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R E S E A R C H A R T I C L E

Formulation and Evaluation of Nanocrystals to Improve Oral Bioavailability

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A B S T R A C T

The development of nanocrystal-based drug formulations has yielded the opportunities to address and treat challenging diseases. Nanocrystals vary in size but are generally ranging from 100 to 500 nm. Through the manipulation of size, surface characteristics and material used, the nanocrystal can be developed into smart systems, encasing therapeutic and imaging agents as well as bearing stealth property. Further, these systems can deliver drug to specific tissues and provide controlled release therapy. This targeted and sustained drug delivery decreases the drug related toxicity and increase patient's compliance with less frequent dosing. Nanotechnology has proven beneficial in the treatment of cancer, AIDS and many other disease, also providing advancement in diagnostic testing. In the presant work selected anti viral drugs Asunaprevir and evaluated

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A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Nanocrystals are crystalline nanoparticles with size ranging from 200 to 500 nm stabilized by surface stabilizers. They increase the saturation solubility, dissolution rate and probably the mucoadhesion resulting in improved oral bioavailability of drugs exhibiting dissolution rate dependent bioavailability. 6 Drug nanocrystals constitute a versatile formulation approach to enhance the pharmacokinetic and pharmacodynamic properties of poorly soluble drugs.

2. Materials and Methods

Asunaprevir, Ibrutinib, PLGA, TPGS, Acetone, Dialysis membrane all the chemicals used were laboratory grade.

Methodology

Method of Preparation of Asunaprevir Loaded Nanocrystals:

Solvent dispersion (Nanoprecipitation):

The nanocrystals are prepared by dissolving the drug in organic phase along with the polymer (PLGA) and added to the aqueous solution containing TPGS which acts as an emulsifier. The solution of organic phase was added in drop wise into aqueous phase under homogenization at 11,000 rpm. The dispersion was kept under magnetic stirring for 4hrs at room temperature. The solution is kept under reduced pressure for about 2-3min. This process forms nanocrystals loaded with drug.

3. Results and Discussion

HPLC Method

Samples collected in diffusion studies were analyzed by HPLC technique. For this purpose a standard plot was plotted in HPLC by using reference standard of Asunaprevir.

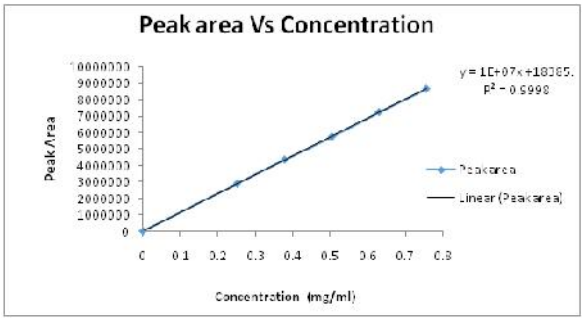

Figure 1: Standard plot of Asunaprevir by using HPLC

Evaluation Parameters

The first part of the plan of work was to optimize the concentration of surfactant to be used in the formulation of nanocrystals. To achieve this, the first three formulations were planned with TPGS concentrations 0.015%, 0.03% and 0.06% respectively. The optimization of surfactant concentration was done on the basis of particle size and entrapment efficiency of nanocrystals obtained.

As the least particle size and best entrapment efficiency was obtained for F2 formulation when compared to F1 and F3, it was decided that the 0.03% of TPGS was the optimum concentration to be used in further formulations. The next part of the plan of work was to optimize the drug polymer ratio. For this, 5 batches were planned (F4 to F8) using the drug polymer ratios of 1:5, 1:10, 1:15, 1:20 and 1:25 respectively. The optimum drug polymer ratio was selected on the basis of entrapment efficiency of the polymer. The entrapment efficiency was found to be very low for 1:5 (21%) and 1:10 (38%) drug polymer ratio. In case of F6, F7 and F8 formulations the entrapment efficiencies were found to be 65%, 82% and 98% respectively. It indicated that there was no further increase in entrapment efficiency even when the polymer concentration was increased. Therefore, it was decided to perform in vitro diffusion studies for all these three (F6, F7 and F8) batches.

Optimized formulations: Based on the entrapment efficiency, a set of formulations (F6, F7 and F8) were considered as optimized compositions which can be taken up further studies and evaluated for the diffusion studies.

