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Formulation and Development of Nanosuspension as an Alternative Approach for Solubility and Dissolution Enhancement of Aceclofenac

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Abstract

Main objectives to develop Aceclofenac Nanosuspension are to enhance solubility and dissolution rate of poorly soluble Drug (Aceclofenac), substantially leading to its bioavailability enhancement &Improvement of aqueous and saturation solubility in turn rapid release of Drug which leads to enhancing therapeutic efficacy. Aceclofenac Nanosuspension was prepared by quasi solvent evaporation method with help of different polymer and concentration. There was changes polymer ratio, volume of organic solvent and stirring speed. Aceclofenac nanosuspension gives immediate release. Aceclofenac nanosuspension were showing highest dissolution rate within 10 minutes comparison with marketed formulations. Aceclofenac Nanosuspension compacts may enhance aqueous solubility and dissolution rate in compare to other solubility enhancement technique hence, this research work may be useful to formulate Aceclofenac Nanosuspension which may give rapid onset of action by rapid absorption, maximize efficacy, dose frequency & hence increase patient Compliance.

Keywords: Aceclofenac, Nanosuspension, solubility enhancement, dissolution enhancement.

1. Introduction

Method by which solution is passing on can essentially influence its feasibility. Some drug has perfect obsession range inside most compelling favourable position. On other hand, moderate progression in ampleness of treatment of couple diseases, and suggested growing need of multidisciplinary approach to manage movement of therapeutics to center in tissue. From this new thought to controlling pharmacokinetics, pharmacodynamic, non-specific destructiveness, immunogenicity to be made. This new thought called as novel solution transport system. In it is planned to achieve reliable movement of pharmaceutical at obvious and reproducible vitality over opened up time span accessible for use. Blend of bolstered release and controlled release property in movement structure would encourage improve remedial effigy.

1.2 Introduction to Nanotechnology

Nanotechnology is craft of matter and material that game plan with particle size in nanometres. "Nano" is gotten from Latin word, which suggests diminutive person ($1nm=10^{-9}m$). Nanomedicine oversees extensive checking, control, improvement, and repair, monitor and upgrade human natural structure at nuclear level using outlined nanostructures and Nano devices. Pharmaceutical

nanotechnology handles employments of nanoscience to medication store as nanomaterials, and as devices like medicine movement, decisive, imaging and biosensor materials. Pharmaceutical nanotechnology has given all more balanced conclusion and focused treatment of ailment at sub-nuclear level. It helps in perceiving antigen associated with diseases, for instance, harm, diabetes mellitus, neurodegenerative sicknesses, and likewise distinguishing microorganisms and disease associated with pollutions. In medication store size decline has crucial application as prescriptions in nanometre size achieve overhaul execution in arrangement of estimations structures.

Nanotechnology gives number of positive circumstances in medication store by;

1) Extended surface locale
2) Enhanced dissolvability
3) Extended rate of crumbling
4) Extended in oral bioavailability
5) Less measure of dose required and lessens amount of doses
6) Protection of prescription from corruption
7) All quicker onset of medicinal action
8) Achievement of prescription concentrating on
9) Latent centering of drugs to macrophages present in liver and spleen.

Diverse sorts of DDS using Nanotechnology Principle:

1) Nanosuspension
2) Solid lipid nanosuspensions
3) Nanocrystals
4) Nanosuspensions
5) Nanoemulsion

1.3 Introduction to Nanosuspension:

Pharmaceutical Nanosuspension is portrayed as finely scattered solid drug particles in liquid vehicle. Atom size in Nanosuspension extends some place around 200 and 600nm. Scrambling of drug Nanocrystals in liquid media prompts "Nanosuspension". Scrambling media can be water, liquid plans or non-watery media (e.g., liquid polyethylene glycol (PEG), oils).

Nanosuspensions have positive circumstances of:

1) Increase in crumbling pace and drenching dissolvability of medicine
2) Improved natural execution
3) Ease of creation and scale-up
4) Long-term physical soundness
5) Versatility
6) Increase in oral absorption
7) Improved dose proportionality [1-2]

1.3.1 Method of Nanosuspensions

Basically there are two methods for course of action of Nanosuspensions. Customary procedures for precipitation (Hydrosols) are called 'Base up development'.'Top down Technologies' are separating methodologies and are favored over precipitation strategies. ‘Top down Technologies' fuse Media Milling (Nanocrystals), High Pressure Homogenization in water (Disso cubes), High Pressure Homogenization in non-watery media (Nano pure) and mix of Precipitation and High-Pressure Homogenization (Nanodegree).

1) Bottom-up advancement
2) Top-down advancement

1.4 Formulation Consideration

1.4.1 Stabilizer

Essential limit of stabilizer is to wet prescription particles totally, and to keep Ostwald’s developing and agglomeration of Nanosuspensions with particular finished objective to yield physically stable arrangement by giving steric or ionic deterrent. Sort and apportion of parity pronouncedly influences physical strength and in vivo behavior of Nanosuspension. Stabilizers that have been used so far are poloxamer, polysorbate, cellulosic, procurements, and lecithin. Lecithin is stabilizer of choice if one intends to develop parentally commendable and auto-clavable Nanosuspension.

1.4.2 Organic Solvent

Characteristic solvents are used as part of arrangement of Nanosuspension if emulsions or littler scale emulsions are used as organization. pharmaceutically acceptable less risky water miscible dissolvable, for instance, methanol, ethanol, chloroform, isopropanol, and generally water miscible solvents ethyl acidic corrosive deduction, ethyl format, butyl lactate, triacetin, propylene carbonate, benzyl alcohol are height in arrangement over conventional dangerous solvents, for instance, dichloromethane.

1.4.3 Co-Surfactants

Choice of co-surfactant is fundamental while using littler scale emulsions to characterize Nanosuspensions. Since co-surfactants can extraordinarily affect stage direct effect of co-surfactant on uptake of internal stage for picked scaled down scale emulsion course of action and taking drugs stacking should be investigated. In spite of way that written work depicts use of bile salts and dipotassium glycerrhizinate as co-surfactants, distinctive solubilizes, for instance, Transcutol, glycofurol, ethanol and isopropanol, can be safely used as co-surfactants as part of enumerating of scaled down scale emulsions.

1.4.4 Other additives

Nanosuspensions may contain included substances, for instance, supports, salts, polyols, osmogent and Cryoprotectant.

