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FORMULATION AND EVALUATION OF NANOCAPSULE OF BUDESONIDE FOR CROHN'S DISEASE

Pharmacy

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ABSTRACT

Nanocapsules can be likened to vesicular systems in which a drug is confined in a cavity consisting of an inner liquid core surrounded by a polymeric membrane.Nanocapsules show promise as active vectors due to their capacity to release drugs; their sub cellular size allows relatively higher intracellular uptake than other particulate systems.

The nanocapsule can be targeted to specific cells and locations within the body after intravenous and subcutaneous routes of injections. The structure of nanocapsule is a hybrid between polymeric nanocapsule and liposome because their oily core which is surrounded by a tension-active rigid membrane. The benefit of encapsulation method are of protection of these substances to protect in the adverse environment, for controlled release and for precision targeting.

Budesonide is also known active substance of corticoid series which is employed in particular for the treatment of bronchial disorders, but also in case of inflammatory bowel disorder such as Crohn's disease and, in particular ulcerative colitis. Budesonide is a glucocorticoid that has high affinity for the glucocorticoid receptor but low systemic activity due to extensive first-pass metabolism in liver. Its use in variety of steroid-responsive illnesses. Budesonide is an anti-inflammatory corticosteroid that exhibits potent glucocorticoid activity & weak minralocorticosteroids. Inflammation in asthma, Crohn's disease or ulcerative colitis is not known. Because budesonide undergoes significant first-pass elimination, the both oral preparation are formulated as an extended release tablet.

KEYWORDS

GENERAL INFORMATION

Nanocapsules can be likened to vesicular systems in which a drug is confined in a cavity consisting of an inner liquid core surrounded by a polymeric membrane. It can be defined as Nano-vesicular systems that exhibit a typical core-shell structure in which the drug is confined to a reservoir or within a cavity surrounded by a polymer membrane or coating. Nanocapsules, existing in miniscule size, range from 10 nm to 1000 nm. Nanocapsule show promise as active vectors due to their capacity to release drugs; their sub cellular size allows relatively higher intracellular uptake than other particulate systems.

The nanocapsule can be targeted to specific cells and locations within the body after intravenous and subcutaneous routes of injections. Nanocapsules comprise of an oily or an aqueous core, which is surrounded by a thin polymer membrane. The nanocapsule core may be aqueous or composed of lipophilic solvent usually oil. The structure of nanocapsule is a hybrid between polymeric nanocapsule and liposome because their oily core which is surrounded by a tension-active rigid membrane. The benefit of encapsulation method are of protection of these substances to protect in the adverse environment , for controlled release and for precision targeting.

COMPONENT OF NANOCAPSULE:

- Lipids
- Oils
- Surfactant
- Water

METHOD OF PREPARATION OF NANOCAPSULE:

- Emulsification-diffusion
- Nano-precipitation
- Emulsification-conservation
- Layer by layer
- Phase inversion
- Solvent evaporation
- Double emulsification

CONTROL RELEASE DRUG DELIVERY

Controlled release dosage forms cover a wide range of prolong action which provide continous release of their ingredient at a predetermined time. The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration i.e. the drug delivery system should deliver drug at a rate indicated by the needs of the body over a specified period of treatment. Two aspects of drug delivery spatial placement and temporal delivery of a drug. Spatial placement relates to the targeting a drug to a specific organ or tissue, while temporal deliver refers to controlling the rate of drug delivery to the target tissue. It is one which immediately attains desired therapeutics concentration and maintains it constant for the entire duration of treatment.

ADVANTAGE OF CONTROL RELEASE:

- Optimal use of drug and improve the patient compliance.
- Controlled rate and site of release.
- Reduced dose frequency.
- Improved patient compliance.
- The maintenance of drug level within a desired range.
- Better drug utilization.
- Decrease toxicity.
- More consistent and prolonged therapeutic effect.

DISADVANTAGE OF CONTROL RELEASE:

- Stability problem.
- Undesirable by product of degradation.
- The chance of patient discomfort from the delivery device for instance if any surgery required to implant or remove the system.
- Higher cost of controlled release system compared with traditional pharmaceutical formulation.
- Toxicity due to dose dumping.
- More rapid development of tolerance.
- Increased Cost.

INFLAMMATORY BOWEL DISEASE (IBD)

IBD is a group of inflammatory conditions of the colon and small intestine.IBD is class of auto immune disease, in which the body's own immune system attacks the elements of the digestive system.