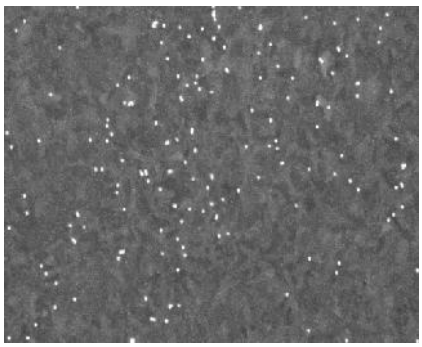

Figure 2: SEM image of optimized formulation

The in vitro diffusion studies were performed in pH 7.4 buffer using Dialysis membrane for 240 hours. Initially the release of drug from all the three batches was found to be about 25-35% in 24 hours. This was due to the release of adsorbed drug from the surface of Nanocrystals . Later on a constant and slow drug release was observed for 240hrs. The drug diffusion for F6, F7 and F8 formulations was found to be approximately same i.e., 96.4%, 91.5% and 85.4% respectively. Therefore the F8 formulation which had drug polymer ratio of 1:25 was decided to be the optimized formulation.

Diffusion study profile for F6, F7 and F8 formulations

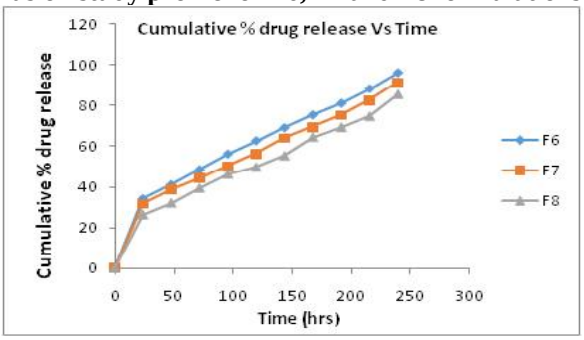

Figure 3: Diffusion study profile Cumulative % release Vs Time (hrs)

