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Fabricating and Optimizing Ophthalmic Nanoparticles for Treating Ocular Viral Infection.

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ABSTRACT

Herps zoster is a viral infection and a common disorder of the eyes. It is caused by herpes simplex virus: herpes virus type 1(HSV-1) & herpes virus type 2 (HSV-2). Topical acyclovir is currently the only and safe pharmacologic treatment of severe viral infection of the eyes. Nanoparticulate formulations have advantage of improving residence time over ocular surface, reduced size increasing permeation across corneal surface for intraocular activity. The objective of this study was to prepare and evaluate Acyclovir (Acy) nanoparticles (NPs) for the treatment of Herps zoster infection of the eyes. So, the present study was designed with the primary aim to prepare nanoparticles by nanoprecipitation method using cationic polymer Eudragit E100 and secondly to study the effect of variables on the behaviour of nanoparticles. Preliminary study showed the compatibility of the drug with the formulation. Optimization was done using full factorial design with independent variables such as Drug to polymer ratio, Organic: aqueous phase ratio and effect of type and concentration of stabilizer. The dependent variables were determined such as particle size, drug entrapment, % drug release selected as the levels. F21 formulation was selected as the final optimized formulation.

Keywords: Acyclovir, Eudragit E 100, nano precipitation, drug entrapment, ocular delivery.

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INTRODUCTION

Herpes simplex keratitis (in the most severe cases) is characterized by the spread of the virus into the deeper corneal layers, leading to damage of the stromal cells. Therefore, treatment requires a suitable permeation of the antiviral drug through the epithelium in order to reduce the virus load. Acyclovir is used as topical antiviral as it inhibits the herpes DNA polymerase, thereby it selectively inhibits herpes virus DNA replication.The topical application of acyclovir is limited by the low corneal penetration of the drug and by poor water solubility of antiviral drugs. The anatomy, physiology and biochemistry of the eye render this organ highly impervious to foreign substances[1] It is a common knowledge that, the ocular bioavailability of drug applied topically as eye drop is very poor. The absorption of drug in the eye is severely limited by some protective mechanisms that ensure the proper functioning of eye and other concomitant factor, for example- Drainage of eye solution Lacrymation and tear turnover, Metabolism, Tear evaporation, Non-productive absorption / adsorption, Limited corneal area and poor corneal permeability. Moreover, the anatomy, physiology and barrier function of the cornea compromise the rapid absorption of drugs. Upon instillation of the eye drops only 1–10% of the drug is made bioavailable while the rest is drained out of the eye through lacrimal secretions. To overcome this problem various approaches have been reported, such as ointments, inserts and aqueous gels, to increase the ocular residence time of topically applied medication.

Controlled drug delivery to the eye offer several advantages over conventional therapies like drug solutions or suspensions in the form of eye drops. The increase of both the residence time of the drug in the precorneal area or cul-de-sac and the absorption would improve its therapeutic effect. One of the significant efforts towards this aim has been the use of colloidal drug delivery systems such as liposomes, micro- or nanoparticles. Nanoparticle systems are able to encapsulate and protect the drug, improve tolerance, penetration efficiency and increase corneal uptake. Nanoparticles have been used as ophthalmic delivery systems because they are able to penetrate into the corneal or conjunctival tissue by an endocytotic mechanism. Nanoparticles are prepared using poorly water soluble drugs without any matrix material suspended in dispersion. These can be used to enhance the solubility of drugs that are poorly soluble in water as well as lipid media. It also improves drug stability as well as bioavailability of poorly soluble drug[2-9]

Nanoparticles, because of their polymeric nature, present some important advantages over other colloidal carriers for ophthalmic applications, that is, a high storage stability, controlled release of the encapsulated drug, and a prolonged residence time in the precorneal area, particularly in

the case of ocular inflammation and/or infection.