1.5 Post-Production Processing

After era treatment of Nanosuspensions gets opportunity to be key when drug contender is exceptionally defenseless to hydrolytic cleavage or compound corruption. Taking care of may in like manner is required when best stabilizer is not prepared to offset Nanosuspension for more drawn out time period or there are pleasantness impediments concerning fancied course. Considering these perspectives, techniques, for instance, lyophilisation or sprinkle drying may be used to make dry powder of Nano-sized prescription particles. Sound decision must be made in these unit operations considering pharmaceutical properties and monetary points. Generally, shower drying is more calm and accommodating than Lyophilization. Effect of postproduction planning on particle size of Nanosuspension and suddenness substance of dried nano measured drug should be given due thought.[3-10]

1.6 Applications of Nanosuspension

1.6.1 Oral

Oral pharmaceutical movement is most comprehensively supported course of association of meds. In any case, couple of meds has issue of compelled bioavailability as result of poor dissolvability and absorption which finally reduces its sufficiency. In such cases, Nanosuspension can deal with issue as it associates in improving deterioration rate and ingestion in light of extended surface range and updated adhesiveness. Nanosuspension can provoke extended mucoadhesion which can increase gastrointestinal travel time and incite extended bioavailability. Redesign in oral bioavailability can be credited to extended surface zone, drenching dissolvability and adhesiveness of solution Nanosuspension. Taste covering of particulate structure is moreover successfully possible.

1.6.2 Parenteral

Nanosuspensions can be used to change ineffectually dissolvable non-injectable meds into definition suitable for intravenous association. Notwithstanding way that production of Nanosuspension for parenteral use is fundamental, current headways in this development have shown its utility as injectable definitions. Strategies used for availability of Nano-suspension are as of now precisely controlled, and can make uniform particles with better control over most great atom size. Distinctive examination reports are open which stretch fittingness of Nanosuspensions for parenteral association.

1.6.3 Ocular delivery

Nanosuspension can wind up being safe house for solutions that show poor dissolvability in lachrymal fluids. Nanosuspensions address flawless strategy for visual movement of hydrophobic solutions in light of their natural ability to upgrade drenching dissolvability of prescriptions. Kassem *et al* have made Nanosuspension transport structure for certain glucocorticoid drugs.

1.6.4 Pulmonary

Nanosuspensions can be beneficial for passing on sedates that show poor dissolvability in pneumonic discharge. At present open procedures for aspiratory movement, for instance, pressurized canned items or dry powder inhalers have certain deterrents, for instance, compelled spread at required site, less living plan time et cetera, which can be overcome by Nanosuspensions. Fluticasone and budesonide have been successfully nitty gritty as Nanosuspension for aspiratory Delivery

1.6.5 Dermal

Nano-crystalline structure has extended drenching dissolvability achieving redesigned dispersal of medicine into skin. Nanocrystals also indicate distinctive properties, for instance, extended penetration into film, overhauled pervasion and bio-adhesiveness which could be to great degree accommodating for dermal application.

1.6.6 Targeting

Uptake of pharmaceutical nanosuspensions depends on upon their atom size. By changing surface properties of nanosuspensions, their in vivo behavior can be balanced and can be used as engaged transport system. phagocytosis uptake of nano-valuable stones can be avoided by preparing stealth nanocrystals or by arranging keen pearls i.e. drug particles underneath atom size of 100nm, which can be used as concentrated taking drugs movement structure. In light of methodology ease, change of nanosuspension is mechanically suitable decision for cantered transport. Surface properties of particles, for instance, surface hydrophobicity, charge, closeness and centralization of certain valuable social occasions choose its organ flow. Atovaquone nano-valuable stones secured with Tween 80 were used to treat toxoplasmosis; parasites could be successfully pulverized in cerebrum.

1.7 Mucoadhesion of Nanosuspensions

Nanosuspensions orally controlled as suspension diffuse into liquid media and rapidly encounter mucosal surface. Particles are immobilized at intestinal surface by hold framework insinuated as "bio connection." From this moment on concentrated suspension goes about as supply of particles and adsorption process happens rapidly. Prompt contact of particles with intestinal cells through bio-paste stage is underlying stride before atom ingestion.[11-15]

1.8 Marketed products

Table: 1 Marketed products of Nanosuspension

Product	Drug	Indication	Company
Triglide	Fenofibrae	Treatment of hypercholesterolemia	Firsthorizon pharmaceutical
Tricor	Fenofibrae	Treatment of hypercholesterolemia	Abbott
MEgace	Megestrol Aceyate	Appetite Stimulant	PAR Pharmaceutical
Rapamune	Sirolims	Immunosuppress	Wyeth

2. Materials and methods

Aceclofenac was received as a gift sample from Torrent Pharmaceutical Ltd., Ahmedabad. PVA, PVP K-30, Tween-80 & Acetone were received as a gift sample from Laboratory Sulab Reagent, Vadodara. Pluronic F68 was received as a gift sample from Colorcon Pt. Ltd., Goa.

2.1 Method of Preparation of Aceclofenac Nanosuspension

Nanosuspensions were prepared by Quasi-Emulsion Solvent Evaporation Method. Aceclofenac was dissolved in acetone at room temperature. This was poured into fixed amount of water containing fixed amount of different stabilizers at room temperature and subsequently stirred on magnetic stirrer to allow volatile solvent to evaporate (Remi, magnetic stirrer, India). Addition of organic solvents by means of syringe positioned with needle directly into stabilizer containing water. Organic solvents were left to evaporate off under slow magnetic stirring of Nanosuspension at room temperature for 1 hour.[39]

2.2 Characterization of Aceclofenac Nanosuspension

2.2.1 Particle size

Particle size was determined by photon correlation spectroscopy (PCS) using Zetasizer 3000 (Malvern Instruments, UK). This analysis yields mean diameter (z-average, measuring range: 20–1000 nm). All data presented are mean values of three independent samples produced under identical production conditions.

2.2.2 Drug content

Prepared Nanosuspensions was analyzed for drug content by UV spectroscopic method. Different batches of nanosuspensions equivalent to 20 mg of Aceclofenac weighed accurately and dissolve in 10 ml acetone. Stock solutions will be diluted with distilled water and analyze by UV spectroscopy at 275 nm.

2.2.3 Saturation solubility studies

Saturation solubility measurements were analyzed by ultraviolet absorbance determination at 275 nm using Shimadzu UV-Visible spectrophotometer. Saturation solubility studies will be carried out for both unprocessed pure drug and different batches of lyophilize Nanosuspension. 10 mg of unprocessed pure drug and Nanosuspension equivalent to 10 mg of Aceclofenac will be weighed and separately introduce into 25 ml stoppered conical flask containing 10 ml distilled water. Flasks will be seal and place in rotary shaker for 24 hrs at 37°C and equilibrate for 2 days. Samples will be collect after specific time interval, and it is filter and analyze. Dilute samples was analysed using UV spectrophotometer at 238 nm. Results were analyzed in triplicate and standard deviations are report.

