

OPTIMIZATION AND EVALUATION OF NANOPARTICULATE SUSTAINED RELEASE DOSAGE FORM OF ANTISPASMODIC DRUG

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ABSTRACT

This study aimed to select or modify the polymer matrix composition to achieve high entrapment of hydrophilic drugs in polymeric nanoparticles (NPs). Alverine Citrate, the bioactive drug, was selected as a representative hydrophilic drug. The nanoprecipitation method was used for the preparation of Alverine Citrate -loaded NPs. Concentrations of polymers were optimized by a 3-level factorial design, which affected the particle size and encapsulation efficiency. The formulation was subjected to morphological, physicochemical, and in vitro drug release studies. The mean particle size of nanoparticles was around 114.8 nm, as estimated with a particle size analyzer. TEM observations revealed a spherical shape. Fourier transform infrared analysis did not reveal any chemical interaction between the drug and polymers used for the nano-formulation. A release study conducted in vitro over a period of 24 h indicated primarily burst release after that sustained release of Alverine Citrate from the formulation. The release kinetics studies revealed that drug release from the nanoparticles follows the Korsmeyer–Peppas model. The study demonstrates the benefit of excipient screening techniques in improving entrapment efficiency of a hydrophilic drug.

KEYWORDS: Polymeric Nanoparticles, nano-precipitation, polydispersity index, zeta potential, entrapment efficiency.

1. INTRODUCTION

Transport and delivery of drug molecules specifically, gradually, or securely to the site of action with the help of effective drug delivery systems (DDS) is becoming a highly important research area for pharmaceutical researchers. Nanotechnology provides an effective DDS for delivering to targeted sites and releasing therapeutic compounds in a controlled manner. Nanoencapsulation is the formation of drug-loaded NPs that increase the efficiency and efficacy, protect drugs from degradation, and maintain the functional activity of encapsulated drugs.¹ Unique features of nanosized particles expand the scope of nanotechnology to many fields, especially in nanomedicine, which includes diagnosis, prevention, and treatment of disease. Several nanoscale DDS have been used, such as liposomes, solid-lipid nanoparticles, micelles, hydrogels, and dendrimers. Therefore, NPs are fabricated from natural and synthetic macromolecules for the fulfillment of drug delivery purposes.^{2,3} Polysaccharides have gained attention due to mucoadhesive properties, biodegradability, sustainability, lack of toxicity, and non-antigenicity; therefore, they are the most abundant industrial raw material for pharmaceutical applications.⁴ For polymeric nanoparticulate systems, several natural polymers like chitosan, starch, dextran, albumin, gelatin, alginate, gums, and synthetic polymers like polylactic acid (PLA), poly-(lactide-co-glycolide) (PLGA), polyanhydrides, and polycaprolactone (PCL) have been used.⁵ The polymeric materials that are to be engulfed, injected, or implanted in the body should have the properties of biodegradability and biocompatibility. Several reports exist in the literature wherein polymeric NPs have been used for delivery of formulation to the oral⁶, parental⁷, and ocular purpose⁸. The polymeric material can protect the labile molecule from enzymatic and hydrolytic degradation in the gastrointestinal tract and enhance transport across the systemic circulation⁹. Some researchers have used polymers in conjugation with other polymers so as to enhance solubility and encapsulation efficiency, reducing the degradation of the drug and prolonging the residence time by increasing the contact time between the therapeutic molecule and the biomembrane at the target or absorption site.¹⁰

Nanoprecipitation is a method for the encapsulation of molecules, using natural polysaccharides: chitosan and HPMC K100M. Chitosan and HPMC K100M are naturally occurring polysaccharides, basically polycationic and polyanionic, respectively, and are biodegradable, biocompatible, and less toxic.¹¹ The cationic nature of chitosan shows good mucoadhesive and membrane permeability properties. The mechanisms of mucoadhesion and membrane permeation are based on the interaction of positive charges with the negatively charged cell membrane

and prevent structural recognition by membrane-associated gate proteins, respectively.¹² The negative charge of HPMC K100M helps in the interaction with positively charged chitosan polymer, leading to the formation of a polymer matrix for encapsulation. Along with these polymers, a surfactant is also used for enhancing the interaction and for reducing the particle size.¹³