Two major types of inflammatory bowel diseases are ulcerative colitis and crohn's diseases.

- **Ulcerative colitis:** it involves only the colon starting from the anal canal. It can remain restricted to the rectum or extended proximally in a contiguous manner to variable extent upto caecum. The lession are mucosal and may be diffuse or confluent.The first definitive description of this condition was made in **1909** and in certain aspects it resembles Crohn's diseases
- **Crohn's disease:** in crohn's diseases lesions are patchy and transmural ;may involve any part of the g.i.t from mouth to the anus .majority of patient have ileocaecal diseases upto ascending colon , but in some it may be restricted to the small intestine . Because the lesions are transmuara, complication like perforation, abscess, fistula,strictures ,etc .

Crohn's disease is less amenable to medical therapy than ulcerative colitis. Crohn's disease, also known as Crohn's syndrome and regional enteritis. It may affect any part of the gastrointestinal tract from mouth to anus.

Crohn's disease is a chronic, or long lasting, disease that causes inflammation, irritation or swelling in the gastrointestinal tract (GIT).

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Fig1.4 : Inflammation in colon

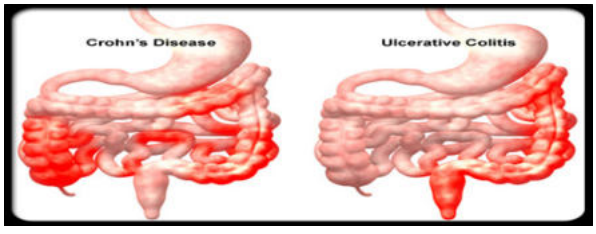


Fig:Different location of crohn's disease and ulcerative colitis

SYMPTOMS OF INFLAMMATORY BOWEL DISEASES:

Diarrhoea,
Abdominal pain and cramping
Mouth sores
Fever and fatigue
Blood in your stool
Reduced appetite and weight loss

TREATMENT AND DRUG USE IN IBD:

Anti-inflammatory drug

- 5-amino salicylic acid (5-ASA)compound
- Corticosteroid
- Immunosuppressant

Sulfasalazine:These drug may be help in crohn's disease affect the colon, but they aren't helpful in treating in the small intestine. They include sulfasalazine which contain sulfa , and mesalmine .the beneficial effect of sulfasalazine is clearly not due to any antibacterial action.

The sulfapyridine emoiety only serve to carry 5-ASA to the colon without being absorbed proximally. Most of the adverse effect like nausea, fever, joint pain, headache, malaise and anaemia etc .

Higher dose of mesalmine may induce remission in mild cases of crohn's colitis as well,but efficacy is uncertain. It is not useful in maintaining remission in crohn's disease.

Corticosteroid: Corticosteroid such as prednisolone can help in reduces inflammation anywhere in your body, but they have numerous side effects, which include a puffy face, excessive facial hair, hyperactivity .more serious side effects include diabetes, cataracts, and glaucoma. Deflazacort work by reducing inflammation in the colon. Doctor's generally use them only if you don't respond to other treatment.

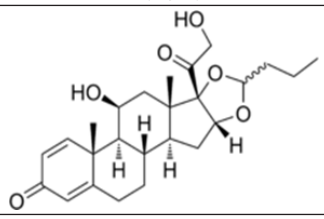
Immunosuppresant: They are use for long term management of IBD. For eg. Azathioprine, this purine metabolite is the most effective use immunosuppressant in IBD. Some patient experience bone marrow toxicity of azathoprine.

DRUG: BUDESONIDE

Budesonide is also known active substance of corticoid series which is employed in particular for the treatment of bronchial disorders, but also in case of inflammatory bowel disorder such as Crohn's disease and, in particular ulcerative colitis.

Budesonide is a glucocorticoid that has high affinity for the glucocorticoid receptor but low systemic activity due to extensive first-pass metabolism in liver. Its use in variety of steroid-responsive illnesses.