2.3 *In vitro* Drug Release Study

Table: 2 In-vitro drug release study

Instrument	USP Dissolution apparatus
Type	Paddle method
Medium	900 ml Phosphate buffer pH 7.4
Temperature	37± 0.5ºC
RPM	50
Testing time	60 min
Amount withdrawn	5 ml
Λ max	275 nm
Sample	Nanosuspensions
Sample Withdrawal interval	Every 5 Min

2.4 Stability Analysis

Drug or dosage form quality may affect under impact of by varying temperature, humidity and light with time which can be found out by stability testing. It can be carried out at 25°C ± 2°C/ 60% RH ± 5% RH and 40°C ± 2°C/ 75% RH ± 5% RH for selected formulation for three months. Samples were withdrawn on 0^{th}, 10^{th}, 20^{th} and 30^{th} day and were analyzed for physical appearance and drug content.[34,39,40]

3. Result and Discussion

3.1 Solubility study of Aceclofenac

Aqueous solubility of Aceclofenac in powder form was determined by shake-flask method. Briefly, 20mg of Aceclofenac was suspended in 10ml of water; and suspensions were shaken at 37°C. Aliquots were withdrawn and filtered through0.22µm Whatmen filter. Filtered solution was suitably diluted and Aceclofenac concentration infiltrate was analyzed by UV analysis method at 275nm (Systronic 2203, Japan). For other solvents such as acetate buffer, phosphate buffer, HCl, acetone, methanol same procedure was followed.

Table 1: Solubility of aceclofenac in various surfactant and co-surfactant

Solvents	Solubility (mg/10 ml) (Mean ± S.D.) (n = 3)	Interpretation
Distilled Water	0.0079±0.08	Very Slightly soluble
pH 4.5 Acetate Buffer	0.131±0.14	Slightly soluble
pH 6.8 Phosphate Buffer	2.51±0.15	Soluble
pH 7.4 Phosphate Buffer	4.019±0.07	Soluble
0.1NHCL	0.039±0.25	Slightly soluble
Acetone	9.78±0.012	Freely soluble
Ethanol	8.48±0.079	Freely soluble

Aceclofenac was completely dissolved in Acetone and slightly dissolved in Water.

3.2 Identification of Drug- Aceclofenac by FT-IR Spectroscopy

Fourier–transform infrared (FT–IR) spectra of moisture free powdered samples of SS, its lyophilized nanosuspensions and PVPK-30 were obtained using spectrophotometer (FTIR- Shimadzu Co., Kyoto, Japan) by potassium bromide (KBr) pellet method. Scanning range was 400–4000 cm-1 and resolution was 1 cm^{-1}.

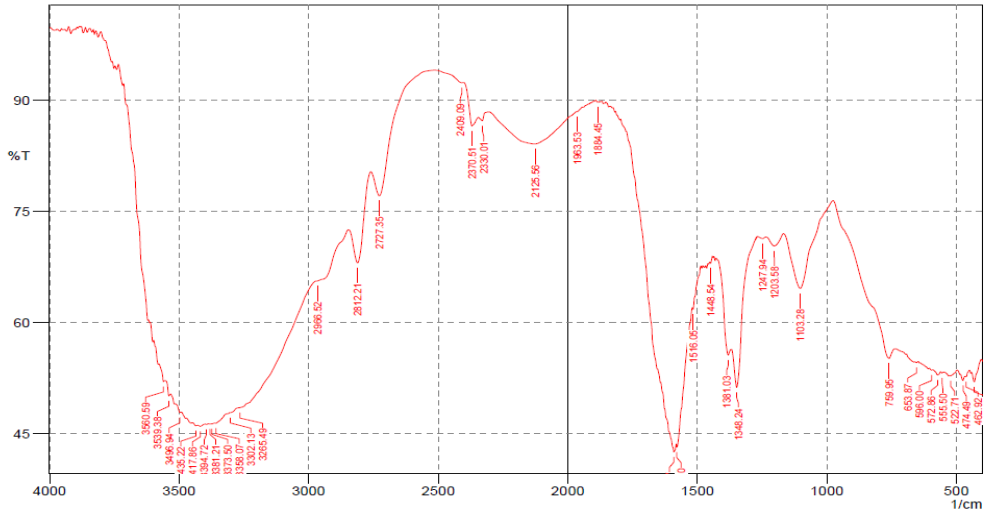


Figure 1: Identification of Pure Drug Aceclofenac + PVP + Tween 80 by IR Spectrum

3.3 Drug Compatibility Studies by DSC

DSC curves of commercial Aceclofenac Figure 5.4 shows broad endotherm ranging from 30 to 120°C indicating loss of water and sharp endotherm at 156.46 °C might be due to melting point of Aceclofenac. Obtained FT-IR spectrum and DSC graph compiles with standard data which further confirms identity and purity of Drug.

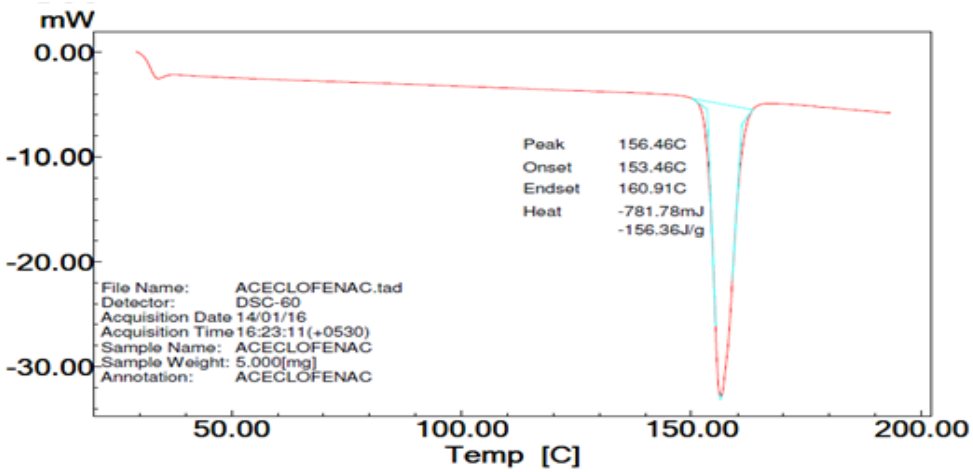


Figure 2: Drug Compatibility Studies by DSC

3.4 Preliminary Selection of Stabilizer Concentration

Table 4: Preliminary Selection of Stabilizer Concentration for Aceclofenac Nanosuspension

Batch	Type of Stabilizer	Stabilizer Concentration	Type of Organic Phase (mL)	Volume of Organic Phase (mL)	Stirring Speed (R.P.M.)
Preliminary selection of concentration of stabilizers					
ANS1	**Pluronic F- 68: Tween 80**	**2:1**	Acetone	5	1500
ANS2	**Pluronic F- 68: Tween 80**	**3:1**	Acetone	5	1500
ANS3	**Pluronic F- 68: Tween 80**	**4:1**	Acetone	5	1500
ANS4	**PVP K30: Tween 80**	**2:1**	Acetone	5	1500
ANS5	**PVP K30: Tween 80**	**3:1**	Acetone	5	1500
ANS6	**PVP K30: Tween 80**	**4:1**	Acetone	5	1500

