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Dissolution Enhancement of BCS Class 4 Dssrugs Using Quality by Design Approach with Solid Dispersion Technique

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Abstract: *Solid dispersion is one of the vastly accepted and practically economical processes in bioavailability enhancement study. The present investigation deals mostly with increase in solubility and dissolution rate of BCS class 4 drugs for enhancement of oral bioavailability. For the same solid dispersion were prepared and analyzed for appropriate concentration of drug polymer ratio by phase solubility analysis. The solvent evaporation study widely accepted due to its efficient solid dispersion in lesser efforts. The study designs were prepared with specific concentration of drug and polymer ratio with the help of high throughput model i.e. Central Composite Design (by Design Expert trial copy) by specific design of experiment with full factorial design (DOE). The fixed variables were concentration of polymers and dependant variables were dissolution and permeability across bio-membrane in in-vitro model. The prepared dispersion investigated for dissolution and permeability improvement using USP Type II apparatus and modified everted gut sac model which leads to improvement of quality of whole formulation with Quality by design efficiently.*

Keywords: *Cefuroxime axetil, Bioavailability enhancement, Dissolution study, Solid Dispersion, Quality by Design (QbD), Design of Experiments (DOE).*

I. Introduction

BCS class 4 drugs are the drugs having low solubility and permeability according to classification system given by Amidon et. al. in 1995. These drugs are formulated such that it is made more permeable and more soluble with help of different formulation techniques. Solid dispersion is the technique primarily used vastly by various researcher due to its simplicity, economical with Solvent evaporation method[1]. BCS class 4 drugs used for present study are Cefuroxime Axetil, Cefpodoxime Proxetil, Furosemide. Cefuroxime Axetil is the second generation Beta Lactam antibiotic is acted with binding to specific penicillin-binding proteins which are generally located inside the bacterial cell wall. The proteins generally inhibit the bacterial cell wall synthesis and causes cell lysis by interfering with an autolysin inhibitor. Cefpodoxime proxetil is an orally administered, extended spectrum, semi-synthetic antibiotic of the cephalosporin class. It inhibits bacterial cell wall synthesis by interfering by its ability of covalently binding to the penicillin-binding proteins. Furosemide inhibits primarily the reabsorption of sodium and chloride not only in the proximal and distal tubules but also in the loop of Henle these drugs are selected as they include in BCS class 4 drugs[2,14].

The DOE with full factorial design is widely used for optimization of pharmaceutical dosage formulation process and excipients concentration depends upon response surface plot of individual polymer responsibility in optimized result. DOE is systematic tool for increasing efficiency development of pharmaceutical dosage forms and helps in improvement of research and development work. The Factorial designs advantageous statistical method as all the factors are studied in all possible combinations for estimating the influence of individual variables and their interactions using minimum experiments. The factorial design applies relationship in dependant variables and their interrelations and study systematically its affect on independant variable and ultimately quality of output in pharmaceutical formulation development[3]. The graphical representation can be evaluated with the help of surface response and contour plot, which generated by statistical software gives a visual representation of the results of the desired responses and it also gives model equation shows response as a function of the different variables. Solid Dispersion is the carrier linked drug delivery system. The objective of the present study to design and develop highly solubilised formulation of BCS class 4 drugs. The formulation designed such that release of the drug enhanced and was evaluated for in vitro drug release, permeability and drug content. The known mechanism behind solid dispersion is incorporation of drug into a water-soluble polymer matrix which can leads to increase in drug dissolution rate and their saturation solubility in the gastrointestinal fluids. The Analysis of variance (ANOVA) can be neglected over design of experiments as ANOVA is generally used for compare the relativity of the two different ingradients in formulation considerations but use of factorial design experiment is indicating the relative significance of a number of variables in the formulation. In addition, it offers way of analyzing the results to decide on most significant variables. The maximum outcome can be drawn out of with the use of a

small number of experiments possible due to factorial design. In addition, they allow a means of assessing interactions which exist between different variables over the response[4-6].

The wetting and water solubilising carrier is Polyvinylpyrolidone K30 adsorbs on surface of drug and enhance the bioavailability[13].

The other used amphiphilic chemical polymeric solubilizer is Soluplus, which was particularly developed for solid solutions. The action of soluplus of increased in bioavailability is due to formation of a matrix polymer for solid solutions on the other, it is capable of solubilizing poorly soluble drugs in aqueous media. Soluplus is a polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft co- polymer[7]

Materials

Cefuroxime Axetil, Cefpodoxime Proxetil, Furosemide was a generous gift from Swapnroop Drugs. PVPK and soluplus was obtained from Anuradha College of Pharmacy, Chikhli. All excipients and solvents used were of analytical grade.

Methods

Determination of drug content

The percent drug content of each solid dispersion, was determined using powder equivalent to 10 mg API and was dissolved in minimum amount of methanol and volume was made up to mark 100 ml using buffer. The solution was then filtered through Whatman filter paper and required dilution were being made and assayed for drug content using UV double beam spectrometer (Shimadzu Corporation, Japan. UV -1601). Three replicates were prepared and average value was reported[8, 9].

FTIR studies

The FTIR spectra of the drug, polymers and solid dispersion in specific ratio were recorded with FTIR spectrophotometer. The FT-IR spectra of the agglomerates and the pure drug were recorded on Shimadzu IRAffinity-1S spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Background spectrum was obtained under identical conditions. Each spectrum was derived from single scan collected in the region 400 – 4000 cm^{-1} at a spectral resolution of 2 cm^{-2} and rationed against background scan of KBr. The samples also mixed and trturated with KBr and forms a mass in DRS unit attached to IR spectrophotometer.

DSC Studies

Differential scanning calorimetric (DSC) analysis of the samples was carried out with a DSC analyzer (SIIO, Japan, Model: SIIO 6300 with Auto-sampler). A sample (3-7 mg) was sealed in an aluminum pan with a perforated lid and heated under nitrogen atmosphere at a heating rate of 10°C/min over the temperature range of 40 – 400°C. The thermograms were obtained and recorded with heat flow and temperature with empty pan as reference.

XRD Studies

The XRPD patterns of the samples were monitored with an X-ray diffractometer (Model: D8 Advance Make: BRUKER, Germany) using Ni filtered CuKα radiation. The samples were analyzed over 2 range of 5.010-39.990^o with scanning step size of 0.020° (2θ) and scan step time of one second.[9]

Mathematical kinetic assessment for drug release mechanism

Release kinetics is an integral part for the development of a dosage form because if the kinetics of drug release is known, one can also established *in vivo in vitro* (IVIVC) correlation. Mathematical approach is one of scientific methods to optimize and evaluate the error in terms of deviation in the release profiles of formulated products during the formulation development stage. Mathematical model approach important in research and development because of its simplicity and their interrelationships may minimize the number of trials in final optimization, thereby improving the formulation development process. The dissolution profile of the optimized batch was fitted to the different kinetic models.

In vitro drug release data were fitted to kinetic models Q_t versus t (zero order) Log (Q_0-Q_t) versus t (first order) Q_t versus square root of t (Higuchi) log %Q_t versus log %t (Korsmeymer-Peppas) Where Q_t is the amount of drug released at time t. The criteria for selecting the most appropriate model are highest R^2 value. Highest R^2 value indicates linearity of dissolution data.[13]

Phase solubility Studies

Solubility measurements were performed in triplicate using the method reported by Higuchi and Connors. An excess amount of drug of about (100 mg) was added to 25 ml distilled water containing increasing concentrations of the Polymer (i.e., 0.1%, 0.2%, 0.3%, 0.4%, 0.5% w/v). The flasks were sealed and shaken at

room temperature (28°C) for 24 h on a shaker, and the samples were filtered through a 0.22-µm whatman filter paper. The filtrate was suitably diluted and analyzed at UV-Vis. spectrophotometer (model no: 1601, Shimadzu, Japan) at specified wavelength [15].

