DEVELOPMENT AND EVALUATION OF ALCOHOL RESISTANT FORMULATION BY USING DIFFERENT POLYMERS TO AVOID DOSE DUMPING

Lalit Garg*[1], Dr. Amul Mishra[2]

[1] Faculty of Pharmacy, BN Institute of Pharmaceutical Sciences, BN University, Udaipur, Rajasthan, INDIA.

[2]Associate Professor & Head of Pharmaceutics, BN Institute of Pharmaceutical Sciences, BN University, Udaipur, Rajasthan, INDIA.

***Corresponding author:** Lalit Garg

Abstract

The objective of the proposed research work is to develop a novel technology for alcohol resistant modified release (MR) formulation, to overcome the problems of fast dissolution of MR formulation when taken with alcohol. To maintain the desired retention time of MR formulation in body irrespective of in-take fluid with which drug is administered and implementation of full factorial design to optimize the formulation.

Preformulation studies which include various identification tests like organoleptic properties, solubility analysis, melting point determination, Evaluation of different grades polymers like compatibility studies with the selected model drugs. Formulation design and optimization without impacting the therapeutic efficacy of drug product.

Dose dumping is a phenomenon of drug metabolism in which environmental factors can cause the premature and exaggerated release of a drug. This can greatly increase the concentration of a drug in the body and thereby produce adverse effects or even drug-induced toxicity.

In the present study Alcohol Dose Dumping resistant formulations were designed and evaluated. Dimethyl fumarate was selected as model drug because of its early dissolution in 40% Ethanol. The purpose of study is to prevent dissolution of Dimethyl fumarate in 40% Alcohol.

Out of 3 grades (LFR 5/60, CR8133, and CR8223) selected for coating of Dimethyl fumarate mini-tablets, LFR 5/60 found best among all 3 grades. The % Drug Release in 40% Ethanol of LFR5/60 Coated Mini-Tablets was found to be 4.834%.

For optimization of LFR 5/60 as coating material, 32 full factorial designs were employed using % weight gain and concentration of PEG-6000 (independent variables). The percentage drug release in-vitro in 40% Alcohol was selected as dependent variables and the best formulation was selected by the design expert software version 11. Optimized formulation with 10% weight gain and PEG-6000 concentration 5.93% was found to be best formulation. Moreover, design expert software also suggests that best % weight gain is 12.717% and PEG-6000 concentration is 4.9735.

From the above results it can be stated that additional coating of Sod. Alginate will be helpful in preventing ADD in 40% Alcohol. The newly formulated mini-tablets have better alcohol resistive activity than the branded Innovator.

Keywords: Immediate release min-tablets, Extended-release tablet, enteric coating, alcohol resistant coating.

1. INTRODUCTION

According to the CDC, most adults of the U.S. consume alcohol, whether in moderate or large quantities and also take medications, at least occasionally. As a result, many of them ingest a medication while alcohol is present in their body or vice versa. A large number of medications—both those available only by prescription and those available over the counter (OTC)—have the potential to interact with alcohol. Those interactions can alter the metabolism or activity of the medication and alcohol metabolism, resulting in potentially serious medical consequences.

Dose dumping refers to the rapid release of the entire dose or a significant fraction thereof in a short period. Depending upon the therapeutic index, the pharmacokinetics, and the therapeutic indication of the API, critical side effects or even fatality can result. Dose dumping resulting from consuming alcoholic beverages with timely connection the administration of medication referred to as "alcohol-induced dose dumping" (ADD).[1]

In comparison to an immediate release (IR) dosage form, of a drug as an oral controlled release (CR), modified-release (MR) or extended-release (ER) product is a simplified approach to ensuring convenience of dosing and sustained therapeutic blood levels over a prolonged time interval (12–24 h). Thus, errors in dosing compliance by the patient or breakthrough pain in the case of pain medications commonly observed with multiple daily dosing (i.e. every 4–6 h) of an IR dosage form, can be easily mitigated by ingestion of a single tablet or capsule formulated as a CR, MR or ER product. These advantages have led to a large number of drugs being formulated and marketed as MR dosage forms. In contrast to an IR tablet or capsule, MR dosage forms contain more significant amounts of active pharmaceutical ingredient and different excipients that allow the drug to release in a slow modulated fashion. As MR medication remains for a longer period in the body, so the chances of ADD are high as a patient should not consume alcoholic beverages during this period. The general time of alcohol absorption is at least 30 minutes, so no alcoholic beverages should be consumed before administration of drug. [2]

Alcohol consumption during the presence of medication in the stomach can cause Alcohol-Drug interaction which leads to various side effects like Nausea, Vomiting, Headaches, Drowsiness, Dizziness, Fainting, Changes in blood pressure, Abnormal behavior, Loss of coordination, also various serious complications like Liver damage, Heart problems, Internal bleeding, Impaired breathing, Depression. The ADD requirements of the European Medical Agency (EMA) and the Food and Drug Administration (FDA) are not fully harmonized and sometimes even conflicting. The table below shows some differences between them for three major topics.

Topic	FDA	EMA
Methodological requirements	Dissolution medium: 0.1N HCl Alcohol concentrations: 0%, 5%, 20%, and 40% Time: every 15 minutes until 2hrs	Dissolution medium: same as that proposed for routine testing Alcohol concentrations: 5%, 10%, and 20% Time: not defined
Products to be tested	At least all (generic) versions for modified-release opioid drug products; more preferably for (all) modified-release drug products with risk of alcohol-induced dose dumping	All oral modified-release applications
Acceptance criterion	Generic drug formulation should show rugged performance in alcohol If a generic drug formulation releases more rapidly in alcohol, the rate should be comparable to that of reference product.	If *in-vitro* alcohol incompatibility of the drug product is demonstrated, product should be reformulated. If alcohol effect cannot be avoided and is present also in the reference product, applicant should justify or demonstrate that it lacks clinical relevance.

Fig.1. Comparison of FDA and EMA requirements with regards to in-vitro testing formulations at risk of ADD

MR dosage forms are meant for release drug in a controlled manner for a longer period, and there is a risk associated with it that it should not release the drug rapidly. Several studies found that MR preparations of theophylline show higher serum levels in the fed state (food-induced change) as compare to the fasted state. Due to rapid drug release from an MR dosage form, can be termed as dose dumping, results in the administration of a single bolus dose chances of exposure levels, safety issues and adverse reactions chances were increased. This situation is dangerous with drugs that have a narrow therapeutic index or centrally acting drugs and will impact clinical efficacy. As a result, CDER (Center for Drug Evaluation and Research) published a guidance document on the design of clinical studies to assess the effects of food on the rate and extent of Absorption of a drug under fed and fasted conditions. [3]

Fumaric Acid Derivatives are simple organic acid of fumaric acid which are derived from the earth smoke plant (*Fumaria officinalis*). In the late 1950s, fumaric acid derivatives were first used for the treatment of psoriasis. Dimethyl fumarate combined with three other fumaric acid esters (FAEs) is solely licensed in Germany as an oral therapy for psoriasis. Systemic treatment of severe psoriasis with fumaric acid esters has been approved in Germany since 1995.[4]

Due to presence of fumaric group in Dimethyl fumarate it is noted in hazardous materials list. It is harmful if inhaled, can cause serious eye irritation, may cause respiratory irritation, may cause drowsiness or dizziness; it can also cause damage to organs through prolonged or repeated exposure, and is flammable.[5]

Alginic acid, also called algin, is a polysaccharide found in the cell walls of brown algae which are hydrophilic in nature and forms a viscous gum when hydrated. With metals such as sodium and calcium, its salts are known as alginates. It is a significant component of the biofilms produced by the bacterium Pseudomonas aeruginosa, a major pathogen found in the lungs of some people who have cystic fibrosis. Its color ranges from white to yellowish-brown. It is sold in filamentous, granular, or powdered forms. [6]

Alginates from different species of brown seaweed often have variations in their chemical structure, resulting in different physical properties. For example, some may yield an alginate that gives a strong gel, another a weaker gel, some may readily give a cream or white alginate, while others are difficult to gel and are best used for technical applications where color does not matter. [7]

There are following 3 types of Sodium Alginate Salts

- ✓ **Sodium alginate** is the sodium salt of alginic acid. Its empirical formula is $NaC_6H_7O_6$. Sodium alginate is a gum, extracted from the cell walls of brown algae.
- ✓ **Potassium alginate** is a chemical compound that is the potassium salt of alginic acid. It is an extract of seaweed. Its empirical chemical formula is $KC_6H_7O_6$.
- ✓ **Calcium alginate**, made from sodium alginate from which the sodium ion has been removed and replaced with calcium, has the chemical formula $C_{12}H_{14}CaO_{12}$.

Out of these three salts Sodium alginate has majorly used due to its wide applications and easily availability.

2. MATERIAL & METHODS

Sr. No	Materials	Sources
1.	Dimethyl Fumarate	Sun pharmaceutical Industries Limited, Gurugram
2.	Lactose Supertab-11SD	DFE Pharma
3.	Croscarmellose Sodium	FMC Biopolymer
4.	Colloidal Silicon Dioxide	Cabot Sanmar
5.	Magnesium Stearate	Peter Greven
6.	Eudragit L-100	Evonik Nutrition & Care GmbH
7.	Tri-ethyl Citrate (TEC)	Vertellus Perf. Mat. Inc. Greens
8.	Talc	Luzenac Pharma
9.	Poly Ethylene Glycol-6000	Viswat Chemicals Ltd.
10.	Sodium Alginate LFR 5/60	FMC Biopolymer
11.	Iso Propyl Alcohol (IPA)	Thermofisher
12.	Sodium Alginate CR8133	FMC Biopolymer
13.	Sodium Alginate CR8223	FMC Biopolymer
14.	Ethanol	Changhu hingsheng Fine Chemicals Ltd.

