FORMULATION AND INVITRO EVALUATION OF APREMILST NANOSPONGES

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Abstract

The Aim of this work is to the development and evaluation of Nanosponges drug delivery system of APREMILAST by using solvent evaporation method. APREMILAST is a BCS class-IV drug, having a half-life of 9hrs, which wasn't suitable for maintaining constant plasma concentrations. So APREMILAST was formulated as a Nano sponge formulation for effective drug release. APREMILAST Nanosponges was formulated using Pluronic F127, ethyl cellulose, β-cyclodextrin with four different drugs. polymer rations, FTIR spectroscopy analyses indicated the chemically stable, amorphous nature of the drug in these Nano sponge. SEM photographs revealed the spherical nature of the Nanosponge in all variations. The formulation F9 has better results than remaining formulations. **F9** formulation containing (APREMILAST: Ethyl cellulose in 1:4 ratio) shows better entrapment efficiency than other formulations, drug release 99.52 % in 7 hours, and follows zero order with super case II transport mechanism.

Keywords: APREMILAST, Ethyl cellulose, Pluronic F127, β-cyclodextrin, FTIR.

Introduction

In recent years, there has been considerable emphasis given to the development of novel Nano sponge base drug delivery systems, in order to modify and control the release behavior of the drugs. By incorporation into a carrier system, it is possible to alter the therapeutic index and duration of the activity of drugs.

Nanosponges are novel class of hyper cross-linked polymer based colloidal structures consisting of solid nanoparticles with colloidal sizes and nanosized cavities. They enhance stability, reduce side effects and modify drug release. The outer surface is typically porous, allowing sustain release of drug. They are mostly use for topical drug delivery. Size range of Nano sponge is 50nm-100nm[1].This technology is being used in cosmetics.

They can be used for targeting drugs to specific sites, prevent drug and protein degradation. These tiny sponges can circulate around the body until they encounter the specific target site and stick on the surface and began to release the drug in a controlled and predictable manner. It is possible to control the size of Nano sponge. To varying the portion of cross-linkers and polymers, the Nano sponge particles can be made larger or smaller. These particles are capable of carrying both lipophilic and hydrophilic substances and of improving the solubility of poorly water-soluble molecules.

Advantages [16, 17, 18, 19]:

1) This technology provides entrapment of active contents and side effects are less.
2) It provides improved stability, elegance and formulation flexibility.
3) It is non-mutagenic.
4) Non-irritating, non-toxic.
5) It provides extended release condition which is continuous action up to 12hr.
6) Drug is protected from degradation.
7) Therapeutic provide onset of action. Formulations are cost effective.
8) It can be used to mask unpleasant flavours and to convert liquid substances to solids[2]. Less harmful side effects (since smaller quantities of the drug have contact with healthy tissue).
9) Nanosponge particles are soluble in water, so encapsulation can be done within the Nano sponge, by the addition of chemical called an adjuvant reagent.
10) Particles can be made smaller or larger by varying the proportion of cross-linker to polymer.

Disadvantages:

1) Nanosponges include only small molecules.
2) Depend only upon loading capacities.

Factors influence nanosponges[33,34]:

1) Type of polymer:

Type of polymer is used which can influence formation as well as performance of nanosponges. For complexation, cavity size of nana sponges should be suitable.

2) Temperature:

Temperature changes can affect drug/ nanasponges complexation. Increase in temperature decreases the magnitude of apparent stability constant of drug due to result of possible reduction of drug interaction forces.

3) Method of preparation:

The method of loading drug into nanasponges can affect drug complexation. Effectiveness of method depends on nature of drug and polymer.

4) Degree of substitution:

Nanosponges are greatly affected by type, number, position of substituent on parent molecule & due to this affects its complexation.

MATEIALS AND METHODOLOGY:

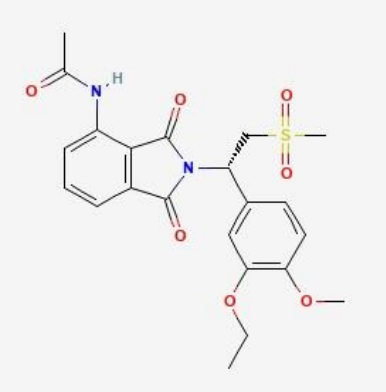
1Drug Profile: APREMILAST

IUPAC Name: N-{2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-methanesulfonylethyl]-1, 3-dioxo-2, 3-dihydro-1H-isoindol-4-yl} acetamide

Molecular formula: $C_{22}H_{24}NO_7S$

Molecular weight: 460.5gm/mol

CAS Number: 608141-41-9



Structure of Apremilast

Mechanism of action:

It is known that apremilast is an inhibitor of phosphodiesterase 4 (PDE4), which mediates the activity of cyclic adenosine monophosphate (cAMP), a second messenger.5 The inhibition of PDE4 by apremilast leads to increased intracellular cAMP levels.14 An increase in cAMP results in the suppression of inflammation by decreasing the expression of TNF-α, IL-17, IL-23, and other inflammatory mediators. The above inflammatory mediators have been implicated in various psoriatic conditions as well as Bechet's disease, leading to their undesirable inflammatory symptoms such as mouth ulcers, skin lesions, and arthritis. Apremilast administration leads to a cascade which eventually decreases the levels of the above mediators, relieving inflammatory symptoms.

Absorption:

An oral dose of apremilast is well-absorbed and the absolute bioavailability is approximately 73%. Tmax is approximately 2.5 hours14 and Cmax has been reported to be approximately 584 ng/mL in one pharmacokinetic study.

Volume of distribution:

The average apparent volume of distribution (VD) is about 87 L, suggesting that apremilast is distributed in the extravascular compartment

Protein-binding:

The plasma protein binding of apremilast is about 68%

Route of elimination:

Only 3% and 7% of an apremilast dose are detected in the urine and feces as unchanged drug, respectively, indicating extensive metabolism and high absorption.

Half-life:

The average elimination half-life of this drug ranges from 6-9 hours.

Metabolism:

Apremilast is heavily metabolized by various pathways, which include oxidation, hydrolysis, in addition to conjugation. About 23 metabolites are produced from its metabolism.3 The CYP3A4 primarily mediates the oxidative metabolism of this drug, with smaller contributions from CYP1A2 and CYP2A6 enzymes.13 The main metabolite of apremilast, M12, is an inactive glucuronide conjugate form of the O-demethylated drug.13 Some other major metabolites, M14 and M16, are significantly less active in the inhibition of PDE4 and inflammatory mediators than their parent drug, apremilast. After an oral dose, unchanged apremilast (45%) and the inactive metabolite, O-desmethyl apremilast glucuronide (39%) are found in the plasma. 10 Minor metabolites M7 and M17 are active, but are only present in about 2% or less of apremilast concentrations, and likely not significant contributors to the actions of apremilast.

