Original Article

DESIGN DEVELOPMENT AND CHARACTERIZATION OF OMEPRAZOLE LOADED NANOSUSPENSION

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Abstract

The purpose of this study was to develop omeprazole nanosuspension with enhanced solubility and bioavailability; it was prepared by precipitation ultrasonication method. To ensure the quality of the omeprazole nanosuspensions, the selected preliminary trial formulation (F10) with particle size 198.6nm, entrapment efficiency 88.9% and *in vitro* drug release 82.2% at 60 min) was subjected to 2^2 factorial design. The optimum composition obtained using a 2-factor, 2-level Factorial design was as follows: polyvinyl alcohol (90 mg), sonication time of 40 min. The constant regression values for particle size was 175nm, zeta potential -24.5mV, *in vitro* drug release 87.3%, entrapment efficiency 92.7%. From the data it was observed that **R2** formulation was the best formulation.Scanning Electron Microscopy revealed that the particles were prismatic in shape. Stability studies performed for a period of 3 months indicated that there were no significant changes in the *in vitro* drug release pattern and entrapment efficiency.

Keywords: Omeprazole, nanosuspension, solubility, bioavailability

INTRODUCTION

Omeprazole is a substituted benzimidazole, which is a proton pump inhibitor used in the treatment of peptic ulcers, reflux esophagitis and the Zollinger-Ellison syndrome. Biopharmaceutical Classification System (BCS) classifies it as a class II compound with high permeability (Log P = 3.51) and low aqueous solubility. In acid solutions, the drug is chemically unstable and sensitive to heat, humidity, light, and organic solvents. Due to its instability, poor water solubility, short biological half-life, rapid metabolism, and rapid elimination, Omeprazole's therapeutic applications are limited. In order to overcome these limitations, resveratrol has been incorporated into a number of different types of products, including polymeric nanoparticles, solid lipid nanoparticles, self-emulsifying drugs, nanoemulsions, liposomes, nanosuspensions, and nanofibers..[1]

A nanosuspension is a colloidal dispersion of nano-sized drug particles stabilized by a polymer, a surfactant, or both. Drugs with low solubility in aqueous or lipid solvents can be upgraded with nanosuspensions. Because nanoparticles have a large surface area, nanosuspensions increase the solubility and dissolution rate of low soluble drugs. This mechanism enables nanosuspension formulations of BCS Class II and IV compounds to display improved bioavailability, quick action, and other biopharmaceutical effects. A nanosuspension can be planned to prepare in two different ways, top-down and bottom-up technology. Using top-down methods such as milling (jet mill and ball mill) and high-pressure homogenization, drug particle size can be reduced without organic solvents Although these top-down techniques produce heat, they can be difficult to use with thermolabile materials because they are high-energy processes. Furthermore, nanoparticles below submicrons

cannot be developed. Additionally, a lot of energy can produce amorphous particles and deform crystals. In the bottom up technique, particles are precipitated from saturated or unsaturated drug solutions. The bottom up methods incorporate different procedures, such as solvent evaporation, supercritical fluid, antisolvent precipitation, and chemical precipitation. The energy requirements of these techniques are moderately lower than those of top down techniques. When applying the most commonly used antisolvent method, residual solvents and particle growth must be controlled. Inadequate understanding of formulation and manufacturing processes leads to improper particle growth control. Thus, there has been a requirement for development of robust processes that do not involve additional harsh processes for organic solvent removal to prepare a nanosuspension. These processes should be perceived utilizing scientific and systematic methods to successfully develop an efficient manufacturing method to produce the final drug product with the desired best quality. From this point of view, the present study focused on application of quality by design (QbD) approach to the development of omeprazole nanosuspension at the formulation step as well as the preparation process.[2]

METHODOLOGY

Materials

Omeprazole was obtained from Sun Ridges Health Care Pondicherry, Poloxamer 407, PVA, and HPMC K100M was obtained from Yarrow Chem Products, Mumbai.

Methods

Preparation of nanosuspension : Precititation Ultrasonication method

Omeprazole was completely dissolved in methanol to form organic phase and deionised water containing different polymers as anti-solvent. Then 1ml of organic solution was quickly injected by a syringe into an anti-solvent phase under sonication for 30 min in an ice cold condition. The samples were placed in a magnetic stirrer for 3h and subsequently placed in refrigerated ultracentrifuge for 1h at 20000rpm. The supernatant was discarded and replaced by same quantity of fresh antisolvent. The solid residue was redispersed by sonication (Table 1).[3]

Table 1: Formulation of omeprazole loaded nanosuspension for preliminary batch

Formulation code	Omeprazole (mg)	Poloxamer 407 (mg)	HPMC K100M (mg)	PVA (mg)
F1	50	50	-	-
F2	50	100	-	-
F3	50	150	-	-
F4	50	200	-	-
F5	50	-	50	-
F6	50		100	-
F7	50	--	150	-
F8	50	-	200	-
F9	50	-	-	50
F10	50	-	-	100
F11	50	-	-	150
F12	50	-	-	200

Evaluation of Nan suspension

Mean Particle size intensity and Zeta Potential

Mean Particle size (MPS) and zeta potential were determined using Malvern size analyser and Zeta sizer Nano ZS at 25±0.5°C.[4]

Entrapment efficiency

The prepared nanosuspension was ultracentrifuged, the amount of free omeprazole present in the clear supernatant was measured using a UV spectrophotometer.