All the reagents used in present study were of analytical grade.

2.1 Characterization of Drug

The selected drug was subjected for investigation of characterization parameters such as: Organoleptic properties, melting point, solubility, partition coefficient, pH détermination, ultra-violet (UV) spectroscopy.[10]

2.2 Organoleptic Properties

The organoleptic properties like general appearance, nature, color, odor, etc. were performed by visual observation and compared with standards of drug given in pharmacopoeia for identification.

Color: Small quantity of drug was taken on butter paper and viewed in well illuminated place.

2.3 Determination of Solubility

The solubility of drug was determined by adding 100mg of drug in of each of distilled water, 0.1 N HCl, Chloroform, Ethanol and Phosphate buffer pH 6.8 and shaken at constant temperature 25°C over a period of 24 hr. The resultant solution was checked for solubility.

2.4 Determination of Melting Point

Melting point of Dimethyl Fumarate was determined by taking small amount of drug in a capillary tube closed at one end and placed in a melting apparatus and the temperature at which drug melts was recorded. This was performed in triplicate and average value was recorded. Also, Melting Point of Dimethyl Fumarate was recorded using DSC Thermogram.

2.5 FTIR Spectroscopic Analysis of Dimethyl Fumarate and Sodium Alginate

Fourier Transform Infrared (FTIR) Spectra of moisture free samples of Dimethyl Fumarate and Sodium Alginate were recorded on IR spectrophotometer. Infrared spectroscopy of different compounds was performed for identification of that particular compound. Various peaks in FTIR spectrum were interpreted for identification of different group in the structure of Dimethyl Fumarate and Sodium Alginate. FTIR Spectroscopy can also be used to investigate and predict any physicochemical interactions between different components. The scanning range varies from 4000 – 400 cm^{-1}.

2.6 Analytical Method Development

2.6.1 Preparation of Standard Curve in 0.1N HCl

From the sub-stock solution 1, 2, 3, 4 and 5 ml were transferred to 10 ml volumetric flasks and were diluted with 0.1N HCl up to the mark to obtain concentrations of 10, 20, 30, 40 and 50µg/ml respectively. Absorbance of each solution was measured at 210nm. The absorbance was plotted against concentrations (concentration on x-axis and absorbance on y-axis) and graph with straight line equation and R^2 values were obtained.

2.6.2 Preparation of Standard Curve in Phosphate buffer 6.8

From the sub-stock solution 1, 2, 3, 4 and 5 ml were transferred to 10 ml volumetric flasks and were diluted with 6.8 phosphate buffer up to the mark to obtain concentrations

of 10, 20, 30, 40 and 50µg/ml respectively. Absorbance of each solution was measured at 210nm. The absorbance was plotted against concentrations (concentration on x-axis and absorbance on y-axis) and graph with straight line equation and R^2 value were obtained.

2.7 Drug Excipients Compatibility Studies

2.7.1 Physical method

Binary mixture containing drug substance and excipient in the ratio of 1:1 was prepared by physical mixing. 2g of mixture in equal quantity was taken into 10ml glass bottle and capped and placed (40°C / 75% RH) for 1 month in stability chamber.

SAMPLE	RATIO
Drug + Lactose Supertab-11SD	1:1
Drug + Croscarmellose Sodium	1:1
Drug + Colloidal Silicon Dioxide	1:1
Drug + Magnesium Stearate	1:1
Drug + Eudragit L-100	1:1
Drug + Tri-ethyl Citrate (TEC)	1:1
Drug + Poly Ethylene Glycol-6000	1:1
Drug + Talc	1:1
Drug + Water	1:1
Drug +Iso Propyl Alcohol (IPA)	1:1
Drug + Sodium Alginate LFR 5/60	1:1
Drug + Sodium Alginate CR8133	1:1
Drug + Sodium Alginate CR8223	1:1

Table 1. Detail of samples of drug excipient compatibility study

2.8 Formulation of Mini-Tablets of Dimethyl Fumarate

2.8.1 Compression of Mini-Tablet

Mini tablets were formulated via direct compression. Composition of Mini tablets is given in table

Step 1 – **Weighing**: Appropriate quantities of Dimethyl Fumarate and excipients like Lactose Supertab-11SD, Croscarmellose Sodium, Colloidal Silicon Dioxide and Magnesium Stearate were measured accurately in different polybags as per formula described below.

Step 2 – **Shifting:**

Dimethyl Fumarate, Lactose Supertab-11SD and Croscarmellose Sodium were shifted through Sieve no. #22 and Colloidal Silicon Dioxide was shifted through Sieve no. #60.

Step 3– **Blending:**

The above sifted materials were mixed using V-blender for 10 minutes at 10 RPM.

Step 4 – Compression:

The above granules were compressed into tablets by CADMACH multi station tablet compression machine by using 5.75 mm multi-tip punch containing 8 needles.

S.No.	Ingredients	Amount in tablet (%)	Weight per 10 Mini-Tablets (mg)
1	Dimethyl Fumarate	40	120
2	Lactose supertab-11SD	53.5	160.5
3	Croscarmellose Sodium	4	12
4	Colloidal Sillicondioxide	1	3
5	Magnesium Stearate	1.5	4.5

Table 2. Formula used for preparation of Core Tablets

2.8.2 Application of Seal Coating to Mini-Tablet [8]

Dimethyl Fumarate (API) will sublimate at high temperature into fumes so before application of Alcohol resistant polymeric coat the mini-tablets are sealed with use of IPA and Water based polymer to avoid sublimation of the drug. Due to presence of IPA in coating solution the bed temperature required during coating process is low.

Procedure for Preparation of Coating Solution

1. Isopropyl Alcohol (IPA), Water, Eudragit L-100 and Triethyl Cellulose (TEC) are accurately weighed.
2. Water and IPA were taken into beaker and with continuous stirring Eudragit L-100 is added slowly into beaker.
3. The stirring was continued till the dispersion was clear.
4. After that weighed amount of TEC was assed to the dispersion and stirring continues for further 30 minutes.

*5%w/w dispersion was prepared.

*IPA and Water were taken in the ratio of 9:1.

S. No.	Ingredients	Quantity (%)
1	Eudragit L-100	5
2	Triethyl Cellulose (TEC)	1
3	Isopropyl Alcohol (IPA)	q.s.
4	Water	q.s.

Table 3. Formula used for preparation of Coating Solution

2.8.3 Application of Coating with different Grades of Sodium Alginate

3 different grades of sodium alginate **PROTANAL LFR5/60, PROTANAL CR8133,** and **PROTANAL CR8223** based on different molecular weight and viscosity were selected to check for their alcohol resistant activity and find which of the following grade have best resistivity towards alcohol.

Molecular weights of Protanal LFR 5/60, Protanal CR 8133, and Protanal CR 8223 are 20-60kDa, 90-180kDa, and 250-350kDa respectively. Viscosity of Protanal LFR 5/60 is 300-700 (10%), Protanal CR 8133 is 100-300 (2%), and Protanal CR 8223 is 600-900 (1.25%).

2.8.4 Preparation of different Coating Solution with different Sodium Alginate

Grades

1 Sodium Alginate, Polyethylene Glycol (PEG-6000), Talc and Water were accurately weighed.

2 Sodium Alginate, Polyethylene Glycol (PEG-6000), Talc were sieved from ASTM #25 and mixed.

3 The mixture was slowly added to water with continuous stirring. The stirring was continued till the dispersion homogenized.

*10%w/w dispersion was prepared.

*30% extra solution was prepared for compensating loss during process.

Ingredients	**Quantity of Various Formulation Codes (%)**		
	C_A	**C_B**	**C_C**
LFR 5/60	40.33	-	-
CR 8133	-	40.33	-
CR 8223	-	-	40.33
Talc	53.74	53.74	53.74
PEG-6000	5.93	5.93	5.93
Water	q.s.	q.s.	q.s.

Table 4. Formula used for preparation of Coating Solution of Sodium Alginate Grades

2.8.5 Selection of Best Sodium Alginate Grade

3 different batches C_A, C_B, C_C obtained are evaluated via *In-vitro* dissolution study to check for best alcohol resistant activity. The grade with best alcohol resistant activity was choose and used further for optimization of formulation.

Optimization of Mini-Tablets of Dimethyl Fumarate Using 3^2 Full Factorial Designs

- **Selection of experimental design**

A full factorial design was selected for the formulation development because in an experimental design it is useful to measure the response of every possible combination of factors and factor levels. These responses are analyzed to provide information about every main and interaction effect. A full factorial DOE is practical when fewer than five factors are being analyzed.

- **Selection and levels of independent and dependent variables**

Percentage Weight Gain and Poly Ethyl Glycol-6000(PEG-6000) were selected as independent variables. These variables helped in selection of optimum percentage weight gain and selection of coating formula for tablets coating. *In-vitro* dissolution is considered as dependent variable. Limits of independent variables are shown in Table 4.8.