Alverine Citrate is an antispasmodic drug characterized by its low aqueous solubility, a major obstacle in drug formulation development to improve its bioavailability. To overcome the problem of poor aqueous solubility of Alverine Citrate, various approaches have been investigated, including physical and chemical modifications of the drug.¹⁴

The present work is undertaken with an aim to form Alverine Citrate-loaded nanoformulation for drug delivery with increased efficacy and sustained release, using biocompatible polymers. The nanoprecipitation method was used for the preparation of nanoformulations, and optimization of the concentrations of the polymers chitosan and HPMC K100M was done with the help of factorial design, with the aim of minimizing particle size (PS) and maximizing encapsulation efficiency (EE). Synthesized Alverine Citrate-loaded polymeric NPs were further characterized by FTIR (Fourier Transform Infrared Spectroscopy) for interactive studies and TEM (Transmission Electron Microscopy) for microscopic evaluation.

2. Experimental

2.1. Materials:

Alverine Citrate was a kind gift sample from Rajesh Chemicals (Mumbai, India). Chitosan was procured from Sisco Research Laboratories. Dimethyl sulfoxide was purchased from Modern Industries. Acetone was purchased from Merck Specialities Private Limited. All other chemicals used were of the highest available grade.

Mechanism of action:

Alverine citrate acts as a direct spasmolytic on smooth muscle, primarily used for irritable bowel syndrome (IBS) and dysmenorrhea by reducing muscle spasms. It works by inhibiting calcium influx into smooth muscle cells, blocking 5-HT_{1A} receptors, and reducing the sensitivity of contractile proteins, thus reducing gut contraction.

2.2. Protocol for nanoparticle synthesis:

Nanoparticles were prepared according to the nanoprecipitation method with slight modification. Briefly, 200 mg of polymer was dissolved in 25 mL of acetone separately. Alverine citrate 50 mg was dissolved in 2 mL of dimethylsulfoxide. Both solutions were mixed, and then 50 mL of water was added and stirred for half an hour. Acetone was eliminated by evaporation under reduced pressure using a rotary evaporator, and the final volume of the suspension was adjusted to 10 mL. Then this suspension was centrifuged at 15000 rpm at 4°C for half an hour. The supernatant was discarded, and the precipitate was washed 3 times with distilled water. The nanoparticles thus obtained were dried overnight in an oven at 60°C and stored in a desiccator. The prepared formulations were characterized for loading efficiency, entrapment efficiency, particle size, particle size distribution, polydispersity index, zeta potential, and drug-excipient compatibility studies.

Table 1: Actual values of parameters for Alverine citrate-loaded NPs

Coded values	Actual values	
	Concentration of Chitosan (%)	Concentration of HPMC K100M (%)
-1	1	0.1
0	1.3	0.15
1	1.5	0.2

2.3. Optimization by factorial statistical design

The factorial design was applied for the optimization of formulation ingredients to develop a nanoformulation. Based on the preliminary experimental observations, concentrations of polymers were found to be more effective on PS and EE. A 3-factor design was chosen for optimization of the nanoformulation to achieve minimum PS and maximum EE. The concentration parameters for the factorial design of Alverine citrate-loaded polymeric NPs are depicted in Table 1. Design-Expert software version 8.0.7.1 was used for the data analysis and for plotting contour plots and 3-D response surface plots.

3. Characterization:

3.1. Drug content:

The total drug content in nanoparticles is quantified by Spectrophotometric analysis. 1 mg equivalent of Alverine Citrate polymeric nanoparticle is dissolved in 1 mL of 0.1 M HCl, and the volume is made up to 100 mL to make a 10 µg / mL concentration, and the absorbance is measured at 209 nm (λ_{max}) using a UV spectrophotometer.