***In vitro* drug release by using Dialysis sac**

The *invitro* release of various nanosuspension formulations were performed by dialysis bag diffusion technique. The sac was hermetically sealed and filled with pH 7.4 phosphate buffer and emptied for leaks. The receptor compartment contained 100 mL of pH 7.4 phosphate buffer maintained at 37 ± 0.5^0C under agitation at 500rpm using a magnetic stirrer. At specific time intervals, aliquots of 1 mL were withdrawn and immediately restored with the same volume of fresh pH 7.4 phosphate buffer. The amount of drug released was assessed by measuring the absorbance at 300 nm using a single beam UV spectrophotometer.[5]

Statistical Optimization

A 2^2 factorial design was used to optimize the variables in the present study. In this design, 2 factors: concentration of PVA and sonication time evaluated, each at 2 levels: low and high and experimental trials were performed at all 4 possible combinations. Various concentrations of PVA (X1) and sonication timewere selected as independent variables. Mean particle size (Y1), Zeta potential (Y2) Entrapment efficiency and *in vitro* drug release were selected as dependent variables. Data obtained from all formulations were analyzed using Mintab 18-V 12.20.

Kinetic Analysis of Release Data

The obtained dissolution data were fitted to zero order, first order, Higuchi- Crowell and Korsmeyer-Peppas equations to understand the rate of drug release from the prepared formulations. The correlation coefficient values were calculated and used to find the fitness of the data.[6][7]. Interpretation is provided in Table 2.[8][9]

Table 2: Interpretation of diffusion release mechanisms from a polymeric film

Exponent "n"	Mechanism
<0.45	Fickian diffusion
0.45-0.89	Anomalous transport (Non-Fickian diffusion)
>0.89-1	Case 1 transport
>1	Super case 11 transport

Scanning Electron Microscopic Study of Optimized Formulation

The optimized formulation was centrifuged, filtered and dried to convert to powder form. The dried powder was evaluated for SEM analysis. The morphology of omeprazole loaded nanosuspension was studied using SEM (S-4800, Hitachi technologies corporation, Japan). Prior to the examination, the sample is mounted onto metal stubs using a double sided adhesive tape and sputtered with a thin layer of gold under vacuum. The scanning electron microscope is operated at an acceleration voltage of 1.5KV. [10][11][12]

HPLC-analysis

The omeprazole concentrations were determined by using an high performance liquid chromatography (HPLC) method. The mobile phase was based on the method of the European Pharmacopoeia: 1.4 g of di-sodium hydrogen phosphate were added to 1000 ml MilliQ-water and the pH was adjusted with phosphoric acid to 7.6. Seventy-three parts of this buffer were mixed with 27 parts of HPLC-grade acetonitrile. Flow rate was 1 ml/min; the UV detector was operated at a wavelength of 280 nm. A Eurosphere 100, C18, 5 mm column was used in the HPLC hardware from Kontron Instruments (Germany). The nanosuspension samples were prepared by diluting 10 ml of nanosuspension to 10 ml with mobile phase. A freshly prepared standard was checked with every run. To compare the stability of the nanosuspension with the omeprazole solution a reference specimen was prepared by dissolving an exact amount (5 mg) of omeprazole in 8.4% sodium bicarbonate solution. The concentration of this reference was assayed directly without dilution.[13]

Stability Study

A short term (3 months) stability studies were performed for the final optimized nanosuspension. The temperature was maintained at 40^0C / 75% RH, to monitor the extend of entrapment efficiency and *invitro* drug release.[14]

RESULTS

Compatability Study of Drug and Excipients - FT-IR (Confirmation of purity)

FTIR spectrum of the pure drug omeprazole and powder mixtures of pure drug and polymers are represented in Figs. 1 - 4. Omeprazole as well as the powder mixtures give spectra indicating presence of absorption peak due to presence of sulfoxide, aromatic amine and vinyl ether, suggesting that these functionalities are also present in both[15]. The characteristic aromatic amine of the drug exhibited absorption peak at 1311 cm^{-1}, vinyl ether at 1205 cm^{-1}, sulfoxide at 1076cm^{-1}.These are also the characteristics of powder mixtures. Hence, it is concluded that the drug is present in free state in the powder mixtures and not in the form of reaction product.