Variable	**Independent**	
Levels	**Weight Gain (%)**	**Poly Ethylene Glycol-6000**

	(X_1)	**(X_2)**
Low (-1)	5%	3.93
Medium (0)	10%	5.93
High (+1)	15%	7.93

Table 5. Selection and levels of independent variables

Ingredients	**Quantity of Various Formulation Codes (%)**					
	C_1	**C_2**	**C_3**	**C_4**	**C_5**	**C_6**
LFR 5/60	40.33	40.33	40.33	40.33	40.33	40.33
Talc	55.74	55.74	51.74	51.74	53.74	53.74
PEG-6000	3.93	3.93	7.93	7.93	5.93	5.93
Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
% Weight Buildup	5	15	5	15	10	10

Table 6. Composition of Different tablet Formulations

- ***In-vitro* drug release study [11]**

In-vitro drug release rate of Dimethyl Fumarate mini-tablets was carried out using USP dissolution testing apparatus type-II. The dissolution test was carried out using 500ml of 0.1N HCl, 5%, 20%, 40% ethanol with 0.1N HCl and 6.8 pH phosphate buffers, at 37°±0.5°C and stirred at 100 rpm. 10 ml of samples were withdrawn at different time intervals (5, 10, 15, 30, 45, 90 and 120 min. for 0.1N HCl and 5, 10, 15, 20, 25 and 30 min. for pH 6.8 Phosphate Buffer) and are filtered with the help of 0.45µ Nylon-66 filters and an equivalent volume of media (pre warmed at 37°C) was added to maintain constant volume. Withdrawn sample were analyzed at 210nm using UV-Visible Spectrophotometer with cuvette path length of 1mm.

2.8.6 Selection of Optimized Formulation

The optimized formulation was selected on the basis of dependent variable *In-vitro* drug release study full factorial design by Design Expert version 11.

2.8.7 Fluid Uptake Efficiency

50 Mini-Tablets from each Trial and for each buffer solution are taken and initial weights were noted and dissolved in beakers previously filled with buffer solutions and left undisturbed for 8 Hours then Tablets were removed and are soaked with tissue paper, the weight was noted. After weighing them again they were dipped into their respective buffer solutions for further 16 more Hours. The % Fluid uptake was measured with following formula:

$$\% \text{ Fluid Uptake} = \frac{\text{Final Weight- Initial Weight}}{\text{Initial Weight}}$$

3. RESULTS AND DISCUSSION

3.1 Pre-formulation Studies

3.1.1 Characterization of Drug

3.1.2 Organoleptic properties: An odorless, white to off-white, crystalline, powder in appearance.

3.1.3 Solubility Studies: Dimethyl Fumarate found to be highly soluble in water. Also, it is highly soluble in acetone as well as in chloroform.

3.1.4 Melting point: The melting point of Dimethyl Fumarate found between 104°C - 106°C similar to innovator value indicating that the sample is almost pure.

The DSC thermogram showed sharp exothermic peak corresponding to Dimethyl Fumarate melting point 105.5°C. The DSC thermogram of Dimethyl Fumarate is shown below.

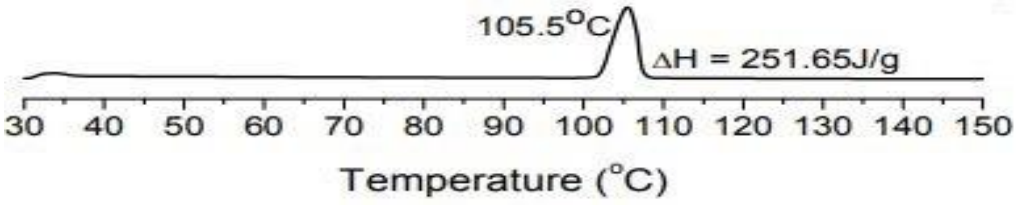


Fig.1. DSC of Dimethyl Fumarate

3.2 FTIR Spectroscopic Analysis of Dimethyl Fumarate and Sodium Alginate

FTIR spectroscopic analysis was carried out to characterize drug and to check purity of drug. The FTIR spectra obtained was compared with that given in pharmacopoeia for Dimethyl Fumarate. Diagnostic peaks and finger print regions were found identical. These characteristics peaks are useful in identification of drug. FTIR of Sodium Alginate was done to characterize and check purity for Excipient.

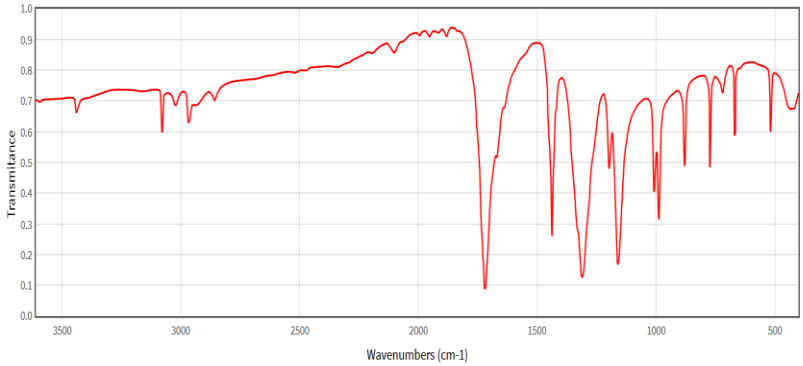


Fig.2. FTIR of Dimethyl Fumarate

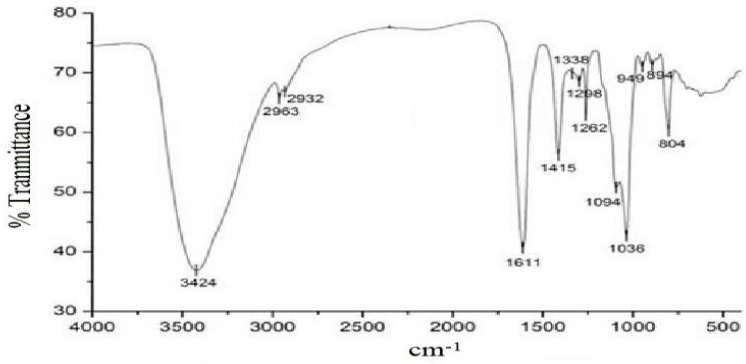


Fig 3. FTIR of Sodium Alginate

3.3 Analytical Method

- **UV- Spectrophotometry:** Dimethyl Fumarate sample was scanned in the range of 400 to 200nm in 0.1N HCl. The λ_{max} found to be 210 nm. The scanned graph generated, as depicted in figure 5.1.

Different concentrations of a standard solution prepared and their absorbance measured in UV-Visible spectroscopy: concentrations and their respective absorbance shown in table 5.1 and 5.2. The standard curve was plotted and shown in figure 5.3 and 5.4. The calibration equation for the straight line observed to be **y=0.1028x+0.0704** with co-relation coefficient as **0.9959 f**or 0.1N HCl and **y=0.1111x+0.4219** with co-relation coefficient as **0.9998** for 6.8 phosphate buffer as it follows Beers Lambert Law and this further used for determination of the concentration of unknown samples.

6.1.1. Preparation of Calibration Curve in 0.1N HCl

Sr. No.	Concentration (µg/ml)	Absorbance (nm)
1	10	0.182
2	20	0.268
3.	30	0.367
4.	40	0.494
5.	50	0.583

Table 7. Concentration and Corresponding Absorbance of Dimethyl Fumarate in 0.1N HCl

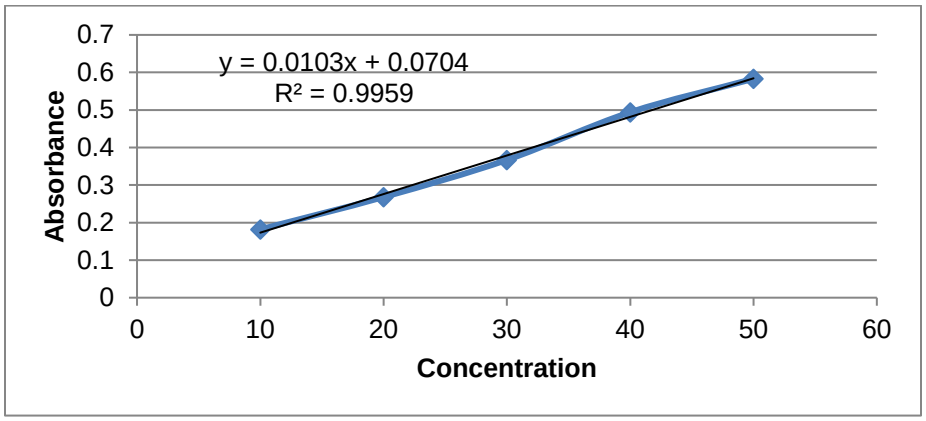


Fig.4. Calibration Curve of Dimethyl Fumarate in 0.1N HCl

6.1.2. Preparation of Calibration Curve in 6.8 Phosphate Buffer

Sr. No.	Concentration (µg/ml)	Absorbance (nm)
1	10	0.535
2	20	0.644
3.	30	0.751
4.	40	0.867

5.	50	0.979

Table.8 Concentration and Corresponding Absorbance of Dimethyl Fumarate in 6.8 Phosphate buffer