Table 5: Characterization of Trial batches (ANS1 – ANS6) for Aceclofenac NSs

Batch No.	Drug Content (mg) (Mean± S.D.) (n=3)	Entrapment Efficiency (%) (Mean±S.D.) (n=3)	Free Drug Content (%)	% CDR (Mean ±S.D.) (n=3)
Preliminary selection of stabilizer concentration				
ANS1	15.704±0.26	70.52±1.41	29.48	78.26±0.43
ANS2	**17.478±0.56**	**79.41±1.59**	**20.59**	**85.98±0.69**
ANS3	**18.042±0.42**	**82.21±1.39**	**17.79**	**92.54±0.53**
ANS4	11.178±0.29	47.89±1.51	52.11	75.25±0.42
ANS5	13.646±0.41	60.23±1.35	39.77	81.69±0.54
ANS6	12.468±0.27	54.34±1.24	45.66	86.27±0.38

Nanoparticulate DDS regularly utilize stabilizers. Without suitable stabilizer, high surface vitality of particles in nano-reach can total to shape bigger particles, along these lines rendering definition shaky. Primary capacity of stabilizer is to wet medication molecule totally to repress agglomeration with another molecule. It gives stearic or ionic boundaries by method for which between particulate associations in nanosuspensions are counteracted. Be that as it may, convergence of stabilizer in Nanosuspension must be ideal. PVA has been observed to be extremely successful stabilizer for Nanoparticles created by different techniques. PVA or Polyvinyl Alcohol has both hydrophobic and hydrophilic parts compare acetic acid derivation and hydroxyl bunches separately. These assistance in getting retained and arranged at interface. Truth be told, viability of PVA in lessening interfacial strain is such that it even elevates to keeping up low molecule size of Nanoparticles. Indeed, even surfactants like Pluronic F-68 and Tween 80 have been used for decreasing interfacial pressure at surface of nanoparticles. Thus, ANS 2 and ANS3 have been chosen as improved clusters.

3.5 Preliminary Selection of Organic Solvent Volume

Table 6: Preliminary Selection of Organic Solvent Volume for Aceclofenac Nanosuspension

Batch	Type of Stabilizer	Stabilizer Concentration	Type of Organic Phase (mL)	Volume of Organic Phase (mL)	Stirring Speed (R.P.M.)
Preliminary selection of organic phase volume					
ANS7	Pluronic F- 68: Tween 80	3:1	Acetone	**5**	1500
ANS8	Pluronic F- 68: Tween 80	3:1	Acetone	**10**	1500
ANS9	Pluronic F- 68: Tween 80	3:1	Acetone	**20**	1500

Table 7: Characterization of Trial batches (ANS7 – ANS9) for Aceclofenac Nanosuspension

Batch No.	Drug Content (mg) (Mean± S.D.) (n=3)	Entrapment Efficiency (%) (Mean±S.D.) (n=3)	Free Drug Content (%)	% CDR (Mean ±S.D.) (n=3)
Effect of organic phase volume				
ANS7	16.446±0.48	72.23±1.33	27.77	86.57±0.38
ANS8	**16.908±0.32**	**74.54±1.21**	**25.46**	**89.68±0.62**
ANS9	13.476±0.36	67.38±1.56	32.62	88.17±0.48

Natural solvents are regularly utilized in definition of nanoparticles by assortment of strategies. Nonetheless, since numerous natural chemicals can be risky in nature, adequacy of natural dissolvable being utilized in detailing must be remembered as for its poisonous quality potential and simplicity of its expulsion from plan. Solvents, for example, ethanol, isopropanol, ethyl acetic acid derivation, butyl lactate, CH3)2CO, and triacetin and benzyl liquor are some which are thought to be satisfactory and accordingly utilized in definition. Thus, ANS 8 has been chosen as enhanced cluster. One of most essential things to be remembered while using natural solvents is their miscibility with water. More dissolvable is miscible and promptly diffusible with water, more compelling will be development of nanosuspension.

3.6 Preliminary Selection of Stirring Speed (RPM)

Table 8: Preliminary Selection of Speed (RPM) for Aceclofenac Nanosuspension

Batch	Type of Stabilizer	Stabilizer Concentration	Type of Organic Phase (mL)	Volume of Organic Phase (mL)	Stirring Speed (R.P.M.)
Preliminary selection of speed (R.P.M.)					
ANS10	Pluronic F- 68: Tween 80	3:1	Acetone	5	**1000**
ANS11	Pluronic F- 68: Tween 80	3:1	Acetone	5	**1500**
ANS12	Pluronic F- 68: Tween 80	3:1	Acetone	5	**2000**

Table 9: Characterization of Trial batches (ANS10 – ANS12) for Aceclofenac Nanosuspension

Batch No.	Drug Content (mg) (Mean±S.D.) (n=3)	Entrapment efficiency (%) (Mean±S.D.) (n=3)	Free Drug Content (%)	%CDR (Mean±S.D.) (n=3)
Selection of speed				
ANS10	17.124±0.56	77.62±1.28	22.38	87.69±1.35
ANS11	**18.444±0.48**	**84.22±1.37**	**15.78**	**90.87±1.42**
ANS12	**17.64±0.36**	**80.23±1.31**	**19.77**	**91.87±1.48**

Stirring speed is also important formulation variable. It has been observed that on average, increasing speed of stirring leads to reduction in particle size towards Nano sized range. However, it has been noted that operating instruments at high speed conditions is not always optimum and average speed has to be maintained. This is because higher agitation speeds often lead to formation of huge amount of foam in suspension which often leads to early separation of solid nanoparticles from aqueous medium. As result, this can lead to ineffective size reduction and insufficient formation of Nanosuspension. Hence, ANS 11 and ANS 12 have been selected as optimized batches.