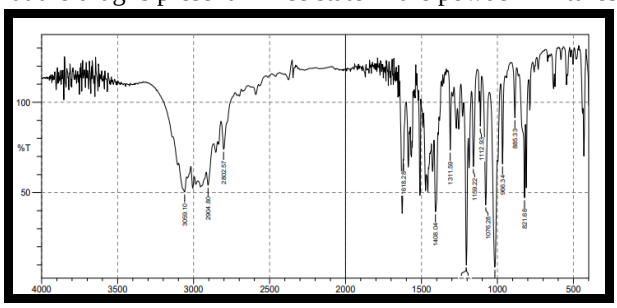


Fig 2: FT-IR Spectrum of drug and HPMC K100M

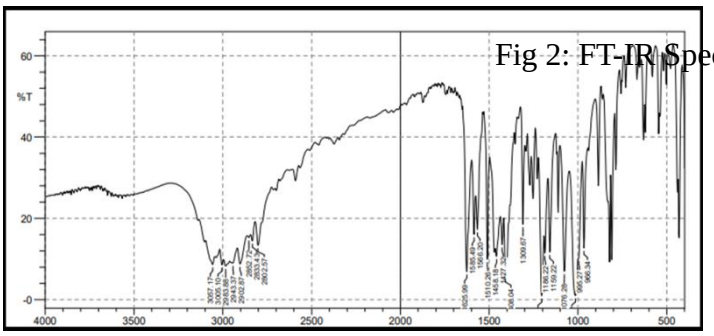
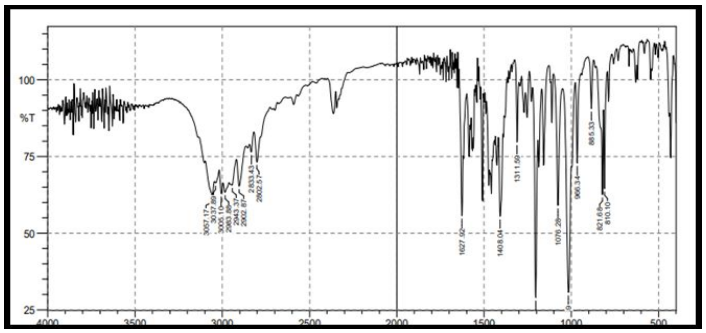


Fig 1:FT-IR Spectrum of pure drug Omeprazole



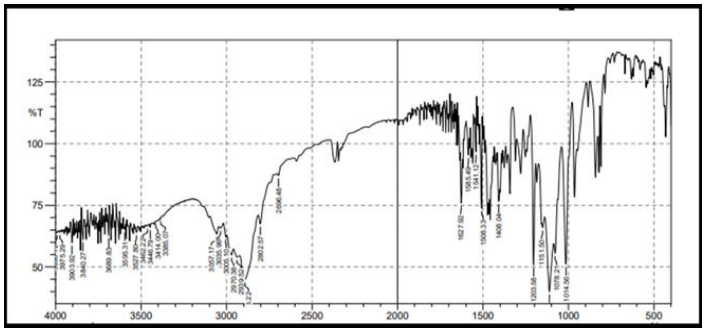


Fig 3: FT-IR study of drug and PVA

Fig 4:FT-IR study of drug and Poloxamer 407

Fig 5: Omeprazole loaded nanosuspension

Evaluation of the Preliminary trial

Mean Particle size

The nanosuspension batches containing different stabilizers may influence the droplet size upon aqueous dispersion. The average particle size distribution data obtained was in the range of 198 – 310 nm nm. Among the set of nanosuspensions, prepared using three different polymers lower particle sizes obtained were 245 ± 1.50 nm for Poloxamer 407 (F3), 298.3 ± 1.02 nm for HPMC K100M (F6), 198.6 ± 1.12 nm for PVA (F10). On comparison, F10 formulation prepared using PVA tend to show lowest particle size (Table 3).

Table 3: Particle size of the Prepared loaded nanosuspension

Formulation Code	Particle Size (nm)
F1	278.3
F2	268.5
F3	245
F4	284.5
F5	310.2
F6	298.3
F7	328.9
F8	345.2
F9	211.9
F10	198.6
F11	257.4
F12	283.5

Entrapment Efficiency

The entrapment efficiency of the formulations was in the range of 68.2 – 78.8%. When concentration of polymer is increased, the platform for binding the drug to the core is increasing[16]. Among the set of nanosuspensions, prepared using three different polymers lower entrapment efficiency obtained were 84.7 ± 0.2% for Poloxamer 407 (F3), 298.3 ± 1.02 nm 72.2 ± 1.4% for HPMC K100M (F6), 89.09 ± 0.6% for PVA (F10). On comparison, F10 was found to have highest entrapment efficiency (100mg PVA) (Table 4).