Acyclovir (ACY) is categorized as a class III drug according to the Biopharmaceutical Classification System (BCS) because of its high solubility and low permeability. Acyclovir is a polar drug with short plasma half life of 2–3h, therefore 4–5 times application is required when administered as ophthalmic ointment. Many attempts have been made to improve the ocular bioavailability and the therapeutic effectiveness of acyclovir, e.g., chemical modification of the drug and its incorporation into colloidal systems such as liposomes or nanoparticles .

Eudragit E 100 is insoluble at physiological pH values and capable of swelling, thus representing good materials for the dispersions of drugs. They are commonly used for the enteric coating of tablets. They showed an interesting size distribution and a positive surface charge, along with good stability, that make them potential candidates for use in ocular drug delivery systems. In particular, their positive surface charge (ζ-potential) can allow a longer residence time of nanoparticles on the cornea surface, with a consequent slower drug release and higher drug concentrations in the aqueous humor, compared with classical eye drops.

Literature review suggest that acyclovir was formulated as a nanoparticle drug delivery system by using different methods like insitu polymerization, nanoprecipitation and using polymers like cyanoacrylate, PLGA,PLA etc. However in the present study is an attempt to prepare and evaluate nanoparticles of the acyclovir by using Eudragit E 100 polymer by nanoprecipitation method which can be used for ophthalmic therapy.

MATERIALS AND METHODS

Materials:

Acyclovir was obtained as a gift sample from Ajanta Pharmaceuticals Limited Mumbai; Eudragit E 100 was obtained from Evonic industries, Mumbai. Also Pluronic F 68 and Pluronic L 81 and Tween 80 purchased from LOBA Chem. Pvt. Ltd., Pluronic F 127 was obtained from Sigma Aldrich Pvt. Ltd, Acetone, Ethanol and Isopropyl Alcohol were obtained from Research Lab. Fine Chem. Industries, Mumbai.

Methods

Formulation of Acyclovir loaded Eudragit E 100 Nanoparticles:

Acyclovir loaded nanoparticles was prepared using Eudragit E 100 as polymer by the solvent displacement (nanoprecipitation) method [12] Organic phase was prepared by solubilising the Eudragit E 100 in alcohol for 10 mins, various types of organic solvents were used to prepare the organic phase like isopropyl alcohol, acetone, ethanol. Aqueous phase was prepared by

dissolving the drug in the aqueous solvent containing hydrophilic surfactant. After preparation of these two phases, aqueous phase was poured into organic phase drop wise under magnetic stirring, and then instantaneously the nanoparticles were formed by continuous addition of one phase to the other, so that milky colloidal nanosuspension was formed. After this the organic solvent was evaporated by continuous stirring. Then resulting nano formulation was taken for further evaluation. Nanoparticles were initially prepared using drug to polymer ratio 1:1 and aqueous to organic ratio 1:10 and 1% concentration of stabilizer to optimize the variables like effect of solvents(isopropyl alcohol, acetone and ethanol), effect of RPM (300,600 & 900), effect of stirring time (2hr, 6hr, 12hr, 24hr) along with effect of stabilizer (pluronic f 68, pluronic f 127, tween 80) to get highest drug entrapment and smallest mean particle size.

Experimental design:

Use of experimental design allows for testing a large number of factors simultaneously and precludes the use of a huge number of independent runs when the traditional step-by-step approach is used. Systematic optimization procedures are carried out by selecting an objective function, finding the most important or contributing factors and investigating the relationship between responses and factors by the so-called response surface methodology.

Optimization technique using 4 levels and 3 factors was employed for the optimization study. This design is suitable for exploration of second order polynomial models, thus helping in optimizing a process using several experimental runs with design expert (version 8.0.1,sta ease inc.) A design matrix comprising of several experimental runs was constructed, for which the coded levels of independent variables where organic: aqueous phase ratio (X1), polymer: drug ratio (X2), concentration of stabilizer (X3), and type of stabilizer (X4).

