Drugs and Excipients	Description and conditions						
		Initial	At room temperature (in days)			At 40ºC ± 2ºand 75%RH± 5% (in days)		
			10	20	30	10	20	30
1.	celecoxib	White powder	NC	NC	NC	NC	NC	NC
2.	GMO	Pale yellow oily phase	NC	NC	NC	NC	NC	NC
3.	Poloxamer 407	White powder	NC	NC	NC	NC	NC	NC
4.	Carbopol 934	White powder	NC	NC	NC	NC	NC	NC
5.	Drug +GMO+poloxamer407	Liquid crystalline dispersion	NC	NC	NC	NC	NC	NC
6.	Drug +GMO+poloxamer407 + carbopol 934	Gelling form	NC	NC	NC	NC	NC	NC

*NC-No Change

Inference

The physical compatibility is shown in Table They were evaluated for 10, 20 and 30 days at room temperature. There was no change in colour. Therefore the drug and excipients are physically compatible with each other. The excipients which are compatible with the drug are selected for formulation.

10.4.1 UV spectrum of celecoxib using PBS 7.4

The maximum absorbance of the celecoxib was studied. The maximum absorbance of the drug celecoxib was found to be 252 nm. Hence the wavelength of 252nm was selected for analysis of drug in dissolution media.

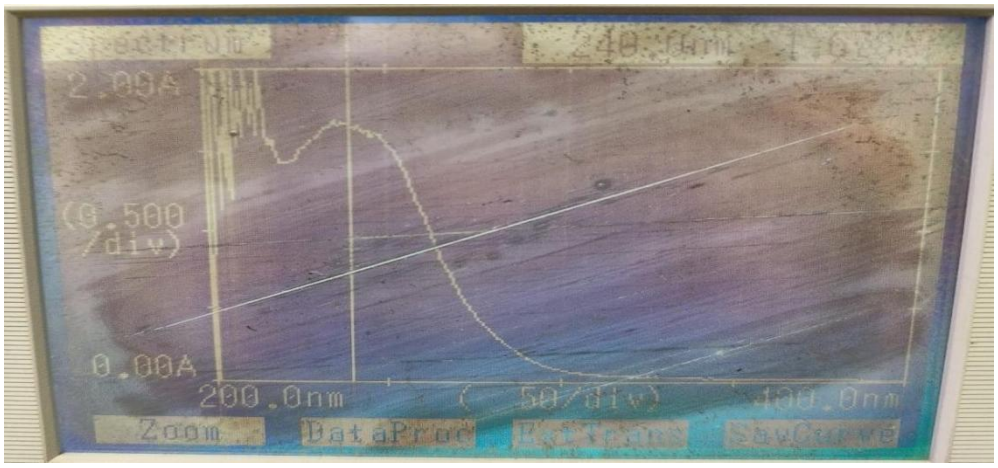


Fig. No. 9.1: Uv Spectrum Celecoxib Using Pbs 7.4.

10.5. Determination of λ max for celecoxib

10.5.1 UV spectrum of celecoxib using methanol

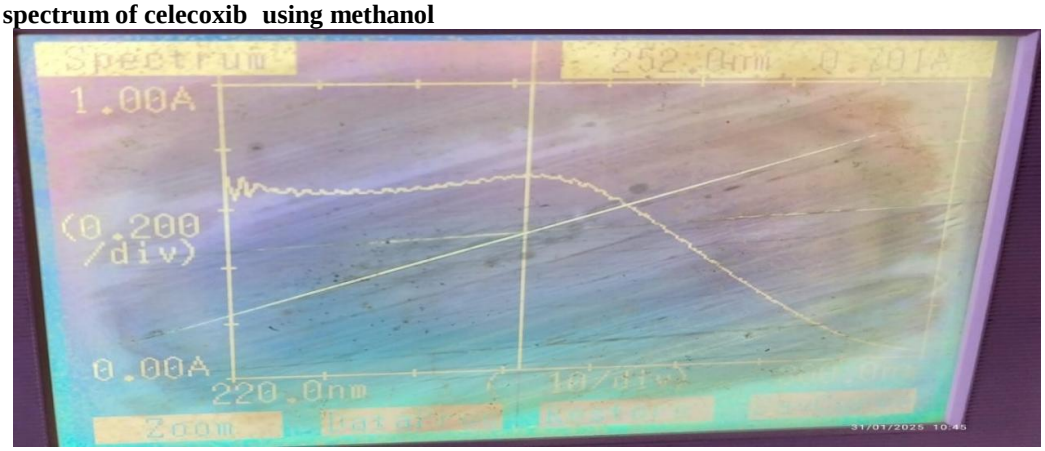


Fig. No. 9.2: UV Spectrum Celecoxib Using Methanol.

10.6.1 Standard Calibration Curve of Celecoxib in phosphate buffer pH 7.4 and methanol

The Ultraviolet Spectrophotometric method was used to analyze the calibration curve of celecoxib. The absorbance of the drug in of concentration ranging from 1-5 µg/ml was measured at a wavelength of 240 and 260nm against blank.

Table No. 12.1: Data for calibration curve of celecoxib in phosphate buffer pH 7.4.

S.NO	CONCENTRATION (MCG/ML)	ABSORBANCE
1.	1	0.177
2.	2	0.342
3.	3	0.501
4.	4	0.652
5.	5	0.819

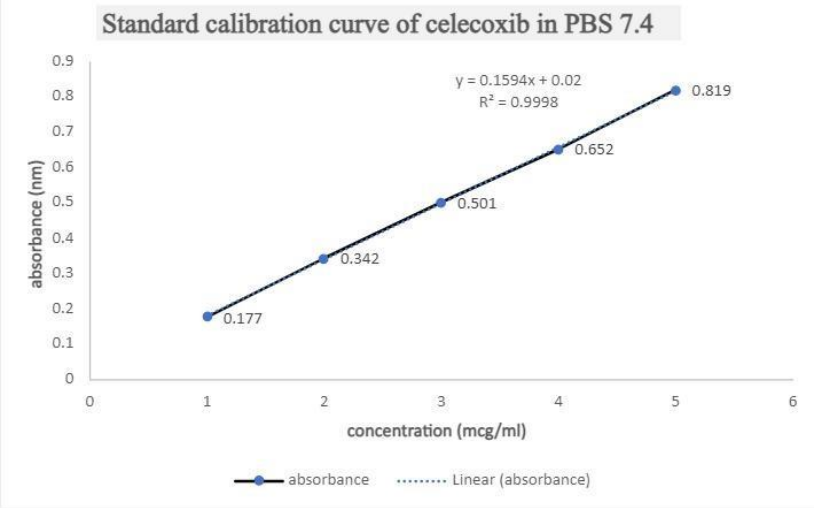


Fig. NO. 10.1: Calibration Curve of Pbs 7.4.

Inference

It was found that the solutions show linearity (R2 = 0.9998) at a concentration of 1-5 µg/ml and obeys Beer Lambert's law.

10.6.2. Standard Calibration Curve of Celecoxib in methanol

The Ultraviolet Spectrophotometric method was used to analyze the calibration curve of celecoxib. The absorbance of the drug in of concentration ranging from 2-10 µg/ml was measured at a wavelength of 240 and 260nm against blank.

Table No. 12.2: Data for calibration curve of celecoxib in methanol.

S.NO	CONCENTRATION (MCG/ML)	ABSORBANCE
1.	2	0.141
2.	4	0.288
3.	6	0.424
4.	8	0.565
5.	10	0.701

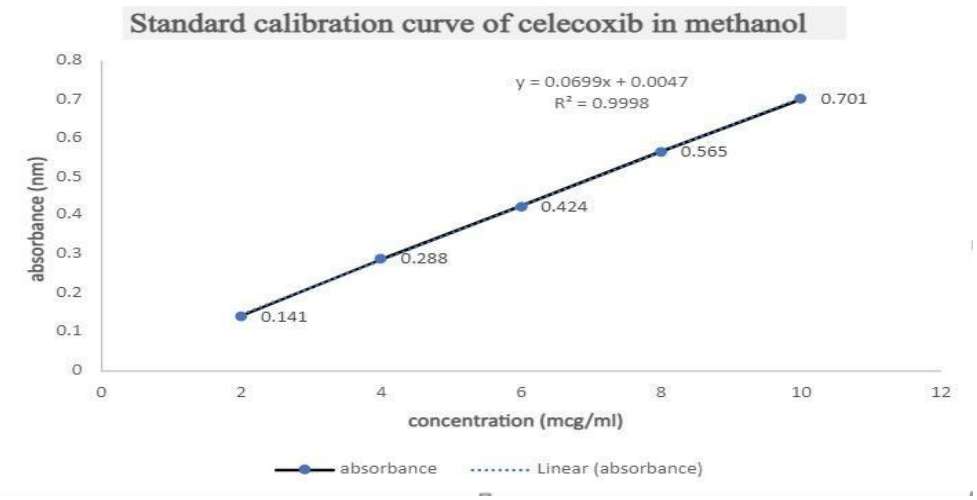


Fig. No. 10.2: Calibration Curve of Methanol.

Inference

It was found that the solutions show linearity (R2 = 0.9998) at a concentration of 2-10µg/ml and obeys Beer Lambert's law.

10.7 Determination of drug -polymer compatibility

A] By FTIR Spectroscopy

The FTIR spectrum of celecoxib with polymers (GMO and poloxamer 407) used in formulations were showed in fig 11, and their interpretations were represented in table 13.