Toxicity: The oral LD50 in mice was greater than 2000 mg/kg in mice. In rats, oral LD50 was 2000 mg/kg males and 300 mg/kg in females.

Adverse effect:

- ✓ Diarrhea,
- ✓ Nausea/Vomiting,
- ✓ Loss of Appetite,
- ✓ Headache
- ✓ Weight Loss

EXCIPIENTS PROFILE:

1.β –CYCLODEXTRIN:

Synonyms: β-Cyclodextrin, β-Cycloamylose, β-Dextrin, Cycloheptaamylose; Cycloheptaglucan, Cyclomaltoheptose.

Nonproprietary names

BP: Betadex PhEur: Betadexum USPNF: Betadex

Chemical name and CAS registry number: β-Cyclodextrin [7585-39-9]

Empirical formula and molecular weight: β-Cyclodextrin $C_{42}H_{70}O_{35}$= 1135

Description:

Cyclodextrinsoccuraswhite, practicallyodorless, finecrystallinepowders, having a slightly sweet taste. Some cyclodextrin derivatives occur as amorphous powders.

Compressibility: 21.0–44.0% Density (bulk): 0.523 g/cm^3

Density (tapped): 0.754 g/cm^3

Melting point: 255–265°C

Solubility:

It Is soluble 1 in 200 parts of propyleneglycol , 1 in 50parts of water at 20°C,1 in 20parts of waterat50°C.It is practically insoluble in acetone,ethanol(95%v/v),and methylene chloride.

Functional category:

Solubilizing agent; stabilizing agent.

Incompatibilities:

The activity of some antimicrobial preservatives in aqueous solution can be reduced in the presence of hydroxypropyl-β-cyclodextrin.

Safety:

B-Cyclodextrins are considered to be nontoxic when administered orally.

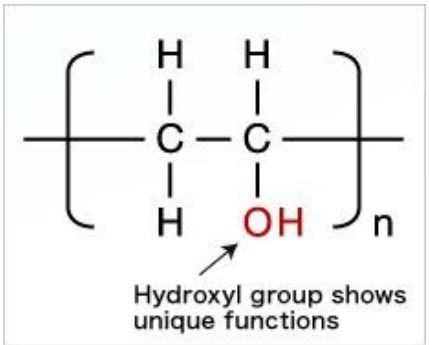
2. POLYVINYL ALCOHOL (PVA):

Polyvinyl Alcohol (PVA) is an environmental friendly and water soluble synthetic polymer with excellent film forming property, and emulsifying properties and outstanding resistance to oil, grease, and solvents. It has been extensively used in adhesive, in textile warp sizing and finishing, in paper size and coating, in the manufacturing of PVAc emulsion, in the suspension polymerization of PVC, and as binder for ceramics, foundry cores and various of pigment.

Polyvinyl alcohol (PVOH, PVA, or PVAl) is a water-soluble synthetic polymer. It has the idealized formula [CH2CH (OH)] n. It is used in papermaking, textiles, and a variety of coatings. It is white (colorless) and odorless. It is sometimes supplied as beads or as solutions in water.

Uses:

Polyvinyl alcohol is used as an emulsion polymerization aid, as protective colloid, to make polyvinyl acetate dispersions. This is the largest market application in China. In Japan its major use is vinylon fiber production. As a surfactant for the formation of polymer encapsulated nanobeads.



Structure of PVA

3. ETHYL CELLULOSE:

Synonyms:

Aquacoat ECD; Aqualon; Ashacel; E462; Ethocel; ethylcellulosum; Surelease.

Chemical name and CAS registry number:

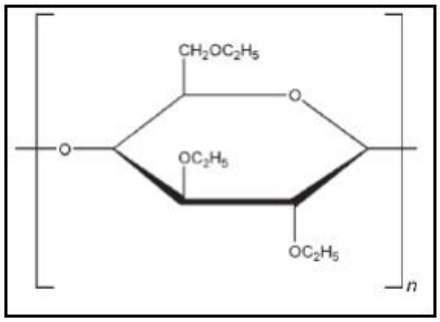
Cellulose ethyl ether [9004-57-3]

Empirical formula and molecular weight:

Ethyl cellulose is partially ethoxylated. Ethyl cellulose with complete ethoxyl substitution (DS = 3) is C12H23O6(C12H22O5)nC12H23O5 where n can vary to provide a wide variety of molecular weights. Ethyl cellulose, an ethyl ether of cellulose, is a long-chain polymer of hydro glucose units joined together by acetal linkages.

Description:

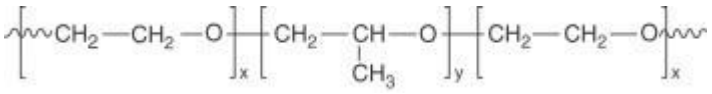
Ethyl cellulose is a tasteless, free-flowing, white to light tan-colored powder. Stability and storage conditions ethyl cellulose is a stable, slightly hygroscopic. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic materials than are cellulose esters. Ethylcellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures. This may be prevented by the use of antioxidant and chemical additives that absorb light in the 230–340 nm range. Ethylcellulose should be stored at a temperature not exceeding 320C (900 F) in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.



Structure of Ethyl Cellulose

4.Pluronic F127

Pluronics represent an important class of biomedical polymers. They are unique materials composed of triblock PEO–PPO–PEO copolymers of poly (ethylene oxide) (PEO) and poly (propylene oxide) (PPO). The Pluronic PEO block is hydrophilic and water soluble while the PPO block is hydrophobic and water insoluble. In an aqueous environment, these block copolymers self-assemble into micelles with a hydrophobic PPO center core and a hydrophilic PEO outer shell that interfaces with water. Since these micelles are amphiphilic, they are able to accommodate lipophilic molecules in the central hydrophobic core area. Consequently, pluronic micelles are effectively used as drug carriers because their assemblies can act as passive drug containers. These assemblies deliver drugs into subcellular compartments by slowly releasing hydrophilic–hydrophobic encapsulated excipients into physiological fluids.



Structure of Pluronic F127

Description: Non-ioniccontains100ppmBHT.