Also the dependant variables evaluated were particle size (Y1), % drug entrapment (Y2), % drug release (Y3). These dependant variables described as coded forms in Table (1), and coded variables of formulations F1 to F40 described in Table (2).

Table 1.variable levels in coded forms

Independent	**Coded Levels of dependant variables**				
Variables	1.682	1	0	-1	-1.682
X1(ratio org:aq.)	47.50:10	40:10	10:10	18:10	10:10
X2(ratio poly:drug	91.53:10	75:10	50.75:10	26.50:10	-
X3(con.of stabilizer	3.74%	3%	1.92%	0.84%	0.10%
X4(typeof stabilizer	-	Pluronic L 81	-	Pluronic F 127	-

CHARACTERIZATION OF NANOPARTICLES:

Particle size analysis and zeta potential measurement:

Particle size, size distribution and zeta potential was measured by zetasizer, Malvern instrument.

The size distribution analysis was performed at a scattering angle of 90^0 and at a temperature of 25^0 C, Whereas zeta potential was measured using a disposable zeta cuvette.

Table 2.Results of coded variables for formulation F1 to F40

Batch code	Variable levels in coded form			
	X1	X2	X3	X4
F 1	0	0	0	-1
F 2	1.682	0	0	1
F3	-1.682	0	0	1
F4	0	-1.682	0	1
F5	0	1.682	0	-1
F6	1	-1	1	-1
F7	-1	1	1	1
F8	0	0	-1.682	1
F9	0	0	0	1
F10	1	1	1	-1
F11	0	0	0	1
F12	1	1	-1	-1
F13	1	1	1	1
F14	0	-1.682	0	-1
F15	0	0	1.682	1
F16	-1	1	-1	-1
F17	-1	-1	1	1
F18	0	0	1.682	-1
F19	1	-1	-1	1
F20	-1.682	0	0	-1
F21	-1	-1	1	-1
F22	1	-1	1	1
F23	0	0	0	1
F24	0	0	0	-1
F25	1	1	-1	1
F26	-1	1	-1	1
F27	0	0	0	-1
F28	0	0	0	1
F29	0	0	0	-1
F30	1.682	0	0	-1
F31	0	0	0	-1
F32	0	1.682	0	1
F33	1	-1	-1	-1
F34	-1	-1	-1	-1
F35	-1	1	1	-1
F36	0	0	0	1
F37	-1	-1	-1	1
F38	0	0	0	-1
F39	0	0	-1.682	-1
F40	0	0	0	1

Drug entrapment efficiency:

Freshly prepared nanosuspension was centrifuged at 19000 rpm for 2 hrs. at 2 to 4 degree celcius temperature using refrigerated super speed centrifuge, REMI Electrotechnik Limited(Instruments Division) Vasai. The amount of incorporated drug was measured by taking the absorbance of appropriately diluted supernatant solution at 256 nm using single beam UV spectrophotometer. Drug entrapment efficiency was calculated by subtracting the unentrapped drug from the initial drug content.

In-vitro Drug release

The Franz diffusion cell was used for studying the *in vitro* release of the nanosuspension. A cellulose acetate membrane (dialysis membrane with a molecular weight cut off value of 12,000-14,000, was adapted to the terminal portion of the cylindrical donor compartment. A 10 mL portion of the nanosuspension containing drug, sufficient for establishing sink conditions for the assay was placed into the donor compartment. The receptor compartment contained 25 mL of phosphate buffer solution of pH 7.4 maintained at 37°C under mild agitation using a magnetic stirrer. At specific time intervals, aliquots of 1mL were withdrawn and immediately restored with the same volume of fresh phosphate buffer. The amount of drug released was assessed by measuring the absorbance at 256 nm using a single beam UV spectrophotometer (Shimadzu UV 1800).