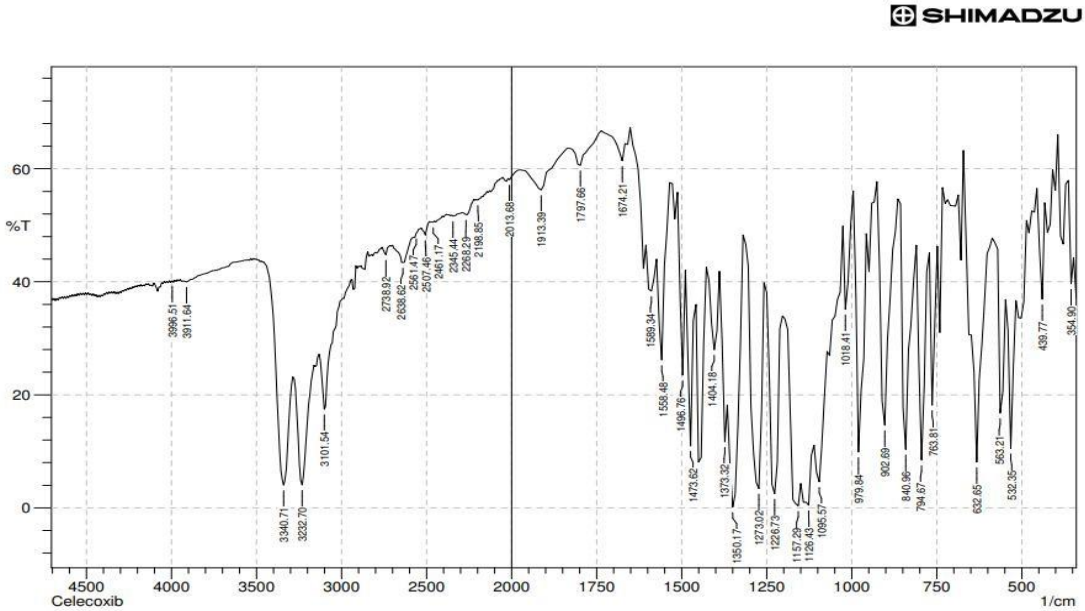


Fig. No. 11: 1 FTIR Spectrum of celecoxib drug.

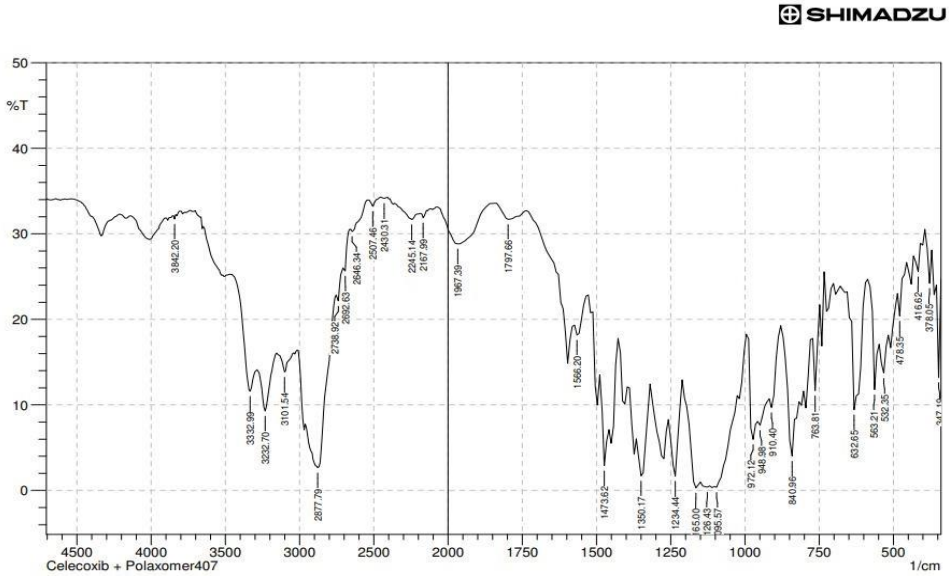


Fig. no. 11.2: FTIR Spectrum of drug and poloxamer 407.

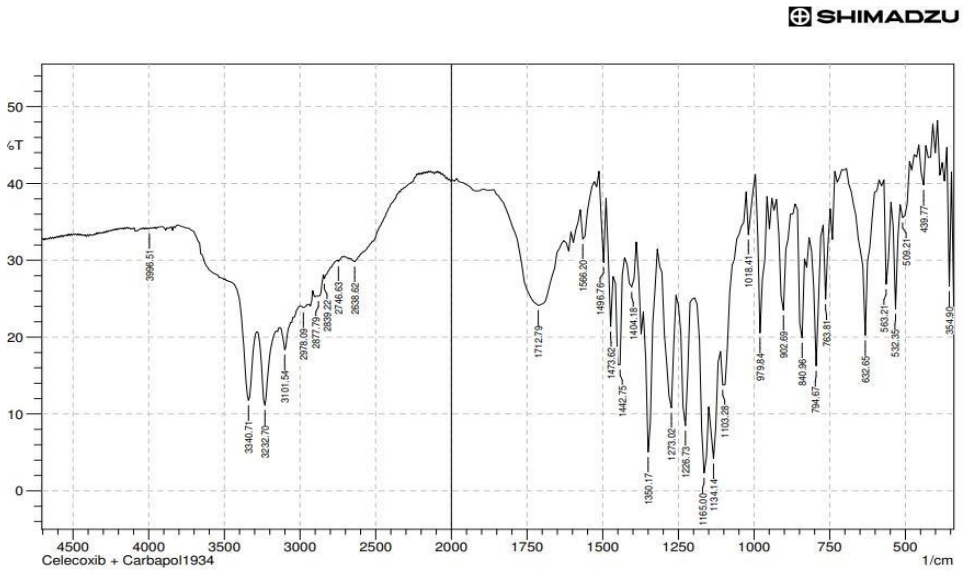


Fig No. 11.3: FTIR spectrum of drug and carbopol 934.

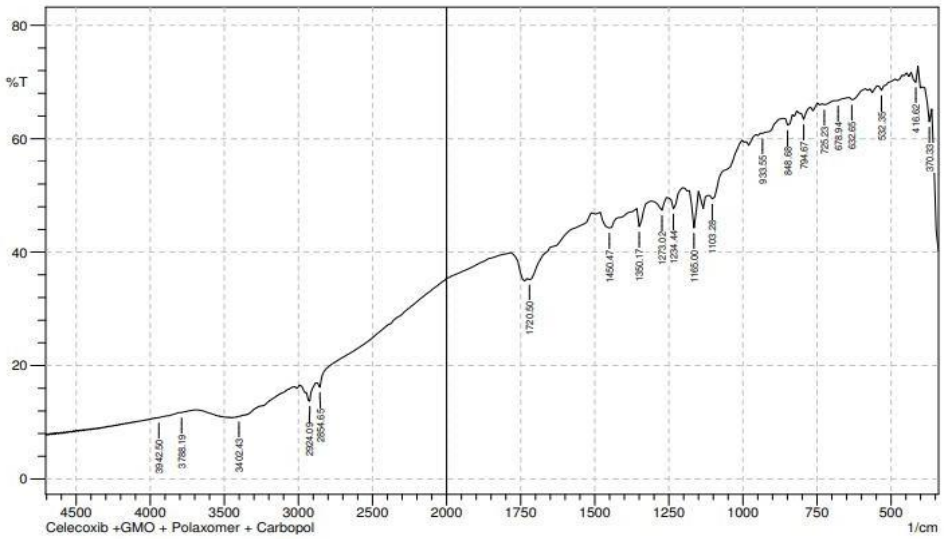


Fig No. 11.4: FTIR spectrum of drug and all polymers.

Table 13: Ftir Spectral Interpretation Of Drug And Polymers.

Specification	Celecoxib drug	Celecoxib with poloxamer 407	Celecoxib with carbabol 934	Celecoxib with pol407, Car934 and GMO	Inference
Wave No .(cm^{-1})					
780-820cm^{-1}	763.81, 794.67	763.81	763.81, 794.67	794.67	Aromatic CH stretching
1150-	1157.29,	1166.00,	1165.00,	1165.00,	S=O
1350cm^{-1}	1226.73,	1234.44	1226.73,	1234.44,	stretching
	1273.02		1273.02	1273.02	
1550-	1558.48,	1566.20	1566.20	1450.47	H
1600cm^{-1}	1589.34				stretching
3200-3500cm^{-1}	3232.70, 3340.71	3232.70, 3332.99	3232.70, 3340.71	3402.43	NH_2 stretching

10. OPTIMIZATION OF CELECOXIB LOADED CUBOSOME BY FACTORIAL DESIGN

A three factors and two level factorial design was used to optimize the celecoxib loaded cubosome formulation. At three factors and two levels, factorial design requires 8 experimental runs to determine the optimized

formulation. A total of 8 experimental runs were generated and evaluated using Design Expert Software. The significant response factors were used to assess the quality of the formulation including Percentage Entrapment Efficiency and Cumulative Percentage Drug Release.

Table No. 14: Variables used in Factorial Design.

VARIABLE	LOW	HIGH
A:STIRRING SPEED(RPM)	500	1000
B: CONCENTRATION OF SURFACTANT [g]	0.2	0.3
C:CONCENTRATION OF LIPID (g)	1.5	2.5

11.1 PREPARATION OF CUBOSOMAL DISPERSION

Nine different formulation of cubosomal dispersion were prepared by top-down method, by using a drug and with polymers in different concentration. polymers GMO and poloxmer407 were used in formulation. The formulation were designed as F1 ,F2, F3, F4, F5, F6, F7 and F8 respectively. All the formulated dispersion were taken for further evaluation.