Linear Formula: (C3H6O·C2H4O)x

CAS Number: 9003-11-6

Molecular weight: 12600g/mol

METHODOLOGY:

Pre-formulation studies:

Prior to the development of nanosponge dosage form, it is essential that certain fundamental physical and chemical properties of the drug molecule alone and when combined with excipients are determined. This first learning phase is known as pre-formulation. The overall objective of the pre-formulation is to generate information useful to the formulator in

developing stable and bioavailable dosage forms which can be mass produced.

The goals of pre-formulation studies are:

- To evaluate the drug substance analytically and determine its necessary characteristics
- To establish its compatibility with different excipients.

Solubility studies:

Solubility of Apremilast was carried out in different solvents like- 0.1N HCL, 7.4pH buffer and 6.8 pH buffer, and also in organic solvents like Ethanol, Dichloromethane, DMSO. Solubility studies were performed by taking excess amount of drug in different beakers containing the solvents. The mixtures were shaken for 24 hrs at regular intervals. The solutions were filtered by using whatmann's filter paper grade no. 41. The filtered solutions were analyzed spectrophotometrically.

Determination of Melting Point:

Melting point of Apremilast was determined by capillary method. Fine powder of Apremilast was filled in glass capillary tube (previously sealed at one end). The capillary tube was tied to thermo meter and the thermometer was placed in the Thais tube and this tube was placed on fire. The powder at what temperature it melted was noticed.

Determination of absorption maximum (λ_{max}):

The wavelength at which maximum absorption of radiation takes place is called as λ_{max}. This λ_{max} is characteristic or unique for every substance and useful in identifying the substance. For accurate analytical work, it is important to determine the absorption maxima of the substance under study. Most drugs absorb radiation in ultraviolet region, as they are aromatic or contain double bonds.

Accurately weighed 10 mg Apremilast separately was dissolved in 10 ml of dichloromethane in a clean 10ml volumetric flask. The volume was made up to 10ml with the same which will give stock solution-I with concentration 1000µg/ml. From the stock solution-I, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using 6.8 pH buffer to obtain stock solution-II with a concentration 100 µg/ml. From stock solution-II, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using 6.8 pH buffer to get a concentration of 10µg/ml. This solution was then scanned at 200-400nm in UV-Visible double beam spectrophotometer to attain the absorption maximum (λ-max).

Construction of calibration curve using 6.8pH buffer :

Accurately weighed 10mg Apremilast was dissolved in dichloromethane taken in a clean 10ml volumetric flask. The volume was made up to 10ml with 6.8 pH buffer which gives a concentration of 1000µg/ml. From this standard solution, 1ml was pipette out in 10ml

volumetric flask and volume was made up to 10ml using 6.8 pH buffer to obtain a concentration of 100µg/ml. From the above stock solution, aliquots of 0.5, 1.0, 1.5, 2.0 , 2.5 and 3 ml each was transferred to a separate 10ml volumetric flask and solution was made up to 10ml using 6.8 pH buffer to obtain a concentration of 5, 10, 15, 20, 25, & 30µg/ml respectively. The absorbance of each solution was measured at 230 nm.

Construction of calibration curve using 0.1N HCL:

Accurately weighed 10mg Apremilast was dissolved in dichloromethane taken in a clean 10ml volumetric flask. The volume was made up to 10ml with 0.1N HCl which gives a concentration of 1000µg/ml. From this standard solution, 1ml was pipette out in 10ml volumetric flask and volume was made up to 10ml using 0.1N HCl to obtain a concentration of 100µg/ml. From the above stock solution, aliquots of .5, 1.0, 1.5, 2.0 , 2.5 and 3 ml each was transferred to a separate 10ml volumetric flask and solution was made up to 10ml using 0.1N HCl to obtain a concentration of 5, 10, 15, 20, 25, & 30 µg/ml respectively. The absorbance of each solution was measured at 230 nm.

Drug excipient compatibility study:

The drug and excipient compatibility was observed using Fourier Transform – Infra Red spectroscopy (FT-IR). The FT-IR spectra obtained from Bruker FT-IR Germany (Alpha T) was utilized in determining any possible interaction between the pure drug and the excipients in the solid state. The potassium bromide pellets were prepared on KBr press by grounding the solid powder sample with 100 times the quantity of KBr in a mortar. The finely grounded powder was then introduced into a stainless steel die and was compressed between polished steel anvils at a pressure of about $8t/in^2$. The spectra were recorded over the wave number of 4000 to $400cm^{-1}$.

Method of Preparation of Nanosponges by solvent Evaporation method:

Nanosponges using different proportions of β-cyclodextrin, ethyl cellulose, Pluronic F127 as rate retarding polymer and co-polymers like polyvinyl alcohol were prepared by solvent evaporation method. Disperse phase consisting of Apremilast and requisite quantity of PVA dissolved in 20 ml solvent (Dichloromethane) was slowly added to a definite amount of PVA in 40ml of aqueous continuous phase, prepared by using magnetic stirrer. The reaction mixture was stirred at 1000 RPM on a magnetic stirrer for 2 hours and kept on hot plate upto complete removal of organic solvent from the formulation. The Nanosponges formed were collected by filtration through whatman filter paper and dried.

Formulation table of Apremilast loaded Nanosponges using solvent evaporation method

Excipients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Apremilast (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
β-cyclodextrin (g)	0.5	1.0	1.5	2.0	--	--	--	--	--	--	--	--
Ethyl Cellulose (g)	--	--	--	--	0.5	1.0	1.5	2.0	--	--	--	--
Pluronic F127 (g)	--	--	--	--	--	--	--	--	0.5	1.0	1.5	2.0
PVA (mg)	500	500	500	500	500	500	500	500	500	500	500	500
Dichloromethane	20	20	20	20	20	20	20	20	20	20	20	20
Water (mL)	40	40	40	40	40	40	40	40	40	40	40	40

Evaluation of Nanosponges:

Entrapment efficiency;

The 30mg of the Apremilast weight equivalent nanosponge was analyzed by dissolving the sample in 10ml of dichloromethane. After the drug was dissolved 10ml of clear layer of dissolved drug is taken. Thereafter the amount of drug in the water phase was detected by a UV-Spectrophotometric method at 230 nm (U.V Spectrophotometer). The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase from the total amount of the drug in the Nanosponges. The entrapment efficiency (%) of drug was calculated by the following equation.

$$\% \text{ of Drug entrapment} = \frac{\text{Mass of drug in nanosponge}}{\text{Mass of drug used in formulation}} \times 100$$

Particle size measurement:

The particle size was determined using the particle size analyzer (Zeta sizer Nano series, UK). The formulations were diluted with an appropriate volume of phosphate buffer solution (PBS, pH 6.8). The measurements were carried out three times where the mean value was used.