DRUG PROFILE:

Drug	Budesonide
State	Solid
Formula	$C_{25}H_{34}O_6$
Structure	
Category	Corticosteroid
Indication	The oral tablet is used for the treatment of mild to moderate active crohn's disease. The oral tablet is used for induction of remission in patients with active mild to moderate ulcerative colitis.
Molecular weight	430.53 gm/mole
IUPAC Name	16,17-(butylidenebis(oxy))-11,21-dihydroxy-(11-B,16-a)-pregna-1,4-dine3,20-dine
Mechanism	Budesonide is an anti-inflammatory corticosteroid that exhibits potent gulcocorticoid activity & weak minralocorticosteroids. Inflammation in asthma, crohn's disease or ulcerative colitis is not known. Because budesonide undergoes significant first-pass elimination, the both oral preparation are formulated as an extended release tablet.
C_{max}	13.3 ± 5.9 hours
T_{max}	1.35 ± 0.96 ng/ml
Route of Administration	Intravenous, Oral
Bioavailability	10-20%
Protein binding	85-90%
Dose	Capsule oral-3mg Tablet -9mg
Route of elimination	Excreted in urine & feces in the form of metabolites.
Half life	2.0-3.6 hrs
Melting point	226^{0}C
Log P	1.9
PKa	Acidic -13.74 basic- - 2.9
Water solubility	Insoluble

INTRODUCTION TO EXCIPIENTS:

- **Polysorbate 80:**
- **Synonyms :** Armotan PMO 20, Capmul POE-O, Cremophore PS 80, Glycosperse O-20, Liposorb O-20, Liposorb O-20K, Montanox 80, polyxyethylene 20 oleate, Tego SMO 80, Tego SMO 80V,Tween 80
- **Chemical Name:** Polyoxy ethylene 20 sorbitan monooleate
- **Emperical Formula:** $C_{64}H_{124}O_{26}$
- **CAS registry No:** 9005-65-6
- **Molecular Weight:** 1310 g/mol

Structural formula:

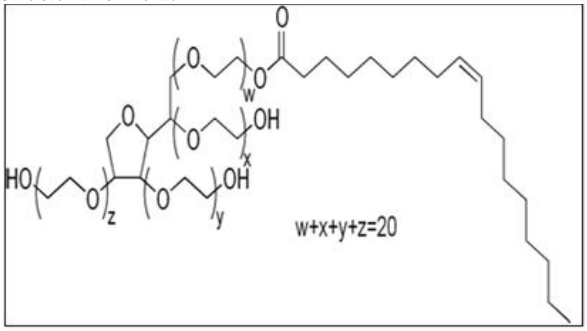
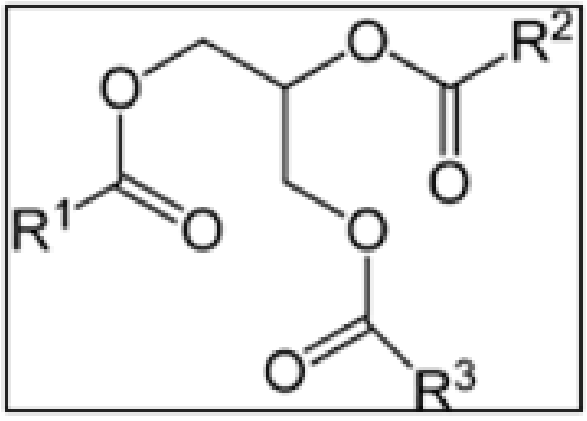


Figure: Structure of polyoxyethylene sorbitan trimester

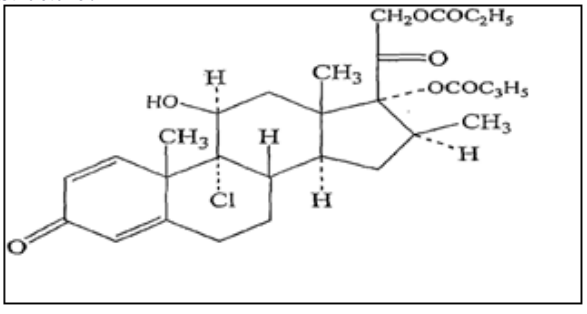
- **Functional Category:** Emollient, dispersing agent, emulsifying agent, nonionic surfactant, plasticizing agent, solubilizing agent, suspending agent.
- **Description:** It is a yellow oily liquid characteristic odour.
- **Typical properties:**

Acid value: 2
HLB value: 15
Moisture content: 3
Saponification value: 45-55
Specific gravity: 1.08
Solubility: Soluble in water and ethanol while insoluble in mineral oil and vegetable oil.