Formulation with 3^2 factorial design (central composite design)

A central composite design is used to systematically study the influence of the individual and combined effect of independent variables i.e. numeric factors which can control by us in formulation. They are X_1 and X_2. From this independent variables affect on dependant variables i.e. drug dissolution after 2 hrs (D2) and Permeability after 4 hours (P4). In present study, two independant factors are evaluated, and experimental trials are performed at all nine possible combinations and record their dependant variables. Statistical model central composite design was selected as two independant variable to study in development of the dosage form in order to find their individual and combined effects on the dependent variables. Response surface and contour plots shows the interaction between different variables. A mathematical quadratic regression model was developed for predicting system responses within selected experimental conditions. Experimental data were fitted according to the following polynomial equation calculated by multiple regression analysis:

$$Y= b_0+ b_1X_1 +b_2X_2+ b_{12}X_1X_2 + b_{11}X_1^2+ b_{22}X_2^2$$

Where Y is the dependent variable, b_0 is the arithmetic mean response of the 9 runs; and b_1 and b_2 are the estimated coefficients for the independent factors X_1 and X_2, respectively. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction term (X_1X_2) shows how the response changes when factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are including investigating nonlinearity. A statistical model, which consists of interactive and polynomial terms, was utilized to evaluate the responses. The responses were analyzed using analysis of variance (ANOVA) and the individual response parameters were evaluated using F test and polynomial equation was generated for each response using multiple linear regression analysis.[15]

Preparation of solid dispersion

Physical mixtures were prepared by mixing accurate weight of API with PVPK, Soluplus in drug: polymer ratio of 1:1 (1:2 for polymers ratio) as per table 1. The powder blend was passed through sieve no 40. The resultant mixture was further kept for evaporation by stirring under mechanical stirrer using a three blade propeller to form a thick and high viscous form and transferred into petri plate, then dried under reduced pressure. The dried mass was pulverized, passed through 60 mesh sieve size, then weighed, transferred to sealed pouch. Stored in a desiccators. The procedure was repeated with different concentration of polymers according to the factorial design.[17]

Optimization of solid dispersion using 3^2 full factorial designs

Response surface methodology (RSM) is characteristically employed to relate a response variable to the levels of the input

Code	Polymer	-1	0	+1
X1	PVPK 30	116.66	166.66	216.66
X2	Soluplus	33.33	83.33	133.33
API	Cefuroxime Axetil, / Cefpodoxime Proxetil/ Furosemide	250		

Table 1: Batch code Variable levels in coded form Drug: Polymer Actual quantity of drug and polymer

Taking drug polymer ratio 1:2 at middle step (i.e at position "0" and ±10 for PVPK and ±10 for SOLUPLUS (2:1 polymer ratio in complex). A 3^2 full factorial design(central composite design) was employed to study the effect of independent variables, i.e., PVPK 30 (X_1) and Soluplus (X_2) on dependent variables permeability; drug released up to 2 h. Contour or RSM plots for each response were generated using the DESIGN EXPERT (STAT-EASE) demo version software. Variables level Low (−1) Medium (0) High (+1).

Run Number	Trial Code Run	
1	-1	0
2	-1	1
3	-1	-1
4	0	1
5	0	-1
6	0	0
7	1	0
8	1	-1
9	1	1

Table 2: Runs for Coded levels of factors

***In vitro* drug release studies**

In vitro drug release profile of the drug from Solid dispersion carried out by the dissolution test according to USP 23. Dissolution studies performed using USP apparatus type-I i.e., basket type at a temperature of 37 ± 0.5°C. Studies were carried out in 900 ml of phosphate buffer pH 7, at 50 rpm and for cefuroxime axetil. 900ml of Glycine Buffer pH 3.0, RPM 75 prepared for cefpodoxime proxetil. For Furosemide dissolution media was phosphate buffer pH 5.8, volume 900 mL. at 50 rpm. The dissolution was continued in phosphate buffer till 2 h. Samples were withdrawn at a predetermined time intervals and replaced with fresh media. Samples were filtered and then analyzed using ultraviolet-visible spectrophotometer at λmax 280 nm, 233 nm, 274 nm for cefuroxime axetil , cefpodoxime proxetil, furosemide respectively for determination of drug content.

Drug permeability study

Drug permeability study was performed using Modified Wilson and Krane apparatus. Solid dispersion containing 50 mg drug were taken and the powdered dispersion were dispersed in 5ml of respective buffer solution and added in the outer receiver compartment, prepared gut sac of chicken ileum which tied to thread at one end and glass canula at other. It was suspended in receiver compartment having canula at the top filed with buffer above 1-2 cm from height in canula the whole assembly maintained at 37°±0.5°C and. After specific time period the samples were collected and analyzed for the drug content [16].

II. Results And Discussion

Actual Drug Content

The percent drug content of each solid dispersion was determined to measure the dose uniformity of the formulation using powder equivalent to 10 mg API and assayed by UV spectrophotometer for drug concentration and reported in table.

Sr. No.	PVPK	SOLUPLUS	Actual Drug Content (%)		
			Cefuroxime Axetil	Cefpodoxime proxetil	Furosemide
1	0	-1	99.53±0.686	98.47±0.729	96.6± 0.636
2	0	1	98.87±0.729	98.47±0.569	97.76±0.177
3	-1	1	99.51±1.090	99.7±0.6497	100.45±0.578
4	1	1	100.58±0.654	100.67±0.663	98.57±0.7
5	1	-1	98.32±1.026	99.68±0.519	99.77±0.559
6	-1	0	99.92±0.971	99.33±0.505	100.64±0.41
7	-1	-1	99.29±1.236	97.49±1.050	100.54±0.661
8	1	0	98.99±1.0154	99.36±0.2325	100.17±0.428
9	0	0	99.62±1.141	98.28±1.28	98.29±0.690

Table 3: Actual Drug Content

FTIR studies

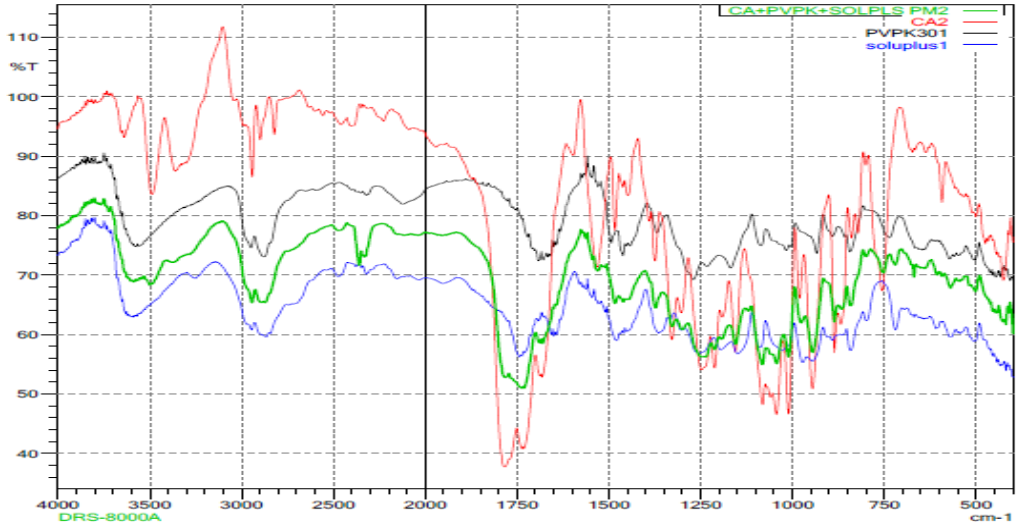

Fig 1. Overlay FTIR Spectra of Cefuroxime Axetil Solid Dispersion

Sr No	Functional Group	Range	Actual Value
1	-OH	3500-3650cm^{-1}	3649.32 cm^{-1}
2	-NH Streching	3450-3500cm^{-1}	3491.16 cm^{-1}
3	Aromatic- CH(Streching)	3000- 3100cm^{-1}	3041.74cm^{-1}
4	Aliphatic –CH(Streching)	2850-2900cm^{-1}	2825.72 cm^{-1}
5	Amide (cefum ring)	1750-1800cm^{-1}	1786.08 cm^{-1}
6	-COOR	1725-1750cm^{-1}	1734.01 cm^{-1}
7	Carbonyl Group	1650–1700 cm^{-1}	1681.93 cm^{-1}
8	-C=O (out of plane)	1200-1400cm^{-1}	1303.88 cm^{-1}
9	-C-N	1080-1200 cm^{-1}	1151.50 cm^{-1}
10	-C-S	600-700 cm^{-1}	673.16cm^{-1}

Table 4: FTIR Cefuroxime Axetil

Sr No	Functional Group	Range	Actual Value
1	Aromatic- CH(Streching)	3000-3100 cm^{-1}	3037.89 cm^{-1}
2	Aliphatic –CH(Streching)	2850-2900cm^{-1}	2885.51 cm^{-1}
3	Amide	1750-1800cm^{-1}	1780.30 cm^{-1}
4	-COOR	1725-1750cm^{-1}	1732.08 cm^{-1}
5	Carbonyl Group	1650 – 1700 cm^{-1}	1685.79 cm^{-1}
6	-C=O (out of plane)	1200-1400 cm^{-1}	1300.02 cm^{-1}
7	-C-N	1080-1200 cm^{-1}	1155.36 cm^{-1}
8	-C-S	600-700 cm^{-1}	669.30 cm^{-1}

Table 5: FTIR Solid Dispersion of Cefuroxime Axetil

From above peak positions distinct peaks of hydroxyl group and aromatic group is low intense which can conclude that the formation of solid dispersion i.e physical complex between excipient and cefuroxime axetil. Also other than above characteristic peaks are absent which suggest no interaction between cefuroxime axetil and excipients in formulation.