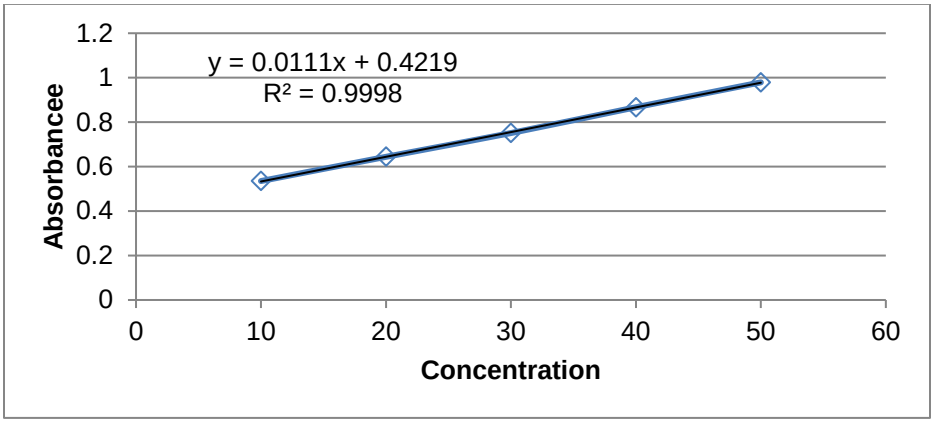


Fig.5. Calibration Curve of Dimethyl Fumarate in phosphate buffer 6.8

3.4 Drug Excipients Compatibility Studies

6.1.3. Physical method

Sr. No.	API	Excipients	Ratio	Result
1	Dimethyl Fumarate	Lactose Supertab-11SD	1:1	White-Off White Powder
2	Dimethyl Fumarate	Croscarmellose Sodium	1:1	White-Off White Powder
3	Dimethyl Fumarate	Colloidal Silicon Dioxide	1:1	White-Off White Powder
4	Dimethyl Fumarate	Magnesium Stearate	1:1	White-Off White Powder
5	Dimethyl Fumarate	Eudragit L-100	1:1	White-Off White Powder
6	Dimethyl Fumarate	Tri-ethyl Citrate (TEC)	1:1	White liquid
7	Dimethyl Fumarate	Sodium Alginate LFR 5/60	1:1	Slight Yellowish Powder
8	Dimethyl Fumarate	Poly Ethyl Glycol-6000(PEG-6000)	1:1	White-Off White Powder
9	Dimethyl Fumarate	Talc	1:1	White-Off White Powder
10	Dimethyl Fumarate	Water	1:1	Whitish Liquid
11	Dimethyl Fumarate	Iso Propyl Alcohol (IPA)	1:1	Whitish Liquid

12	Dimethyl Fumarate	Sodium Alginate CR8133	1:1	Slight Yellowish Powder
13	Dimethyl Fumarate	Sodium Alginate CR8223	1:1	Slight Yellowish Powder

Table.9. Drug-Excipient Compatibility Study @40ºC/75%RH for 1M

3.5 *In-Vitro* Dissolution Study

6.1.4. *In-vitro* Dissolution Study of Enteric Coat Mini-Tablet

Time(min)	% Drug Released			
	0% Ethanol	5% Ethanol	20% Ethanol	40% Ethanol
0	0.000	0.000	0.000	0.000
15	0.002	0.454	1.632	75.414
30	0.005	0.572	1.995	91.972
45	0.006	0.738	4.206	94.253
60	0.009	0.985	8.099	95.419
90	0.011	3.215	24.711	96.617
120	0.013	3.313	45.613	96.921

Table 10. *In-vitro* Dissolution Study of Enteric Coat

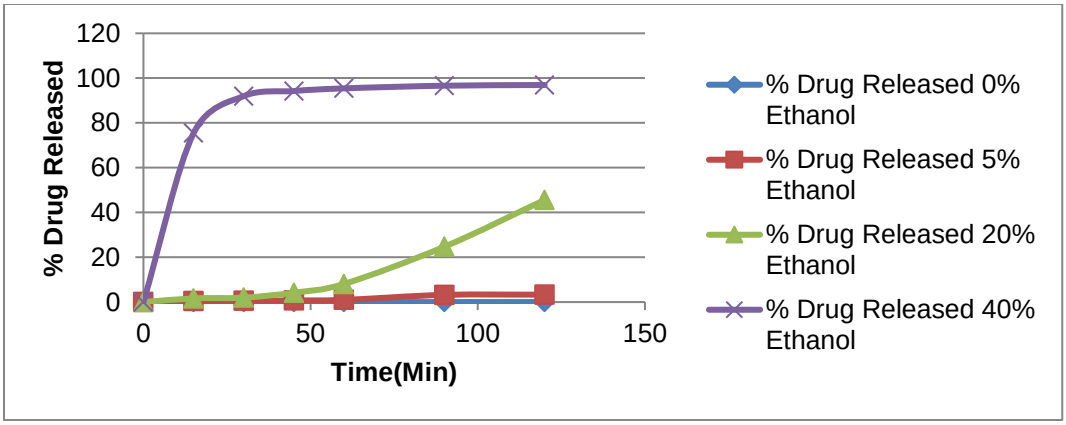


Fig.6. *In-vitro* Dissolution Study of Enteric Coat

6.1.5. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%) {(CA)}

Time(min)	% Drug Released			
	0% Ethanol	5% Ethanol	20% Ethanol	40% Ethanol
0	0.000	0.000	0.000	0.000
15	0.001	0.000	0.205	0.462
30	0.003	0.001	0.318	0.974
45	0.004	0.002	0.442	1.497
60	0.006	0.002	0.675	2.302
90	0.009	0.003	1.101	3.603
120	0.011	0.005	2.290	4.834

Table 11. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%)

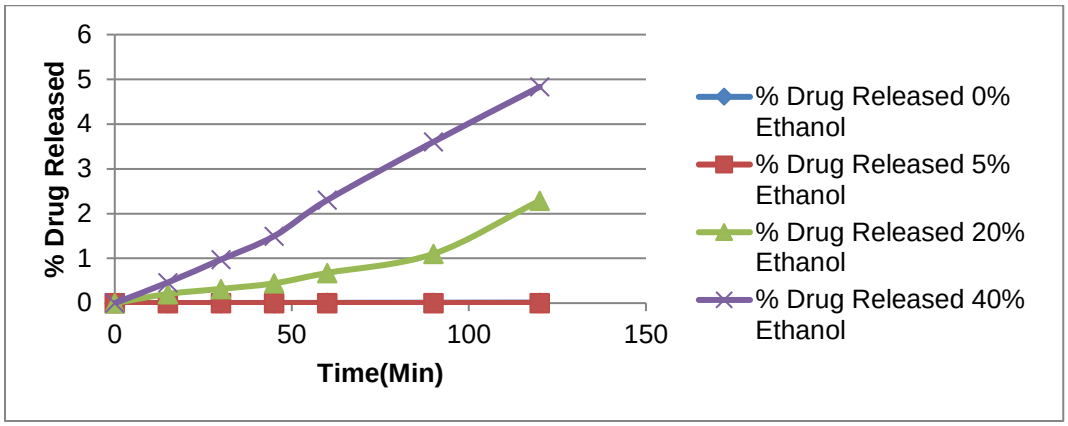


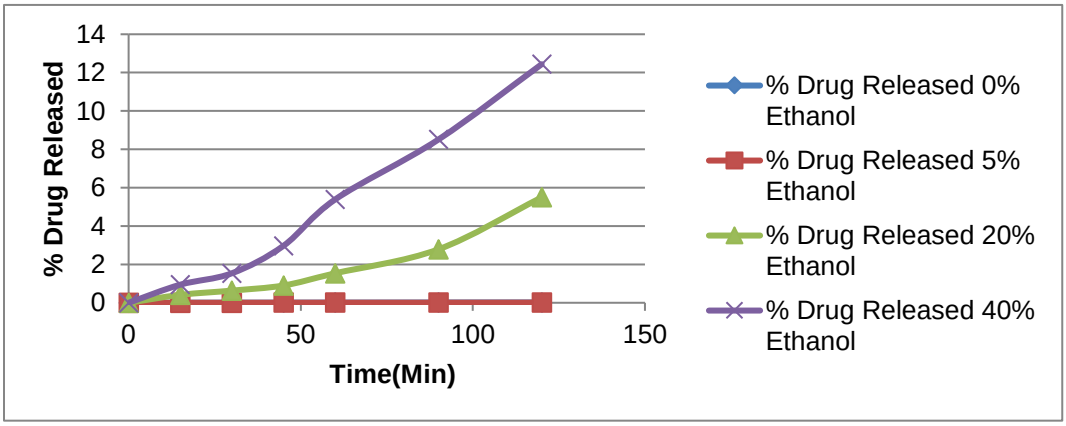
Fig.7. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%)

6.1.6. *In-vitro* Dissolution Study with Sodium Alginate CR 8133 Coat (10%) {(C_B)}

Time(min)	% Drug Released			
	0% Ethanol	**5% Ethanol**	**20% Ethanol**	**40% Ethanol**
0	0.000	0.000	0.000	0.000
15	0.004	0.001	0.412	0.943
30	0.005	0.002	0.634	1.537
45	0.007	0.004	0.901	2.972
60	0.009	0.005	1.537	5.386
90	0.014	0.006	2.786	8.512
120	0.017	0.009	5.495	12.439