Olive Oil
Structure:

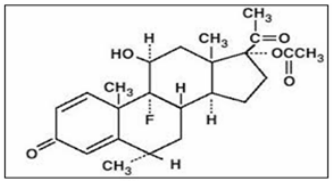


Formula: $CH_3(CH)CH{=}CH(CH_2)_7COOH$
Total saturated fat: Palmitic acid -13.0%
Stearic acid -1.5%
Unsaturated fat: 85%
Melting point: -6.0 (21.2°f)
Boiling point: 300°C
Viscosity: 84 Cp
Saponification: 184-196
CAS registry No: 8001-25-0
Functional category: Cosmetic, pharmaceutical, soaps
Lipoid S 75
Structure :

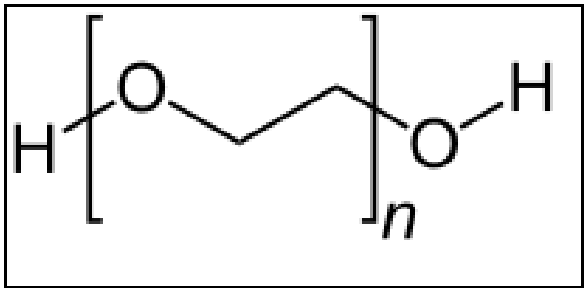


Soya bean phospholipids with 70% phosphotidycholine

Storage: -20+/-5°C
Iodine value: 85-95
Consistency: Coarse agglomerates
Colour: Yellow brownish
Melting point: 75°C
CAS registry No: 8030-76-0
Molecular weight: 800 g/mol
Storage: Keep in well closed in cold area
Sodium Chloride:
Structure:



Formula: NacL
Molar mass: 58.44 $gmol^{-1}$
Appearance: Colorless crystals
Odor: odorless
Density: 2.165 g/cm^3
Melting point: 801°C
Solubility in water: 354 g/L
CAS registry No: 7440-23-5
Functional category: Salinity, preservative
Storage: Strore in well closed container
PEG 600:
Structure:



Molecular formula: $C_{2n}H_{4n+2}O_{n+1}$
Molecular weight: 570-630
Density: 1.1258 g/ml
Melting point: 15-25
Viscosity: 10.8cP
CAS registry No: 25322-68-3
Functional category: Non-ionic surfactant, emulsifying agent.

MATERIALS USED IN PRESENT WORK:

Table: Materials used in Present Work

Category	Materials & reagents	Supplier
Drug	Budesonide	Sun Pharma Pvt. Ltd.
Lipids	Cholesterol	SD fine, Ltd
	Lecithin 100	Sigma Aldrich Chemical. Pvt. Ltd
	Lipoid S 75	Lipoid, Germany
Surfactants	Tween 80	SRL
	PEG 600	SD fine. Ltd
	Cremophore RH 40	Sigma Aldrich Chemical. Pvt. Ltd
Oils	Olive oil	SD fine. Ltd
	Ethyl oleate	SD fine. Ltd
	Oleic acid	SRL
	Castor oil	SRL
Solvents	Ethanol	SD fine. Ltd
	Methanol	SD fine. Ltd
Non-solvent	Sodium chloride	Fisher
	Water	Water supply specialist Pvt .Ltd, , India

EQUIPMENT'S USED IN PRESENT WORK:

Table: Equipments used in Present Work

Equipments	Manufacturer
Digital weight balance	Scaletec Mechatronic Pvt.Ltd.
Magnetic stirrer	Remi Service Pvt. Ltd.
Mechanical stirrer	Remi Service Pvt. Ltd
Lyophillizer	Allied Frost, New Delhi
Malvern zeta seizer	Nano ZS-90 Malvern Instrument UK
U.V Visible spectroscopy	Shimadzu 1800, Japan
Scanning electron microscope	FEI SEM
Fourier Transform infrared spectrophotometer	Bruker Alpha,Germany
Dissolution test apparatus	LAMBINDIA DS 8000
Stability chamber	Remi Instrument Ltd,Mumbai

SUMMARY OF EXPERIMENTAL WORK:

1) Preformulation study

a) Solubility study
b) Melting point determination
c) FTIR spectra of Budesonide
d) Compatibility study by FTIR

2) Calibration curve development

3) Preparation of Lipid nanocapsule
a) Screening of Lipid
b) Screening of Oil
c) Screening of Surfactant
d) Factorial design
e) Check point batch preparation

4) Characterization & evaluation of optimized Lipid nanocapsule
a) Particle size & Polydispersity index
b) Zeta potential
c) Encapsulation efficiency
d) Drug content
e) Scanning electron microscopy

5) In vitro study

6) Stability study

PRE-FORMULATION STUDY: IDENTIFICATION OF DRUG:
Organoleptic Property: As per Indian pharmacopeia 2014 drug was White color, Odorless

Solubility Study: 10 mg drug was taken in the test tube and solvent was added gradually in volume of 10 ml with continuous shaking until it dissolves completely. Solubility of drug was checked in water, ethanol, methanol and acetone. Solubility was calculated as per descriptive term given in Indian Pharmacopeia 2010.