Preliminary compatibility study

FTIR:

IR spectra were taken by using Fourier transform infrared spectrophotometer (Shimadzu IR affinity1). The technique used was attenuated total reflectance and spectra were scanned in range of 4000-400cm^{-1}. FTIR study was carried on pure drug, polymer and formulation to confirm compatibility of drug with polymer used in the preparation nanoformulation**.**

Differential scanning calorimetry:

Differential scanning calorimetry thermogram of acyclovir was recorded using DSC apparatus(Mettler Toledo). The samples were hematically sealed in aluminium pans and heated at a constant rate of 20^{0}C/min. Over temperature range of 100 to 300^{0}C. Inert atmosphere was maintained by purging nitrogen gas at flow rate of 50 ml/min.

RESULTS AND DISCUSSION:

Compatibility study using FTIR:

IR spectrum of pure drug and formulation was recorded in range 400-4000 cm-1 with attenuated

total reflectance scanning technique. For confirmation of stability of drug IR spectra of formulation was taken and compared with pure drug. The results of these studies reveal that there were no definite changes obtained in the bands of drug with that of pure drug.

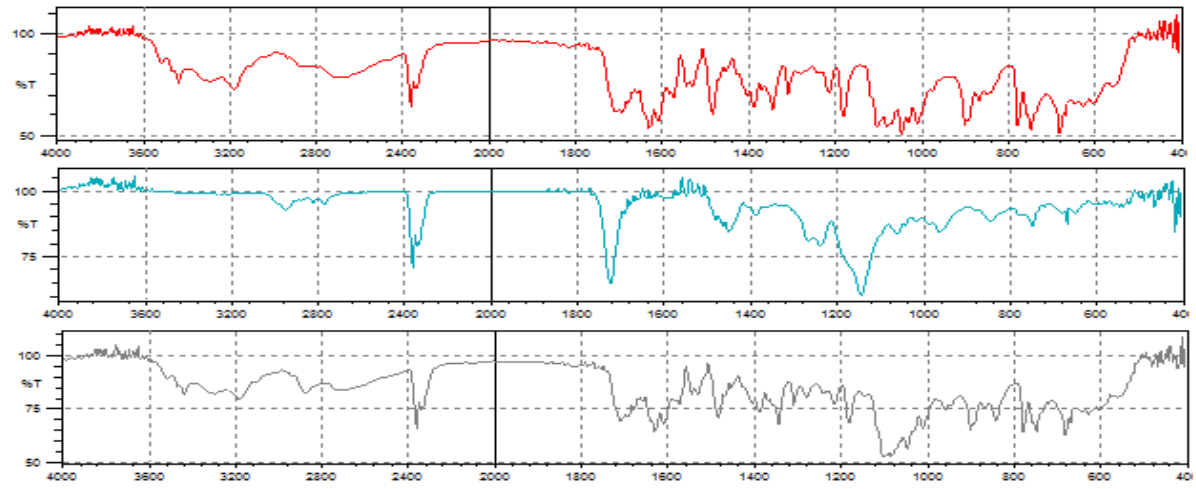

Figure 1. FTIR spectra of drug, polymer and formulation

Compatibility study using DSC

In DSC study also drug was characterized by its melting point & formulation was also showing same results as drug, by these mentioned evidences lead to the conclusion that changes were not seen there was no physical interaction between the drug & polymers