Scanning electron microscopy:

The morphological features of prepared Nanospongess are observed by scanning electron microscopy at different magnifications.

Dissolution study:

For the oral dosage forms the in vitro dissolution study must be conducted in the dissolution medium which simulate the in-vivo conditions (actual physiological conditions). The in vitro drug release studies for the prepared formulation were conducted for a period of 12 hrs using an Electro lab model dissolution tester USP Type-1 apparatus (rotating basket) set at 50 RPM and a temperature of 37± 0.5°C weight equivalent to 30mg of Apremilast nanosponge was filled in capsule and kept in basket apparatus and placed in the 900ml of the medium. At specified intervals 5ml samples were withdrawn from the dissolution medium and replaced with fresh medium to keep the volume constant. The absorbance of the sample solution was analyzed at 230 nm for the presence of model drug, using a UV-visible spectrophotometer.

Zero-order model:

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation

$$\mathbf{Qt = Q0 + K0t}$$

Where Qt is the amount of drug dissolved in time t, Q0 is the initial amount of drug in the solution (most times, Q0 = 0) and K0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

Application: 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 Model:

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

Release behavior generally follows the following first order equation:

$$\textbf{Log C= Log } C_o\textbf{-kt/2.303}$$

Where C is the amount of drug dissolved at time t,

C_o is the amount of drug dissolved at t=0 and

k is the first order rate constant.

A graph of log cumulative of % drug remaining vs time yields a straight line.

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 model: The first example of a mathematical model aimed to describe drug release from a system 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

- 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.

In a general way the Higuchi model is simply expressed by following equation

$$Q = K_H - t^{1/2}$$

Where, K_H is the Higuchi dissolution constant.

The data obtained were plotted as cumulative percentage drug release versus 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.

Korsmeyer-Peppas model: 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, first60% drug release data were fitted in Korsmeyer-Peppas model,

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

where Mt / M∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices.

In this model, the value of n characterizes the release mechanism of drug as described in the following table.

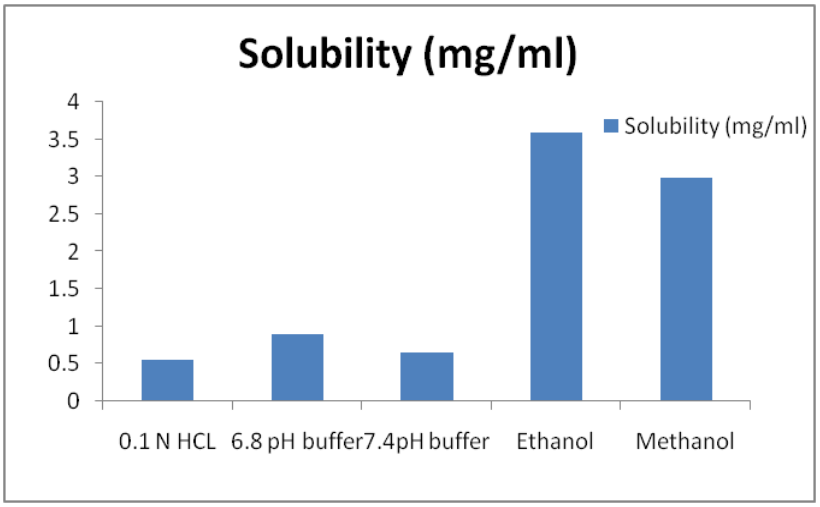
Drug transport mechanisms suggested based on 'n' value.

S. No	Release exponent	Drugtransportmechanism	Rate as a function of time
1	0.5	Fickian diffusion	$t^{-0.5}$
2	0.45 < n = 0.89	Non -Fickian transport	t^{n-1}
3	0.89	Case II transport	Zero order release
4	Higher than 0.89	Super case II transport	t^{n-1}

RESULTS AND DISCUSSION:

Solubility Studies of APREMILAST

Buffer	Solubility (mg/ml)
0.1 N HCL	0.549
6.8 pH buffer	0.896
7.4pH buffer	0.658
Ethanol	3.584
Methanol	2.986

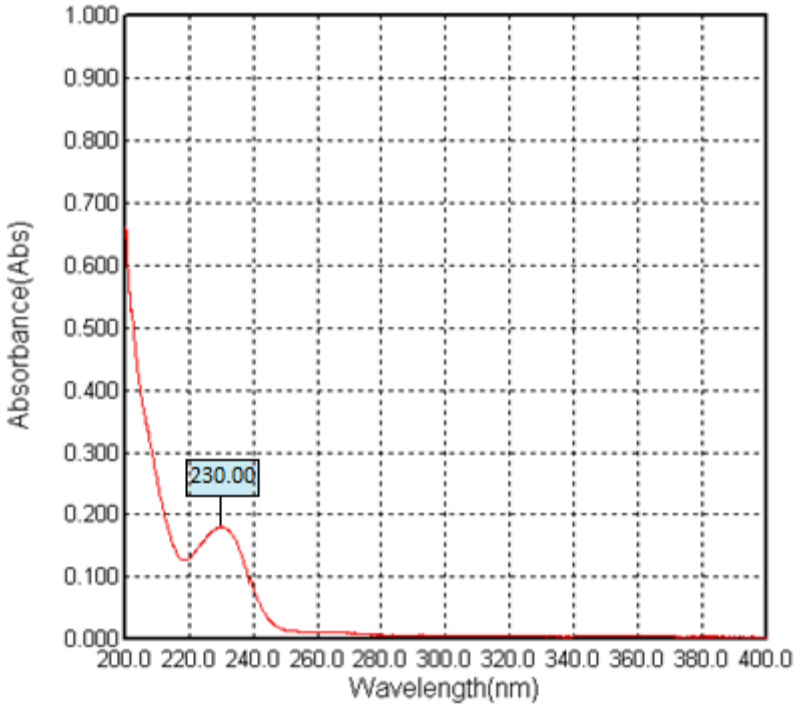


Solubility Studies of APREMILAST

Determination of melting point

The melting point of APREMILAST was found to be 156.1° C which was determined by capillary method.

Determination of absorption maximum (λmax): Determination of Apremilast λ-max was done in 6.8 pH phosphate buffer for accurate quantitative assessment of drug dissolution rate.