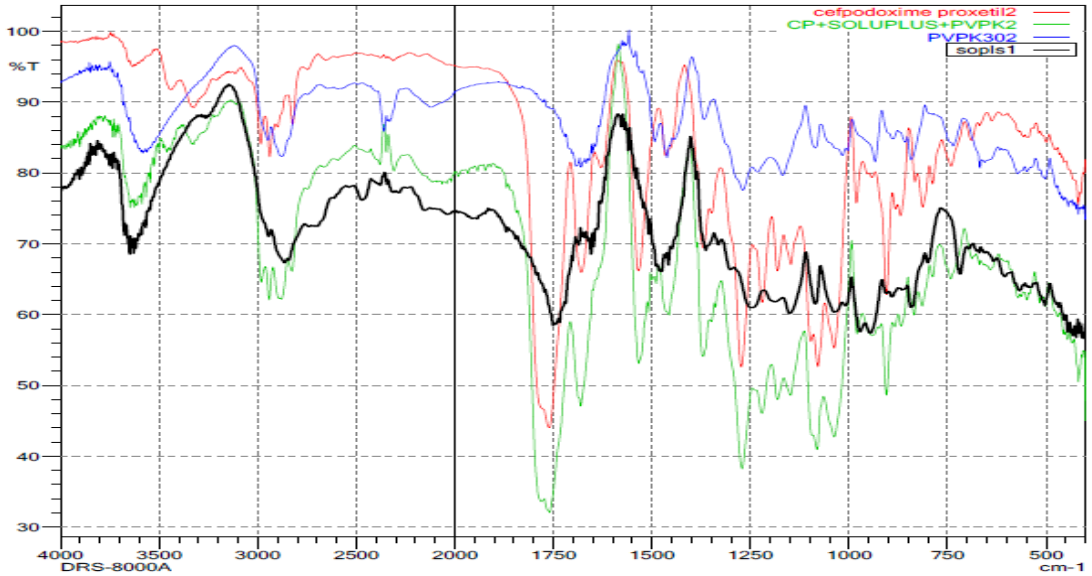

Fig. 2: Overlay Spectra of Cefpodoxime Proxetil Solid Dispersion

Sr No	Functional Group	Range	Actual Value
1	-NH (pri. Amine)	3500-3600cm^{-1}	3560.59 cm^{-1}
2	-NH (sec amine)	3250-3300cm^{-1}	3229.14 cm^{-1}
3	Aromatic- CH	3000-3100 cm^{-1}	3057.17 cm^{-1}
4	Aliphatic –CH	2850-2900cm^{-1}	2893.22 cm^{-1}
5	Amide (cefum ring)	1750-1800cm^{-1}	1786.08 cm^{-1}
6	-COOR	1725-1750cm^{-1}	1737.86 cm^{-1}
7	Carbonyl Group	1650 – 1700 cm^{-1}	1680.00 cm^{-1}
8	-C=O (out of plane)	1200-1400cm^{-1}	1346.31 cm^{-1}
9	-C-N	1080-1200 cm^{-1}	1145.72cm^{-1}
10	-C-S	600-700 cm^{-1}	665.44 cm^{-1}

Table 6: FTIR of Cefpodoxime Proxetil

Sr No	Functional Group	Range	Actual Value
1	Amine (pri. and sec.)	3500-3600 cm^{-1}	3585.67 cm^{-1}
2	Aromatic -CH(Streching)	3000-3100 cm^{-1}	3066.82 cm^{-1}
3	Aliphatic –CH	2850-2900cm^{-1}	2883.58 cm^{-1}
4	Amide	1750-1800cm^{-1}	1786.08 cm^{-1}
5	-COOR	1725-1750cm^{-1}	1726.29 cm^{-1}
6	Carbonyl Group	1650–1700 cm^{-1}	1681.93 cm^{-1}
7	-C-N	1080-1200 cm^{-1}	1145.72 cm^{-1}
8	-C-S	600-700 cm^{-1}	688.59 cm^{-1}

Table 7: FTIR Solid Dispersion of Cefpodoxime Proxetil

No other characteristic peaks were observed in the IR spectra of formulations which indicated the absence of any interaction between drug and polymers

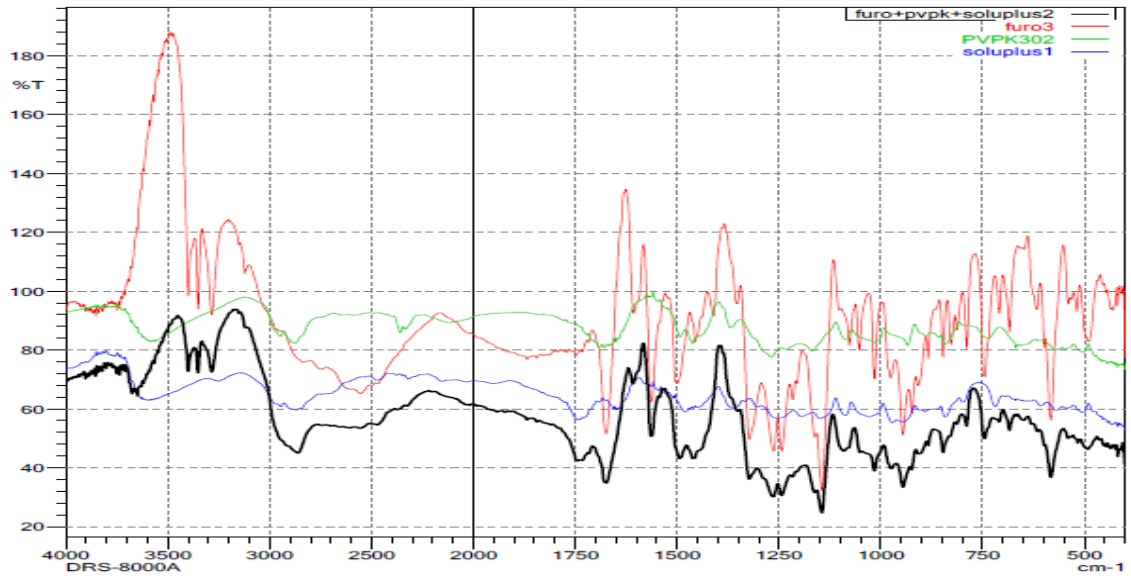

Fig.3: Overlay FTIR Spectra of Furosemide Solid Dispersion

Sr No	Functional Group	Range	Actual Value
1	-NH (pri. And sec.)	3300-3400cm^{-1}	3348.42 cm^{-1}
2	Aromatic- CH	3000-3100 cm^{-1}	3041.74 cm^{-1}
3	Aliphatic –CH	2850-2900cm^{-1}	2835.36 cm^{-1}
4	Carbonyl Group	1650–1700 cm^{-1}	1674.21 cm^{-1}
5	C-O-C	1050-1150cm^{-1}	1074.35 cm^{-1}
6	S=O	1030-1060 cm^{-1}	1053.13 cm^{-1}
7	-C-Cl	600-700 cm^{-1}	644.22 cm^{-1}

Table 8: FTIR Furosemide

Sr No	Functional Group	Range	Actual Value
1	Amine (pri. and sec.)	3300-3400 cm^{-1}	3356.14 cm^{-1}
2	Aromatic –CH(Streching)	2900-3100 cm^{-1}	2958.80 cm^{-1}
3	Aliphatic –CH	2800-2900cm^{-1}	2854.65 cm^{-1}
4	-COOR	1725-1750cm^{-1}	1745.58 cm^{-1}
5	Carbonyl Group	1650 – 1700 cm^{-1}	1674.21 cm^{-1}
6	C-O-C	1050-1150cm^{-1}	1089.78 cm^{-1}
7	S=O	1030-1060 cm^{-1}	1035.77 cm^{-1}
8	-C-Cl	600-700 cm^{-1}	684.73 cm^{-1}

Table 9: FTIR Solid Dispersion of Furosemide

All the above characteristic peaks appear in the spectra of all binary systems at same wave number indicating no modification or interaction between the drug and carrier.