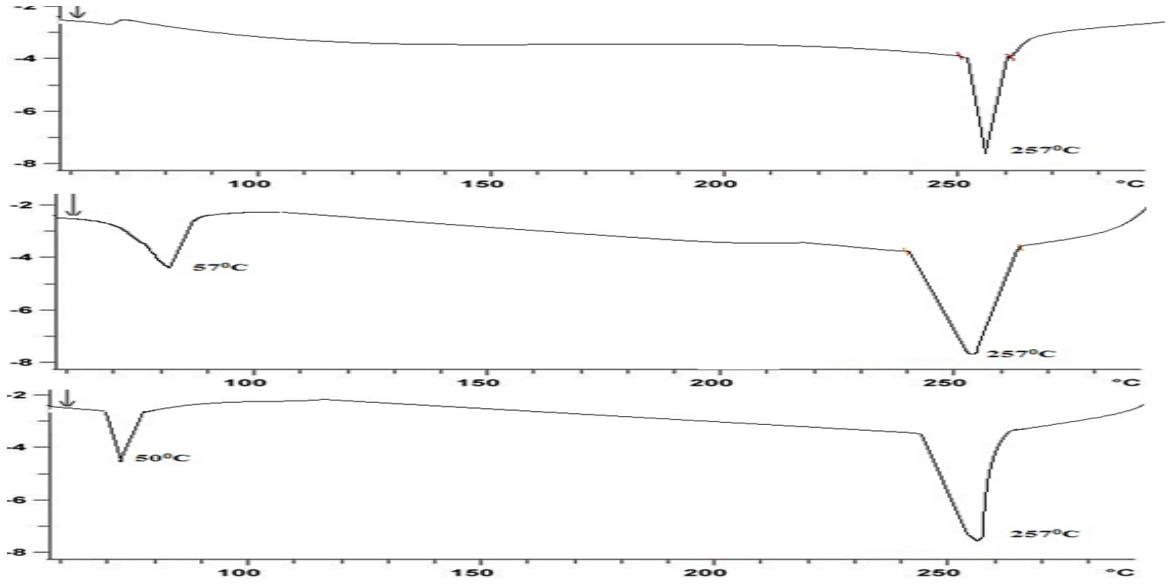

Figure 2. DSC spectra of drug, polymer and formulation

Selection of Formation variables of nanoparticles

From the result of the effect of various organic solvents, the solvent like Isopropyl alcohol was selected due to the highest drug entrapment efficiency 92.68% and smallest mean particle size 390 nm due to the reason of rapid evaporation of the solvent. From the results of variables in

RPM and Stirring time, stirring speed like 600 RPM and stirring time of 6 hrs .was selected which gives smallest particle size like 416 nm. and highest entrapment efficiency 92.71. Selection was done due to the reason of getting the spherical shaped nanoparticles in reduced size. From the results of the various variables, stabilizer like pluronic f 127 was selected. The surfactant concentration in the continuous phase, providing a thin protective layer around the droplets and hence reducing their coalescence. When the concentration of the surfactant is too low, then the aggregation of the polymer droplets occurred.

With the selection of these formulation variables, the nanoparticulate formulations were made according to the full factorial design using design expert software and evaluated. optimization was done.

Optimization

Upon 'trading of' various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, as given by the design software, optimization was done according to 4^3full factorial design so that total several experimental runs was generated from these one optimized formulation was selected due to better drug entrapment and better drug release and reduced particle size as compared to others.

Formulation F21 was selected as the final optimized formulation which gives minimum particle size 414 nm, highest entrapment efficiency 92.76% and highest drug release i.e.98.73%

Table 3.Summary of results of regression analysis for response

Models	R^2	Adjusted R^2	Predicted R^2	S.D.	Remarks
Response(Y1) Linear	0.9846	0.9199	0.8478	0.023	Suggested
Response(Y2) Linear	0.9818	0.9340	0.8148	0.94	Suggested
Response(Y3) Linear	0.9809	0.9215	0.8804	0.26	Suggested

Data analysis and validation of optimization model:

Statistical validation of the polynomial equation generated by design expert was established on the basis of ANOVA provision in the software. The models were evaluated in terms of statistically significant coefficients and R^2 values. Various feasibility and grid searches were conducted to find the compositions of optimized formulations and various 3 D response surface graphs were generated by using Design Expert software. The optimized formulations were evaluated for various response properties. The resultant experimental values of the response were quantitatively compared with that of the predicted values.