Table: Solubility study

Sr. No.	Descriptive terms	Parts of solvent required for part of solute (gm/ml)
1.	Very soluble	Less than 1
2.	Freely soluble	From 1 to 10
3.	Soluble	From 10 to 30
4.	Sparingly soluble	From 30 to 100
5.	Slightly soluble	From 100 to 1000
6.	Very slightly soluble	From 1000 to 10,000
7.	Practically insoluble	10,000 or more

MELTING POINT DETERMINATION:
Determination of melting point by open capillary method.. Powdered drug (Budesonide) was put into the one side open end of thin capillary of about 5 cm length with uniform diameter (1mm width), by tapping gently. Capillary was then placed into the orifice of the melting point apparatus (Veego Model: VMP-D) . The temperature at which the substance started to covert liquid and temperature at which the solid disappeared and completely into liquid was noted from the digital display as melting point range of drug.

IDENTIFICATION BY FTIR:
The FTIR of pure drug, Lipids, Surfactant, Oils (Lipoid S 75, Tween 80, Olive oil) and drug with each ingredient were performed using Fourier Transform Infrared Spectrophotometer (Bruker, ALPHA-T). The amount of each formulation ingredient in the physical mixture was same as that in the optimized batch. The pure drug and physical mixture were then separately mixed with IR and KBr. This mixture was then scanned over a wave number range of 4000 to 400 cm^{-1}.

ESTIMATION OF BUDESONIDE BY UV-VISIBLE SPECTROSCOPY:
Budesonide was estimated by UV-Visible spectroscopic method using phosphate buffer pH 6.8 and 0.1 N Hcl.

PREPARATION OF PHOSPHATE BUFFER PH 6.8:
The ingredient mentioned in Table were weighed accurately and dissolved in 1000 ml of distilled water and pH was adjusted to 6.8.

Table: Formula for phosphate buffer pH 6.8

Ingredient	Quantity
Di sodium hydrogen phosphate	28.8 g
Potassium hydrogen phosphate	11.45 g
Distilled water	1000 ml

DETERMINATION OF λMAX BY UV ABSORBANCE:
25 mg Budesonide was dissolved in 25 ml of methanol and made up the volume with Phosphate buffer pH 6.8 to form 100 µ/ml of stock solution. From stock solution, 5ml of aliquot was taken and diluted up to 100 ml with Phosphate Buffer pH 6.8 to form solution of 5 µg/ml. The final solution was scanned for absorbed between 200-400 nm using UV-Visible spectrophotometer.

Preparation of calibration curve of Budesonide in phosphate buffer pH 6.8
Stock solution was prepared by dissolving 25 mg drug in 25 ml methanol water (1000 µg/ml). From that solution, 2.5 ml was withdrawn and diluted with distill water up to 25 ml (100 µg/ml).From that solution 20 ml was withdrawn and diluted with 100 ml 6.8 buffer. From the above solution(20µg/ml), 2,4,6,8, and 10 ml of the solution was withdrawn and diluted up to 10 ml with distill water to get 4, 8, 12, 16 and 20 µg/ml of solutions. Using UV-Visible Spectrophotometer, the absorbance of each solution was measured at 246 nm against Phosphate buffer pH 6.8 as blank. The results are shown in table.

PREPARATION OF 0 .1 N HCL:
The ingredient mentioned in Table 4.5 were accurately dissolved in 1000 ml distilled water.

Table Formula for 0.1 N HCL

Ingredient	Quantity
HCL	8.5 ml
Distilled water	1000 ml

Preparation of calibration curve of Budesonide in 0.1N HCL
Stock solution was prepared by dissolving 25 mg drug in 25 ml methanol water (1000 µg/ml).From that solution, 5 ml was withdrawn and diluted with 0.1 N HCl water up to 50 ml (100 µg/ml). From that solution 25 ml was withdrawn and diluted with 100 ml 0.1N HCl. From the above solution (25µg/ml), 2, 4, 6, 8, and 10 ml of the solution was withdrawn and diluted up to 10 ml with 0.1 N HCl to get 4, 8, 12, 16 and 20 µg/ml of solutions. Using UV-Visible Spectrophotometer, the absorbance of each solution was measured at 246 nm against 0.1 N HCl as blank. The results are shown in table.