3.7 3^2 Factorial Design Approach

Table 10: 3^2 Factorial Design

Independent Variables of Formulations			
Independent Variables	Low (-1)	Medium (0)	High (+)
Stabilizer Concentration (X10	2:1	3:1	4:1
Speed (RPM)(X2)	1000	1500	2000
Dependent Variables			
Y1= % Entrapment Efficiency			
Y2= %CDR			

3.8 Compositions of Factorial Batches in decoded Form

Table 11: Compositions of Factorial Batches in Coded Form

ACE NS 3^2=9 Batches		
Variable level in decoded form		
Batch No	**Stabilizer Concentration(X1)**	**Speed (R.P.M.) (X2)**
ACNS1	-1	-1
ACNS2	-1	0
ACNS3	-1	+1
ACNS4	0	-1
ACNS5	0	0
ACNS6	0	+1
ACNS7	+1	-1
ACNS8	+1	0
ACNS9	+1	+1

3.9 Formulation Design by 3^2 Factorial Designs

Table12: Formulation Design by 3^2 Factorial Design

Aceclofenac NS 3^2=9 Batches		
Variable level in decoded form		
Batch No	**Stabilizer Concentration(X1)**	**Speed (R.P.M.) (X2)**
ACNS1	2:1	1000
ACNS2	2:1	1500
ACNS3	2:1	2000
ACNS4	3:1	1000
ACNS5	3:1	1500
ACNS6	3:1	2000
ACNS7	4:1	1000
ACNS8	4:1	1500
ACNS9	4:1	2000

Figure 3: Various Aceclofenac loaded Nanosuspension formulation using by 3^2 Factorial Designs

3.10 Characterization of Batches (ACNS1-ACNS9)

Table 13: Characterization of Batches (ACNS1 to ACNS9)

Batch No	Entrapment Efficiency (%)	Drug release (%)
ACNS1	81.70±0.59	90.74±0.26
ACNS2	76.25±.0.72	85.69±0.36
ACNS3	75.55±0.42	84.71±0.42
ACNS4	80.82±0.56	89.29±0.34
ACNS5	85.45±0.68	92.37±0.39
ACNS6	72.98±0.69	82.13±0.40
ACNS7	79.80±0.73	88.28±0.24
ACNS8	77.59±0.86	86.26±0.37
ACNS9	86.14±0.55	93.42±0.42

3.11 Saturation solubility studies

Table: 14 Characterization of Saturation Solubility of Batches (ACNS1 to ACNS9)

Batch No	Saturation Solubility
ACNS1	189.20±0.15
ACNS2	186.62±0.22
ACNS3	196.72±0.27
ACNS4	179.58±0.12
ACNS5	180.43±0.28
ACNS6	202.64±0.26
ACNS7	192.30±0.27
ACNS8	192.83±0.12
ACNS9	192.55±0.20

3.12 Statistical Analysis

Plan master programming rendition 9.0.2.0. Was utilized for Statistical investigation and delivered first request polynomial mathematical statements. From preparatory results, 32 full factorial configuration was used in which two elements were assessed, independently at three levels and conceivable nine mixes were planned. Three level factorial studies were done utilizing two distinct variables. In first factorial outline, measure of Stabilizer focus (X1) and Speed (X2) were taken as free variables while % E.E (Y1), % CDR (Y2) and immersion dissolvability were chosen as needy variables for both factorial plans.

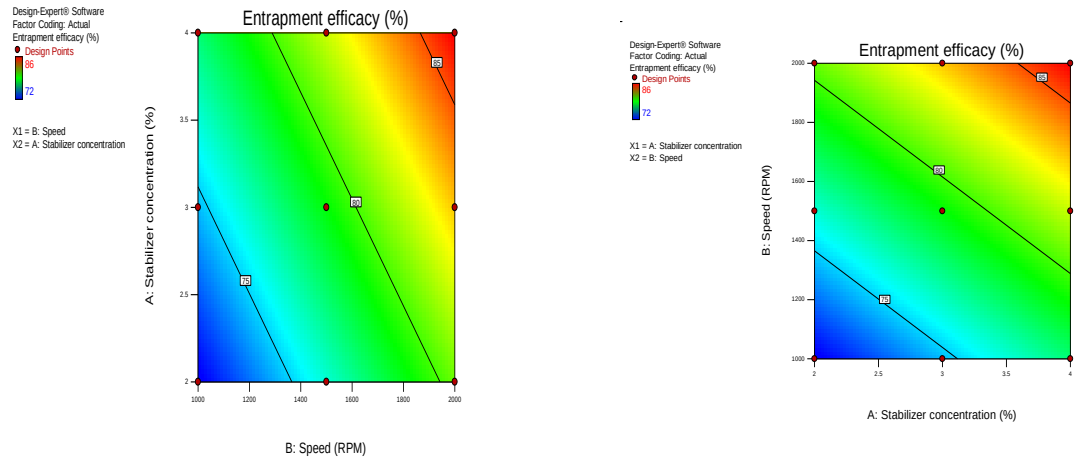
3.13 Effect on Entrapment Efficiency (Y1) - Surface Response Study

Positive quality for coefficient of X1-sort of stabilizer fixation in mathematical statement shows increment in Entrapment Efficiency. Positive estimation of coefficient of X2-Speed shows increment accordingly of Y1 i.e. Entanglement Efficiency. It demonstrates linearity of surface reaction and shape plot as appeared in figure. Full model was discovered immaterial so lessened model was connected for each of the two autonomous variables and definite ANOVA, Response Surface Counter Plot and 3 D plot are as per the following:

$$Y=+79.00+2.83* A+4.33* B$$

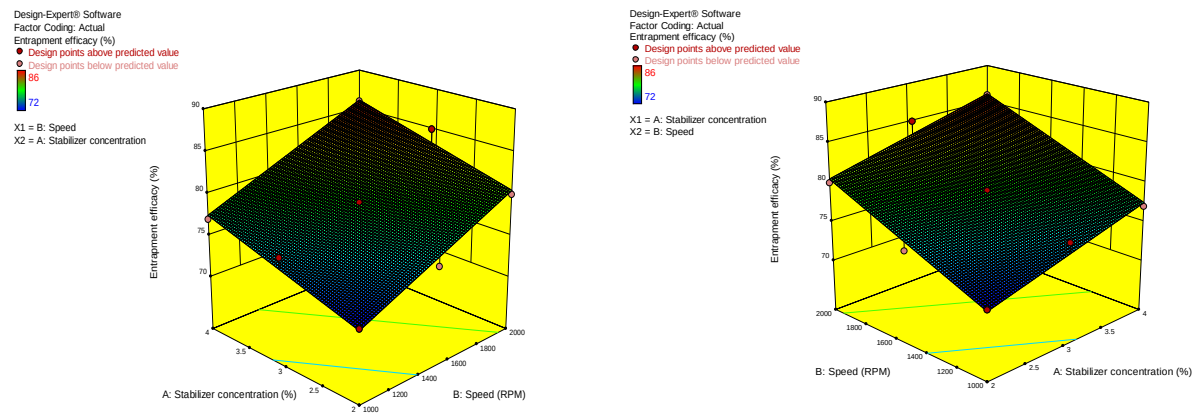
Table 15: ANOVA Table for Response Y1 (Entrapment Efficacy)