Fitting of the data to the model

Fitting of the data for observed responses to various models, it was observed that the best fitted model for all the four dependent variables was linear model. The values of the coefficients for

organic: aqueous ratio, polymer: drug ratio, con. of stabilizer relates to the effects of these factors and their comparative significance on the particle size, entrapment and release of nanoparticulate system. Higher values of the standard deviation (SD) for coefficients indicate the linear nature of the relationship. A positive value in regression equation for a response represents an effect that favors the optimization (synergistic effect), while a negative value indicates an inverse relationship (antagonistic effect) between the factor and the response

Development of Polynomial Equation:

From the data of experimental design and parameters for factorial formulations F1 to F 40, polynomial equations for three dependant variables (particle size, drug entrapment, drug release) have been derived.

1) The equation derived for particle size as shown below:

$Y1 = 1.78X1 + 1.87X2 - 4.57X3$ ……..1

2) The equation derived for drug entrapment shown below

$Y2 = 0.028X1 - 0.043X2 + 0.144X3$ ……..2

3) The equation derived for drug release shown below:

$Y3 = -0.400X1 - 0.280X2 + 0.371X3$ ……..3

In equation (2) negative sign for coefficient of X2 indicates that the drug entrapment of nanoparticles increases when polymer : drug ratio is decreased and positive sign for coefficient of X1 indicates a positive effect of organic to aqueous ratio on drug entrapment.

In equation (3) negative sign for coefficient of X1 and X2 indicates that as the ratio of organic: aqueous phase is increased then response Y3(% drug release) is decreased when concentration of stabilizer is increased.

Response surface analysis:

Response surface plots shows the relationship between the factors even more clearly. These three dimensional model graphs depicting the effects of the pre-determined factors on various responses such as particle size, drug entrapment, and % drug release, based on the model polynomial function, to assess change in the response surface. The response surface plots were used to study the interaction effects of 2 independent variables on the responses or dependent variables, when a third factor is kept at constant level. When these plots were carefully observed, the qualitative effect of each variable on each response parameter could be visualized.

From the response surface graphs of particle size as shown in (figure.3 and figure.4), it was concluded that there was not any change in effect of particle size between the concentration and the type of stabilizer on the particle size and as the concentration of polymer and organic layer

was increased, particle size increased

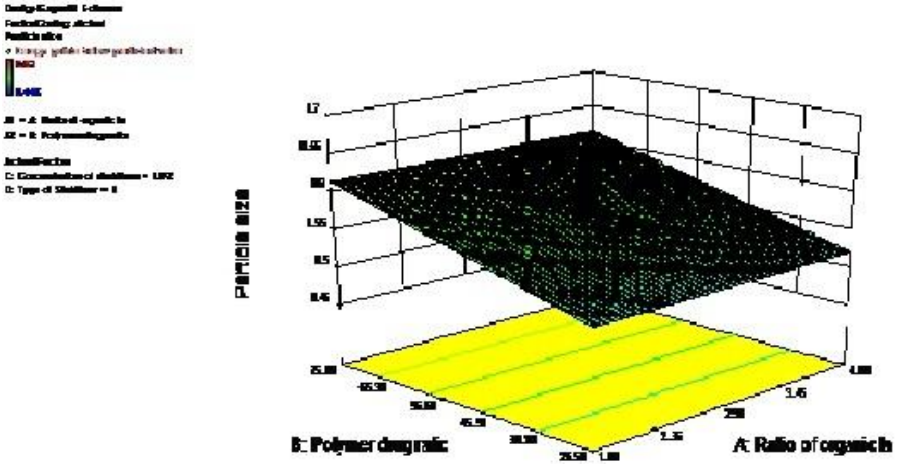

Figure 3. Response surface plot showing the effect of polymer: drug ratio and org:aq. ratio using type of stabilizer(pluronic f 127) on particle size