11.2EVALUATION OF CUBOSOMAL DISPERSION

A] Optical microscopy

Images obtained under an optical microscope confirmed the formation of the crystal cubic structures. Showed in figure no.12. It was found that the formed crystals were spherical and some are in cubic shape.

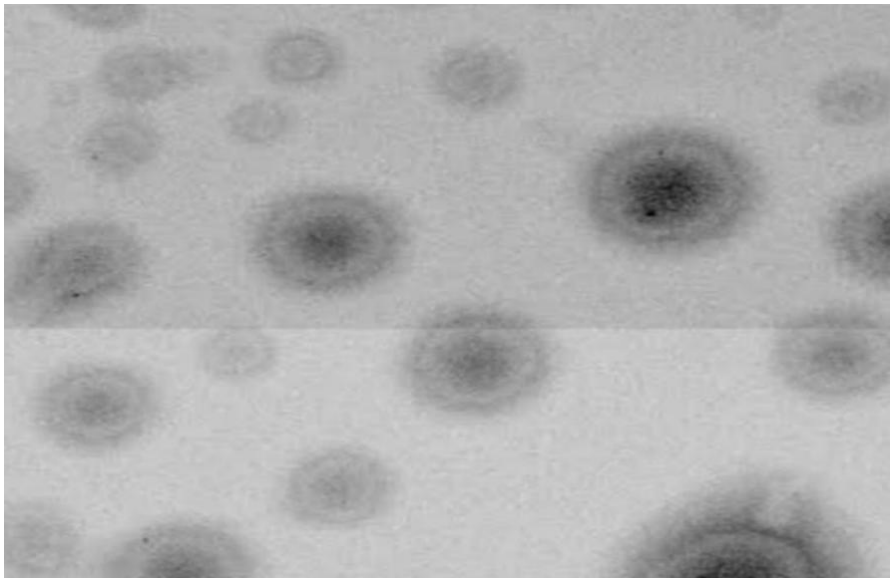


Figure no. 12: Optical microscopy of cubosomal dispersion.

B] Drug Entrapment Studies of Cubosome

Drug entrapment efficiency was determined in order to make sure that the added amount of celecoxib is present in the cubosome dispersion. The EE of all batches is in the range of 79.1-88.2%. The highest EE was found in the batch F7, consisted of 2.5 g of GMO, and 0.2g of poloxamer 407. The EE of celecoxib into cubic nanoparticles was dependent on the concentration of GMO. The result showed that the EE increased, as the amount of lipid and surfactant increased. Increasing amount of GMO was bound to increase the % of EE because of the increased concentration of mono-, di-, and triglycerides, which act as solubilizing agents for celecoxib and provide more space to accommodate excessive drugs. This effect may be observed due to the increased viscosity of the medium, because increasing the amount of lipid resulted in faster solidification of the cubosomal nanoparticles, which would prevent drug diffusion to the external phase of the medium. As the percentage of emulsifier increased, part of the celecoxib was incorporated in the surfactant layer at the surface of the cubosomes, leading to a high entrapment efficency.

Table No. 15: Entrapment Efficiency Of Cubosomal Dispersion.

S.NO	FORMULATION CODE	PERCENTAGE ENTRAPMENT EFFICIENCY
1	F1	83.7
2	F2	81.1
3	F3	79.1
4	F4	85.4
5	F5	86.4
6	F6	81.5
7	F7	88.2
8	F8	83.1

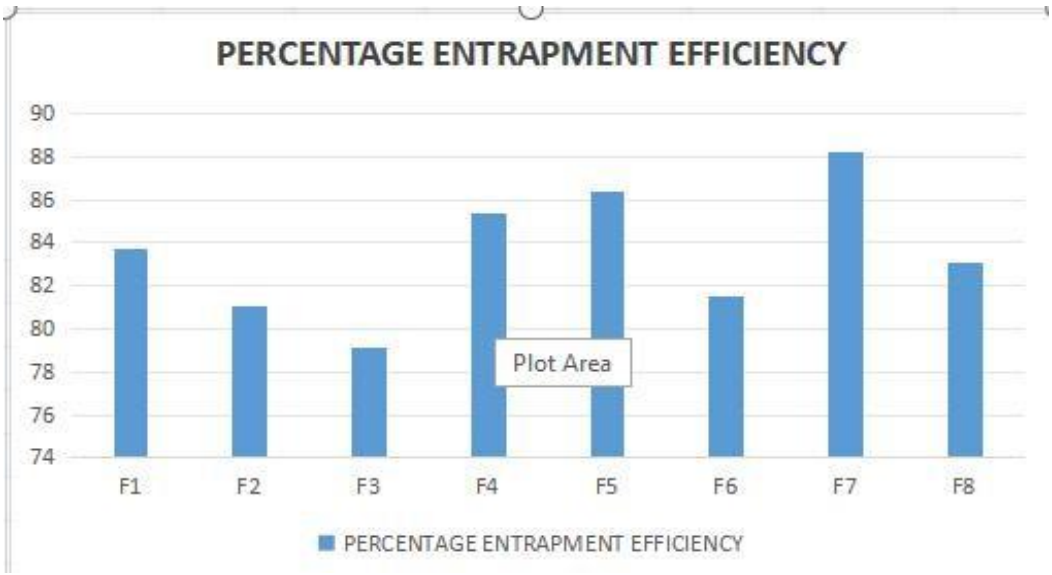


Figure no. 13: Graphical representation of percentage entrapment efficiency.

C] In Vitro Drug Release Studies of Cubosome

The In vitro release characteristics of cubosomal dispersions shows that the drug release is directly proportional to the concentration of GMO and inversely proportional to the concentration of P-407 i.e. the cubosomes showed decrease in percent drug release when using of lower concentration of GMO and higher concentration of P-407 polymer. Here F7 has higher GMO concentration and lower Poloxamer 407 concentration, and it also showed higher percentage of drug release ie, 89.62 %.

Table no. 16: Percentage Drug Release Of Cubosomal Dispersion.

TIME [HOURS]	F1	F2	F3	F4	F5	F6	F7	F8
1	4.60	4.39	3.97	5.85	5.23	3.97	5.85	4.60
2	11.71	10.04	8.57	12.13	11.08	10.24	12.33	9.41
3	21.12	20.07	18.61	21.95	20.70	20.70	21.12	19.23
4	29.69	28.43	25.51	31.15	28.85	28.85	31.15	26.34
5	40.56	40.14	37.84	41.60	39.93	40.56	42.02	39.30
6	52.06	49.97	48.29	52.68	51.01	50.80	54.15	49.76
7	72.96	69.83	68.78	73.80	71.29	70.87	77.56	70.04
8	83.63	81.33	80.70	84.67	83.21	81.54	89.69	81.54

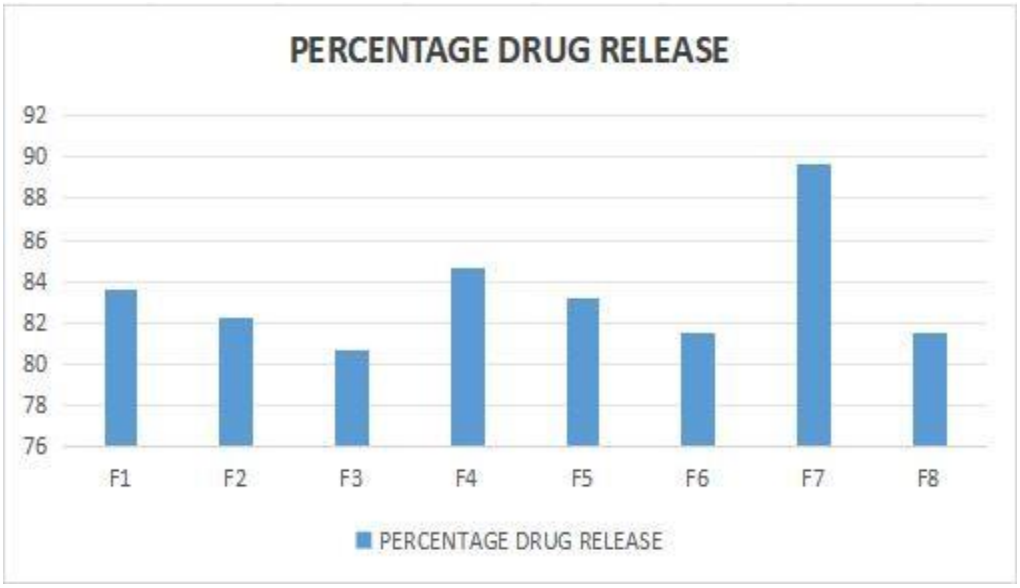


Figure no. 14: Graphical representation of percentage drug release.

D] Drug content estimation of cubosome

The % drug content in various formulations ranged from 83.3-94.6%. The drug content data revealed that there was no significant difference in the uniformity of the drug content in the formulations. So, it indicated that celecoxib was uniformly distributed in vesicular dispersions.

Table No. 17: Drug Content Of Cubosomal Dispersion.