Table 4: Entrapment efficiency of the prepared Omeprazole loaded nanosuspension

Formulation code	Entrapment Efficiency (%)
F1	78.6 ± 0.3
F2	80.3 ± 1.2
F3	84.7 ± 0.2
F4	83.9 ± 0.9
F5	68.2 ± 0.9
F6	72.2 ± 1.4
F7	74.2 ± 0.5
F8	75.5 ± 0.2
F9	85.2 ± 0.7
F10	89.09 ± 0.6

F11	87.1 ± 0.3
F12	87.2 ± 0.5

***In vitro* drug release using Dialysis sac**

Drug release data for all batches of omeprazole loaded nanosuspensions were in the range 52-82%. From the data, it was clear that amount of polymer directly compromises the drug release of prepared formulations. It was observed that the *in vitro* drug release of the F10 formulation was faster and higher than other formulations. The sharp increase and highest rate (82.2% at 60min), in the *in vitro* drug release was due to the increased surface area of the nanosized drug particles. According to Noyes–Whitney equation, an increase in solubility and decrease in particle size lead to an increased in rate of drug release and it has been reported that the solubility increases with decreasing particle size (nanometers in range). The overall release profiles in the present investigation suggests that nanosized drug particles (≈198 nm) found to have profound impact on rate of drug release and drug solubility. The bioavailability of nanosuspension thus can be affected by the rate of drug release, where particle size reduction can significantly improve the performance of the drug.

From the above evaluation parameters F10 has shown lower particle size, higher entrapment efficiency and highest *invitro* drug release rates. So, F10 formulation was considered as the best from the preliminary trial datas.

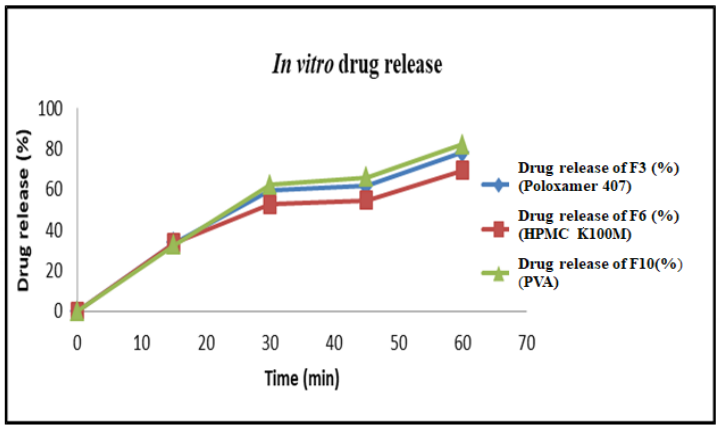


Fig. 6: Dissolution profile of best selected formulation of Omeprazole loaded nanosuspension

Statistical Optimization

A 2^2 factorial design was used to optimize the variables in the present study. In this design, 2 factors: concentration of PVA and sonication time evaluated, each at 2 levels: low and high and experimental trials were performed at all 4 possible combinations. Various concentrations of PVA (X1) such 90mg, 110 mg and sonication time were 20min, 40min were selected as independent variables (Table V). Mean particle size (Y1), Zeta potential (Y2), Entrapment efficiency (Y3) and *in vitro* drug release (Y4) were selected as dependent variables. Data obtained from all formulations were analyzed using Mintab 18-V 12.20. All batches that showed particle size in the range of 175-315 nm, zeta potential -7.32 to -27.1 mV, entrapment efficiency 68.7-92.73%, *in vitro* drug release 55.9-87.3%.The various models fitted for each responses were linear and two- factor

interaction models. Using the ANOVA available in the software, the polynomial equations involving the main effects and interaction factors were determined based on the estimation of various statistical parameters. Obtained data were subjected to multiple regression analysis[17,18].

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2$$

Hence the constant regression values for particle size was 175.2nm, zeta potential -24.5mV, *in vitro* drug release 87.3%, entrapment efficiency 92.7%. From the data it was observed that **R2** formulation was the best formulation.

Table 5: Optimization batches of Nanosuspension using 2^2 Factorial Design

Run Order	X1	X2	Y1	Y2	Y3	Y4
R1	-1	-1	272	-7.32	68.72	62.48
R2	-1	+1	175	-24.5	92.73	87.32
R3	+1	-1	315	-9.81	73.86	55.9
R4	+1	+1	209	-27..1	88.7	78.3

N.B: X1=Concentration of PVA X2=Sonication time
Y1=Particle size Y2=Zeta potential,
Y3=Entrapment efficiency Y4= *In vitro* drug release