ANOVA for Response Surface Linear model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		**Mean**	**F**	**p-value**	
Source	**Squares**	**Df**	**Square**	**Value**	**Prob > F**	
Model	160.83	2	80.42	67.33	< 0.0001	Significant
A-Stabilizer concentration	48.17	1	48.17	40.33	0.0007	
B-Speed	112.67	1	112.67	94.33	< 0.0001	
Residual	7.17	6	1.19			
Cor Total	168.00	8				



(a) **(b)**

Figure 4: Response Surface Plot (a) Stabilizer Concentration and (b) Speed on Entrapment Efficacy (Y1)



(a) **(b)**

Figure5: 3D Surface Plot: (a) stabilizer Concentration and (b) Speed on Entrapment Efficacy (Y1)

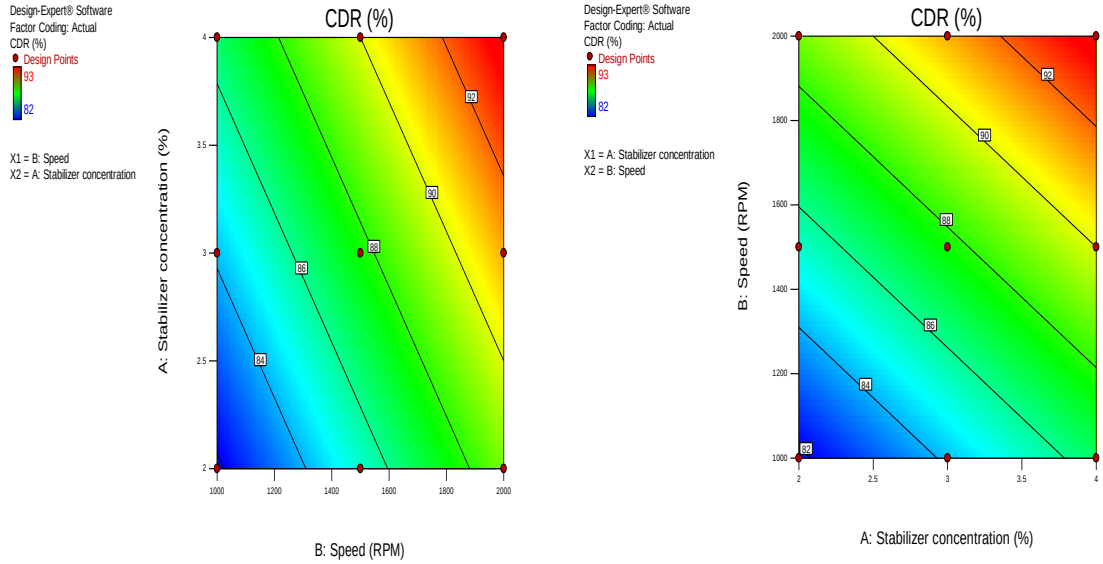
3.14 Effect on % Drug release (Y2) - Surface Response Study

Positive value for coefficient of X1- type of stabilizer Concentration in equation indicates increase in Drug release. Positive value of coefficient of X2-Speed also indicates increase in response of Y2 i.e. % CDR. It indicates linearity of surface response and contour plot as shown in figure.

$$Y=+87.67+2.33* A+3.50* B$$

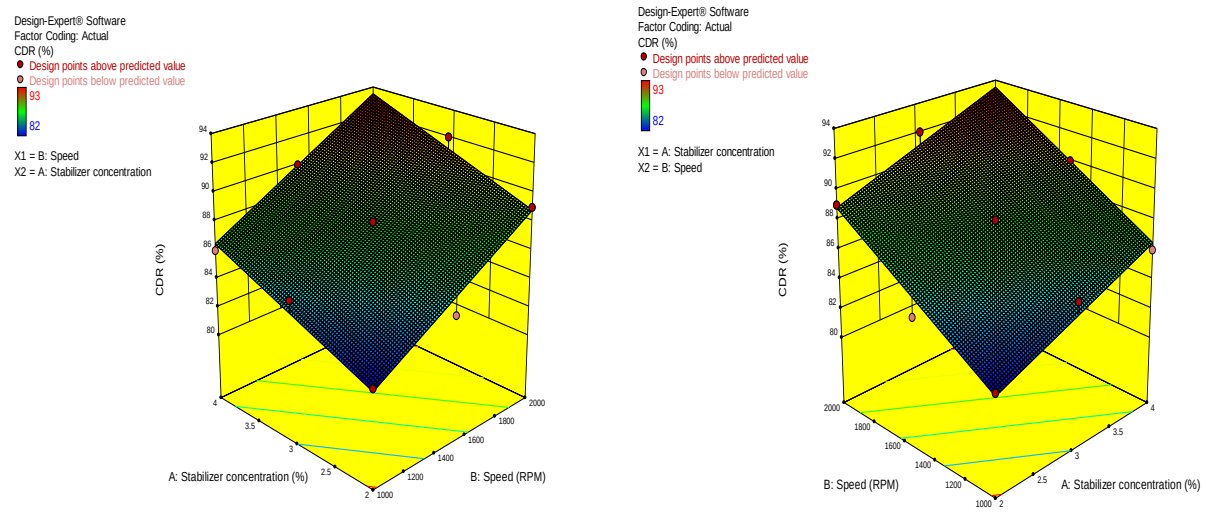
Table: 2ANOVA Table for Response Y2 (%CDR)

ANOVA for Response Surface Linear model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	p-value	
Source	Squares	Df	Square	Value	Prob > F	
Model	106.17	2	53.08	83.09	< 0.0001	significant
A-Stabilizer concentration	32.67	1	32.67	51.13	0.0004	
B-Speed	73.50	1	73.50	115.04	< 0.0001	
Residual	3.83	6	0.64			
Cor Total	110.00	8				



(a) **(b)**

Figure: 6 Response Surface Plot: (a) Speed (b) Stabilizer Concentration on % CDR (Y2)



(a) **(b)**

Figure 7: 3D Surface Plot: (a) Stabilizer Concentration and (b) Speed on % CDR (Y2)