- **SELECTION OF EXCIPIENTS**
- **SCREENING OF LIPIDS**

Formulation is prepared by taking different lipids, Surfactant and Oil their different concentration. Then making a trial batch of this and evaluate it. Then optimize the formulation by particle size and PDI. This is obtained desired particle size and entrapment efficiency.

- **SCREENING OF OILS**

Formulation is prepared by solubility of drug in Oils. take 10 mg drug and dissolve in 10 ml oil and put in the orbital shaker for 24 hrs after that take absorbance by UV method highly soluble oil is optimized.

- **SCREENING OF SURFACTANT**

Formulation is prepared by taking different surfactant and their different concentration .Then making a trial batch of this and evaluate it. Then optimize the surfactant. This is obtained desired particle size and entrapment efficiency.

- **DRUG - EXCIPIENTS COMPATIBILITY STUDY**

The FTIR of drug, Lipid with drug, Surfactant with drug, Oil with drug, and physical mixture of drug with Lipid, Surfactant, Oil were performed using Fourier Transform Infrared Spectrophotometer (Bruker, ALPHA-T). The drug and physical mixture were separately mixed with IR grade KBr. Then these mixture were scanned at transmission mode in region of 4000 – 400 cm^{-1}.

- **METHOD OF PREPARATION OF LIPID NANOCAPSULE**

PHASE INVERSION METHOD:
The lipid nanocapsule were prepared by the phase inversion method .They were composed of a liquid oily core surrounded by a cohesive interface and dispersed in a aqueous medium. The oil phase composed of oil and lipid. The aqueous phase consists of 2.5 % w/w Nacl and Surfactant. The emulsion is first prepared by mixture of oil and drug. (Step 1)Drug was dissolved into the organic solvent. Then evaporate the solvent by magnetic stirrer. Then add lipid phase in that solvent and temperature raised upto 85°C. Then aqueous phase which was mixture of Nacl and Surfactant were added in to the Oil phase. Three temperature cycles were applied to obtained the inversion process. (85-60-85-60-85°C) cycles were applied. (Step 2) An irreversible shocked is then induced by dilution with cold deionized water (2°C) added to the mixture leading to the formation of stable nanocapsules. Then dry it with Lyophillizer.

OPTIMIZATION OF FORMULATION
OPTIMIZATION BY BOX – BEHNKEN DESIGN:

The use of trial and error for the development of new pharmaceutical formulation leads to a satisfactory formulation. The optimization done on the basis of experimental and statistical analysis of the result can provide and efficient for the prediction of the optimal composition. Design Expert 9.0.4 Software was used for determining influence of the factors on selected response.

To study the combination of all factors at all levels, a three factors, two levels factorial design was constructed in a fully randomized order. Three independent variable, Surfactant (X1), Lipids (X2) , Oil (X3) were set as different levels. Low, Medium and High levels of each variable were coded as -1, 0 , and +1 respectively. The dependent variable measured were particle size (Y1), entrapment efficiency (Y2). Regression analysis was used to find out the control factors that significant affect response variables.

The result of ANOVA and multiple regression analysis that led to describing the effect of independent variables on the selected responses. The levels of significant selected was 5 % ($P<0.05$). Counter plot and response surface plot were conducted. By the overlay plot best optimized formulation was found out on the desired response.

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 \quad \text{.....4.1}$$

Table Factorial Design			
Independent variable	Levels		
	Low (-1)	Medium(0)	High(+1)
X1= Concentration of Surfactant (ml)	0.5	1	1.5
X2= Concentration of Lipids (gm)	1	1.5	2
X3= Concentration of Oils (ml)	1.5	2	2.5
Dependent variable	Constrain		
Y1= Particle size	Minimize		
Y2= Entrapment efficiency	Maximize		

Table: Different batches of Lipid nanocapsule preparation as per factorial design

No. of Runs	Coded value			Uncoded value		
	X1	X2	X3	X1	X2	X3
1	-1	-1	0	0.5	1	2
2	+1	-1	0	1.5	2	2
3	-1	+1	0	0.5	1	2
4	+1	+1	0	1.5	2	2
5	-1	0	-1	0.5	1.5	1.5
6	+1	0	-1	1.5	1.5	1.5
7	-1	0	+1	0.5	1.5	2.5
8	+1	0	+1	1.5	1.5	2.5
9	0	-1	-1	1	1	1.5
10	0	+1	-1	1	1	1.5
11	0	-1	+1	1	2	2.5
12	0	+1	+1	1	2	2.5
13	0	0	0	1	1	2
14	0	0	0	1	1	2
15	0	0	0	1	1	2
16	0	0	0	1	1	2
17	0	0	0	1	1	2

CHECK POINT BATCH PREPARATION:

Check point batch was prepared and evaluated after optimization study to evaluate the model validity and reliability. The method of preparation of formulation was same as mentioned in the optimization.