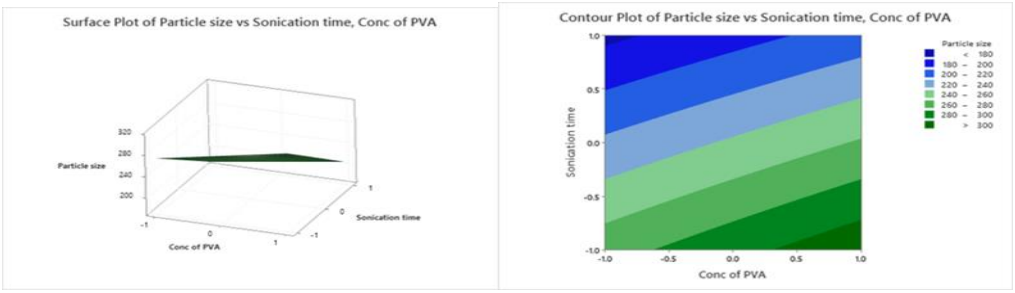


Fig. 7: a) b)

a) Surface Plot of Y1 (Particle size) vs X2(Sonication time), X1 (Conc. Of PVA).
b) Contour plot of Y1 (Particle size) vs X2 (Sonication time), X1 (Conc. Of PVA)

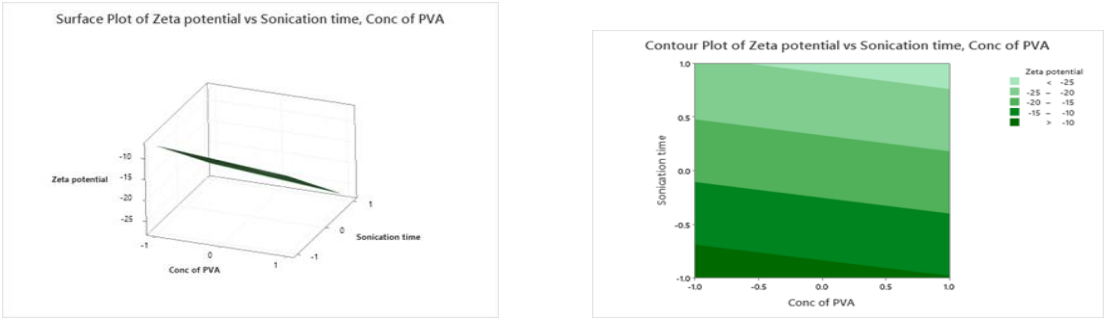
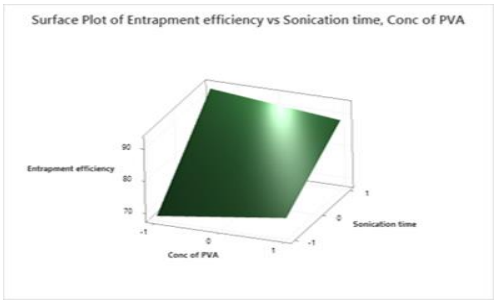


Fig. 8: a) b)

a) Surface Plot of Y2 (Zeta potential) vs X2(Sonication time), X1 (Conc. of PVA)
b) Contour Plot of Y2 (Zeta potential) vs X2(Sonication time), X1 (Conc. of PVA



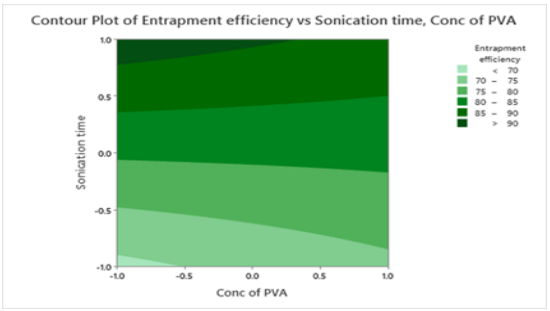
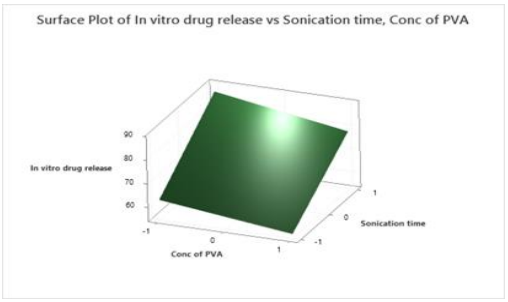


Fig.9: a) b)

a)Surface Plot of Y3 (Entrapment efficiency)vs X2(Conc. Of Sonication time),X1(Conc.of PVA) b)Contour Plot of (Entrapment efficiency) vs X2 (Sonication time), X1 (Conc. Of PVA)