The calculation is performed as follows:

$$\text{Total drug content} = \frac{\text{Vol. Total}}{\text{Vol. Aliquot}} \times \text{Drug amount in aliquot}$$

3.2. Loading Efficiency:

Drug content in the preparation was determined by extracting the drug from the nanoparticles with 0.1 M hydrochloric acid. In this method, the nanoparticles (50 mg) were stirred in 50 mL of 0.1 M hydrochloric acid until dissolved; it was filtered through a Millipore filter, and the drug content was determined, after suitable dilution, at 209 nm by UV spectrophotometry. The loading efficiency (L) of the nanoparticles was calculated.

$$L (\%) = (Q_n / W_n) \times 100$$

Where W_n is the weight of the nanoparticles, and Q_n is the amount of drug present in the nanoparticles.

3.3. Entrapment Efficiency :

For the determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (w) by a UV spectrophotometer at 209 nm. A standard calibration curve of the drug was plotted for this purpose. The amount of drug in the supernatant was then subtracted from the total amount of drug added during the preparation (W). Effectively, (W-w) will give the amount of drug entrapped in the particles. Then, the percentage entrapment of the drug was calculated.

$$\% \text{ Drug Entrapment} = (W-w/W) \times 100$$

3.4. Particle size analysis:

Particle size analysis of Alverine Citrate polymeric nanoparticles was determined using a Zetasizer by Horiba SZ-100. Before the measurement, the nanoparticles were diluted with 0.1 M HCl, and the prepared samples were transferred directly into a cuvette, where the particle size was measured.

Table 2: Factorial design layout for formulation with experimental observations.

Exp. no.	Coded values of CS	Coded values of HPMC K100M	PS (nm) (R1)	EE (%) (R2)
1.	0	-1	205.5	77.96
2.	0	0	184.3	79.80
3.	-1	1	256.3	81.21
4.	-1	0	117.1	83.39
5.	-1	-1	114.8	98.53
6.	1	0	250.5	84.16
7.	0	1	239.3	81.22
8.	1	1	389.2	78.70
9.	1	-1	270.7	80.20

3.5. Polydispersity index:

The prepared polymeric nanoparticles were evaluated for mean particle size and polydispersity index(PDI). Mean particle size and PDI were measured using Horiba SZ-100. The measurement was carried out after diluting the prepared nanoparticles suitably. The polydispersity index (PDI) value is an indication of the uniformity of particle size distribution.

3.6. Zeta potential analysis:

Zeta potential is a measure of surface charge. The Zeta potential of the prepared polymeric nanoparticles was measured to estimate the stability of Alverine Citrate polymeric nanoparticles. Zeta potential measurement was performed using Horiba SZ-100.

3.7. Drug-Excipient Compatibility Studies:

The drug-excipient compatibility studies were performed using an FT-IR spectrophotometer (Jasco FT/IR-4100). The FT-IR spectra of the drug, polymers, and formulations were analyzed separately and then correlated for incompatibility.

3.8. In Vitro Drug Release:

The *in vitro* drug release study of Alverine Citrate polymeric nanoparticle formulations used the dialysis bag diffusion method. Drug-loaded nanoparticles (5 mL) were dispersed into a dialysis bag, and the dialysis bag was then kept in a beaker containing 100 mL of pH 6.8 phosphate buffer. The beaker was placed over a magnetic stirrer, and the temperature of the assembly was maintained at 37 ± 1 °C throughout the experiment. During the experiment,

the rpm was maintained at 100 rpm. Samples (2 mL) were withdrawn at definite time intervals and replaced with equal amounts of fresh pH 6.8 phosphate buffer. After suitable dilutions, the samples were analyzed using a UV–Visible spectrophotometer at 209 nm.

3.9. *In Vitro* Drug Release Kinetics:

The data obtained from the *in vitro* release study were used to fit into kinetic models. This was done to determine the mechanism of drug release from Alverine Citrate polymeric nanoparticles. In order to determine the release model, the *in vitro* release data were analyzed according to zero-order, first-order, matrix, Peppas, and Higuchi-Crowell models. The preference for a certain mechanism was based on the coefficient of determination (r^2) for the parameters studied, where the highest coefficient of determination is preferred for the selection of the order of release.