S.NO	FORMULATION CODE	% DRUG CONTENT
1	F1	92.21
2	F2	90.21
3	F3	87.62
4	F4	92.81
5	F5	91.61
6	F6	88.82
7	F7	94.6
8	F8	83.63

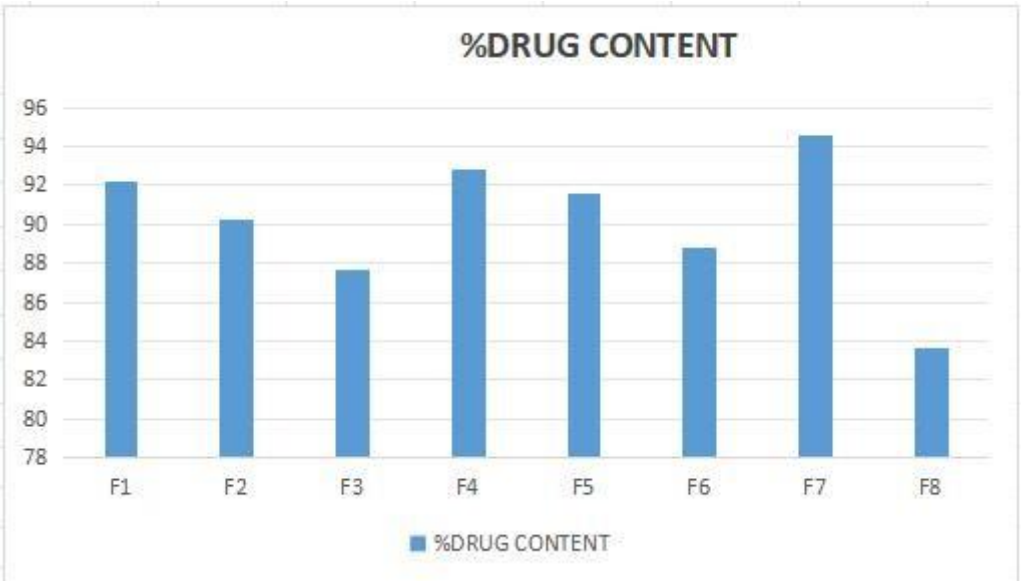


Figure No. 15: Graphical Representation of Drug Content.

11.3 OPTIMIZATION OF CELECOXIB LOADED CUBOSOMES

Table 18: Summary of 2^3 factorial design for celecoxib loded cubosome.

RUNS	Factor 1	Factor 2	Factor 3	Response 1	Response 2
Runs	Stirring Speed (RPM)	Surfactant Concentration (g)	Lipid Concentration (g)	Entrapment Efficiency (%)	Cumulative Percentage Drug Release
1	1000	1.5	0.3	83.7	83.63
2	1000	1.5	0.2	81.1	82.23
3	1000	2.5	0.3	79.1	80.03
4	500	1.5	0.3	85.4	84.83
5	500	2.5	0.2	86.4	83.63
6	500	2.5	0.3	81.5	81.63
7	500	1.5	0.2	88.2	89.62
8	1000	2.5	0.2	83.1	81.43

Table No. 18.1: Design summary.

Factor	Name	Unit	Type	Minimum	Maximum	Coded low	Coded high	Mean
A	Stirring speed	RPM	Numeric	500	1000	-1	+1	750
B	Concen tration of surfact ant	G	Numeric	1.5	2.5	-1	+1	2.0
C	Concen tration of lipid	G	Numeric	0.2	0.3	-1	+1	0.25

Table No. 18.2: Polynomial analysis.

Response	Name	Units	Minimum	Maximum	Mean	Ratio
R1	Entrapment efficiency	%	79.1	88.2	82.77	1.09
R2	Cumulative percentage drug release	%	80.70	89.69	83.48	1.11

Response 1: cumulative % of drug entrapment efficiency

ANOVA for linear model

Table No 19.1: Analysis of variance table (Partial sum of squares – Type III).

Source	Sum of Squares	Df	Mean Square	F- value	p-value	
Model	**39.12**	**1**	**39.12**	**7.96**	**0.0303**	**Significant**
A Concentration of surfactant	39.12	1	39.12	7.96	0.0303	
B Concentration of lipid	39.12	1	39.12			
Residual	29.49	6	4.92			
Cor Total	68.61	7				

The Model F-value of 7.96 implies the model is significant. There is only a 3.03% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B, C are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table No. 19.2: Fit statistics.

User standard deviation	0.0000	**R^2**	0.5701
Standard deviation	2.22	**Adjusted R^2**	0.4985
Mean	85.24	**Predicted R^2**	0.2358
C.V.%	2.60	**Adequate Precision**	3.9895

The **Predicted R^2 0.2358** of is in reasonable agreement with the **Adjusted R^2 of 0.4985**; i.e. the difference is more than 0.2.

Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of indicates an adequate signal. This model can be used to navigate the design space.

Table No 19.3 Coefficients in Terms of Coded Factors.

Factor	Coefficient Estimate	Df	Standard error	95% CI Low	95% CI High	VIF
Intercept	85.24	1	0.7839	83.32	87.15	
B-Concentration of Surfactant	-2.21	1	0.7839	-4.13	-0.2932	1.0000
C-Concentration of lipid	-2.21	1	0.7839	-4.13	-0.2932	1.0000

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1: VIFs greater than 1 indicate multi co linearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Normal plot of residuals for Y1 – Percentage Entrapment Efficiency

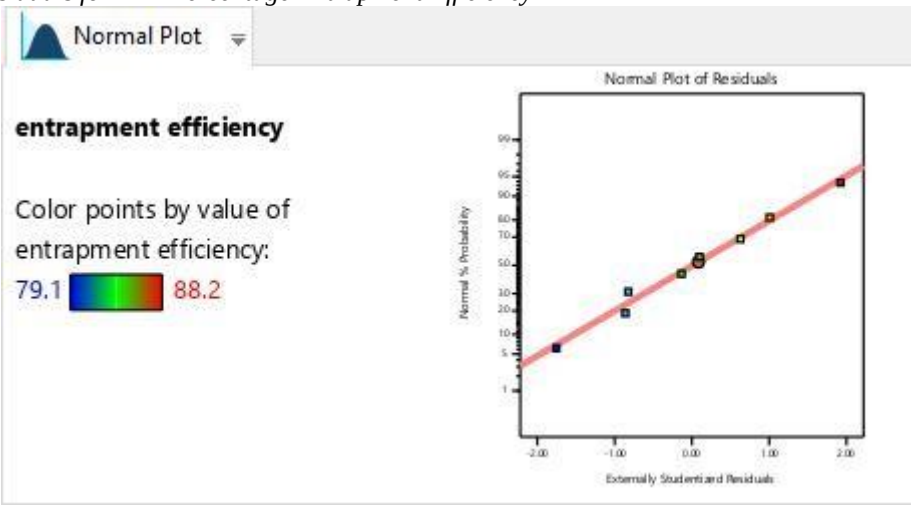


Figure No. 16.1: Normal plot of residuals for Y1.

The normal % probability vs. externally studentized residuals designed that the maximum of the colored points demonstrating the entrapment efficiency was seen around the normal probility line.

The normal plot of residual shows satisfaction. Since, the residuals are move near to the straight line.

Box – cox plot for Y1 – Percentage Entrapment Efficiency

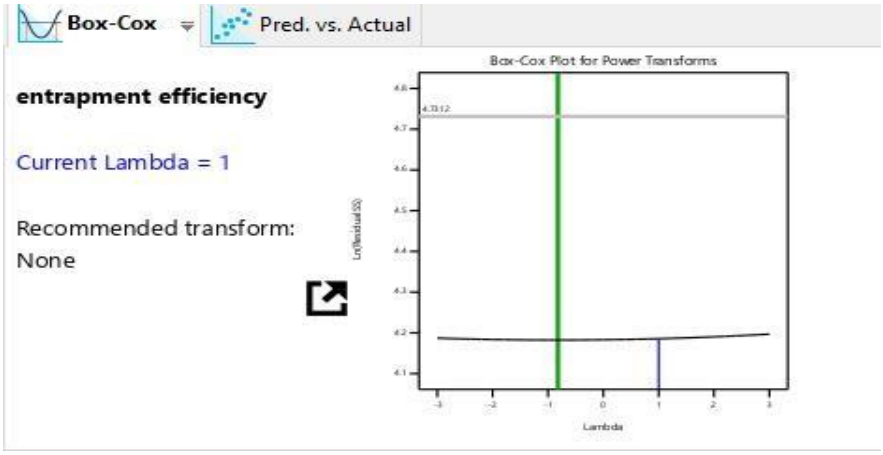


Figure No. 16.2: Box – cox plot for Y1.

The Box – Cox plot for power exposed a linear relationship given in fig 16.2.

Residual vs. Predicted plots for Y1 – Percentage Entrapment Efficiency

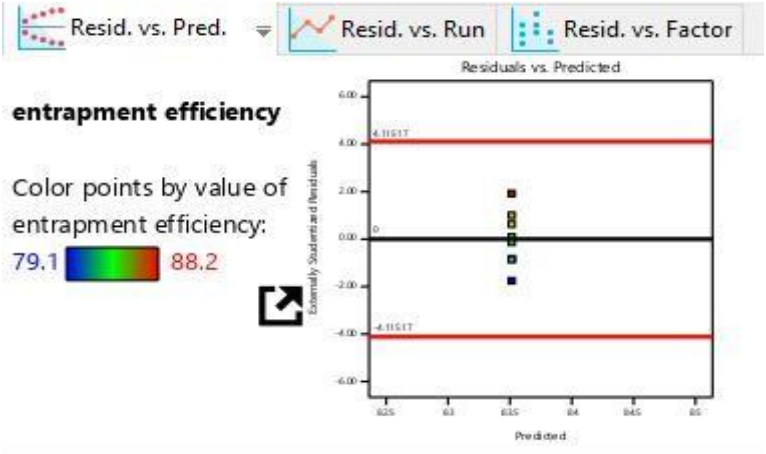


Figure No 16.3 Residual vs. Predicted plots.