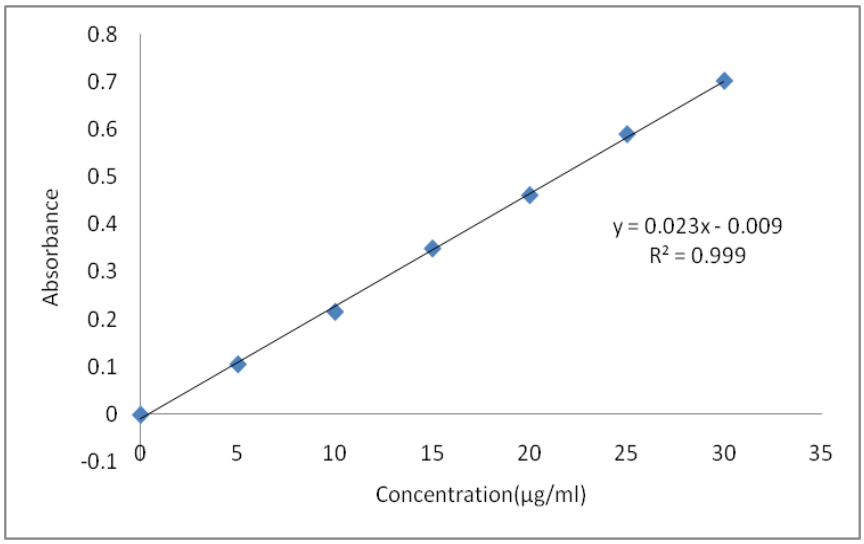


λ-max in 6.8 phosphate buffer

Calibration curve:

Calibration curve data of APREMILAST in 0.1N HCL:

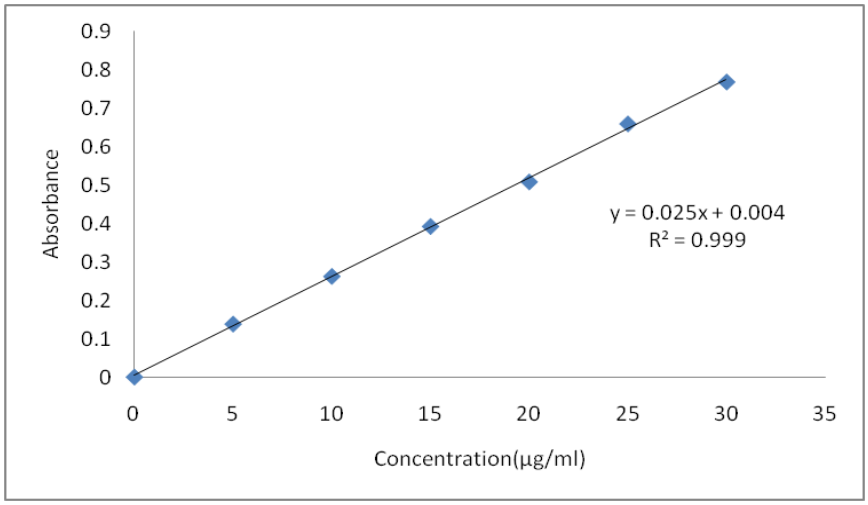
Concentration (µg/mL)	Absorbance
0	0
5	0.106
10	0.216
15	0.349
20	0.461
25	0.589
30	0.701



Calibration curve data of APREMILAST in 0.1N HCL

Calibration curve of APREMILAST in 6.8 pH buffer:

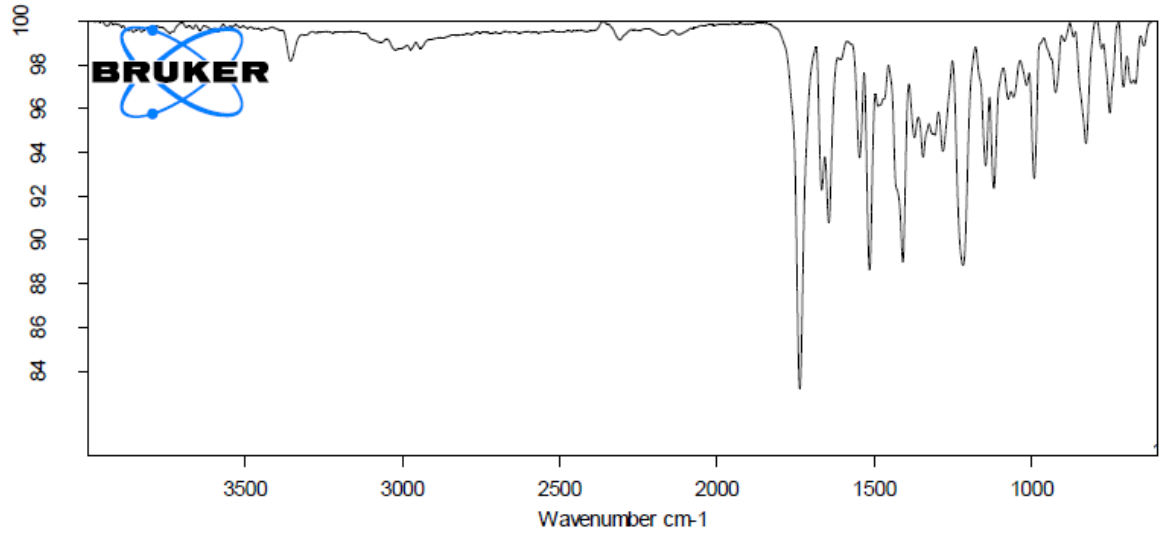
Concentration (µg/mL)	Absorbance
0	0
5	0.138
10	0.262
15	0.392
20	0.508
25	0.659
30	0.768