DSC Studies

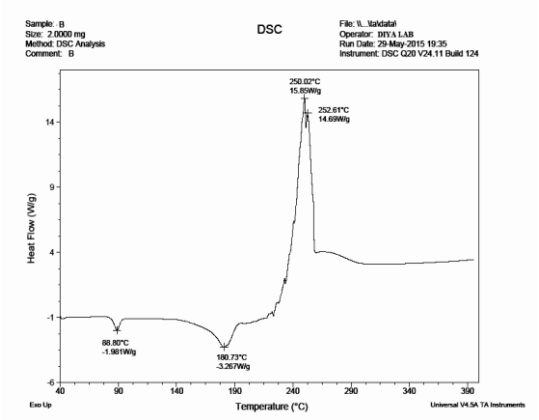

Fig.5: DSC of Cefuroxime Axetil

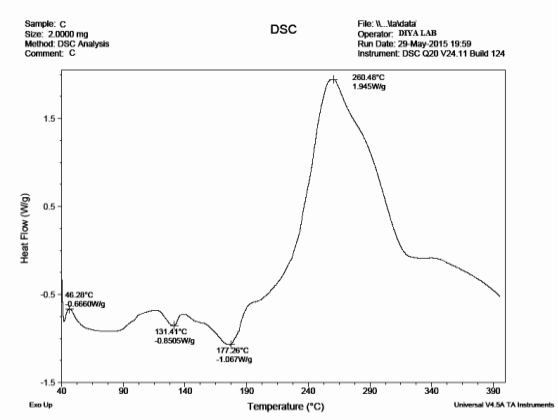

Fig. 6: DSC of SD (Cefuroxime Axetil, PVPK and Soluplus)

The DSC studies performed to understand heat effect on given dispersion. The DSC thermogram of CA exhibited a sharp endothermic peak at 88.80° and 180.73°. Whereas, solid dispersion exhibited an endothermic peak at 131.41° and 177.28° indicates the formation of Solid Dispersion and drug shows exothermic peak at 250.02° and solid dispersion shows broad exothermic peak 260.48°. This suggests drug might formed complex with carrier.

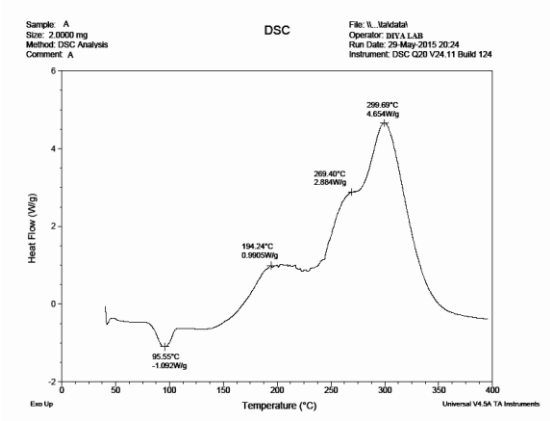

Fig. 7: DSC of Cefpodoxime Proxetil

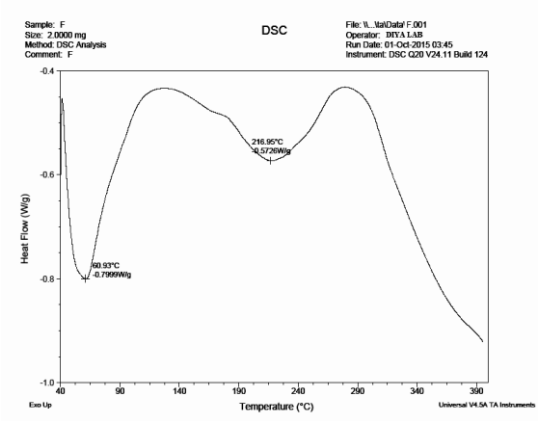

Fig. 8: DSC of SD (Cefpodoxime Proxetil, PVPK and Soluplus)

The results of DSC of pure cefpodoxime proxetil shows broad endotherm at 95.55°, whereas in case formulation shift of melting endotherm to 60.93°, and 216.95° and exotherm could not be differentiated after larger endotherms appeared which shows the formation of solid dispersion.

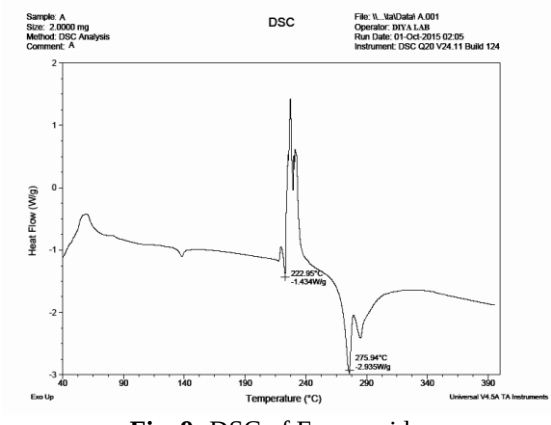

Fig. 9: DSC of Furosemide

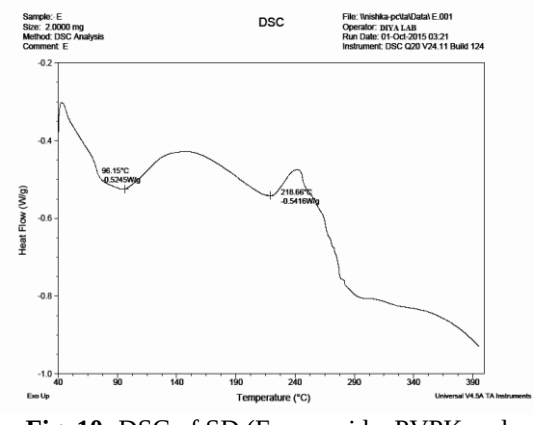

Fig. 10: DSC of SD (Furosemide, PVPK and Soluplus)

The DSC plot of pure furosemide drug powder shows a sharp endothermic peak 222.95°C followed by endotherm at 275.94° C, in the formulaton endothermic peaks were observed at the temperature 96.15°C and 218.66° C which results the solid dispersion formation confirmation.

XRD Studies

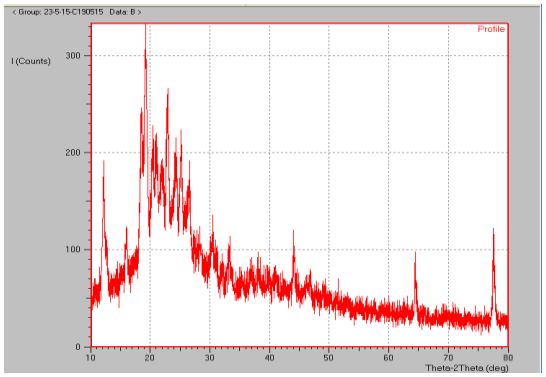

Fig 11: XRD of Cefuroxime Axetil

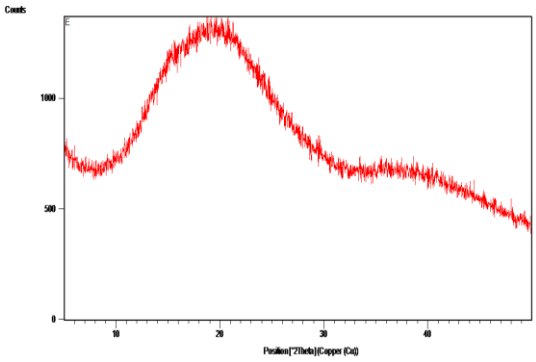

Fig 12: XRD of Cefuroxime Axetil, PVPK-30 and Soluplus

The XRD study performed for determination of crystal habit of pure drug and effect of solid dispersion on drug and polymer. The XRD patterns of drug, showed presence of significant sharp peaks indicated that the drug is in somewhat crystalline state. However, the XRD pattern of SDs showed characteristic reduced intensity of peaks no new intensity peaks were observed. This confirmed that the drug is present in amorphous state in SD.