The externally studentized residuals vs. predicted tenets plot signifies that the colored points of Entrapment efficiency was privileged the limits.

Cook's Distance for Y1 – Percentage Entrapment Efficiency.

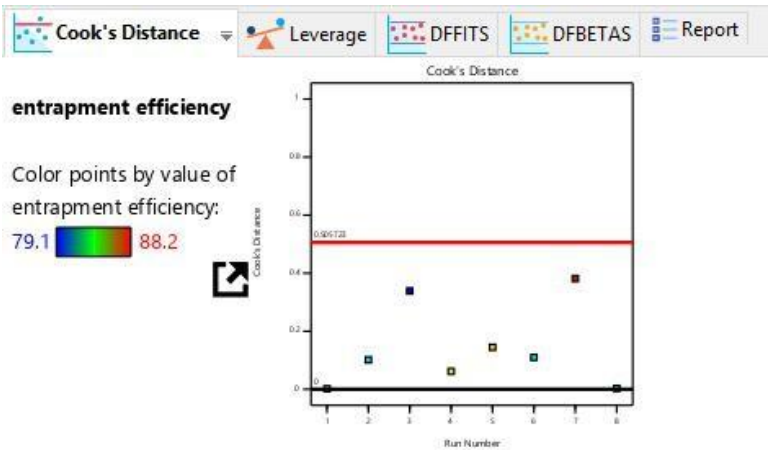


Figure No. 16.4: Cook 's Distance.

The predicted vs. actual plot exposed linear relationship. The color point located near to the straight line. So, this plot indicates pass the desirability limits.

3D Response Plots of Y1 – Entrapment Efficiency

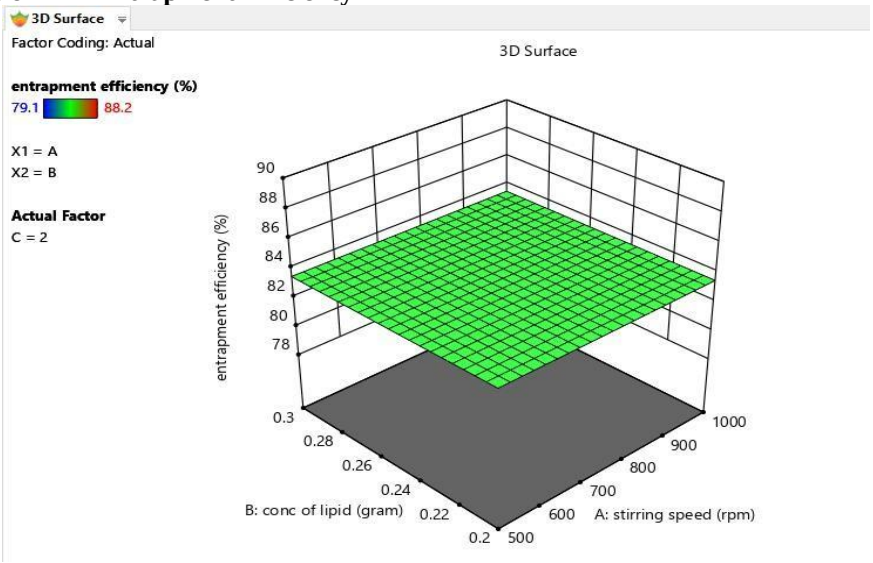


Figure No 16.5 3D Response Plots of Y1.

Response 2: Cumulative % drug release

ANOVA for Linear Model

Table No 20.1 Analysis of variance table [Partial sum of squares - Type III].

Source	Sum of squares	Df	Mean Square	F-Value	p-Value	
Model	**37.10**	**2**	**18.55**	**23.65**	**0.0028**	**Significant**
Stirring speed	7.76	1	7.76	9.90	0.0255	
B-Concentration of surfactant	29.34	1	29.34	37.41	0.0017	
C-Concentration of lipid	29.34	1	0.7842	37.41	0.0017	
Residual	3.92	5				
Cor Total	41.02	7				

The **Model F-value** of 23.65 implies the model is significant. There is only a 0.28 % chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B, C are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Predicted R^2** of 0.7553 is in reasonable agreement with the Adjusted R^2 of 0.8662.I.e. difference is less than 0.2.

Table No. 20.2: Fit statistics.

Standard deviation	0.8856	**R^2**	0.9044
Mean	82.77	**Adjusted R2**	0.8662
C.V.%	1.07	**Predicted R2**	0.7553
		Adequate Precision	10.0054

Table No. 20.3: Coefficients in Terms of Coded Factors.

Factor	Coefficient Estimate	Df	Standard error	95% CI Low	95% CI High	VIF
Intercept	82.77	1	0.3131	81.96	83.57	
A-Stirring speed	-0.9850	1	0.3131	-1.79	-0.1802	1.0000
B-Concentration of Surfactant	-1.92	1	0.3131	-2.72	-1.11	1.0000
C-Concentration of lipid	-1.92	1	0.3131	-2.72	-1.11	1.0000

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1: VIFs greater than 1 indicate multi – co linearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Normal Plots of Residuals for Y2 – Cumulative Percentage drug release

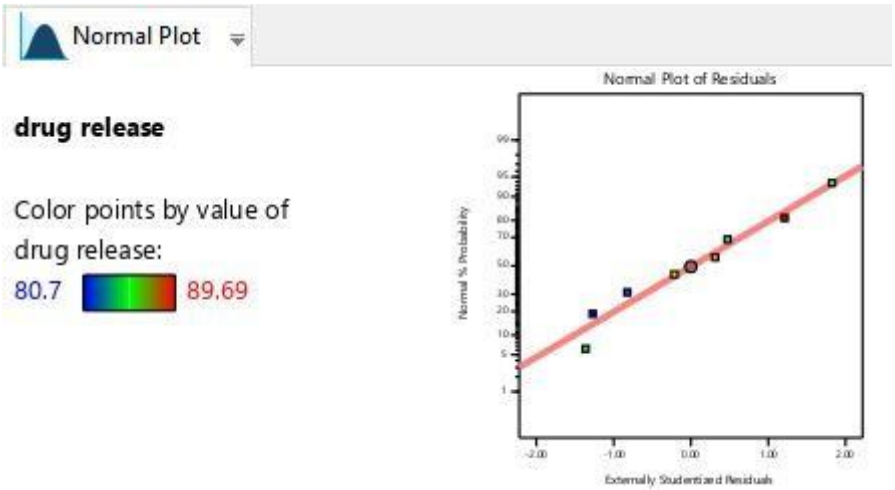


Figure No. 17.1: Normal Plots of Residuals for Y2.

The normal % probability vs. externally studentized residuals designed that the maximum of the colored points demonstrating the DR at the end of 8 hr was seen around the normal probility line.

The normal plot of residual shows satisfaction. Since, the residuals are move near to the straight line.

Residual vs. Predicted plots for Y2 – Cumulative Percentage drug release.

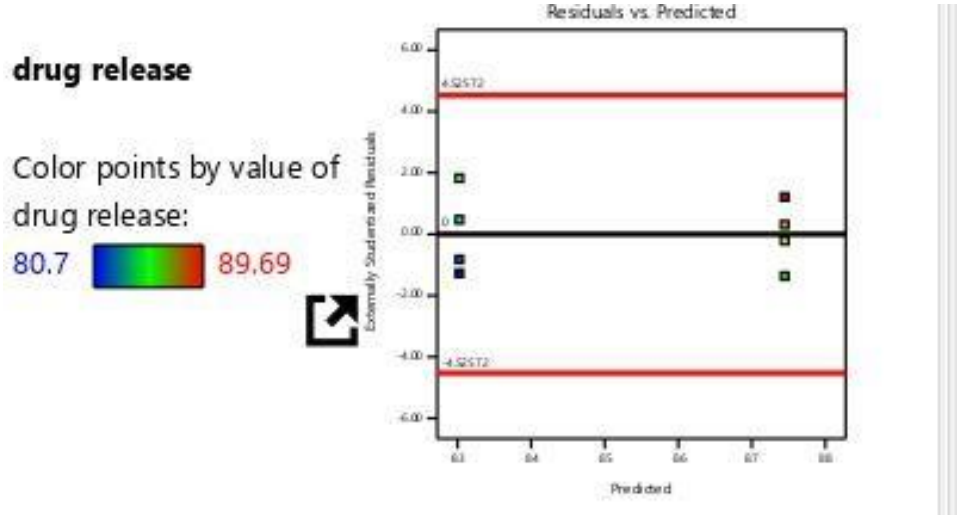


Figure No. 17.2: Residual vs. Predicted plots for Y2.

The externally studentized residuals vs. predicted tenets plot signifies that the coloured points of DR at end of 12 hrs were privileged the limits.