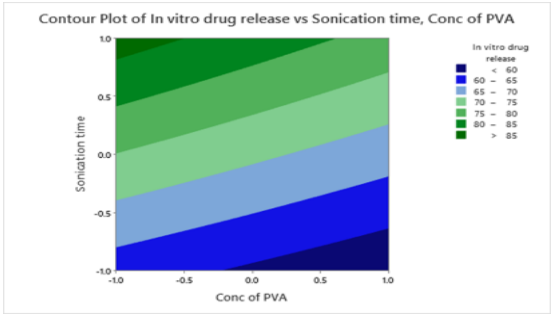


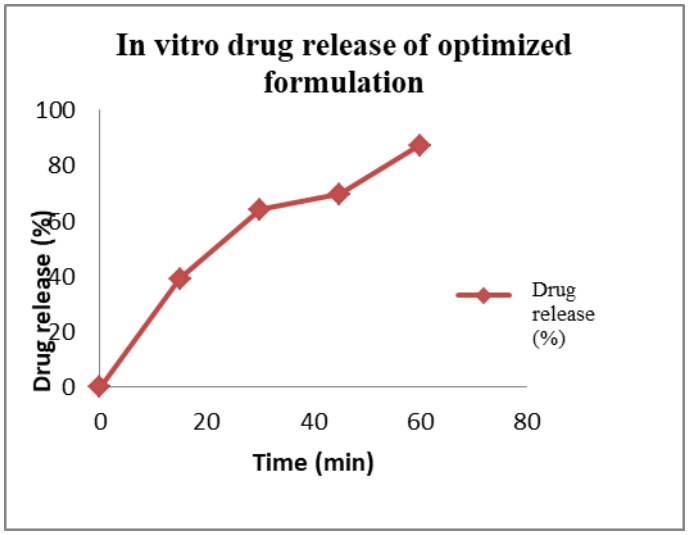
Fig. 10: a) b)

a) Surface Plot of Y4(In vitro Drug release) vs X2(Sonication time), X1 (Conc. Of PVA)

b)Contour Plot of Y4(In vitro Drug release) vs X2(Sonication time), X1 (Conc. Of PVA)

***In Vitro* Dissolution Study of Optimised Formulation (R2)**

It was observed that the *in vitro* drug release of the optimized formulation R2 was faster and higher than pure drug. The sharp increase and highest rate (87.32% at 60 min), (87.35% at 5h) was obtained in comparison with pure drug(44.68% at t_{5h}) and F10 (82.2% at t_{5h}) in the *in vitro* drug release was due to the increased surface area of the nanosized drug particles. The overall release profiles in the present investigation suggests that nanosized drug particles (≈175 nm) found to have profound impact on rate of drug release and drug solubility.



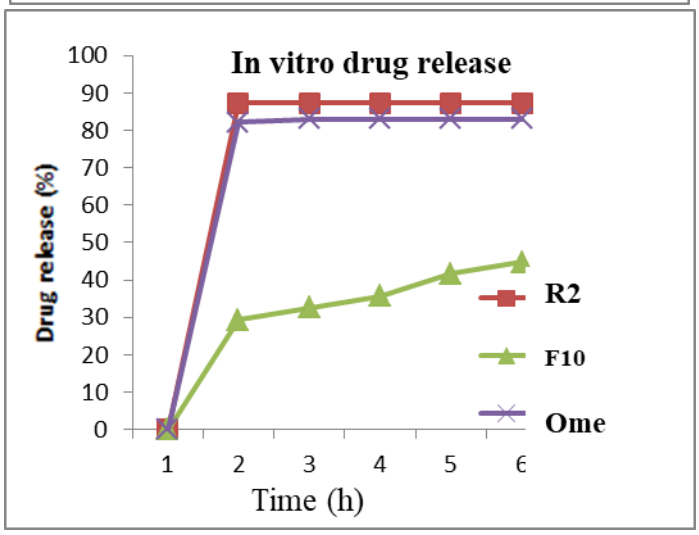


Fig. 11: a) b)

Percentage drug release profile of a) optimized formulation for 60 min b) comparison on R2, F10 and pure drug Omeprazole

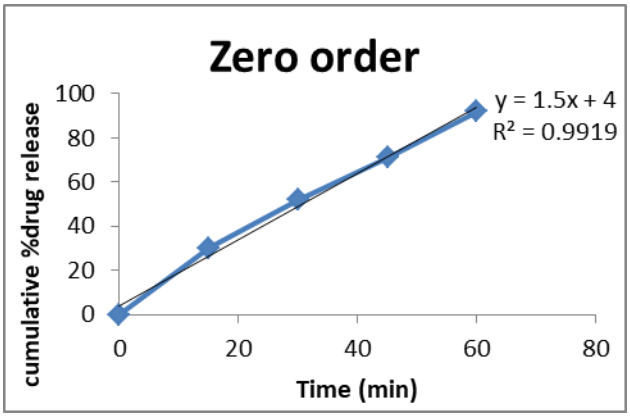
Drug Release Kinetics

The results of In vitro release profile obtained from the formulation code could be plotted in models of data treatment as follows (Table 6):

Table 6: Kinetic study of optimized formulation

Code	Coefficient Of determination($R^{2)}$				Korsmeyer plot (n)	Release Mechanism
	Zero Order R^2	First Order R^2	Higuchi R^2	Korsmeyer Peppas R^2		
R2	0.99	0.88	0.89	0.89	0.9	case II transport

- Zero order kinetics model-Cumulative percentage drug release vs time
- First order kinetics model-log cumulative percent drug remaining vs time
- Higuchi's model-cumulative % drug release vs square root of time
- Korsemeyer equation or Peppa's model-Cumulative % drug released Vs log time[19,20].