Zero order plots for F8 formulation

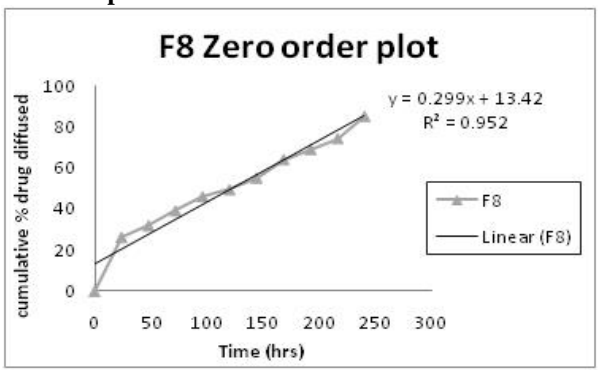

Figure 4: Zero order plot For F8 Formulation

First order plot for F8 formulation

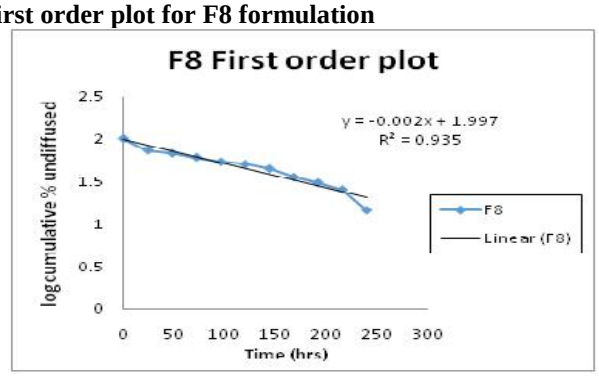

Figure 5: First order plot For F8 Formulation

Higuchi plot for F8 formulation

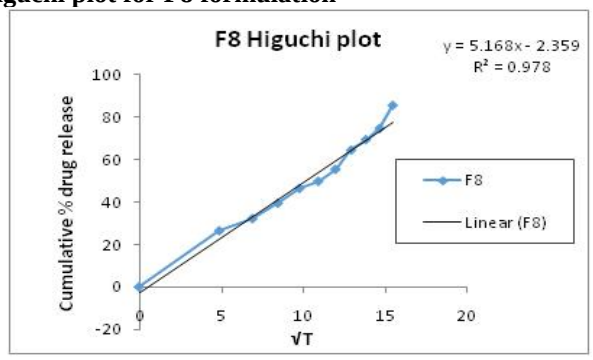

Figure 6: Higuchi plot For F8 Formulation

The drug release from the Nanocrystals was found to follow Zero order release based on the "r" value obtained for Zero order (0.952) and first order (0.935) for F8 formulation. Also, the drug release mechanism was found to be "Diffusion" based on the "r" value of 0.978 obtained for Higuchi's plot. Similarly, the drug release mechanism was found to be of Anomalous diffusion mechanism based on the "n" value of 0.774 obtained for Peppa's equation.

IR studies:

From the IR spectra it is clearly evident that there were no interactions of the drug. IR spectrum of the pure drug and the Drug polymer mixture (1:1) shows the characteristic peaks at 3386.95 cm^{-1} to 709.35cm^{-1}This confines the undisturbed structure of the drug (Table 11). This proves the fact that there is no potential incompatibility of the drug with the polymer used in the formulation. Hence the formula for Asunaprevir can be reproduced in the industrial scale without any apprehension of possible Drug polymer interactions.