3D Response Plots of Y2 – Cumulative Percentage drug release

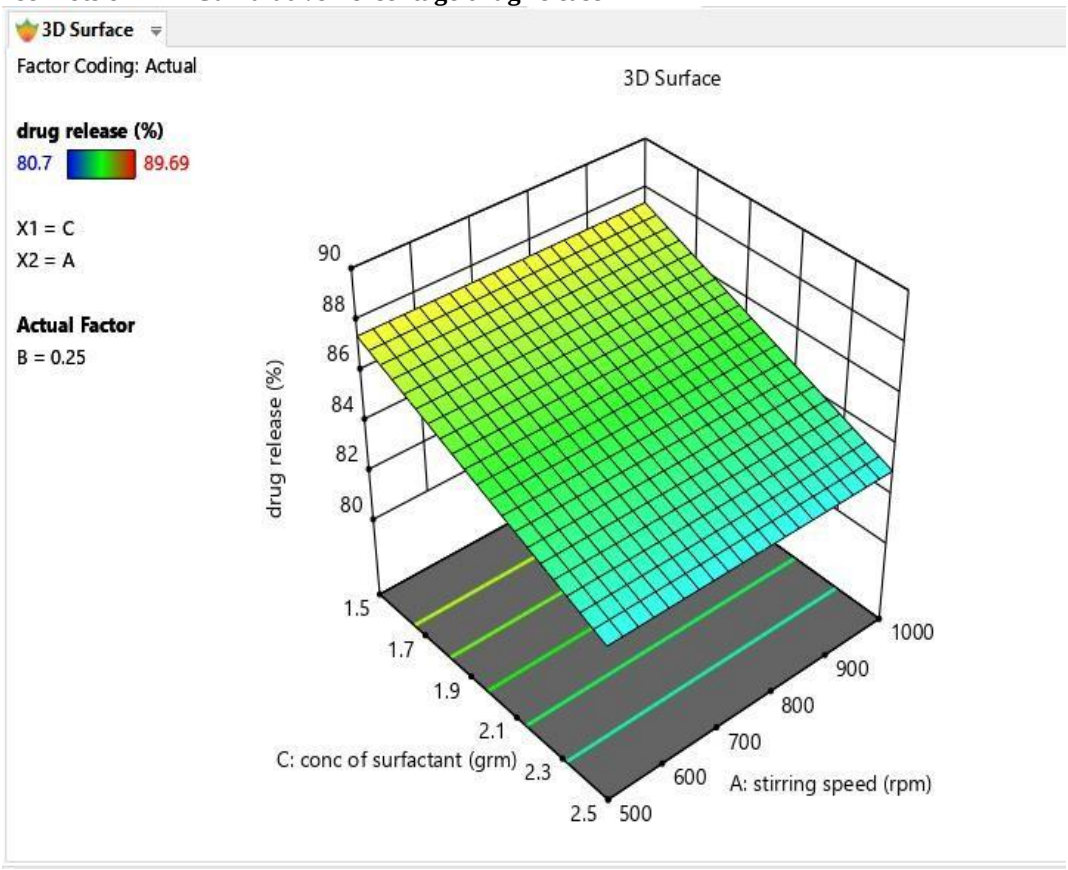


Figure No. 17.3: 3D Response Plots of Y2.

Box-Cox Plot for Y2 – Cumulative Percentage Drug Release

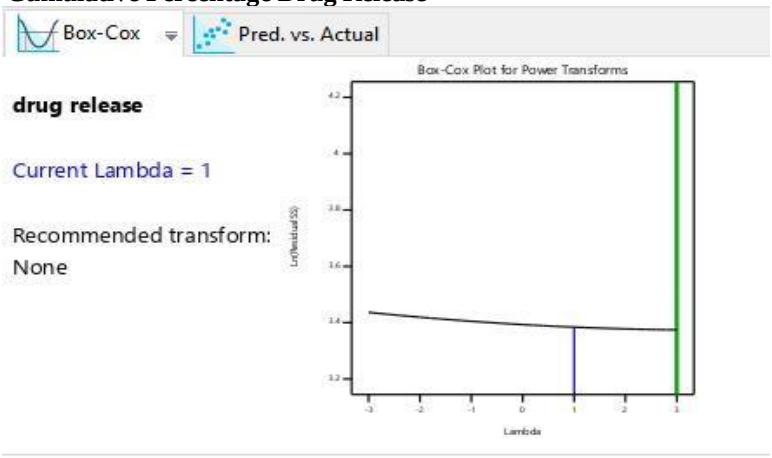


Figure No. 17.4: Box -Cox Plot for Y2.

The cook's distances were plotted and the red line represents DR at the end of 8 hr was in close contact with the predicted values.

Predicted vs. Actual graph for Y2 – Cumulative Percentage Drug Release.

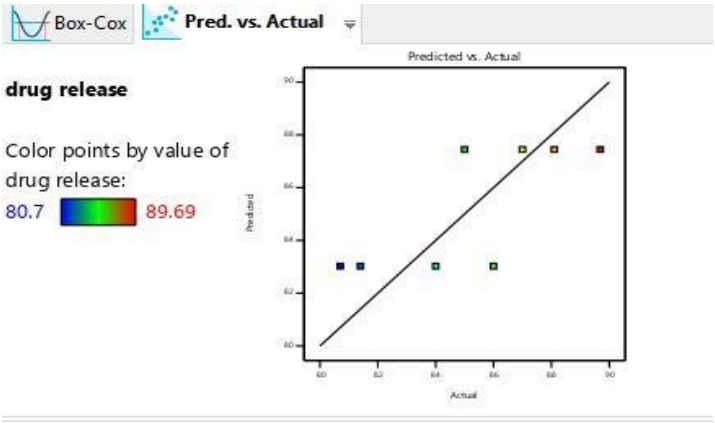


Figure No. 17.5: Predicted vs. Actual graph for Y2.

The predicted vs. actual plot exposed linear relationship. The color point located near to the straight line. So, this plot indicates pass the desirability limits.

E] Scanning electron microscopy[SEM]

Surface morphology and shape characteristics of dispersion for formulation F7 was evaluated by means of scanning electron microscopy. The SEM photograph of the dispersion revealed that the dispersion were smooth, hollow surface, cubic in shape and slightly aggregated. The SEM photograph were provided in figure 18.

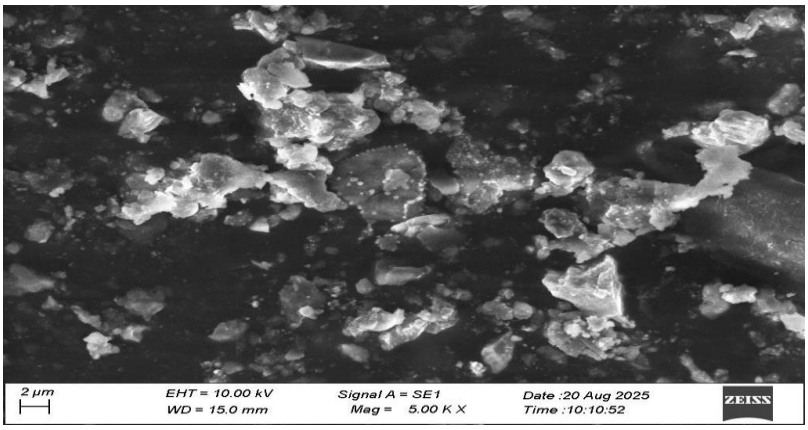


Figure no. 18: SEM image of cubosomal dispersion.

F] Determination of Particle Size and Polydispersity of Optimized Formulation

PDI indicates the particle size distribution, which ranges from 0 to 1. Theoretically, a monodisperse population indicates PDI equal to zero. The low value of PDI signifies the uniformity of particle size within the formulations.

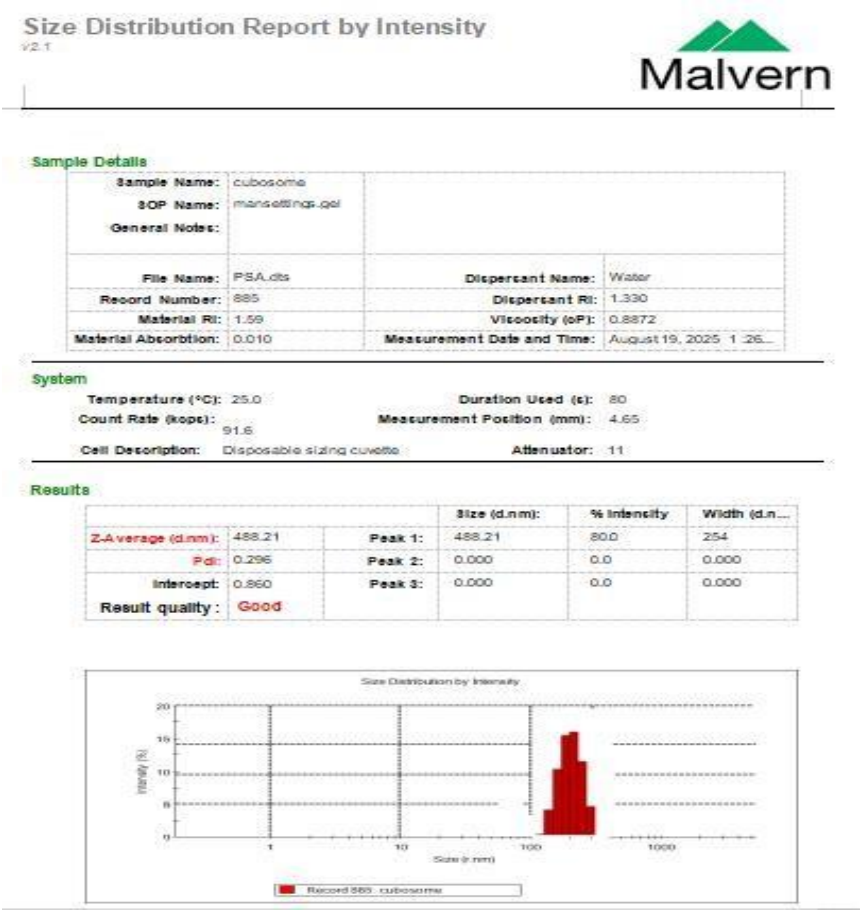


Figure No. 19: Particle Size and Polydispersity of Optimized Formulation Inference.