4. RESULTS AND DISCUSSION

4.1. Protocol for nanoparticle synthesis

Polymeric nanoparticles offer a valuable delivery system of active ingredients because of high encapsulation efficiency, biodegradability, biocompatibility, low toxicity, and high stability. The Alverine Citrate-loaded polymeric NPs were synthesized by the nanoprecipitation method. Particle formation was attained by successive addition of positively charged chitosan and the negatively charged HPMC K100M. Positively charged amine groups (NH_2) of chitosan interact electrostatically with negatively charged carboxyl groups (COO^-) of HPMC K100M and form a mesh-like polymer structure for drug loading.

4.2. Optimization by factorial statistical design

Nine batches of Alverine Citrate-loaded polymeric NPs were obtained using a 3-level factorial design by varying the concentration of CS (A) and HPMC K100M (B), and the experimental observations of PS (R1) and EE (R2) are tabulated in Table 2. The preliminary trial revealed that the concentrations of polymers and surfactant influenced the PS and EE of Alverine Citrate-loaded polymeric NPs. From the observations, it was found that at the near 0 level, minimum PS and maximum EE were achieved. The regression equation for the model relating R1 and R2 as responses is shown in Eqs. (1) and (2), respectively. The positive and negative signs in Eqs. (1) and (2) indicate synergistic and antagonistic effects, respectively. The results of ANOVA analysis of the polynomial equations are portrayed in Table 3, which indicates that the quadratic model was the best fit and significant for the development of two responses (PS and EE). The significance of the model was also depicted by the values of R^2 , which are >0.98 for both responses. However, other factors are also in a reasonable range, which depicts the significance of the model.

$$\text{PS} = +188.86 + 17.73 \times A + 13.03 \times B + 42.67 \times A^2 + 31.27 \times B^2 \quad (1)$$

$$\text{EE} = +98.34 + 4.85 \times A + 4.42 \times B + 1.99 \times A \times B - 4.37 \times A^2 - 9.40 \times B^2 \quad (2)$$

The contour plots, Figs. 1 and 2, render the interaction behavior of the concentrations of the independent factors, CS (A) and HPMC K100M (B), on the response of dependent factors PS (R1) and EE (R2), respectively. The significance of the mutual interaction has been predicted from the shape of the contour plots. Figs. 1 and 2 are elliptical contour plots, meaning the independent factors have significant interaction for PS and EE, respectively.

Table 3: ANOVA analysis summary for PS and EE for Alverine Citrate-loaded polymeric NPs.

Parameters	Response R1(PS)	Response R2(EE)
SS	8502.13	489.07
MS	2125.53	97.81
DF	4	5
F-Value	78.93	80.74
P-Value	0.0005	0.0021
SD	5.19	1.10
CV	2.18	1.23
R^2	0.9875	0.9926
Adj.- R^2	0.9750	0.9803
Pred.- R^2	0.9367	0.9108
Adeq precision	27.070	23.959

SS indicates sum of squares; MS is mean square; DF is degrees of freedom; F is Fisher's ratio; P is probability; SD is standard deviation; CV is coefficient of variation; R is regression coefficient.