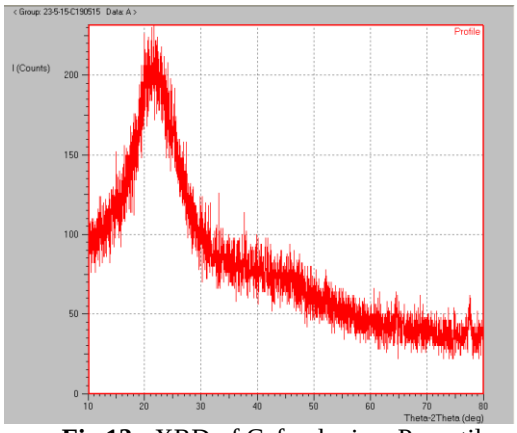

Fig.13 : XRD of Cefpodoxime Proxetil

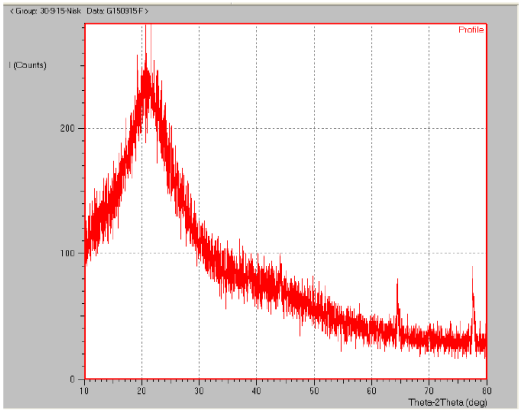

Fig. 14: XRD of SD (Cefpodoxime Proxetil, PVPK and Soluplus)

The diffraction spectra of Cefpodoxime Proxetil doesn't show any intense peaks which leads to conclusion that the drug is present in amorphous state and solid dispersion with soluplus,

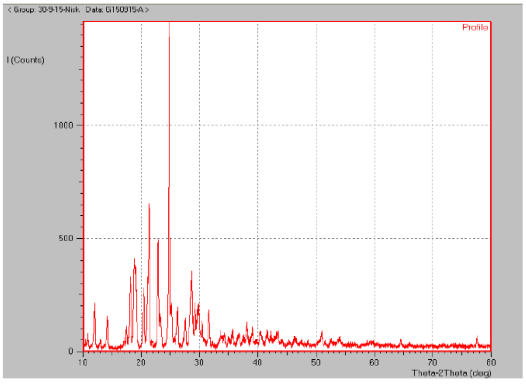

Fig. 15: XRD of Furosemide

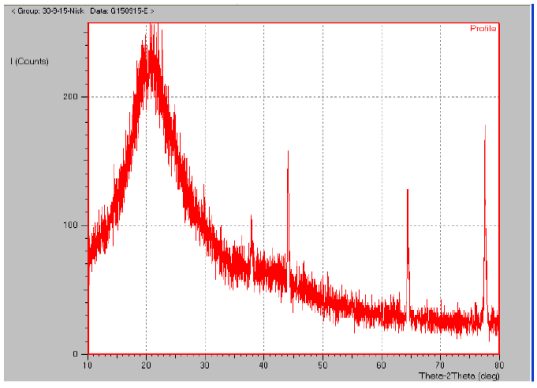

Fig. 16: XRD of SD (Furosemide, PVPK and Soluplus)

The XRD pattern of furosemide shows intense and sharp peaks that prove the crystalline nature of the compound. The solid dispersion of furosemide with PVPK and soluplus show undefined, broad, diffuse peaks of low intensities. This feature indicates the formation of a significant amount of amorphous material.

Standard Calibration Curve

The drug evaluated for formulation before their formulation. The absorbance maxima of the individual drug determined with their respective buffer solutions. Standard Calibration Curve of Cefuroxime Axetil was prepared in 7.0 pH phosphate buffer at λ_{max} 280 nm. Standard Calibration Curve of Cefpodoxime Proxetil was prepared in 3.0 pH phosphate buffer at λ_{max} 233nm.Standerd Calibration Curve of Furosemide was prepared in 5.8 pH phosphate buffer λ_{max} 274nm. Using UV-Visible spectrophotometer. For this stock solution of 1000 µg/ml was prepared. Serial dilutions of 5, 10, 15, 20, 25 µg/ml were prepared and absorbance was taken. Averages of 3 sets of values were taken for standerd calibration curve, and solutions were scanned in the range 200-400 nm against blank. The calibration curve was plotted.

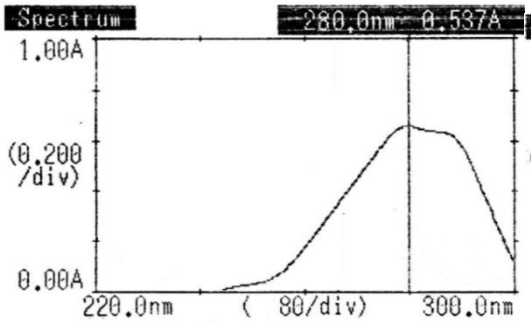

Fig. 17: Absorbance Maxima of Cefuroxime Axetil at 280 nm

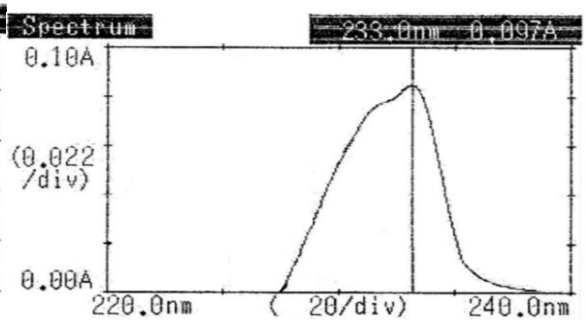

Fig. 18: Absorbance maxima of Cefpodoxime Proxetil at 233 nm

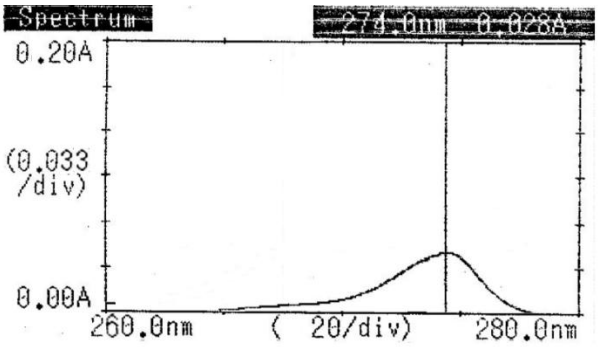

Fig. 19: Absorbance maxima of Furosemide at 274 nm

Standard Calibration Curve of Cefuroxime Axetil

Concentration (µg/ml)	Absorbance (nm)
0	0
5	0.7905±0.012
10	1.6078±0.05
15	2.3699±0.11
20	3.0679±0.15
25	3.9133±0.008

Table 10: Standard Calibration Curve of Cefuroxime Axetil

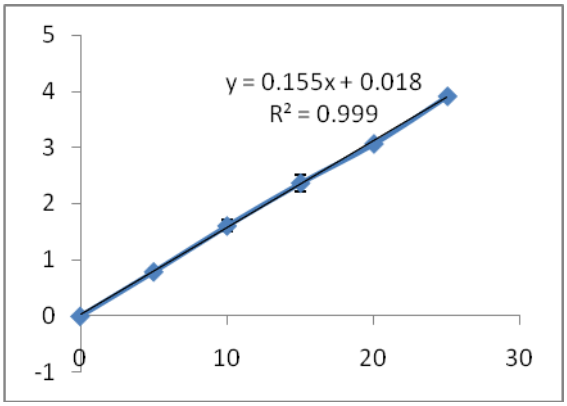

Fig. 20: Standard Calibration Curve of Cefuroxime Axetil

Standard Calibration Curve of Cefpodoxime Proxetil

Concentration (µg/ml)	Absorbance(nm)
0	0
5	0.7905±0.082
10	1.3944±0.157
15	2.1072±0.021
20	2.7445±0.089
25	3.3876±0.28

Table 11: Standard Calibration Curve of Cefpodoxime Proxetil

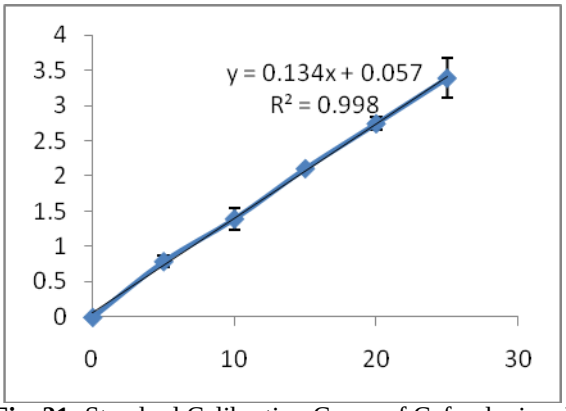

Fig. 21: Standard Calibration Curve of Cefpodoxime Proxetil

Standerd Calibration Curve of Furosemide

Concentration (µg/ml)	Absorbance (nm)
0	0
5	0.5926±0.0362
10	1.312±0.0046
15	1.9876±0.035
20	2.608±0.179
25	3.1937±0.138