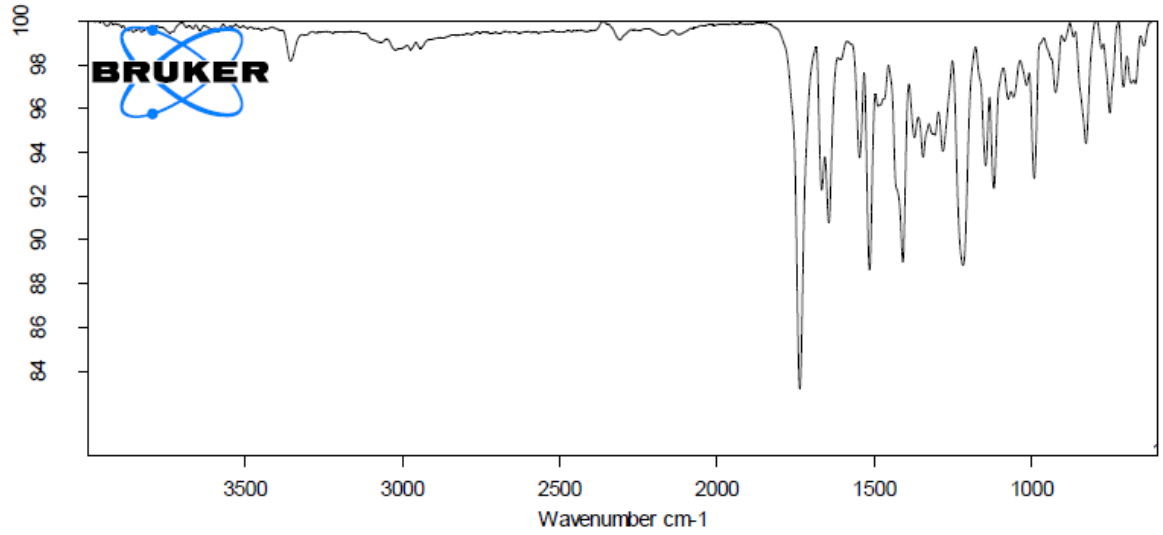
Calibration curve of APREMILAST in 6.8 pH buffer

FTIR Drug excipient compatibility :

Drug and excipient compatibility was confirmed by comparing spectra of FT-IR analysis of Pure drug with that of various excipients used in the formulation.



FTIR Spectra of Pure Drug



FTIR Spectra of Optimized Formulaton

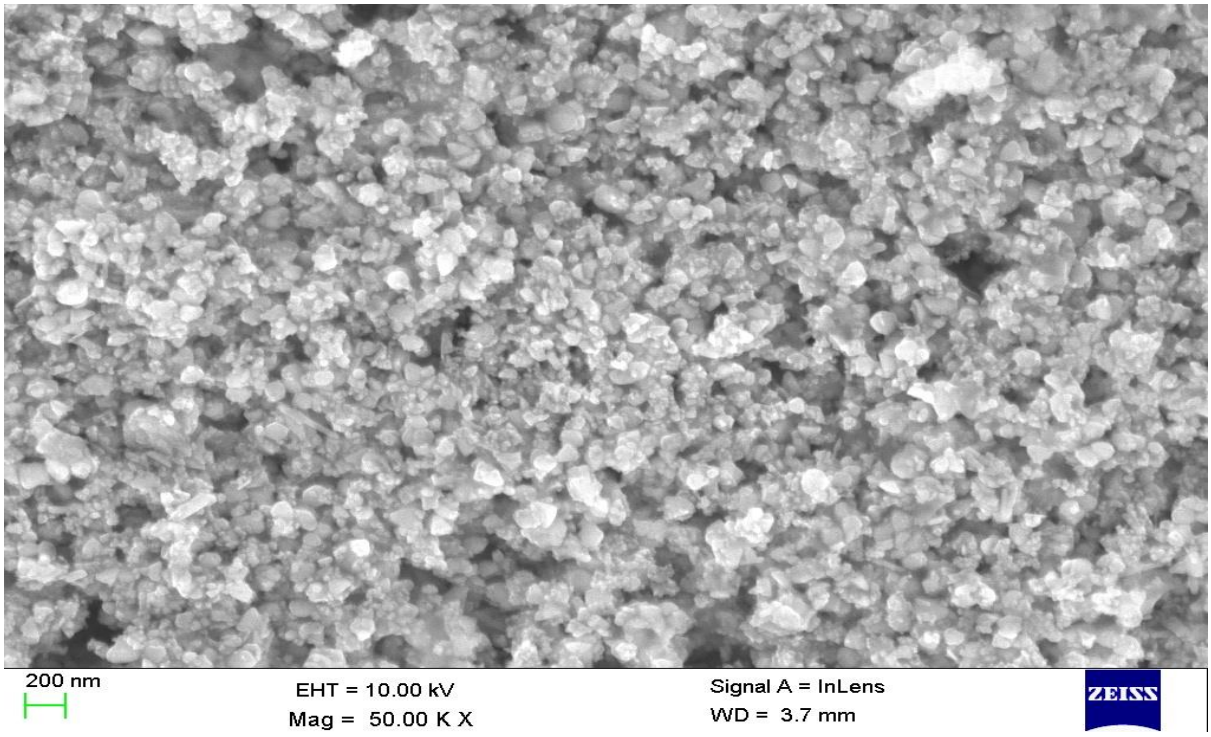
Particle size analysis of Nanosponges:

The particle size of the Nanosponge was determined by optical microscopy and the Nanosponges were found to be uniform in size. The average particle size of all formulations ranges from 200 nm to 600 nm which is in increasing order due to the increase in the concentration of polymer but after certain concentration it was observed that as the ratio of

drug to polymer was increased, the particle size decreased. This could probably be due to the fact that in high drug to polymer ratio, the amount of polymer available per Nanosponge was comparatively less. Probably in high drug-polymer ratios less polymer amounts surround the drug and reducing the thickness of polymer wall and Nanosponges with smaller size were obtained. By performing the particle size analysis, it is concluded that the formulation has the particle size varies with the concentration of polymer drug ratio.

B) Morphology determination by scanning electron microscopy (SEM):

Scanning electron microscopy (SEM) was used to determine the Morphology of the prepared Nanosponges. SEM is useful for characterizing the morphology and size of microscopic specimens with particle size as low as 10 -10 to 10 -12 grams. The sample was placed in an evacuated chamber and scanned in a controlled pattern by an electron beam. Interaction of the electron beam with the specimen produces a variety of physical phenomena that, when detected, are used to form images and provide elemental information about the specimens.