Table 12. *In-vitro* Dissolution Study with Sodium Alginate CR 8133 Coat (10%)

Fig.8. *In-vitro* Dissolution Study with Sodium Alginate CR 8133 Coat (10%)



6.1.7. *In-vitro* Dissolution Study with Sodium Alginate CR 8223 Coat (10%) {(C_C)}

Time(min)	% Drug Released			
	0% Ethanol	**5% Ethanol**	**20% Ethanol**	**40% Ethanol**
0	0.000	0.000	0.000	0.000
15	0.003	0.000	0.261	0.513
30	0.008	0.009	0.491	0.995

45	0.010	0.012	0.547	1.917
60	0.013	0.021	0.976	2.398
90	0.016	0.019	1.989	5.563
120	0.018	0.024	4.185	8.924

Table 13. *In-vitro* Dissolution Study with Sodium Alginate CR 8223 Coat (10%)

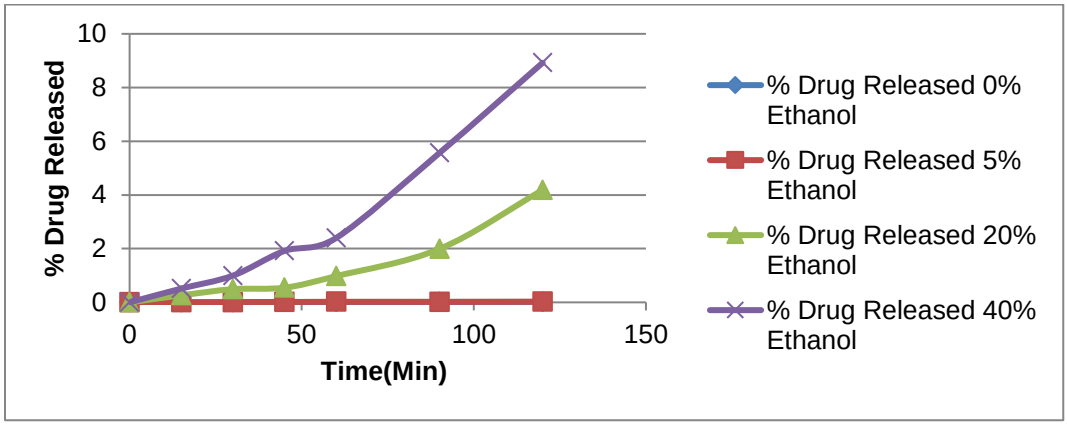


Fig.9. *In-vitro* Dissolution Study with Sodium Alginate CR 8223 Coat (10%)

6.1.8. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (5%) and PEG-6000 (3.93%) {(C_1)}

DOE TRIAL 1 {(C_1)}

Time(min)	% Drug Released			
	0% Ethanol	**5% Ethanol**	**20% Ethanol**	**40% Ethanol**
0	0.000	0.000	0.000	0.000
15	0.000	0.000	2.111	3.257
30	0.003	0.004	4.798	5.749
45	0.004	0.006	7.673	8.982
60	0.007	0.007	10.562	12.976
90	0.008	0.013	15.792	23.670
120	0.010	0.015	22.901	39.181

Table 14. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (5%) and PEG-6000 (3.93%)

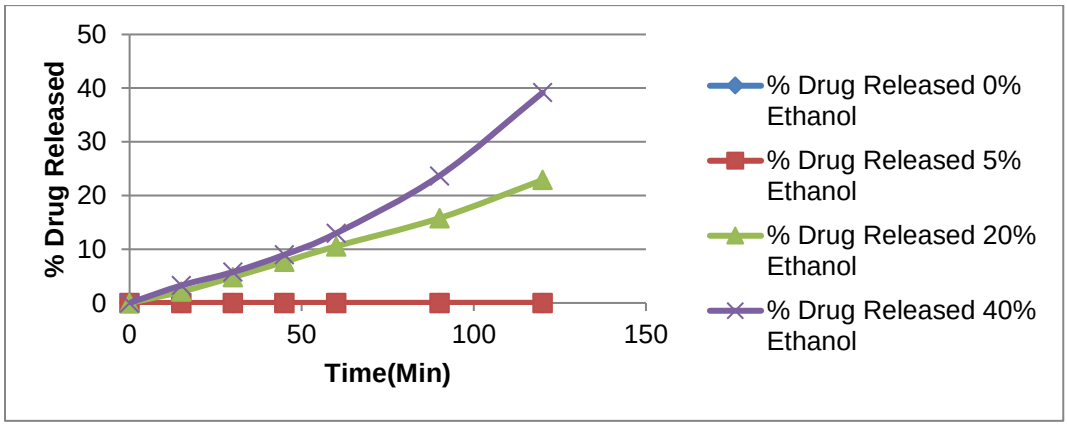


Fig.10. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (5%) and PEG-6000 (3.93%)

6.1.9. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (15%) and PEG-6000 (3.93%) {(C_2)}

DOE TRIAL 2 {(C_2)}

Time(min)	% Drug Released			
	0% Ethanol	**5% Ethanol**	**20% Ethanol**	**40% Ethanol**
0	0.000	0.000	0.000	0.000
15	0.000	0.005	0.121	0.432
30	0.002	0.011	0.215	0.556
45	0.003	0.027	0.487	0.758
60	0.005	0.073	0.563	0.985
90	0.005	0.091	0.869	1.637
120	0.011	0.094	1.018	2.572

Table 15. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (15%) and PEG-6000 (3.93%)

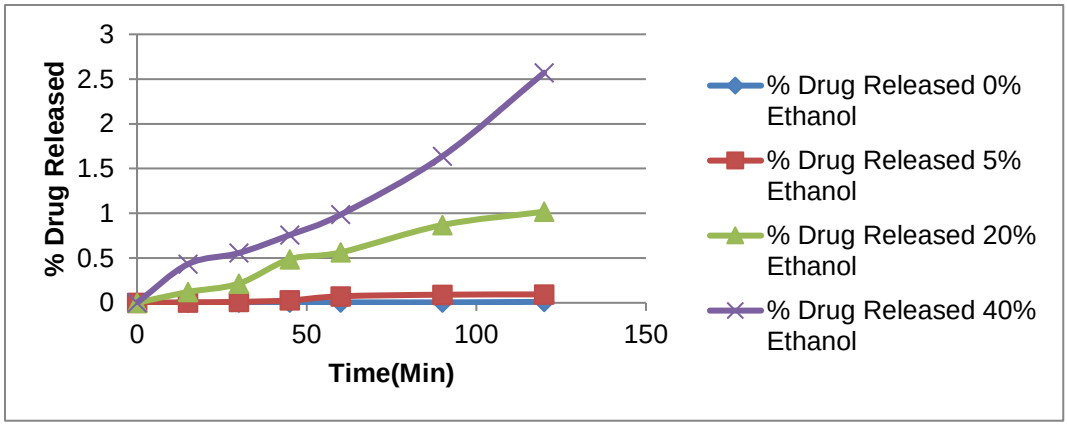


Fig.11. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (15%) and PEG-6000 (3.93%)

6.1.10. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (5%) and PEG-6000 (7.93%) {(C_3)}

DOE TRIAL 3 {(C_3)}

Time(min)	% Drug Released			
	0% Ethanol	**5% Ethanol**	**20% Ethanol**	**40% Ethanol**
0	0.000	0.000	0.000	0.000
15	0.000	0.031	9.181	15.704
30	0.002	0.059	13.877	20.499
45	0.001	0.096	19.603	28.827

60	0.002	1.757	24.091	41.706
90	0.007	4.631	33.206	62.709
120	0.008	6.646	48.708	76.881

Table 16. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (5%) and PEG-6000 (7.93%)

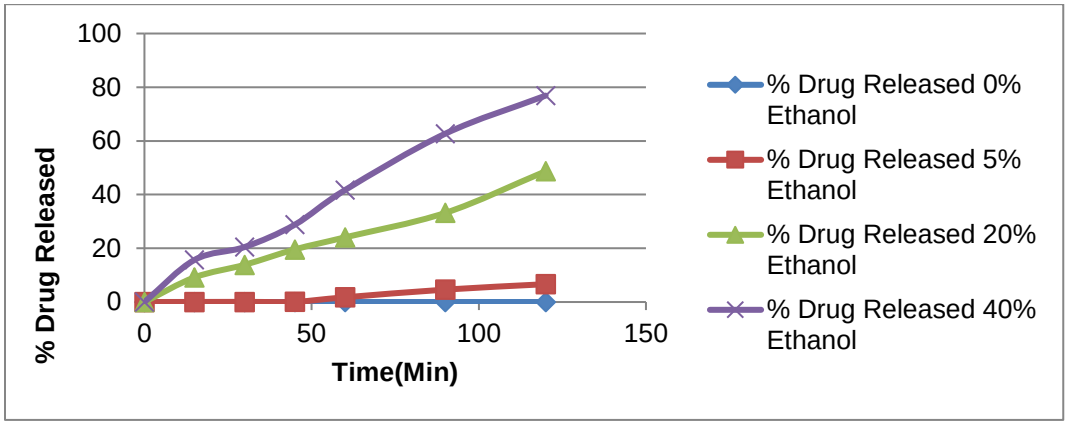


Fig.12. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (5%) and PEG-6000 (7.93%)