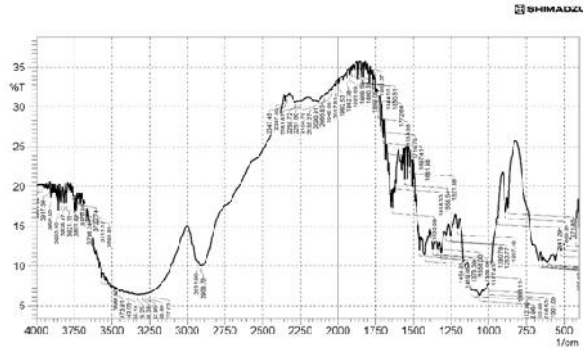

Figure 7: FTIR spectrum of Asunaprevir pure drug

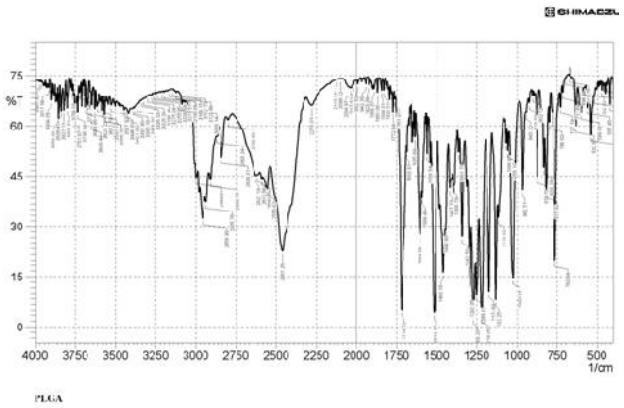

Figure 8: FTIR spectrum of PLGA

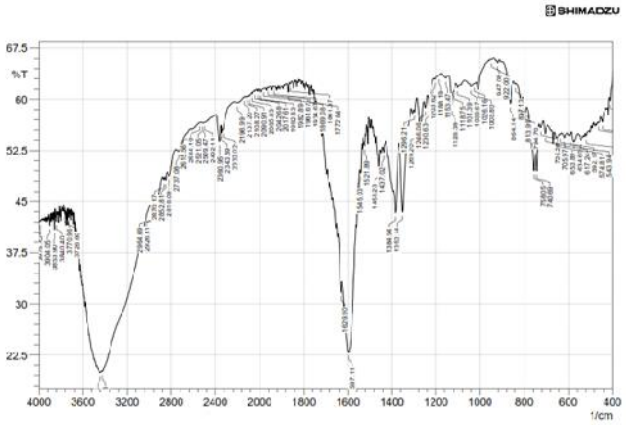

Figure 9: FTIR spectrum of TPGS

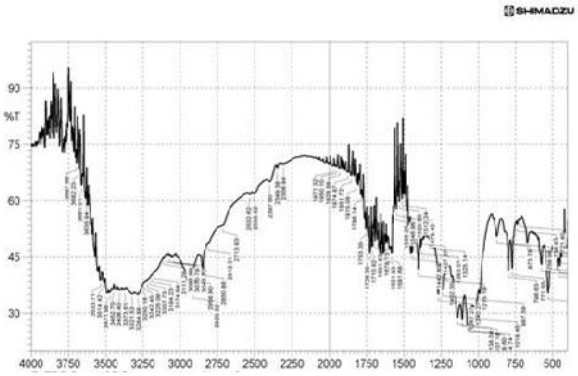

Figure 10: FTIR spectrum of drug + polymer mix

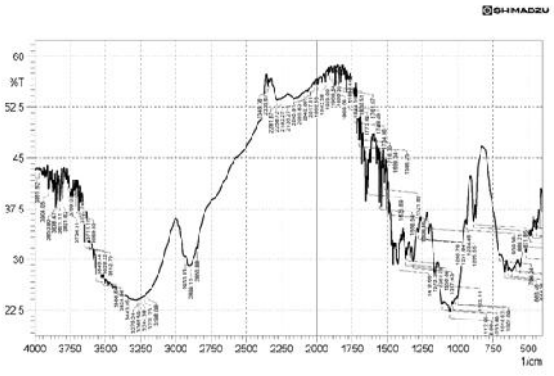

Figure 12: FTIR spectrum of optimized formulation

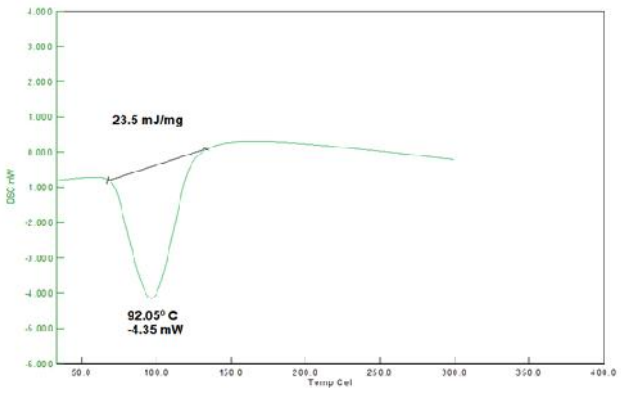

Figure 13: DSC spectrum of pure drug

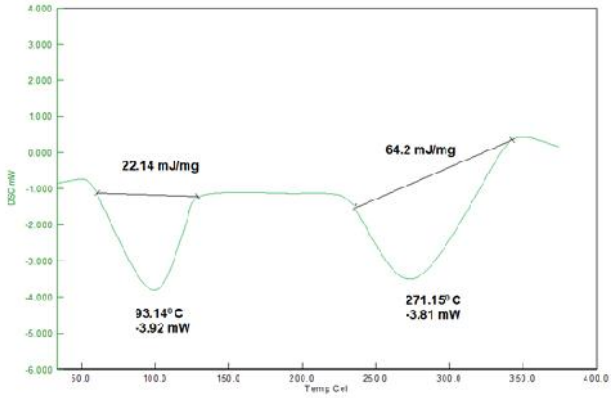

Figure 14: DSC spectrum of optimized formulation

Table 1: Composition of the Nanocrystals

Ingredients	Batch No							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
PLGA (50:50)(mg)	13	13	13	25	50	75	100	125
TPGS(%g/ml)	0.015	0.03	0.06	0.03	0.03	0.03	0.03	0.03
Asunaprevir (mg)	5	5	5	5	5	5	5	5
Acetone (ml)	3	3	3	3	3	3	3	3
Water (ml)	10	10	10	10	10	10	10	10

Note: In above all formulations (F1 to F8) 5mg of the drug was added instead of original dose of the API . The above formulations were prepared and the entrapment efficiency was determined for choosing best formulation.