3.15 Establishing Design Space and Control Strategy

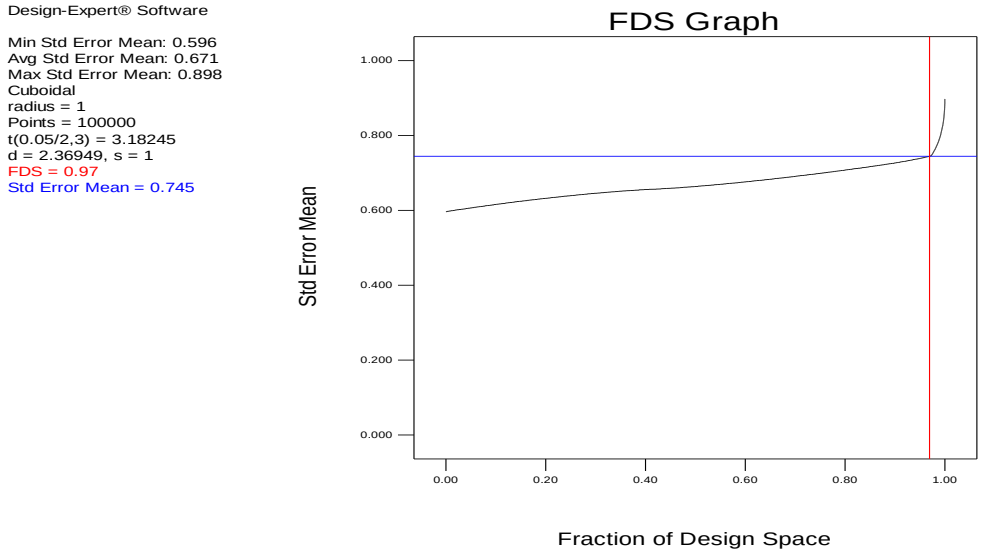


Figure: 8 FDS Graph

FDS bend demonstrates what % part of outline space has given expectation mistake or lower. Great configuration will have compliment and lower bend than poor outline as appeared in figure. Compliment implies general forecast mistake will be consistent. Lower implies general forecast .blunder will be littler. FDS ought to be no less than 0.8 or 80% for investigation, and 100% for strength testing.

3.16 Validation

From polynomial mathematical statements created for reaction, serious network and incorporated inspect was performed over test field utilizing Design Expert Software (9.0.2.0.). During free variable portrayal study, effect of parameters Stabilizer Concentration (mg) and Speed (RPM) were surveyed. Criteria considered of reaction E.E (Y1), Drug discharge (Y2), between, 80-90 %, and 85-95 % separately. This study lead to information space and at last plan space from multidimensional mix of force, dissolvable volume and time prompts satisfactory working reaches for confining adhesive as for target item profile. Plan space appeared in figure likewise called as overlay plot which is shaded locale with yellow shading shows that district of effective working extents.

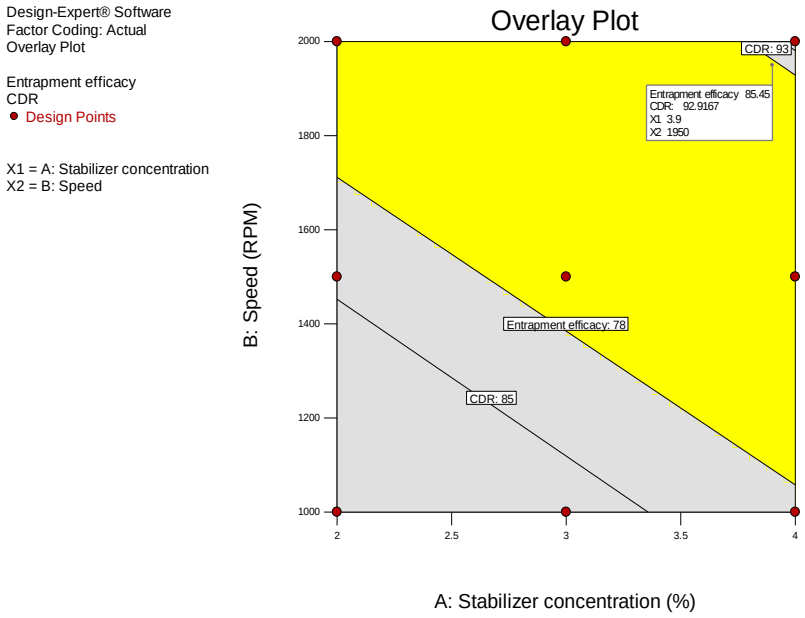


Figure 9: Overlay Plot

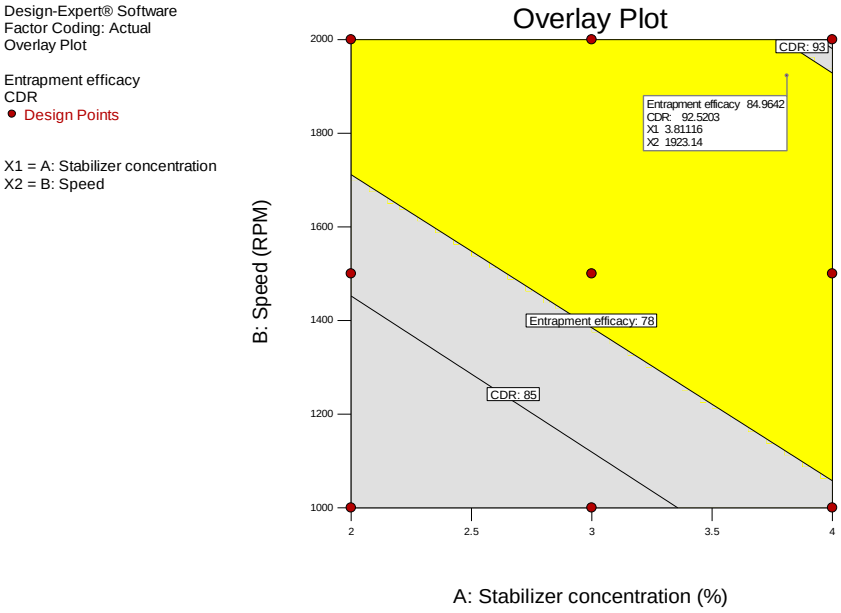


Figure 10: Overlay Plot

3.17 Check point analysis of Validation Batches

ANS10 & ANS11 formulation was made for check point analysis and predicted and experimental values were compared.

Table 17: Validation of Batches ACNS10 & ACNS11: Predicted Response

Batch No	Stabilizer Concentration (X1)	Speed-RPM (X2)	% E.E (Y1)	% CDR (Y2)
ACNS10	3.9	1950	85.45	92.91
ACNS11	3.81	1923	84.96	92.52

Table: 18 Validation Batches ACNS10& ACNS11: Actual Response

Batch No	Stabilizer Concentration (X1)	Speed-RPM (X2)	% E.E (Y1)	% CDR (Y2)
ACNS10	3.9	1950	84.26±1.22	91.84±1.54
ACNS11	3.81	1923	83.76±1.32	91.37±1.04