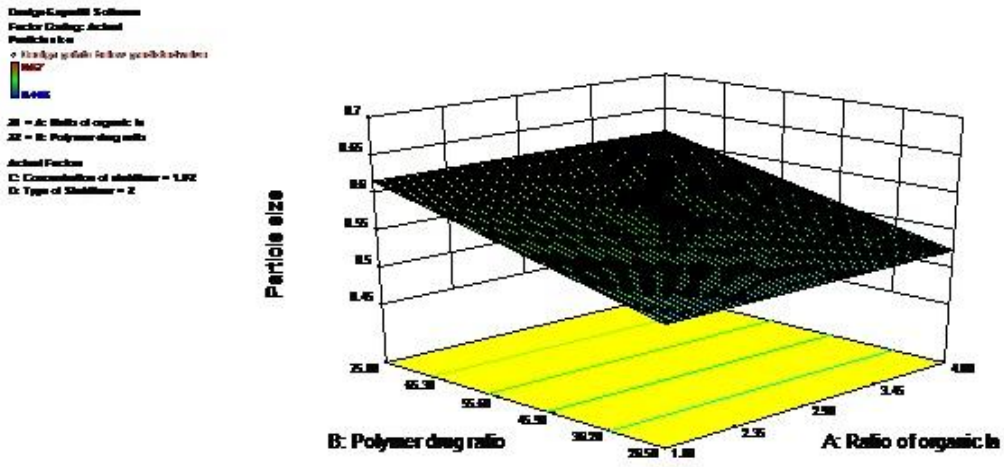

Figure 4. Response surface plot showing the effect of polymer :drug ratio and org: aq phase ratio using type of stabilizer(pluronic l 81) on particle size

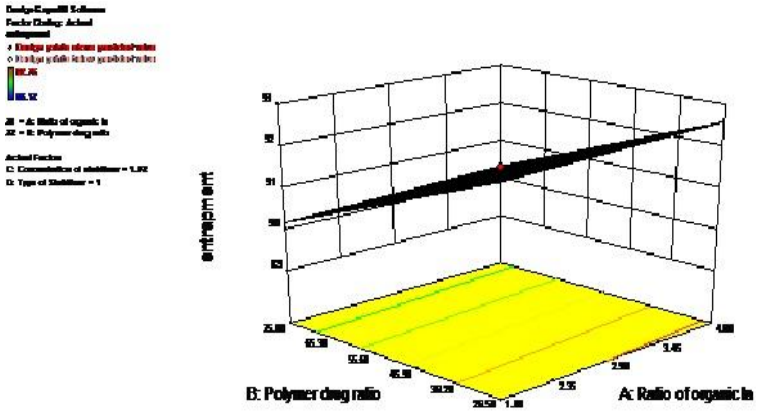

Figure 5. Response surface plot showing the effect of polymer: drug ratio and org: aq phase ratio using type of stabilizer(pluronic f 127) on entrapment

From response surface graphs of entrapment as shown in (figure.5 and figure.6),concluded that as the concentration and type of stabilizer(pluronic f 127) showed maximum drug entrapment as compared to the (pluronic l 81) and as the ratio of organic: aqueous was increased, drug entrapment also increased such as the concentration of polymer increased it decreased the drug entrapment

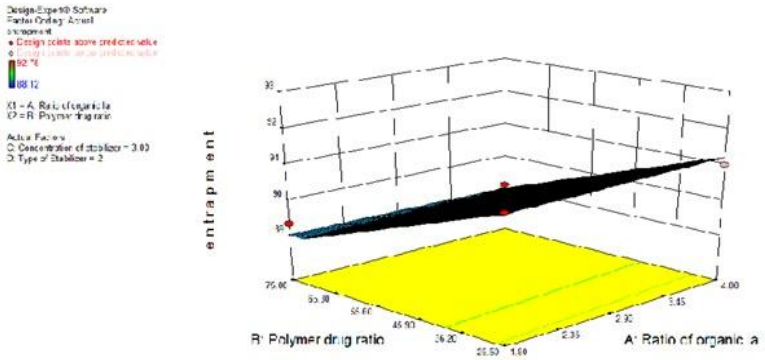

Figure 6. Response surface plot showing the effect of polymer: drug ratio and org: aq phase ratio using type of stabilizer(pluronic l 81) on entrapment