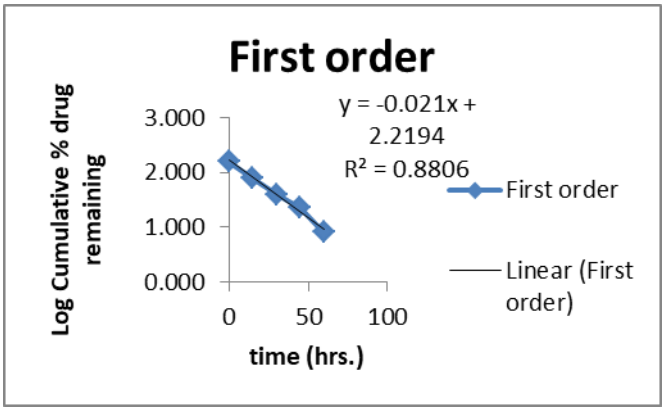


Fig.12: Zero order plot for optimized formulation Fig. 13: First order plot for optimized formulation

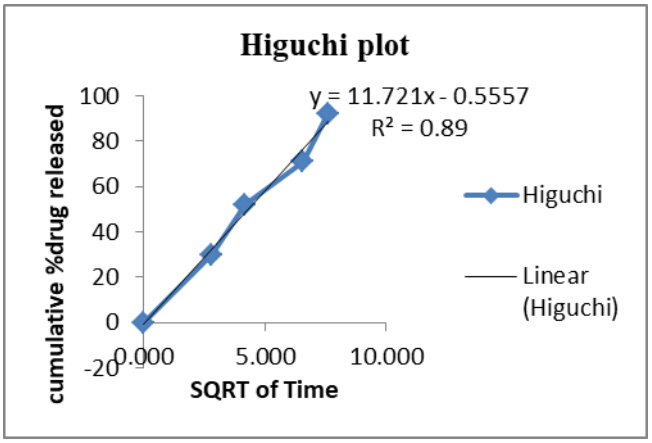


Fig.14: Higuchi plot for the optimized formulation

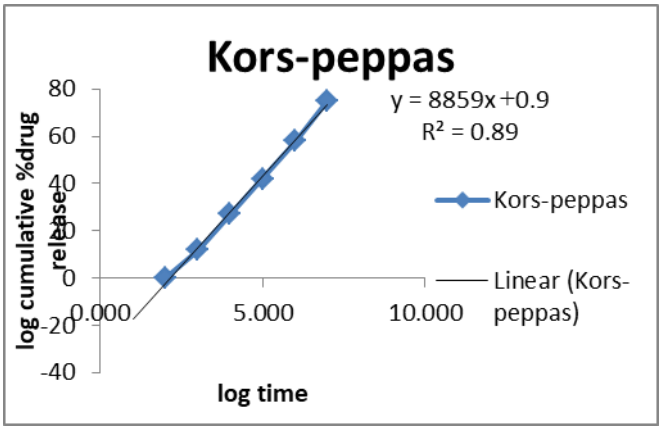


Fig. 15: Korsemeyer peppa's plot

The Korsemeyer peppa's plot n value was found to be 0.9. Hence the optimized formulation was found to be controlled release and obeys Zero order kinetics. The mechanism is Case II transport.

Scanning Electron Microscopy and Zeta Potential of Optimized Formulation

SEM was performed to analyse the morphology of the optimized formulation. The prepared nanosuspension formulation was found to be prismatic in nature (Fig 16)and Zeta potential of the optimized formulation was found to be -24.5mV (Fig 17).