6.1.11. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (15%) and PEG-6000 (7.93%) {(C_4)}

DOE TRIAL 4 {(C_4)}

Time(min)	% Drug Released			
	0% Ethanol	**5% Ethanol**	**20% Ethanol**	**40% Ethanol**
0	0.000	0.000	0.000	0.000
15	0.006	0.013	0.369	0.461
30	0.007	0.022	0.543	0.899
45	0.009	0.029	0.878	1.518
60	0.012	0.032	1.143	2.988
90	0.016	0.041	1.978	5.313
120	0.019	0.044	3.817	8.484

Table 17. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (15%) and PEG-6000 (7.93%)

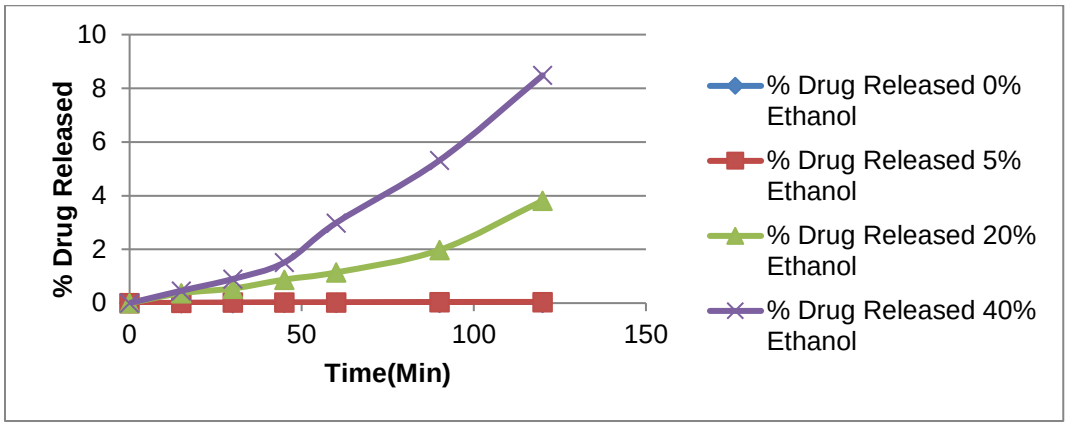


Fig.13. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (15%) and PEG-6000 (7.93%)

***6.1.12. In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%) and PEG-6000 (5.93%) {(C5)}**

DOE TRIAL 5 {(C_5)}

Time(min)	% Drug Released			
	0% Ethanol	**5% Ethanol**	**20% Ethanol**	**40% Ethanol**
0	0.000	0.000	0.000	0.000
15	0.000	0.000	0.065	0.159
30	0.000	0.000	0.135	0.362
45	0.001	0.002	0.189	0.437
60	0.003	0.003	0.348	0.775
90	0.004	0.005	0.469	1.018
120	0.004	0.006	0.531	1.323

Table 18. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%) and PEG-6000 (5.93%)

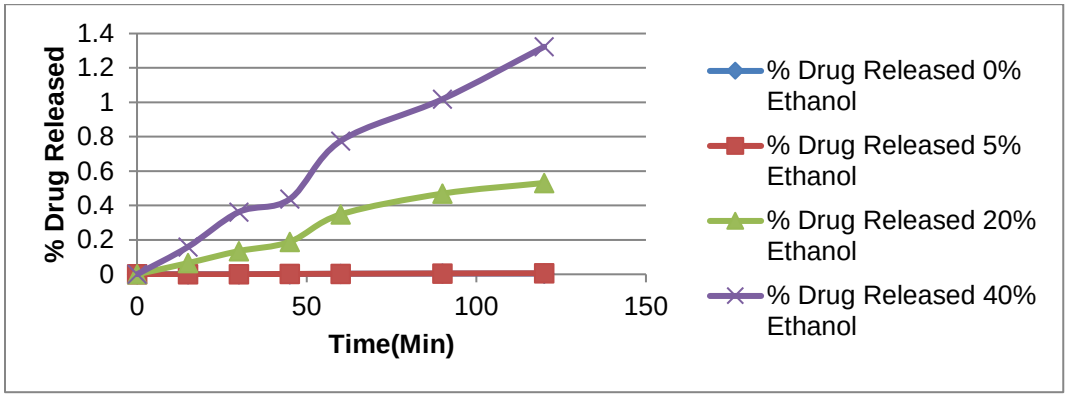


Fig.14. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%) and PEG-6000 (5.93%)

***6.1.13. In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%) and PEG-6000 (5.93%) {(C6)}**

DOE TRIAL 6 {(C_6)}

Time(min)	% Drug Released			
	0% Ethanol	**5% Ethanol**	**20% Ethanol**	**40% Ethanol**
0	0.000	0.000	0.000	0.000
15	0.000	0.000	0.049	0.103
30	0.001	0.001	0.095	0.276
45	0.003	0.002	0.129	0.327
60	0.002	0.003	0.234	0.582
90	0.007	0.005	0.316	0.899
120	0.005	0.006	0.475	1.402

Table 19. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%) and PEG-6000 (5.93%)

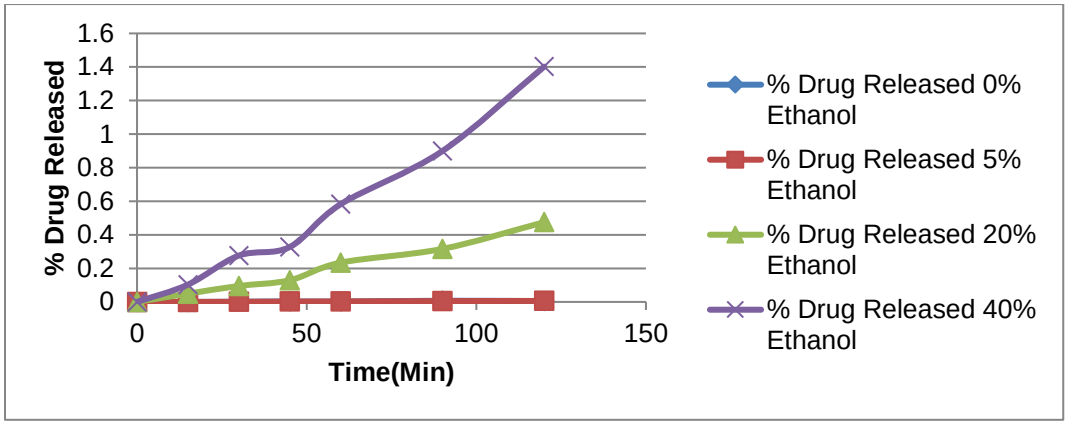


Fig.15. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%) and PEG-6000 (5.93%)

3.6 Fluid Uptake Efficiency

6.1.14. Fluid Uptake Efficiency after 8 Hours in 3 Different Buffer Solutions

Buffer Solution	**Parameters**	**Formulation Code**					
		C_1	**C_2**	**C_3**	**C_4**	**C_5**	**C_6**
0.1N HCl	**Initial Weight (mg)**	334.20	365.80	333.70	365.90	350.20	350.10
	Final Weight (mg)	367.19	461.93	372.54	472.16	422.97	424.11
	% Fluid Uptake	9.87	26.28	11.64	29.04	20.78	21.14
4.5 Acetate Buffer	**Initial Weight (mg)**	334.30	365.70	333.80	365.80	349.50	349.60
	Final Weight (mg)	354.46	427.28	358.8	430.91	388.68	390.78
	% Fluid Uptake	6.03	15.84	7.49	17.8	11.21	11.78
6.8 Phosphate Buffer	**Initial Weight (mg)**	333.50	365.50	334.10	365.40	349.90	349.80
	Final Weight (mg)	341.70	403.00	347.16	407.02	373.62	373.73
	% Fluid Uptake	2.46	10.26	3.91	11.39	6.78	6.84

Table 20. Fluid Uptake Efficiency after 8 Hours

6.1.15. Fluid Uptake Efficiency after 24 Hours in 3 Different Buffer Solutions

Buffer Solution	Parameters	Formulation Code					
		C_1	C_2	C_3	C_4	C_5	C_6
0.1N HCl	Initial Weight (mg)	334.20	365.80	333.70	365.90	350.20	350.10
	Final Weight (mg)	374.94	486.00	384.99	490.42	427.70	428.80
	% Fluid Uptake	12.19	32.86	15.37	34.03	22.13	22.48
4.5 Acetate Buffer	Initial Weight (mg)	334.30	365.70	333.80	365.80	349.50	349.60
	Final Weight (mg)	360.01	429.44	365.58	436.07	397.35	398.79
	% Fluid Uptake	7.69	17.43	9.52	19.21	13.69	14.07
6.8 Phosphate Buffer	Initial Weight (mg)	333.50	365.50	334.10	365.50	349.90	349.80
	Final Weight (mg)	346.77	411.81	354.41	416.27	376.00	377.61
	% Fluid Uptake	3.98	12.67	6.08	13.89	7.46	7.95

Table 21. Fluid Uptake Efficiency after 24 Hours

3.7 Optimization of Formulation Using Design Expert Software

The observed value of response (in-vitro dissolution ADD for 40% @ 2hr) was further analyzed statistically to evaluate the effect of various factors and interaction of factors using DOE. The optimized formulation was selected using statistical screening.[9]