EVALUATION OF LIPID NANOCAPSULES:

PARTICLE SIZE, POLYDISPERSITY INDEX:

The mean size and polydispersityindex of size distribution of lipid nanocapsule was determined by photon correlation spectroscopy using Zetasizer NanoZS90 (Malvern Instrument).Each sample was diluted with distilled water. The result shown in Figure 5.23

ZETA POTENTIAL:

Zeta potential was determined by using Zetasizer. Clear disposable zeta cell cuvette was used for determining zeta potential. The cuvette was filled with using micro pipette. 20 zeta runs made for each sample and temperature was maintained at 25°C. The results are shown in Figure 5.24.

% ENTRAPMENT EFFICIENCY:

% EE was calculated by determined the amount of nanoencapsulated Budesonide in the aqueous surfactant solution. The aqueous medium was separated by using the centrifuge. Volume of 1.5 ml of the Lipid nanocapsule of Budesonide was placed in tube and speed of centrifuge was kept 15,000 rpm for 1 hr. The concentration of Budesonide in the aqueous phase was determined using UV-visible spectrophotometer at max 246nm.

% EE was calculated using following equation:

$$\% EE = \frac{\text{Drug content in nanocapsule}}{\text{Total content of used drug}} \times 100$$

DRUG CONTENT:

The formulation containing 100 mg equivalent quantity of drug was taken in 100 ml volumetric flask, dissolved in PBS pH 6.8 and volume was made up to 100 ml with PBS pH 6.8 and then it was filtered. The absorbance values were measured with suitable dilutions at 246 nm using UV – Visible spectrophotometer in triplicate. The concentrations of drug were calculated from standard calibration curve prepared in PBS pH 6.8.

SCANNING ELECTRON MICROSCOPY:

The morphological characteristics of lipid nanocapsules were determined by using a scanning electron Microscopy (SEM). The surface morphology and appearance of the particles were observed at X2700 magnification and an accelerating voltage of 15kV.

IN-VITRO RELEASE STUDY:

The releases of Budesonide from lipid nanocapsules were performed in 0.1 N HCL and phosphate buffer pH 6.8 using dialysis bag method. Dialysis membrane having pore 2.4 nm and molecular weight 14000 (Dialysis membrane 150, Hi Media, Mumbai, India) was used. The dialysis bag retains lipid nanocapsules and allows the free drug into dissolution media. The bag was soaked in buffer solution 24 hours before use. In vitro diffusion study was carried out by USP type II apparatus using dialysis bag method. 2 ml of lipid nanocapsules was placed in dialysis bag and sealed at both ends. In that 900 ml 0.1 N HCL for 2 hrs and phosphate buffer 6.8 for 8 hrs with 100 rpm at 37± 2°C. Samples were withdrawn with predetermine time intervals and maintained the sink condition by replacing fresh dissolution medium. The content of Budesonide in the sample was determined UV spectrophotometer at λ_{max} 246nm. (1800, Shimadzu, Japan)

RELEASE KINETIC OF LIPID NANOCAPSULES:

To study the release kinetics of Budesonide from lipid nanocapsules, the releasedata were fitted to following equation:

- **Zero order kinetics:**

$f_t = K_0 t$

Where, f_t= fraction of dose released at time t,

K_0= zero order release rate constant

- **First order kinetics:**

$\ln Q_t = \ln Q_0 + K_t$

Where, Q_t = amount of drug remaining to be released at time t,

Q_0= amount of drug remaining to be released at zero hour,

K_t = first order release rate constant

- **Higuchi's model:**

$Q_t = K_H t^{1/2}$

Where K_H = Higuchi release rate constant.