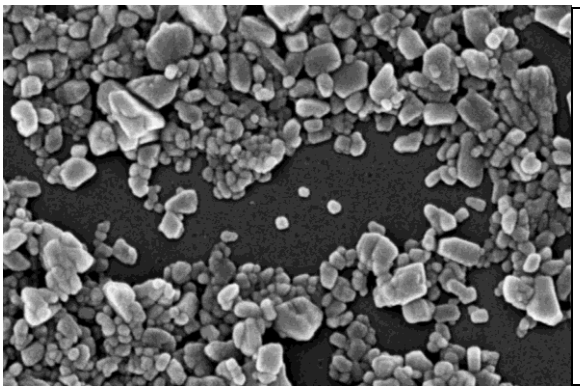


Fig.16: SEM of optimized formulation

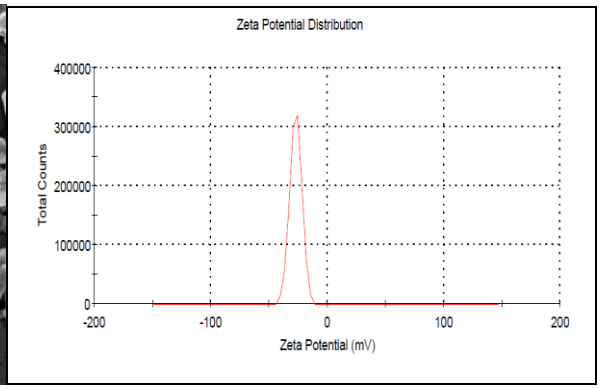


Fig.17: Zeta potential of optimized formulation

HPLC

A reversed phase HPLC method has been developed and validated as per USP and FDA guidelines for determination of omeprazole in pharmaceutical formulations by using a gradient mobile phase comprising 90% aqueous acetonitrile to 100% acetonitrile for 10 minutes at ambient temperature at flow rate of 0.7 mL/min with UV detection at 302 nm. The injection volume was kept at 20 µL for standard and all samples. The retention time of omeprazole was obtained at 3.0 ± 0.2 min.

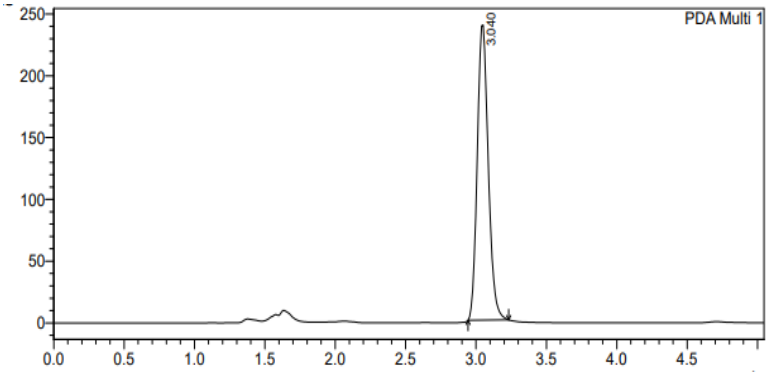


Fig.18. HPLC chromatogram of optimized omeprazole nanosuspension

Short Term Stability Studies

Optimized formulation R2 was kept for Stability studies at 40^0C/75%RH for 3 months. The entrapment efficiency and *in vitro* drug release profile of Optimized formulation of the tablets after 1 month has no change i.e. remained as same as that of the initial formulation. There was only a small difference in the *in vitro* dissolution study. The R^2 value of finished formulation before 3 months was 0.958 and after 3 months was0.959. Hence there was only a slight difference in the R^2 value. The total release after 1 month was found to be 82.2%. Stability study showed that there are no significant changes in the *in vitro* drug release pattern and entrapment efficiency. Hence, the drug was found to be suitable for the long term storage (Table 7, Fig 19).

Table 7: Results of Entrapment efficiency and In vitro drug release after 3 months stability study

Condition	**Entrapment efficiency(%)**	***In vitro* drug release (%)**

Initial - A2 formulation	89.09	82.20
1month 40^0C/75%RH	89.09	82.01

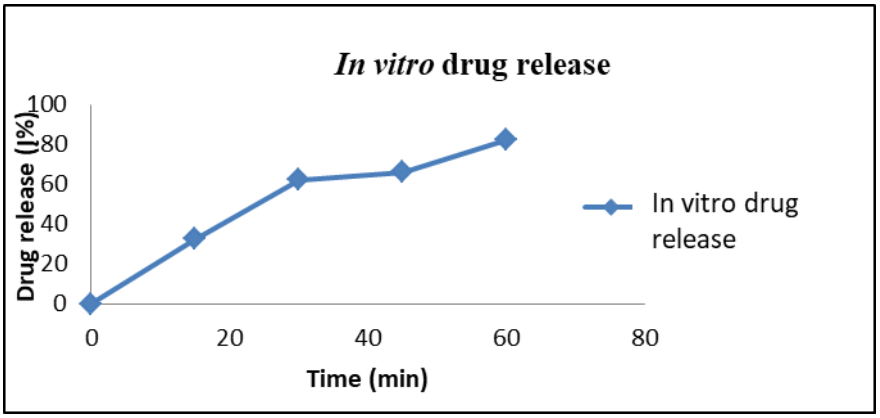


Fig. 19: In vitro drug release profile of Optimized formulation after 1month 40^0C/75%RH

CONCLUSION

In conclusion, research work focused to develop Omeprazole loaded nanosuspension and optimization were carried out in order to enhance solubility, bioavailability and to reduce side effects. The study results indicated that nanosuspension prepared with PVA (F10 formulation) with drug to polymer ratio 1:2 has shown lower particle size, higher entrapment efficiency and higher *in vitro* drug release. On the basis of evaluation parameters, the optimized formulation (R2) can be used once in a day application which is based on the severity of the disease, and age. In conclusion the optimized formulation is suitable for large scale manufacturing. In the near future the optimized nanosuspension prepared with PVA can be extended to produce intravenously injectable nanosuspension, because the polymers are selected in such a way that they are suitable for parenteral administration.

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