Nanosponges structure optimized formulation (F9)

Entrapment efficiency:

It is calculated to know about the efficiency of any method, thus it helps in selection of appropriate method of production. After the preparation of formulations the Practical yield was calculated as Nanosponges recovered from each preparation in relation to the sum of starting material (Theoretical yield). It can be calculated using following formula.

$$\textbf{Entrapment efficiency} = \frac{\text{Practical yield}}{\text{Theoretical yield (drug + polymer)}} \times 100$$

Table: Entrapment Efficiency F1-F12

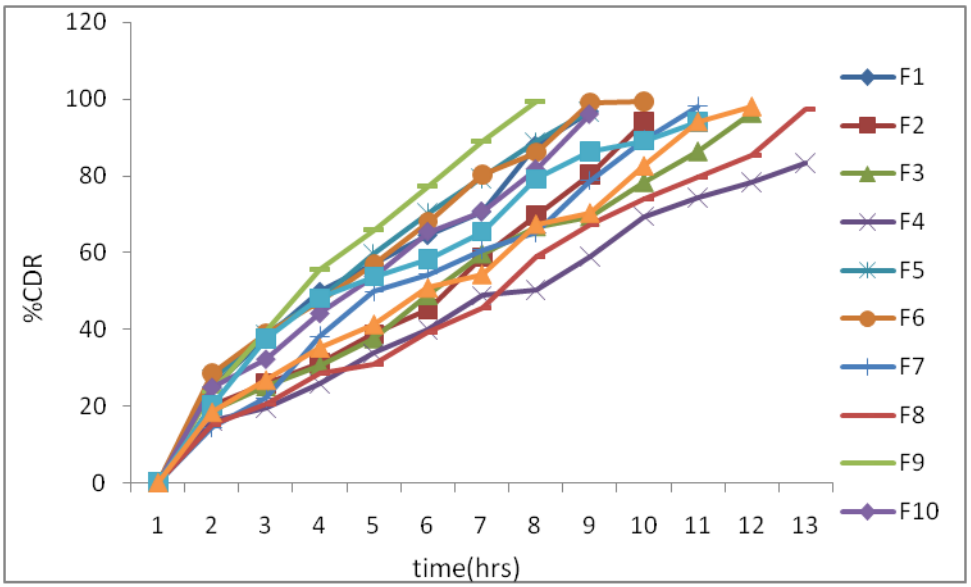
Formulation code	% Entrapment Efficiency
F1	91.68
F2	97.22
F3	95.12
F4	97.88
F5	96.28
F6	95.64
F7	98.88
F8	96.23
F9	97.85
F10	98.66
F11	99.64
F12	99.90

In vitro dissolution studies of prepared Nanosponges:

In vitro release studies were performed in triplicate using USP basket method at 50 rpm and 37±0.2^{o}C in 900ml of 0.1N HCl for 2hrs and remaining hours in phosphate buffer (pH 6.8). 10 mg of the formulated Nanosponges is used for each experiment. Samples were taken at appropriate time intervals for 1,2,3,4,5,6,7,8,9,10,11, & 12 hour. The samples were measured spectrophotometrically at 230 nm. Fresh dissolution medium was replenished each time when sample is withdrawn to compensate the volume.

Table: Percentage of drug release of Nano-sponges (F1-F12)

Time (hrs)	%CDR											
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**
0	0	0	0	0	0	0	0	0	**0**	0	0	0
1	24.87	20.03	18.69	15.94	26.89	28.43	14.45	15.05	**23.63**	24.78	20.09	18.34
2	37.86	25.84	25.13	19.38	38.75	38.97	22.09	20.35	**39.42**	32.12	37.47	26.75
3	49.81	31.17	30.48	25.79	47.89	47.12	38.16	28.43	**55.76**	44.05	48.07	35.12
4	57.09	38.75	37.61	33.63	59.75	56.89	49.89	30.87	**65.98**	53.72	53.63	41.28
5	64.48	45.18	48.82	39.78	70.29	68.06	54.32	39.12	**77.39**	65.34	58.27	50.79
6	70.62	58.78	59.68	48.69	79.42	80.24	60.78	45.68	**89.24**	70.65	65.39	54.31
7	87.91	69.69	66.79	50.12	88.79	86.09	65.25	58.89	**99.52**	81.67	79.15	67.38
8	96.73	80.27	69.39	58.79	96.89	99.06	78.81	67.34		95.95	86.36	70.34
9		94.08	78.32	69.42		99.35	89.39	74.03			89.09	82.63
10			86.41	74.24			98.12	79.69			94.02	94.16
11			96.43	78.23				85.37				98.12
12				83.32				97.42				



Percentage cummulative drug release graph F1-F12

Kinetics Analysis for F9:

Zero order:

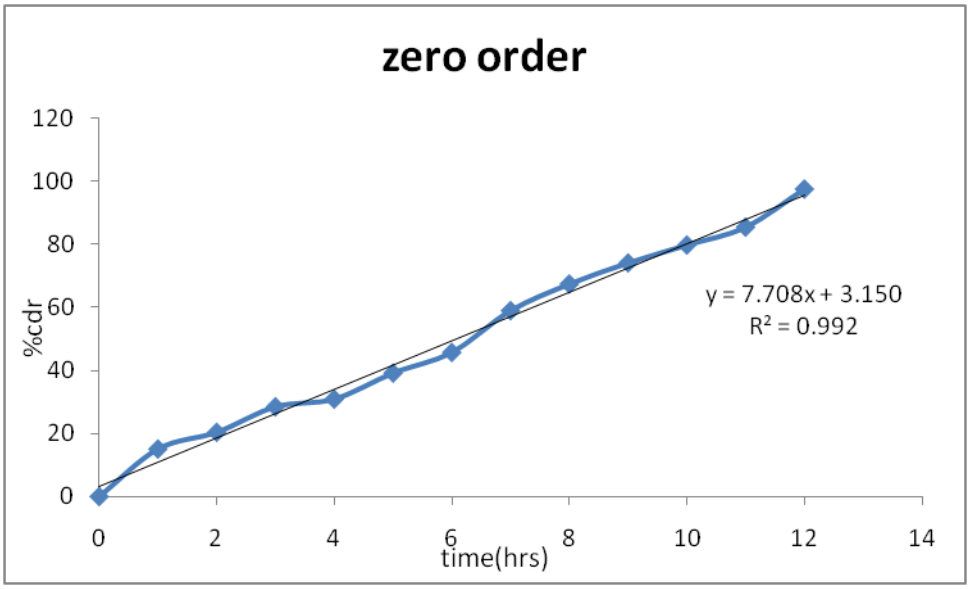


Fig 6.12: Zero Order graph for F9

First order:

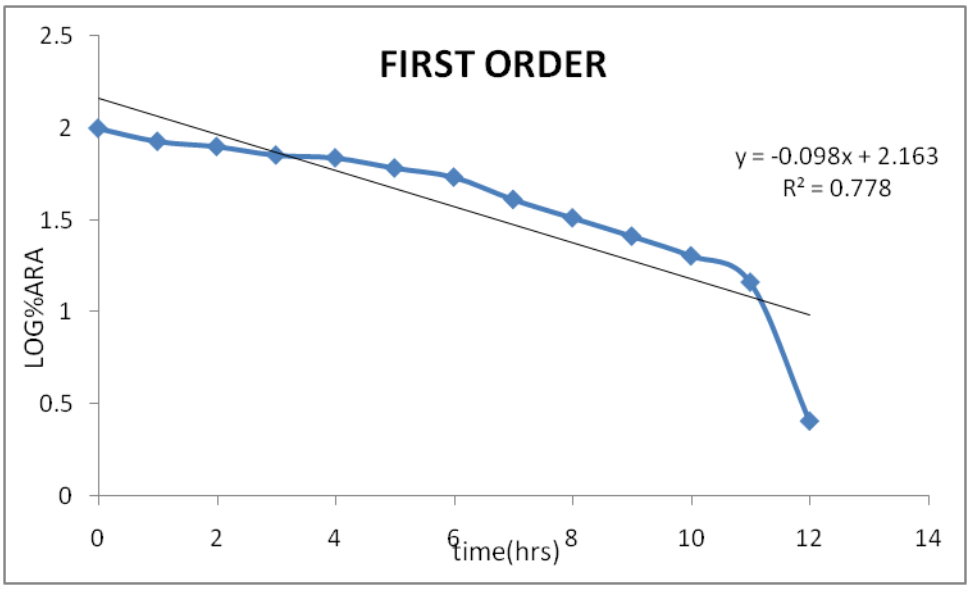


Fig 6.13: First Order graph for F9

Higuchi plot:

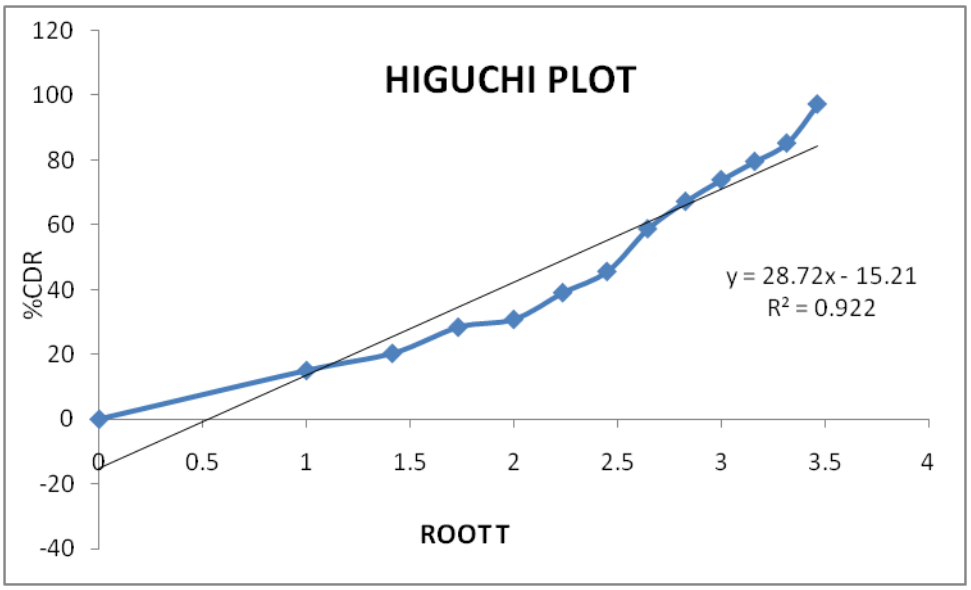


Fig 6.14: Higuchi Plot for F9

Peppas plot:

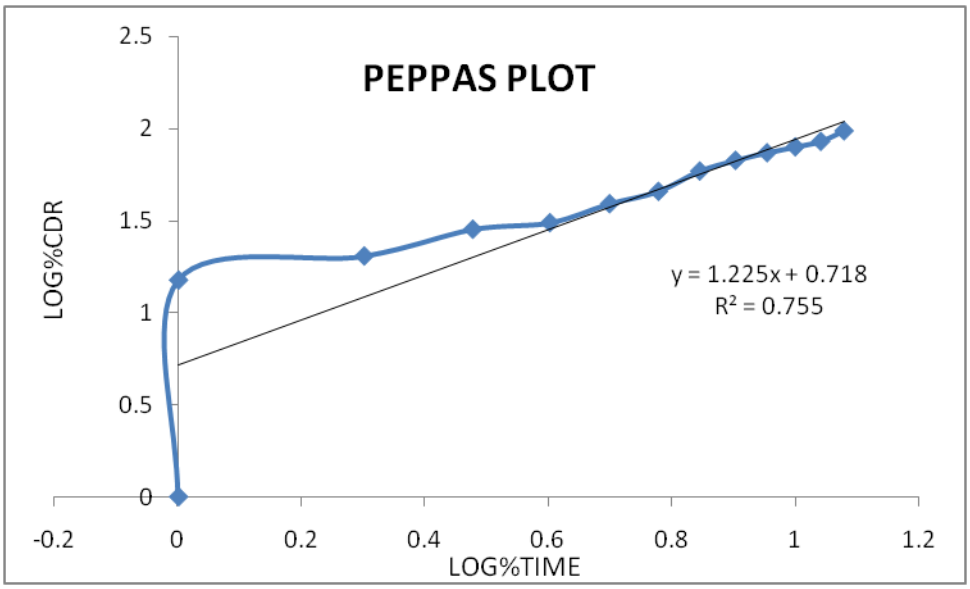


Fig 6.15 : Peppas Plot for F9

Regression values of F9:

Table : Regression values

Formulation Code	Zero order	First order	Higuchi	Peppas	Peppas
	R^2	R^2	R^2	R^2	n
F9	0.992	0.778	0.922	0.755	1.225

SUMMARY & CONCLUSSION:

The Apremilast Nanosponges were prepared by solvent evaporation method using ethyl cellulose, β-cyclodextrin and Pluronic F127, as rate retarding polymers, PVA and methanol as crosslinking agents. **F9** have its entrapment efficiency is 97.85%, drug release 99.52% in 12 hour, The optimized formulation F8 has coefficient of determination (R^2) values of 0.992, 0.778, 0.922, 0.755 for Zero order, First order, Higuchi, Korsmeyer Peppas respectively. A good linearity was observed with the Zero order, the slope of the regression line from the

Higuchi plot indicates the rate of drug release through the mode of diffusion and to further confirm the diffusion mechanism, data was fitted into the Korsmeyer Peppas equation which showed linearity with n value of 1.225 for optimized formulation. Thus 'n' value indicates the supercase II transport mechanism.

Based upon the preliminary data and in vitro dissolution studies of Apremilast Nanosponges it was concluded that the formulation Nanosponges was successfully formulated by using Drug: Ethyl cellulose in 1:4 ratio.

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