Results analyzed by Design-Expert version 11;

- Actual by predicted plot
- Studentized Residuals
  - Summary of Fit
- ANOVA
- Predicted Profiler
- Interaction Profiles
  - Effect Screening
- Normal Plot
- Independent Variables
- Contour Profiler
- Response Grid Slider

6.1.16. Actual by Predicted Plot

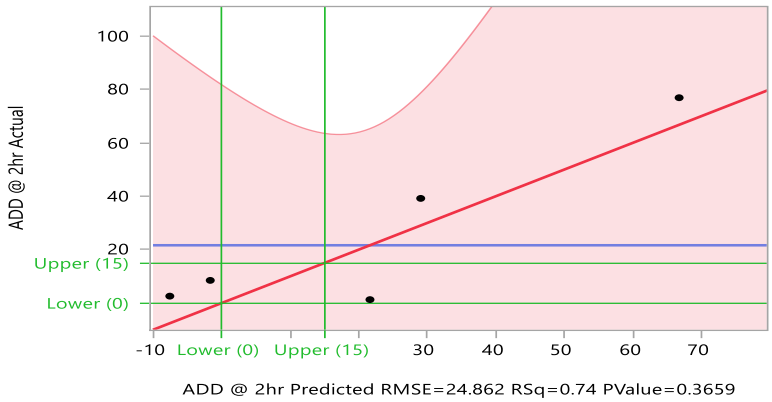


Fig.16. Actual by Predicted Plot

6.1.17. Studentized Residuals

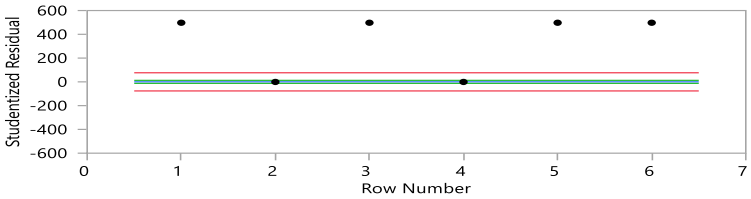


Fig.17. Studentized Residuals

6.1.18. Summary of Fit

R Square	0.738109
R Square Adj	0.345273
Root Mean Square Error	24.86237
Mean of Response	21.65
Observations (or Sum Wgts)	6

Table 22. Summary of Fit

6.1.19. Analysis of Variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	3	3484.3000	1161.43	1.8789
Error	2	1236.2750	618.14	**Prob > F**
C. Total	5	4720.5750		0.3659

Table 23. Analysis of Variance

6.1.20. Prediction Profiler

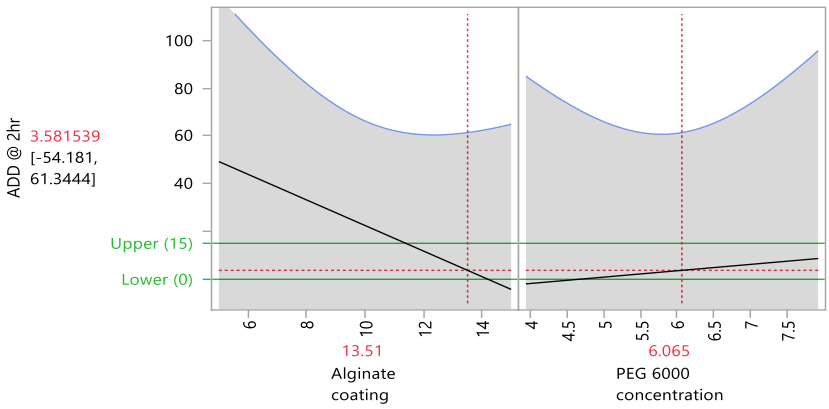


Fig.18. Prediction Profiler

6.1.21. Interaction Profiles

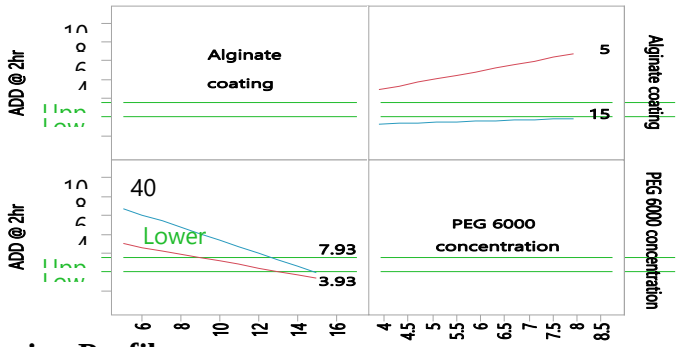


Fig.19. Interaction Profiles

6.1.22. Effect Screening

Using estimates standardized to have equal variances. The parameter estimates are not correlated.

	Lenth PSE
t-Test Scale	1.3152406
Coded Scale	13.349719

Table 24. Effect Screening

6.1.23. Normal Plot

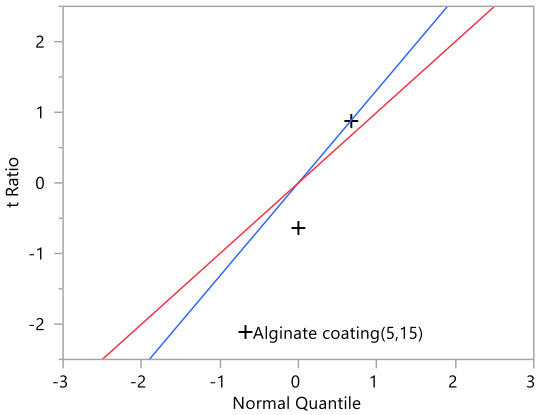


Fig.20. Normal Plot

6.1.24. Contour Profiler

Horiz	Vert	Factor		Current X
○	◉	Alginate coating		12.717
◉	○	PEG 6000 concentration		4.9735

	Response		Contour	Current Y	Lo Limit	Hi Limit
—	ADD @ 2hr		6.91	4.2388793	0	15

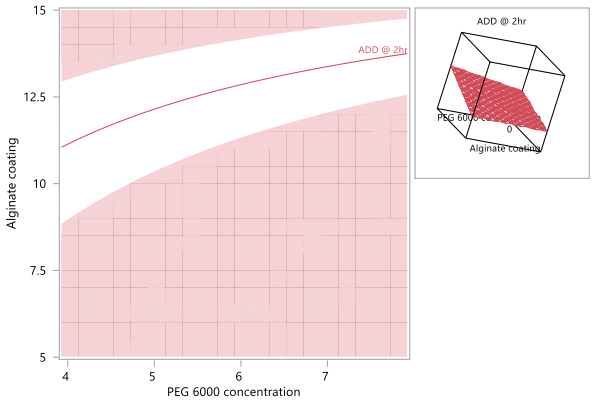


Fig.21. Contour Profiler

6.1.25. Response Grid Slider

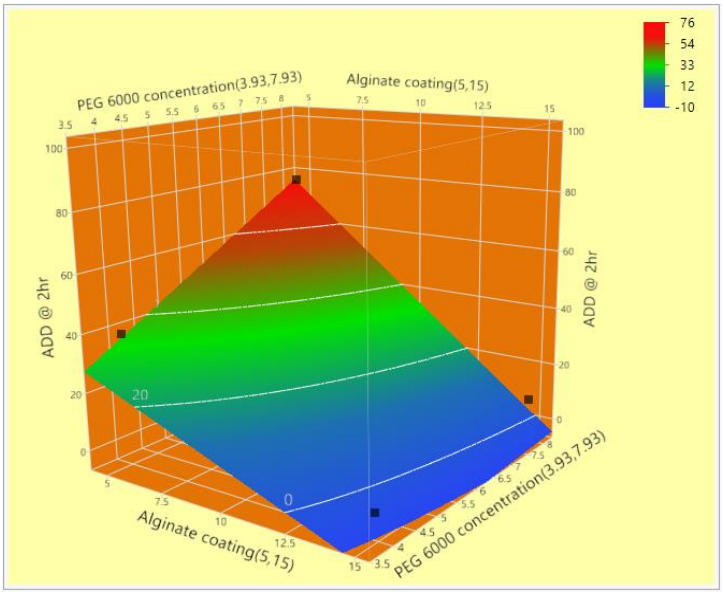


Fig.22. Response Grid Slider

3.8 *In-Vitro* Dissolution of Optimized Formulation

6.1.26. In-vitro Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%) and PEG-6000 (5.93%)

Time(min)	% Drug Released			
	0% Ethanol	**5% Ethanol**	**20% Ethanol**	**40% Ethanol**

0	0.000	0.000	0.000	0.000
15	0.008	0.010	0.093	0.319
30	0.011	0.015	0.149	0.601
45	0.015	0.019	0.237	0.797
60	0.022	0.021	0.478	0.982
90	0.026	0.022	0.816	1.459
120	0.031	0.027	0.995	1.881

Table 25. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%) and PEG-6000 (5.93%)