Table 12: Standerd Calibration Curve of Furosemide

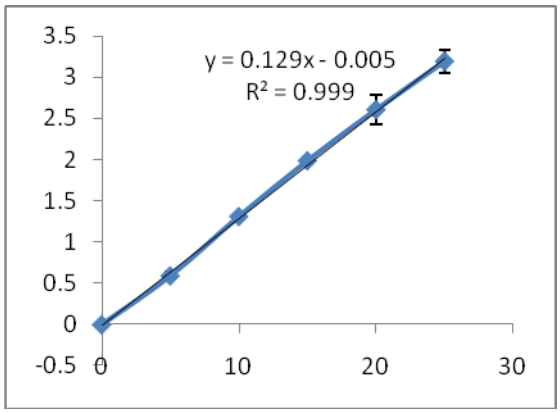

Fig. 22: Standerd Calibration Curve of Furosemide

Solid Dispersion Formulations
Phase Solubility Study

Sr. No.	Polymer Conc. (% w/v)	Drug Concentration (µg/ml)
1	0.1	1.254± 0.32
2	0.2	2.283± 0.83
3	0.3	3.4553± 0.11
4	0.4	5.1047± 0.25
5	0.5	7.8386± 0.093

Table 13: Phase Solubility Study of PVPK-30 and Cefuroxime Axetil

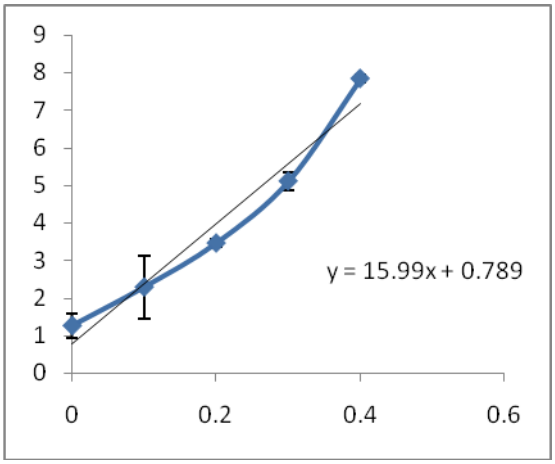

Fig.23 : Phase Solubility Study of PVPK 30 and Cefuroxime Axetil

Sr. No.	PolymerConc (% w/v)	Drug Concentration (µg/ml)
1	0.1	1.254± 0.32
2	0.2	2.276± 0.051
3	0.3	4.594± 0.11
4	0.4	5.412± 0.24
5	0.5	6.58± 0.16

Table 14: Phase solubility Study of Soluplus and Cefuroxime Axetil

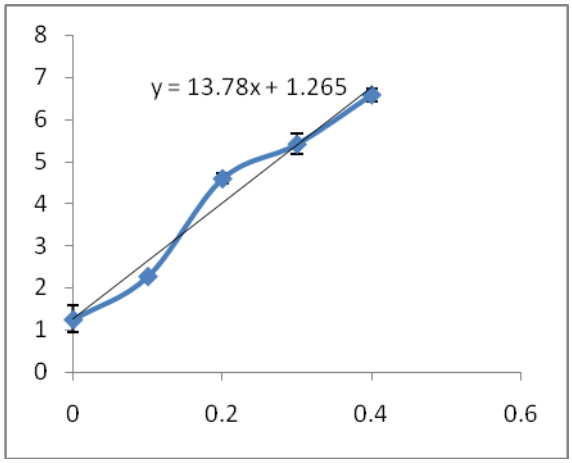

Fig. 24: Phase Solubility Study of Soluplus and Cefuroxime Axetil

Sr No	Polymer Conc (% w/v)	Drug Concentration (µg/ml)
1	0.1	1.081 ± 0.112
2	0.2	1.483± 0.111
3	0.3	2.655± 0.048
4	0.4	4.504± 0.174
5	0.5	6.738± 0.30

Table 15: Phase Solubility Study of PVPK 30 and Cefpodoxime Proxetil

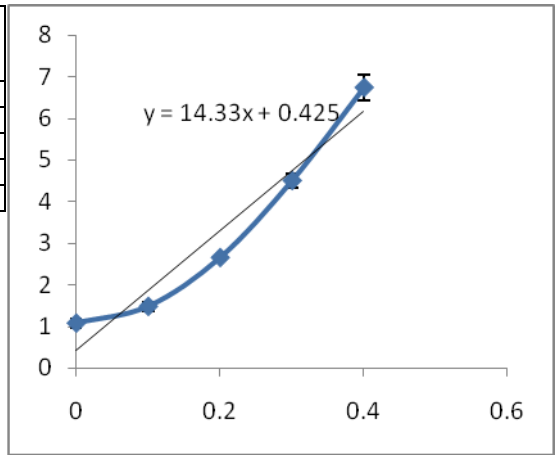

Fig. 25: Phase Solubility Study of PVPK 30 Cefpodoxime Proxetil

Sr No	Polymer Conc (% w/v)	Drug Concentration (µg/ml)
1	0.1	1.081± 0.112
1	0.2	1.776± 0.19
2	0.3	2.872± 0.22
3	0.4	4.268± 0.31
4	0.5	5.526± 0.14

Table 16: Phase solubility Study of Soluplus and Cefpodoxime Proxetil

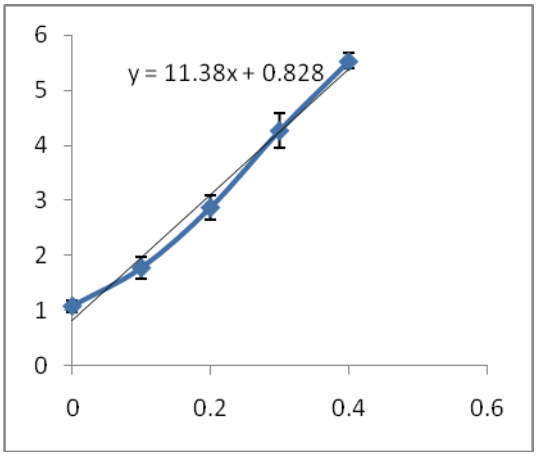

Fig. 26: Phase Solubility Study of Soluplus and Cefpodoxime Proxetil

Sr. No.	Polymer Conc (% w/v)	Drug Concentration (µg/ml)
1	0.1	0.891± 0.072
2	0.2	1.665± 0.138
3	0.3	2.555± 0.085
4	0.4	3.914± 0.124
5	0.5	5.988± 0.145

Table 17: Phase Solubility Study of PVPK-30 and Furosemide

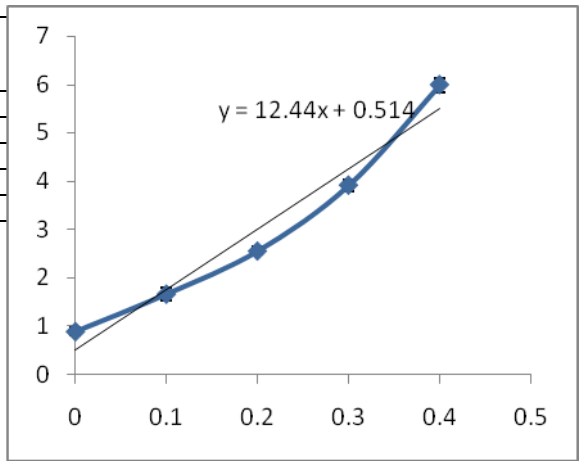

Fig. 27: Phase Solubility Study of PVPK 30 and Furosemide

Sr No	Polymer Conc (% w/v)	Drug Concentration (µg/ml)
1	0.1	0.891± 0.072
2	0.2	1.666± 0.137
3	0.3	2.763 ± 0.124
4	0.4	3.748± 0.081
5	0.5	4.586± 0.112

Table 18: Phase solubility Study of Soluplus and Furosemide

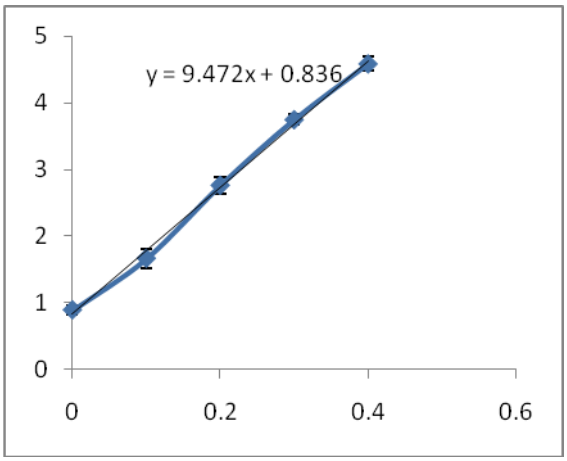

Fig. 28: Phase Solubility Study of Soluplus and Furosemide

The phase solubility study determines capacity of polymer for drug entrapment. The different polymers are used in solubility enhancement are according to their drug entrapment capacity.