The Particle Size and Polydispersity of Optimized Formulation was found to be 488.21nm and 0.296.

11.4 PREPARATION OF CUBOSOMAL GEL

From the drug content, entrapment efficiency and drug release study, it is found that F7 is the best formulation.so it was selected and formulated to gel figure no 20.

Figure no. 20: Preparation of cubosomal gel.

11.5 EVALUATION OF CUBOSOMAL GEL

A] ***Appearance***

It was determined by visual inspection. All the formulations were found to be homogenous.

B] ***pH***

The pH was found to be 5.7, which was close to skin pH.

C] ***Drug Content Estimation of Cubosomal Gel***

Drug content of the gel formulations was found to be 94±0.579%. (The reading is an average of 3 determinations).

Table No. 21: Drug Content of Cubosomal Gel.

S.NO	PERCENTAGE DRUG CONTENT	AVERAGE
1	94.78	94.59
2	94.78	
3	94.21	

D]Ex- vivo Skin Permeation Study:

The gel prepared using optimized cubosomal gel [F7] was used for ex-vivo permeation study using goat's ear

skin and showed 84.83% permeation through the skin. It is shown in table no:22.

Table No. 22: Skin Permeation of Cubosomal Gel.

TIME[HOURS]	ABSORBANCE	PERCENTAGE OF DRUG PERMEATION
1	0.054	10.77
2	0.119	23.75
3	0.172	34.33
4	0.265	52.89
5	0.352	70.25
6	0.425	84.83

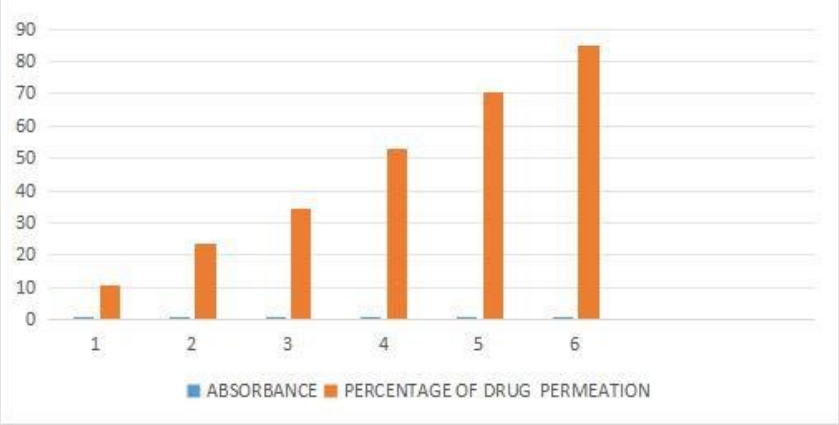


Figure no. 21: Graphical representation drug skin permeation in cubosomal gel.

D] RELEASE KINETICS OF OPTIMIZIED CELECOXIB LOADED CUBOSOMAL GEL

Table No. 23: Release kinetics of Optimized celecoxib loded cubosomal gel.

Time (hours)	Log time	Square root of time	Cumulative % drug release	Cumulative % drug remaining	Log cumulative % drug release	Log cumulative % drug remaining	Cube root of % drug remaining
0	∞	0	0	100	∞	2	4.641
1	0	1	5.88	94.12	0.7693	1.9736	4.5488
2	0.301	1.4142	12.33	87.67	1.0909	1.9428	4.444
3	0.477	1.732	21.12	78.88	1.3246	1.8969	4.2904
4	0.602	2	31.15	68.85	1.4934	1.8363	4.0986
5	0.692	2.236	42.02	57.98	1.6234	1.7632	3.8704
6	0.778	2.4494	54.15	45.85	1.7335	1.6613	3.5739
7	0.845	2.6457	77.56	22.44	1.8896	1.3510	2.8206
8	0.903	2.828	89.69	10.31	1.9527	1.0132	2.176

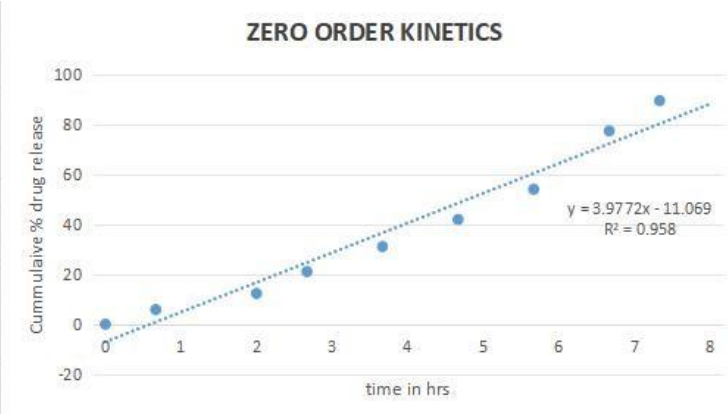


Figure No. 22.1: A plot for zero order kinetics of optimized celecoxib loaded cubosomes.

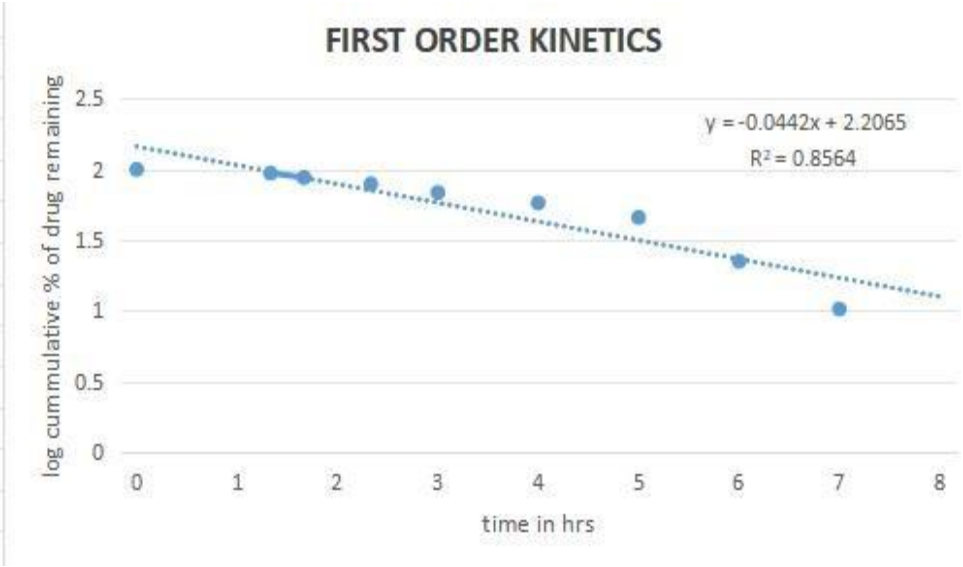


Figure No. 22.2: A plot for first order kinetics of optimized celecoxib loaded cubosomes.

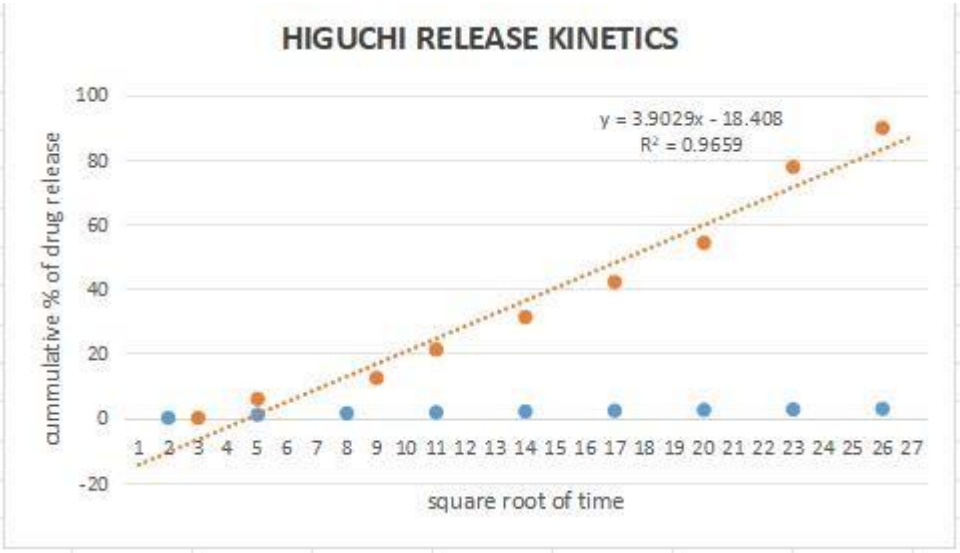


Figure No. 22.3: Higuchi release kinetics of optimized celecoxib loaded cubosomes.

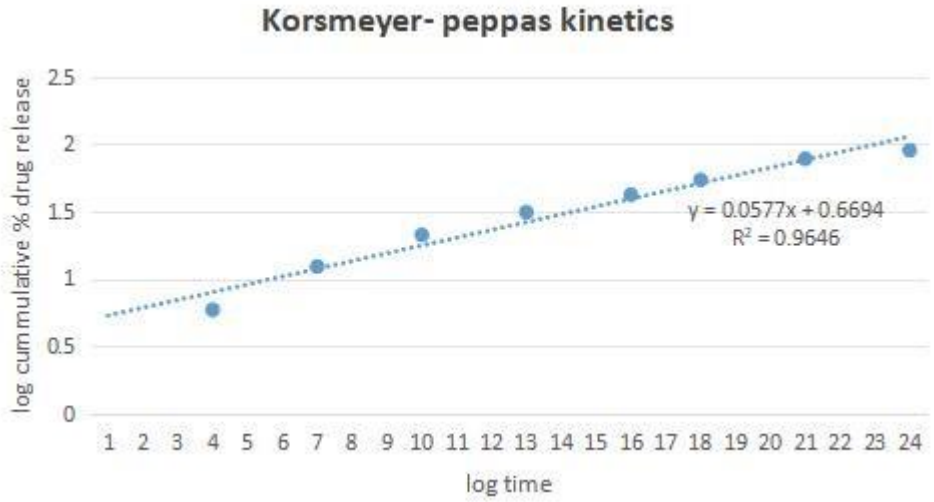


Figure No. 22.4: Korsmeyer- peppas kinetics of optimized celecoxib loaded cubosomes.