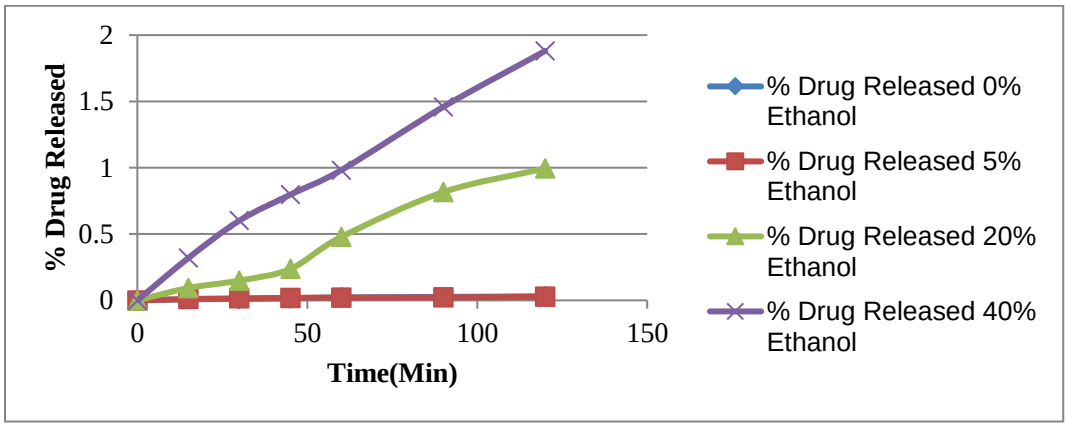


Fig.23. *In-vitro* Dissolution Study with Sodium Alginate LFR 5/60 Coat (10%) and PEG-6000 (5.93%)

6.1.27. In pH 6.8 Phosphate Buffer

The Optimized formulation scanned for *In-vitro* dissolution in pH 6.8 Phosphate Buffer.

S.No.	Time Intervals (Min.)	Cumulative Drug Release (%)
1	0	0
2	5	39.23
3	10	60.76
4	15	78.98
5	20	94.36
6	25	98.04
7	30	98.59

Table 26. *In-vitro* Dissolution Study in pH 6.8 Phosphate Buffer.

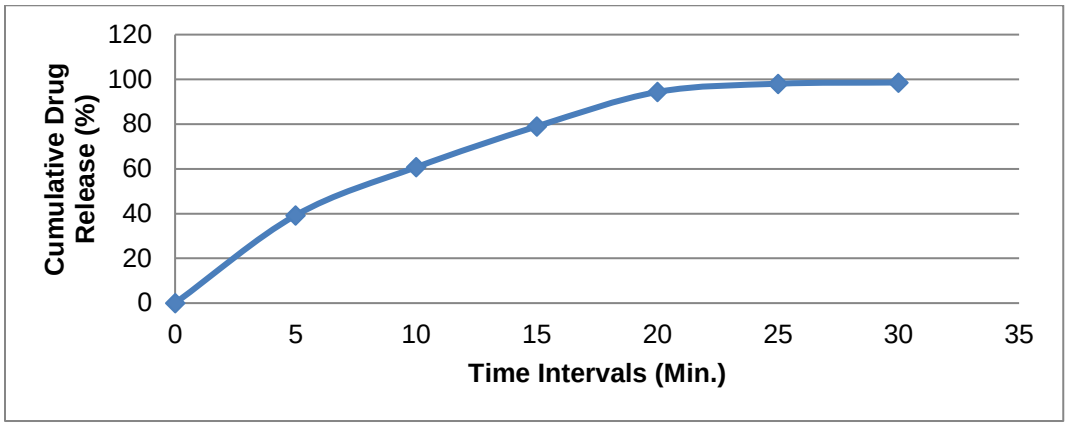


Fig.24. *In-vitro* Dissolution Study in pH 6.8 Phosphate Buffer

6.1.28. *In-Vitro* Dissolution Comparative Study between Innovator, Enteric Coat only and Optimized Formulation (C_5) with 40% Ethanol in 0.1N HCl

Time(min)	% Drug Released in 40% Ethanol		
	Innovator	Enteric Coat Only	C_5
0	0.00	0.000	0.000
15	81.29	75.414	0.159
30	97.22	91.972	0.362
45	98.39	94.253	0.437
60	98.71	95.419	0.775
90	98.65	96.617	1.018
120	98.97	96.921	1.323

Table 27. Comparative Study between Innovator, Enteric Coat only and Optimized Formulation (C_5) with 40% Ethanol in 0.1N HCl

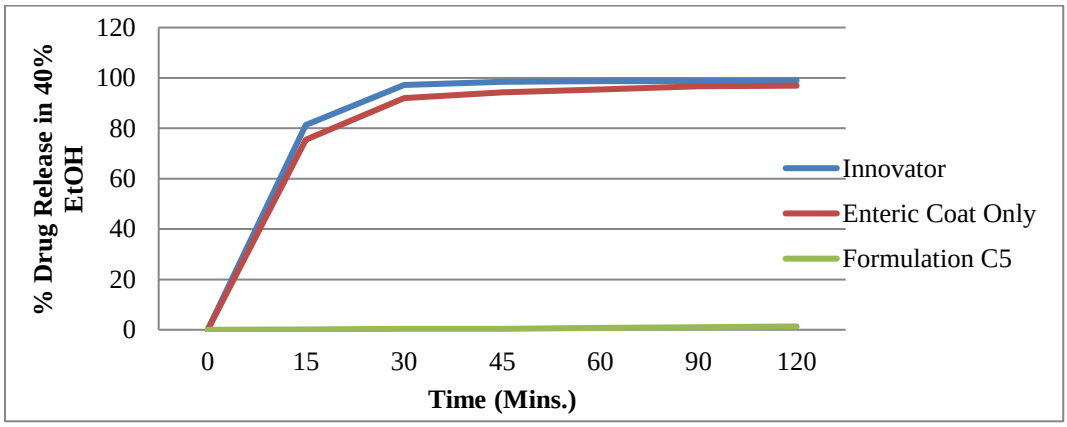


Fig.25. Comparative Study between Innovator, Enteric Coat only and Optimized Formulation (C_5) with 40% Ethanol in 0.1N HCl

4. SUMMARY AND CONCLUSION

In the present study Alcohol Dose Dumping resistant formulations were designed and evaluated. Dimethyl Fumarate was selected as model drug because of its early dissolution

in 40% Ethanol. The purpose of study is to prevent dissolution of Dimethyl Fumarate in 40% Alcohol.

Out of 3 grades (LFR 5/60, CR8133, and CR8223) selected for coating of Dimethyl Fumarate mini-tablets, LFR 5/60 found best among all 3 grades. The % Drug Release in 40% Ethanol of LFR5/60 Coated Mini-Tablets was found to be 4.834%.

For optimization of LFR 5/60 as coating material, 3^2 full factorial designs were employed using % weight gain and concentration of PEG-6000 (independent variables). The percentage drug release in-vitro in 40% Alcohol was selected as dependent variables and the best formulation was selected by the design expert software version 11. Optimized formulation with 10% weight gain and PEG-6000 concentration 5.93% was found to be best formulation. Moreover, design expert software also suggests that best % weight gain is 12.717% and PEG-6000 concentration is 4.9735.

From the above results it can be stated that additional coating of Sod. Alginate will be helpful in preventing ADD in 40% Alcohol. The newly formulated mini-tablets have better alcohol resistive activity than the patented drug with brand name Innovator.

5. BIBLIOGRAPHY

1. Hughes, K., Gray, A., & Thomas, P. T. (2015). Regulatory considerations for alcohol-induced dose dumping of oral modified-release formulations. *Pharmaceutical Technology*, 38(10), 40-46.
2. Cederbaum, A. I. (2012). Alcohol metabolism. *Clinics in Liver Disease*, 16, 667-685.
3. D'Souza, S., Mayock, S., & Salt, A. (2017). A review of in vivo and in vitro aspects of alcohol-induced dose dumping. *AAPS Open*, 3(1). https://doi.org/10.1186/s41120-017-0012-0
4. Mrowietz, U., Altmeyer, P., Bieber, T., Röcken, M., Schopf, R. E., & Sterry, W. (2007). Treatment of psoriasis with fumaric acid esters (Fumaderm®). *Journal der Deutschen Dermatologischen Gesellschaft*, 5(8), 716-717. https://doi.org/10.1111/j.1610-0387.2007.06387.x
5. Avantor Performance Materials. (2014). *Dimethylformamide safety data sheet* (pp. 8-11).
6. Boyd, A., & Chakrabarty, A. M. (1995). Pseudomonas aeruginosa biofilms: Role of the alginate exopolysaccharide. *Journal of Industrial Microbiology*, 15(3), 162-168. https://doi.org/10.1007/BF01569819
7. McHugh, D. J. (2003). *A guide to the seaweed industry*. FAO Fisheries Technical Paper No. 441.
8. Rowe, R. C., Sheskey, P. J., & Owen, S. C. (Eds.). (2006). *Handbook of pharmaceutical excipients* (5th ed., pp. 656-658). London: Pharmaceutical Press.
9. Singh, B., Bhatowa, R., Tripathi, C., & Kapil, R. (2011). Developing micro-/nanoparticulate drug delivery systems using "design of experiments."

International Journal of Pharmaceutical Investigation, 1(2), 75-81. https://doi.org/10.4103/2230-973X.82384

10. Troy, B. D. (Ed.). (2006). *Remington: The science and practice of pharmacy* (21st ed., pp. 929-938). Philadelphia: Lippincott Williams & Wilkins.
11. Subrahmanyam, C. V. S. (2019). *Physical pharmaceutics-I* (1st ed., Vol. 1, pp. 125-127). New Delhi: Vallabh Prakashan