The polymers gives here two types of phase solubility curve they are A_L typre where every polymer shows the increase in solubility of the drug by adding polymer which gives straight line in phase solubility curve. Another type is A_P in this curve due to the formation of secondary complexes of drug and polymer which is more solubilised than the primary complexes hence the ratio of drug to polymer was selected higher in concentration. In above curves the PVPK gives the curve of this type which suggests that the final concentraton to be decided for PVPK-30 is double as it tends to forms secondary complex. Hence in formulation concentration was decided 1:2 (Soluplus : PVPK-30) in drug polymer ratio previously decided 1:1 in solid dispersion preparation.

Solid Dispersion

The solid dispersion of drug Cefuroxime axetil,/ Cefpodoxime Proxetil/ Furosemide and polymer PVPK and soluplus were prepared by solvent evaporation method. The polymer as selected from the phase solubility study for determination of solubility. The complex nature evaluated using hydrophilic polymers, due to formation of soluble complexes and/or wetting action carrier. The stability constant determined by equation

S= slope/Ks(1-slope)

where S is apperant stability constant , Ks is solubility of drug in vehicle without carrier. the stability constant of Cefuroxime axetil is 482.76 mg/mol. in PVPK , 1567.89 mg/mol in soluplus, Cefpodoxime proxetil is 563.90 mg/mol in PVPK, 1391.87 mg/mol in soluplus , And furosemide is 940.83 mg/mol in PVPK, 2399.25 mg/mol in soluplus. The apperant stability constant should be more than 100 for prediction of stability of complex in solution. Solubility of Cefuroxime Axetil in buffer is 0.0671mg/ml without carrier, while

Cefpodoxime Proxetil it is 0.0893 mg/ml and Furosemide it is 0.0436 mg/ml, the solubility constant of drug and carrier complex was above 100 for each complex hence can be said as stable complex of drug and polymer in solution. The phase solubility results indicated a linear increase of Cefuroxime axetil (r^2=0.991, slope=6.214), Cefpodoxime Proxetil (r^2=0.992,slope=7.614), Furosemide (r^2=0.992,slope=6.92) solubility as a function of polymer PVPK-30 concentration indicative of a A_L type solubility diagram. In general, if the rise of concentration of phase diagram linear i.e coefficient of line (R^2) value below near to 1, the complex stoichiometry will be assumed to be 1:1 and such profiles according to Higuchi and Connors are of A_L type. Further in formulation study the central composite design was employed to study the effect of independent variables (PVPK [X_1] and Soluplus [X_2]) on dependent variables dissolution study for 2 hours and permeability up to 4 h. The polynomial equations for each response with their high magnitude of the coefficients and mathematical sign indicate about the fit of the model. The final results were confirmed by outcome of the result in vitro drug release and permeability study of optimised batch and its comparison with pure drug.

Factorial equation for dissolution study

Batch Run	X_1	X_2	Dissolution(%) after 2 hrs	Permeability (mg/ml)
1	1	0	19.71±0.04	1.3313±0.12
2	1	1	18.48±0.04	1.4371±0.17
3	0	0	17.05±0.1	1.1302±0.27
4	0	-1	19.9±0.26	0.6731±0.07
5	-1	1	15.9±0.06	2.1321±0.20
6	1	-1	21.4±0.255	1.2805±0.2
7	-1	0	16.7±0.10	1.3963±0.3
8	-1	-1	18.5±0.36	0.7169±0.1
9	0	1	17.8±0.35	1.5247±0.2

All values are expressed as mean±SD, (number of terms) *n*=3

Table 19: Characteristics of solid dispersion of Cefuroxime Axetil

Dependent variables	Source of variation				Sum of squares	Degree of freedom	F value	P value
	Source	Std. Deviation	R-Square	Predicted R-Square				
Dissolution (T_2)	Linear	0.66	0.8925	0.8294	21.69	2	24.92	0.0012
Permeability	Quadratic	0.032	0.9980	0.9810	0.15	2	74.76	0.0028

Table 20: ANOVA of independent variables

Final Equation in Terms of Coded Factors

The model proposes the following polynomial equation for percentage drug entrapment (Cefuroxime Axetil) by Polymer:

Dissolution (Y) = $18.38 + 1..41X_1 - 1.27X_2$

Permeability = $1.10-0.033X_1+0.40X_2-0.31X_1X_2+0.27X_1^2+8.133E-003X_2^2$

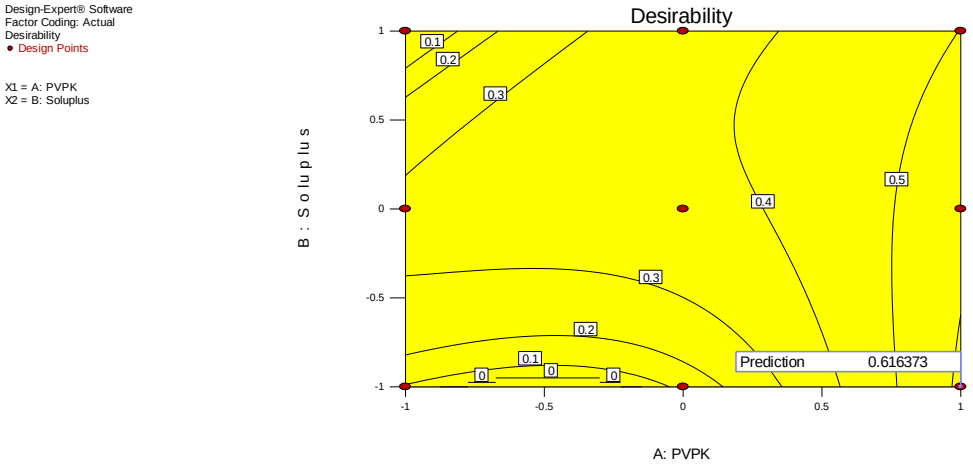

Figure 29: Contour and response surface methodology plot for Desirability study for optimization of the batch indicating graphical and numerical optimization.

Batch Run	X_1	X_2	Dissolution (%) after 2 hrs	Permeability (mg/ml)
1	1	0	20.39 ± 0.19	1.2597± 0.16
2	1	1	20.06 ± 0.09	1.898 ± 0.201
3	0	0	18.71 ± 0.09	1.411 ± 0.255
4	0	-1	22.02 ± 0.76	0.7688± 0.179
5	-1	1	18.31 ± 0.06	2.257 ±0.213
6	1	-1	22.49 ± 0.38	1.093 ± 0.099
7	-1	0	18.22 ± 0.06	1.617 ± 0.108
8	-1	-1	20.21 ± 0.09	0.6298 ± 0.088
9	0	1	19.46 ± 0.04	1.5407 ± 0.178

All values are expressed as mean±SD, (number of terms) *n*=3

Table 21: Characteristics of solid dispersion of Cefpodoxime Proxetil

Dependent variables	Source of variation				Sum of squares	Degree of freedom	F value	P value
	Source	Std. Deviation	R-Square	Predicted R-Square				
Dissolution (T2)	Quadratic	0.43	0.9693	0.7563	3.5	2	9.28	0.0519
Permeability	Linear	0.27	0.7996	0.4701	1.72	2	11.97	0.0081

Table 22: ANOVA of independent variables

Final Equation in Terms of Coded Factors

The model proposes the following polynomial equation for percentage drug entrapment (Cefpodoxime Proxetil) by Polymer:

Dissolution (Y) = $19.18+ 1.03X_1 - 1.15X_2 – 0.13\ X_1X_2 – 0.12\ X_1^2+1.32\ X_2^2$

Permeability (Y) = $+1.39 - 0.042X_1 + 0.53X_2$

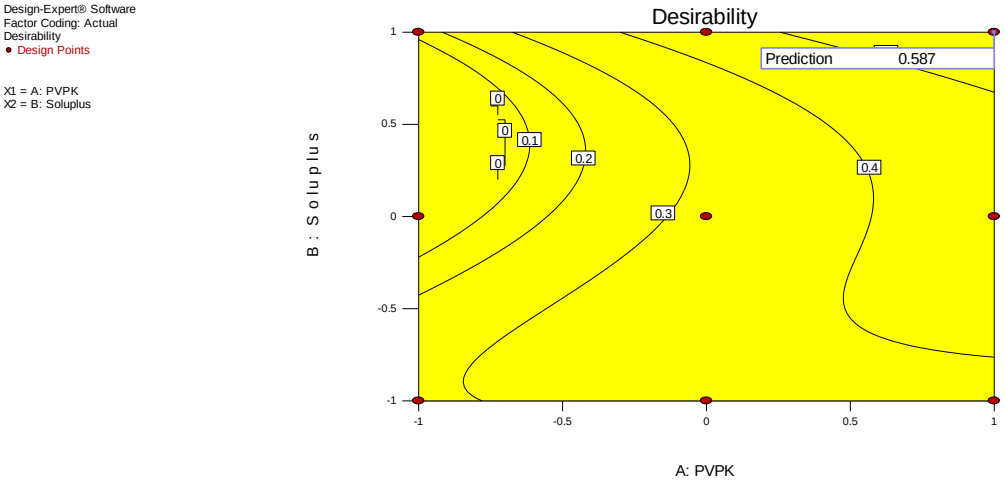

Figure 30: Desirability plot for optimization of the batch indicating graphical and numerical optimization.