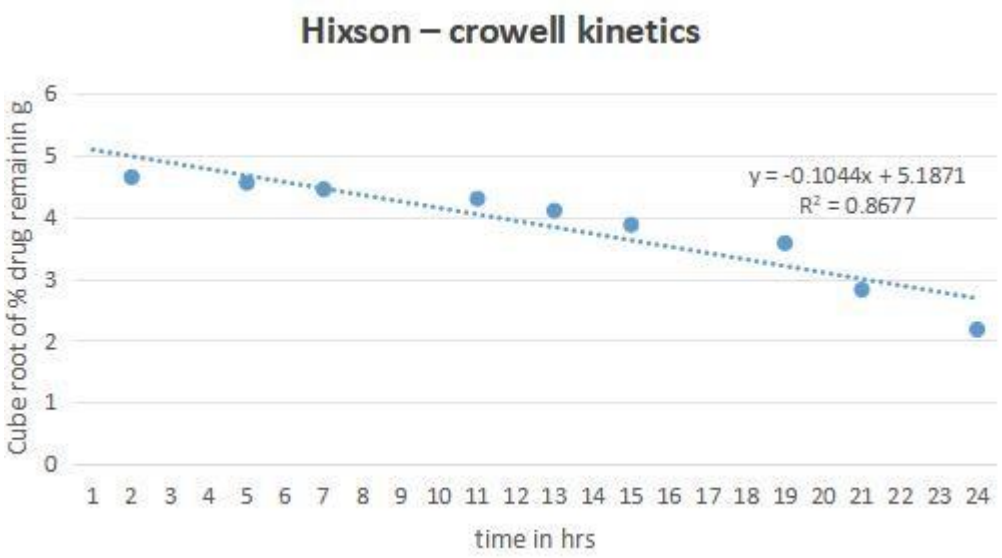


Figure No. 22.5: Hixson – crowell kinetics of optimized celecoxib loaded cubosomes.

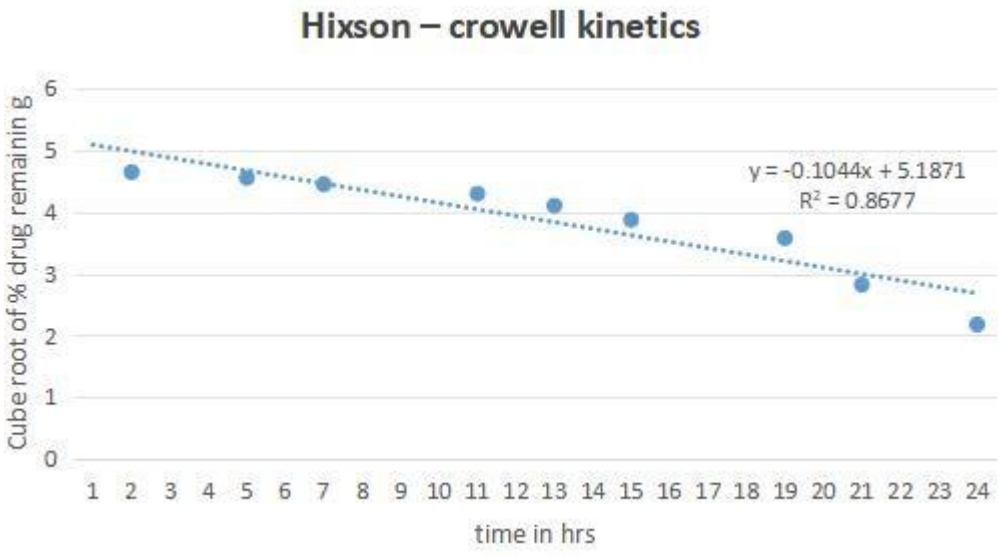
The coefficient of determination (R2) was taken as criteria for choosing the most appropriate model. The R 2 values of various models are given in table no: 23.1.

Table No. 23.1 kinetics Model and its R^2 value.

S.No	Kinetics models	Coefficient of determination (R^2) of optimized formulation
1	First order	0.8564
2	Zero order	0.9581
3	Higuchi	0.9659
4	Korsmeyer and peppas	0.9646
5	Hixson Crowell	0.8677

The data from *in vitro* release of optimized formulation was fit into various kinetic models.

Good linearity was observed with the Higuchi release kinetics (R2=0.9659). Hence the result indicating that the drug release was based on their change in surface area and diameter of the particles.



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The data from *in vitro* release of optimized formulation was fit into various kinetic models.

Good linearity was observed with the Higuchi release kinetics (R2=0.9659). Hence the result indicating that the drug release was based on their change in surface area and diameter of the particles.

E] STABILITY STUDY

PH, Drug content and drug release values are analyzed periodically as per ICH guidelines through accelerated stability studies for optimized gel formulation was shown in Table 24.

Table No. 24: Stability Study of Cubosomal Gel.

Time in days	PH	% Drug content	% Drug release
0	5.7	94.6	89.6
15	5.5	93.2	89.3
30	5.4	92.5	88.5
60	5.3	92.1	87.4

Inference

The results show No Significant Change in P H, Drug release and Drug Content of optimized formulation after two month.

11. SUMMARY AND CONCULSION

The propose of this research was Cubosomes can be prepared by simple combination of biologically compatible lipids (GMO) and water and are thus more suited for pharmaceutical and body tissue. The ability to form cubosomes during the preparation offers enhanced flexibility for product development. The above research specifies that the cubosomal utility as controlled release drug carrier. Prolonged release is achieved when they are formulated as topical gels on maintaining the cubosome structure. This product can be manufactured in large scale and commercialized for the treatment of arthritic patients, as it provides controlled delivery of the drug in human via the non- invasive skin route with more sustaining, less frequent dosing and with more bio-availability when compared to oral delivery.

- Top- down method was employed to produce cubosomal dispersion using GMO and POLOXAMER 407.
- The compatibility study of Celecoxib with excipients was carried out using FTIR Spectrometer. It revealed no interaction between the drug and excipients.
- Calibration curve was plotted for celecoxib and it was found that the solutions show linearity (0.999) and obeyed Beer Lambert's law.
- Formulation study design was developed by using Factorial Design (Design Expert, version 13, Stat Ease., Inc), the formulation design were predicted and statistically analyzed. The independent variables selected were GMO (A), POLOXAMER 407 (B) and Stirring speed (C) and the dependent variable chosen were Percentage Entrapment efficiency (R1) and Cumulative % drug release (R2). The formulations were optimized using Factorial design by comparing the predicted values with the observed values. The design predicted the values of optimized formulation which was then formulated and evaluated.
- The optimized Celecoxib loaded cubosomal dispersion was evaluated for FT-IR study and it is clearly evident that the optimized celecoxib loaded cubosomes showed the presence of characteristics bands of celecoxib. This indicates the absence of chemical interaction between the drug and the excipients.
- The entrapment efficiency of the optimized celecoxib loded cubosoml dispersion was determined and its entrapment efficiency was observed to be 88.2%.
- The UV-visible Spectrophotometric method was used to determine the drug content of optimized celecoxib loaded cubosomes. The drug content was found to be 94.5%.
- The optimized celecoxib loaded cubosomes was characterized for surface morphology, particle size analysis. The shape and surface morphology of optimized celecoxib loaded cubosome was observed in Scanning Electron Microscopy. It showed that the SLN was spherical and discrete in morphology. The particle size distribution and polydispersity study were carried out using particle size analyzer. The mean particle size of optimized celecoxib loaded cubosome was found to be 4 8 8. 2 1 nm. Polydispersity of optimized celecoxib loaded cubosome was found out to be 0.296, indicating uniformity of particle size within formulation.
- The optimized celecoxib loaded cubosome was further formulated as gel.

The optimized formulation was subjected to room temperature and 40ºc. The results shows no significant change in appearance, Ph, drug release and drug content of optimized formulation after two month.

12. FUTURE STUDIES

A] *In-vitro Anti-inflammatory Activity*[13]

The anti-inflammatory activity of cubosomal gel was

studied by using inhibition of albumin denaturation technique. The reaction mixture (5 ml) consisted of 4.5 ml of bovine serum albumin (5% aqueous solution) and 0.5 ml of cubosomal gel, pH was adjusted at 6.3 using a small amount of 1N Hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the sample, 2.5 ml of phosphate buffer solution was added into each test tube. Turbidity was measured spectrophotometrically at 252 nm. For control tests,0.5 ml of distilled water was used instead of cubosomal gel. The percentage inhibition of protein denaturation was calculated as follows.

Percentage inhibition=(Abs Control –Abs Sample) X 100/Abs control. B] In-vitro skin irritation test:[20]

HET-CAM (Hen's Egg Test on the Chorioallantoic Membrane) Test

Incubated eggs of 9 days were collected from hatchery, shells were removed carefully using forceps. Test sample is applied directly to the CAM. Allow the sample for exposure to the CAM for at-least 300 second. The end point is measured by the visual inspections.

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