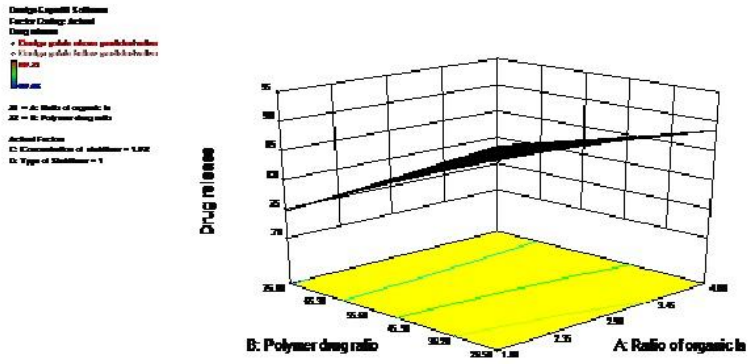

Figure 7. Response surface plot showing the effect of polymer: drug ratio and org: aq phase ratio using type of stabilizer(pluronic f 127) on drug release.

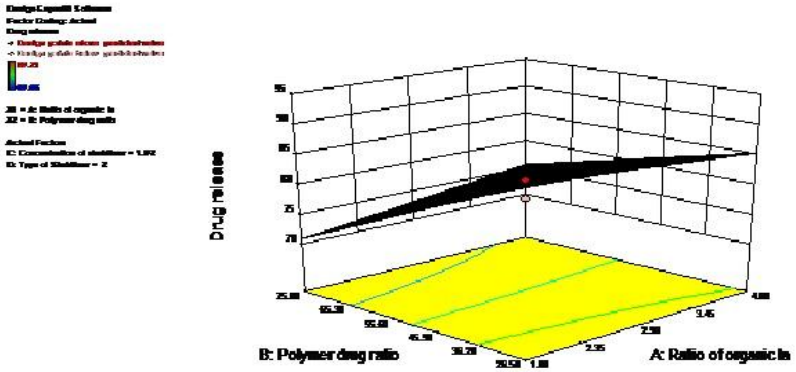

Figure 8. Response surface plot showing the effect of polymer: drug ratio and org: aq phase ratio using type of stabilizer(pluronic l 81) on drug release

From response surface graphs of drug release as shown in (figure.7 & figure.8), it was concluded that, the concentration and type of stabilizer(pluronic f 127) gives maximum drug release as compared to (pluronic l 81).& as the ratio of polymer: drug increased it decreased drug release

In-vitro diffusion study:

The in vitro release study of the optimized formulation in phosphate buffer, pH 7.4 showed an initial burst release of about 10–12% of acyclovir, followed by a more gradual and sustained release phase for the following 24 h. Even after 24 h, about 5–7% of the drug still remained in the nanoparticles, Evaluation of the release profiles of the formulation suggests that the developed nanoparticles can be used as an important platform for sustained drug release. The initial fast release of acyclovir may be due to the rapid hydration of nanoparticles due to the hydrophilic nature of acyclovir and eudragit E 100. In-vitro drug release showed that, formulation F2 gives highest drug release i.e. 99.21%

CONCLUSION:

Formulation of acyclovir loaded eudragit E 100 nanoparticles was done by solvent displacement method. Optimization was done by experimental design with the evaluation of various independent variables as factors on the responses made. It gives the statistical approach for the effective formulation of the nanoparticle with desired particle size, drug entrapment and effective drug release. The influence of the factors such as organic: aqueous phase ratio, polymer: drug ratio, concentration of stabilizer and type of stabilizer with the responses such as particle size, drug entrapment and drug release of drug loaded nanoparticle was studied to evaluate the effective and desirable formulation with desired response

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