Batch Run	X_1	X_2	Dissolution (%) after 2 hrs	Permeability (mg/ml)
1	1	0	16.53± 0.167	0.9568± 0.29
2	1	1	17.1± 0.77	1.224± 0.81
3	0	0	15.22 ± 0.55	1.047 ± 0.40
4	0	-1	18.21 ±0.286	0.6493 ± 0.47
5	-1	1	13.68± 0.236	1.83± 0.012
6	1	-1	17.81 ± 0.355	0.8322± 0.42
7	-1	0	14.34 ± 0.285	1.4272 ± 0.29
8	-1	-1	12.084 ± 0.304	0.6034±0.38
9	0	1	15.76 ± 0.477	1.271±0.83

All values are expressed as mean±SD, (number of terms) *n*=3

Table 23: Characteristics of solid dispersion of Furosemide

Dependent variables	Source of variation				Sum of squares	Degree of freedom	F value	P value
	Source	Std. Deviation	R-Square	Predicted R-Square				
Dissolution (T2)	Linear	1.34	0.6694	0.2167	21.83	2	6.07	0.0367
Permeability	Linear	0.14	0.9197	0.7321	0.17	2	8.83	0.0311

ANOVA: Analysis of variance

Table 24: ANOVA of dependent variables

Final Equation in Terms of Coded Factors:

The model proposes the following polynomial equation for percentage drug entrapment (Furosemide) by Polymer:

Furosemide Dissolution = $15.64+1.89X_1 -0.26X_2$

Permeability = $1.09 - 0.14X_1 + 0.37X_2 - 0.21X_1X_2$

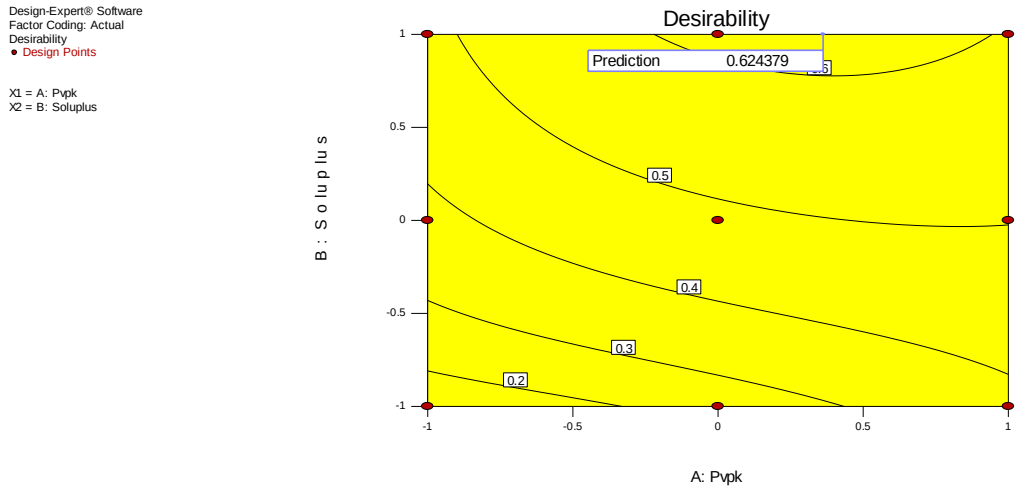

Figure 31: Contour and response surface methodology plot for Desirability study for optimization of the batch indicating graphical and numerical optimization.

The results of the equation showed that X_1 (pvpk-30) has a positive effect on the drug release as shown by the positive coefficient as compared to the X_2 (soluplus) which showed a positive effect on the drug transport across intestinal membrane as shown by the Constraints were set according to the formulation of solid dispersion using the minimum amount of excipients, which would give desired response values i.e., good dissolution and permeability across intestinal membrane. In optimization [Figure 30] maximum desirability was achieved at (1 0.36) coded batch drug polymer ratio. Over lay plot of the desirability give the details of the optimized batch giving the optimum results of the optimized batch, which were very closer to the results obtained by the batch

Drug	Goal	Batch	Predicted Dissolution	Actual Dissolution	Predicted Permeability	Actual Permeability
Cefuroxime Axetil	High Drug Release, High Permeability, In Range PVPK-30, In Range Soluplus	1 -1	21.0672	21.4	1.263	1.2805
Cefpodoxime Proxetil		1 1	20.1286	20.06	1.877	1.898
Furosemide		0.36* 1	16.06	16.11	1.34	1.31

*by taking 0.36=181.66 pvpk frm number line of code

Table 25: Constraints for responses

In vitro Drug Release Study of Pure Drug and Optimised batch

The In vitro drug release study of drug after solid dispersion with optimised batch and pure drug is as follows

Time (min)	CA	CP	Fur	CA (1 -1)	CP (1 1)	Fur (0.36 1)
0	0	0	0	0	0	0
10	0.0034±0.027	0.0048±0.0088	0.0036±0.0091	1.974± 0.254	2.833±0.191	1.086±0.057
20	0.0077±0.054	0.0083±0.010	0.0072±0.0096	4.2 ± 0.293	4.97± 0.155	3.98±0.128
30	0.0115±0.037	0.0126±0.014	0.0103 ±0.021	7.214 ± 0.55	8.26± 0.532	6.04± 0.170
60	0.0779±0.035	0.0894±0.111	0.0762± 0.013	11.98±0.6433	12.98±0.111	10.48±0.103
90	0.0829± 0.15	0.0983±0.104	0.0722± 0.064	17.347±0.510	17.99±0.444	13.07±0.519
120	0.0967± 0.11	0.1893±0.154	0.0783± 0.046	21.4 ± 0.498	20.06±0.121	16.1±0.0283

Table 26: In vitro Drug Release Profile of Optimised Formulation and Drug

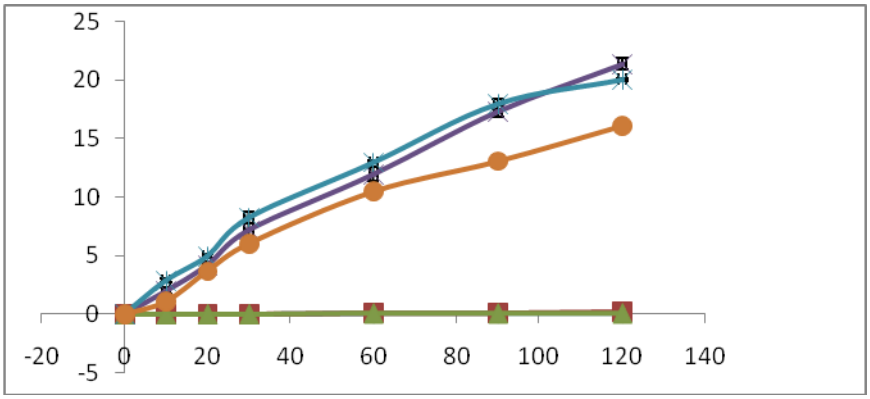

Fig 32: In vitro Drug Release profile of Formulation comparing with pure drug.

Formulation \ Model		Zero Order	First Order	Korse Mayer Peppas	Hickson Krowell	Higuchi
CA	R^2	0.9050	0.9051	0.9050	0.9050	0.7629
CP	R^2	0.9165	0.9164	0.9609	0.9165	0.6886
Furo	R^2	0.8417	0.8417	0.8458	0.8417	0.7412
CA (1 -1)	R^2	0.9879	0.9949	0.9969	0.9930	0.9020
CP (1 1)	R^2	0.9442	0.9635	0.9912	0.9577	0.9440
Furo (0.36 1)	R^2	0.9736	0.9736	0.9871	0.9703	0.9167

Table 27: Model Fitting of Drug release profile

The above table shows model fitting which suggest the drug release kinetics follows Korsemayer peppas model showing R^2 value close to one than other. Hence model with R^2 value near to 1 showing Korsemayer Peppas best fit model for drug release.

III. Conclusion

The results of a 3^2 full factorial design (Central Composite Design) revealed that the pvpk-30 and soluplus significantly affected the dependent variables dissolution after 2 hrs. And drug permeability across intestinal membrane. The solid dispersion of the best batch exhibited a dissolution and permeability is increased (preclinically). The in vitro release studies indicate that the solid dispersion formulation could increase the drug bioavailability due to rise in solubility and permeability of drug.

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