- **Hixson – Crowell model:**

$W_0^{1/3} - W_t^{1/3} = K_S t$

Where, W_0= initial amount of drug present in matrix ,

W_t = amount of drug released at time t

K_S= Hixon – Crowell release rate constant

- **Korsmeyer Peppas model:**

$M_t / M_\infty = Kt^n$

Where, M_t= amount of drug release at time t,

M_∞= amount of drug released at infinite time

K = Korsmeyer- Peppas' release rate costant

n = release exponents

The type of drug transport mechanism		
Diffusional Exponent, n	**Type of transport (release)**	**Time dependent**

n = 0.5	Fickian diffusion	$t^{½}$
n = > 0.5 - < 0. 1	Anomalous transport	t^{n-1}
n = >1.0	Case II transport	time dependent
n >>1.0	Super case II transport	t^{n-1}

EX-VIVO PERMEATION STUDY:

Ex-vivo study was conducted using isolated chick intestine. 10 cm length of intestine was cut and washed by syringe and empty the residual content of intestine. One end of intestine tight with thread. From other open end filled with drug and 0.1 N HCl. Similarly the other intestine filled with optimized formulation with 0.1 N HCl. Both was placed in 50 ml beaker separately containing 50 ml SGF place in magnetic stirrer and 100 rpm. The amount of drug diffuse was find out by withdraw 2 ml sample by 2 hour interval.

STABILITY STUDY:

The optimized Budesonide Lipid nanocapsule formulation was subjected to one month short term stability testing. Optimized Budesonide Lipid nanocapsule formulation was stored in sealed glass vials and placed in a stability chamber for period of 1 month. The stability study of Lipid nanocapsule was carried at 40° C/75% RH, 25°C/60%, RH and 4° as per ICH guideline.

SUMMARY AND CONCLUSION:

Budesonide is a glucocorticoid effective in treatment of Crohn's disease and Ulcerative colitis. The aim of present study was to formulation and evaluation of Nanocapsules of Budesonide for Crohn's disease. Solubility of drug was in different solvent, different lipids and different oils were taken as selection criteria for Lipid nanocapsules. UV spectroscopy method used for spectral analysis of drug and Calibration curve plotted shown linearity of the curve. Melting point was determined by using melting point determination apparatus.

Olive oil gives highest solubility in drug 5 ml oil taken in the test tube and 5 mg drug was added in that oil. Then kept it in orbital shaker for 24 hours and then measured the concentration by UV spectroscopy. It gives highest solubility of drug so Olive oil was selected. Lipoid S 75 (70% Phosphotidylcholin) is highly soluble and selected on the basis of Particle size and PDI so it was selected. Tween 80 gives nanometer size range and highest entrapment efficiency so it was selected. Selected ingredients were taken and Lipid nanocapsules prepared by Phase-Inversion method.

IR spectra was drug recorded and functional group was interpreted by structure and was found appropriate structure of drug. IR spectra of drug and Lipid, Oil, Surfactant were taken and compatibility study was done.

Total 17 batches were prepared using Box-Behnken design with three independent factor of Lipid, Oil, Surfactant were taken. It was evaluated by Particle size and %Entrapment efficiency and Zeta potential, Drug content, In-vitro release were carried out for optimized batch. All the Lipid nanocapsules shows the optimum particle size in range of 20 – 150. The Zeta potential shows that formulation was stable and entrapment efficiency was 78.62± 0.43. SEM images shows that particles are in round spherical shape and smooth surface and in nanometer size range. In vitro release gives the sustain release profile and kinetic study was done. In in-vitro release the zero order release follow so it gives sustain release result.Ex-vivo permeation study was done by the comparison between the pure drug and the optimized formulation. Optimized formulation gives greater release as compared to pure drug.The stability study of Optimized batch was performed at 4°C, 25°C, 40°C for 1 month.

CONCLUSION:

The Lipid nanocapsules made from Lipidic layer and drug was incorporated in inner core of the lipid.Screening of Lipids, Oils and Surfactant were helpful for the selection of Excipients. The lipid nanocapsule prepared by the Phase-inversion method and when it prepare it converted into the dry powder by Lyophillizer. Formulation of Lipid nanocapsule were optimized by Box-Behnken design. The Optimized batch F3 gives the 83.1 nm size and 0.231 PDI. And gives 78.62 % entrapment efficiency. The optimized batch F3 having small Particle size and high entrapment efficiency. Particle size, PDI, Zeta potential, %Entrapment efficiency were the important property of stable Lipid nanocapsules.

Ex-Vivo study done by the comparison between the lipid nanocapsule and pure drug. It increases the permeation. It shows the greater release as compared to pure drug. Thus the formulation of Lipid nanocapsules shows the better for oral drug delivery.

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