Table 2: Standard Curve of Asunaprevir

S.No	Concentration (mg/ml)	Peak area
1	0	0
2	0.2525	2912222
3	0.37875	4364555
4	0.505	5760005
5	0.6312	7265583
6	0.7575	8682663

Table 3: Evaluation Studies of Prepared Nanocrystals: Entrapment Efficiency, Particle size, Zeta Potential and Drug Loading

Batch No	Particle size (nm)	Zeta potential (mV)	Drug Loaded (mg)	Entrapment Efficiency (%)
F1	250.8	-0.272	0.2	4
F2	152.5	-5.16	0.23	4.6
F3	539.9	-1.92	0.21	4.2
F4	---	---	1.05	21
F5	106.8	-24.1	1.9	38
F6	132.3	-24.7	3.25	65
F7	155.5	-25.6	4.1	82
F8	122.4	-27.2	4.9	98

Table 4: Formulations used for in vitro diffusion study

Ingredients (mg)	F6	F7	F8
PLGA (50:50)	75	100	125
TPGS%(g/ml)	0.03	0.03	0.03
Asunaprevir (mg)	5	5	5
Acetone (ml)	3	3	3
Water (ml)	10	10	10

In- vitro drug release of Asunaprevir loaded Nanocrystals

➢ **Name of the drug**	**:**	**Asunaprevir**
➢ **Total no. of time points including zero**	**:**	11
➢ **Diffusion medium**	**:**	pH 7.4 PBS
➢ **RPM**	**:**	200
➢ **Volume of diffusion medium (ml)**	**:**	100
➢ **Volume of sample removed (ml)**	**:**	5
➢ **Formulations**	**:**	F6, F7, F8

Table 5: Diffusion study profiles for F6, F7, F8

Time (Hr)	Cumulative % drug release		
	F6	F7	F8
0	0	0	0
24	34.2	31.6	26.4
48	41.2	38.8	32.1
72	48.6	44.5	39.4
96	56.4	50.3	46.3
120	62.9	56.4	49.7
144	69.3	64.1	55.3
168	75.6	69.7	64.4
192	81.3	75.4	69.3
216	88.5	82.6	74.6
240	96.4	91.5	85.4

Table 6: Drug release kinetics for F6, F7 and F8

Release kinetics		Formulations		
		F6	**F7**	**F8**
zero order	**R^2 value**	0.881	0.936	0.952
	K_0	0.271	0.316	0.299
First order	**R^2 value**	0.873	0.915	0.935
	K_1	0.009	0.007	0.005
Higuchi	**R^2 value**	0.991	0.984	0.978
	K_H	5.906	5.526	5.168
Peppa's	**R^2 value**	0.956	0.958	0.969
	n value	0.798	0.785	0.774

Table 7: Results of stability studies of optimized formulation F8

Formulation code	Parameters	Initial	1st Month	2nd Month	Limits as per specifications
F8	25˚C/60%RH % Release	96.20	96.27	96.78	Not less than 85%
F8	30˚C/75%RH % Release	97.12	96.79	96.80	Not less than 85%
F8	40˚C/75%RH % Release	97.25	97.48	96.83	Not less than 85%
F8	25˚C/60%RH Assay value	97.65	98.19	98.31	Not less than 90% Not more than 110%
F8	30˚C/75%RH Assay value	98.16	98.21	98.32	Not less than 90% Not more than 110%

F8	40˚C/75%RH	Assay value	98.20	98.16	98.22	Not less than 90% Not more than 110%

Table 8: Stability dissolution profile of F8 for 1st and 2nd month

S.No	Time (in minutes)	F8 1st Month	F8 2nd Month
1	0	0	0
2	10	50.25	54.15
3	20	78.62	78.60
4	30	84.80	84.75
5	40	88.60	90.46
6	50	90.77	92.78
7	60	94.15	96.11

4. Conclusion

Asunaprevir

The present research proposed a novel formulation by applying Vitamin E TPGS as an emulsifier to fabricate Nanocrystals by solvent dispersion/nanopresipitation for controlled release of antineoplastic drug Asunaprevir. Investigation of the preparation, characterization and in-vitro release of the Nanocrystals was carried out. The different formulations of with various ratios of drug-polymer and surfactant were evaluated and optimized. Our results demonstrated that vitamin E TPGS could be an efficient emulsifier for fabrication of polymeric nanocrystals, which can achieve excellent effects in drug encapsulation efficiency, size and size distribution and in vitro release kinetics of the nanocrystals .In this research, a drug encapsulation efficiency as high as 98% has been achieved. The particle size and size distribution strongly depends on the amount of TPGS added in the fabrication. Drug release kinetics indicated that drug release was best explained by Higuchi's equation, as these plots showed the highest linearity (r^2=0.978) but a close relationship was also noted with Zero order kinetics (r^2 =0.952)

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