3.18 Particle size Analysis of Optimized Batch ACNS10

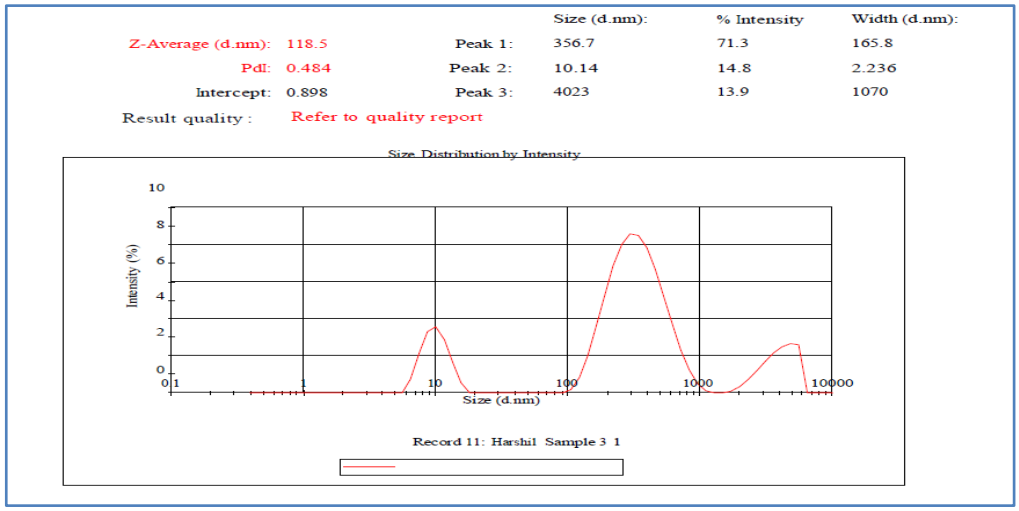


Figure 11: Particle size Analysis of Optimized Batch ACNS10

Pure Aceclofenac used for study was characterized by relatively large particles as reported. nanosuspension prepared after emulsification and solvent evaporation may decrease in particle size when compared to pure drug particles which may have positive effect on drug dissolution rate As per Noyes-Whitney equation. Hence decrease in particle size will have significant effect in drug solubility and dissolution characteristics.

3.19 Zeta potential of optimised Batch ACNS10

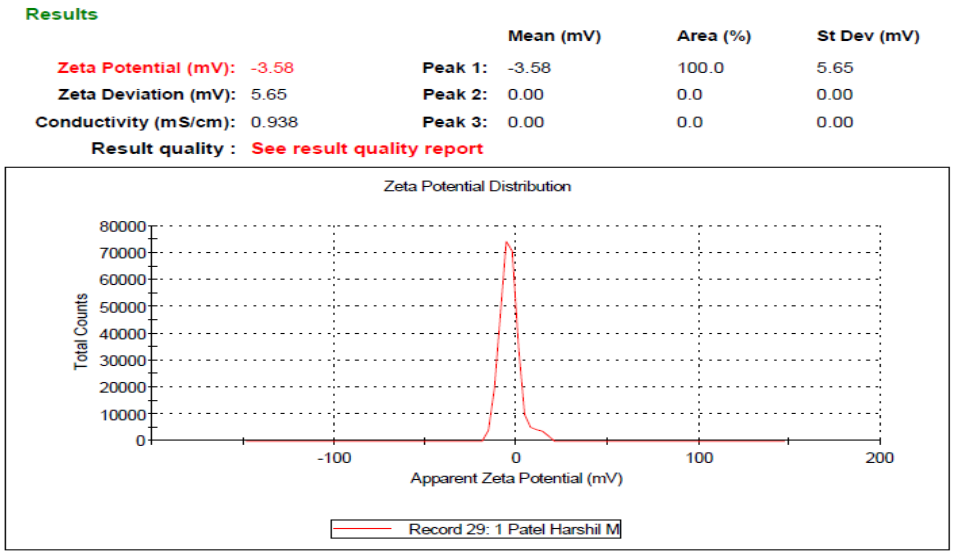


Figure: 12 Zeta potential of optimis-ed Batch ACNS10

Zeta potential was determined by Beckman coulter. Zeta potential of selected formulation (ANS10) was found to be + 5.65. Positive zeta value was obtained due to positive charge of polymer. Mobility distribution graph can be given in Figure.

3.20 Stability Study of ACNS 10 for 1 Month

Table: 19 Stability Study of ACNS 10 for 1 Month

Parameter	Optimized Aceclofenac Nanosuspension			
	Room Temperature			
	0 Day	10 Day	20 Day	30 Day
% E.E	84.26	83.48	84.21	84.91
% CDR	91.84	90.43	91.68	90.57

Comparison study of Nanosuspension optimized formulation and marketed formulation of Aceclofenac Tablets

Table 20: *In-Vitro* Drug release study

Time (min)	% Drug Release		
	Marketed Aceclofenac tablet (Mean± S.D.) (n=3)	ACNS10 (Mean± S.D.) (n=3)	ACNS11 (Mean± S.D.) (n=3)
0	0.00	0.00	0.00
5	7.77±2.12	20.42±2.12	24.88±1.52
10	13.40±1.54	30.92±2.58	41.16±1.25
15	17.30±1.46	50.78±1.97	54.95±1.47
20	23.64±2.28	58.49±2.28	64.37±1.28
25	33.73±1.18	77.25±2.43	76.03±1.34
30	46.03±2.17	91.87±2.62	92.12±1.92

From comparison study of Nanosuspension optimized formulation and market formulation of Aceclofenac Tablets, It can be concluded that% Drug release of Aceclofenac Nanosuspension was better than marketed formulation of Aceclofenac Tablets (Aerosol Tablet).

4. Conclusion

Nanosuspension containing Aceclofenac was prepared by quasi emulsion Solvent Evaporation method using Pluronic F68 using DoE approach. Optimized Batch was subjected for % drug content, % Entrapment efficiency; Particle size Analysis, and in vitro drug release studies, low SD and CV values indicate drug content was uniform and reproducible in all formulations.

IR spectral analysis and DSC suggested compatibility between drug and formulation additive. Drug exists in original form and available for biological action.

Dissolution parameters were studied by using dissolution software PCP DISSO V.3 for Nanosuspension which proved increase in Saturation Solubility and Dissolution rate.

Nanosuspension which gave better physical, morphological and % encapsulation in either of Stabilizers and Excipients. Various Oral formulations with Aceclofenac in free form and in Nanoparticulate delivery system were formulated and *in vitro* release studies were carried out.

By considering all results of Check Point Analysis Nanosuspension It shows that saturation solubility of Nanosuspension is increased up to two to three folds as compared to pure Aceclofenac. Also dissolution rate is increase therefore bioavailability of Aceclofenac is increases. No changes found after stability analysis for period of 1 month.

From study it can be concluded that it is possible to design Nanosuspension for Aceclofenac may increase efficacy and patient compliance which are of prime importance.

Future Perspective

Aceclofenac loaded Nanosuspension based oral drug delivery system may be less side effect with reduction of dose by immediate release at system. Aceclofenac Nanosuspension can be enhance solubility and dissolution and increase bioavailability & hence increase patient Compliance.

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