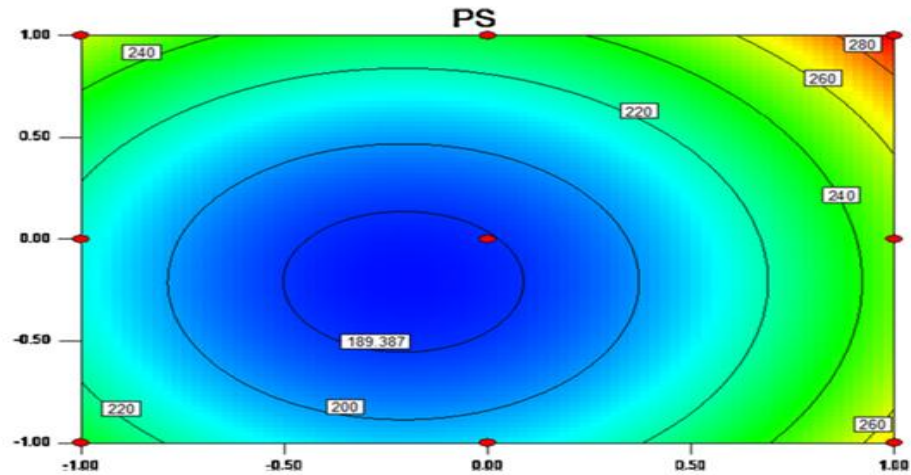


Fig.1: Contour plot for PS

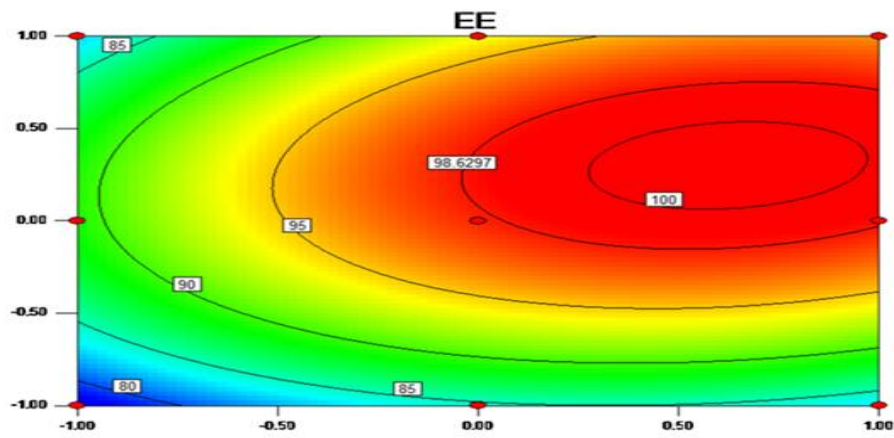


Fig.2: Contour plot for EE

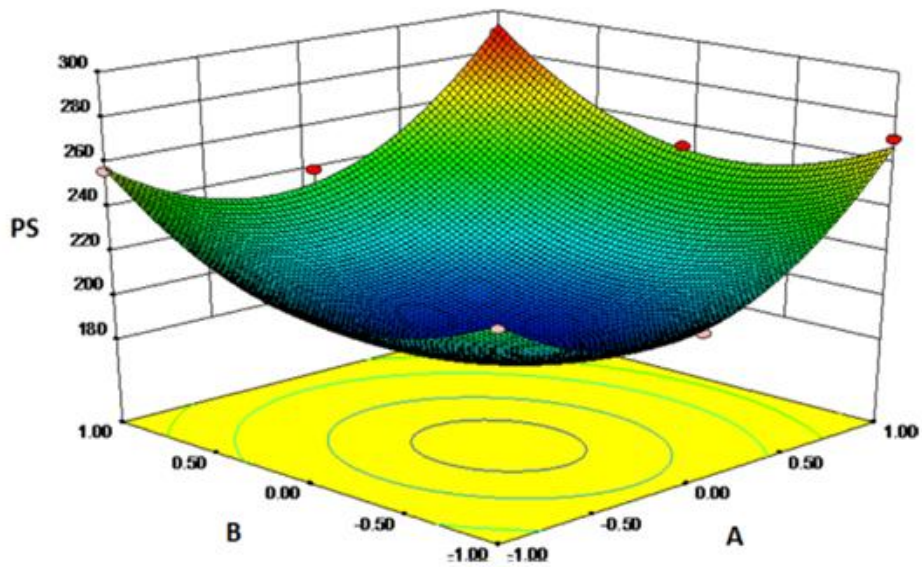


Fig.3: Response surface plot for PS

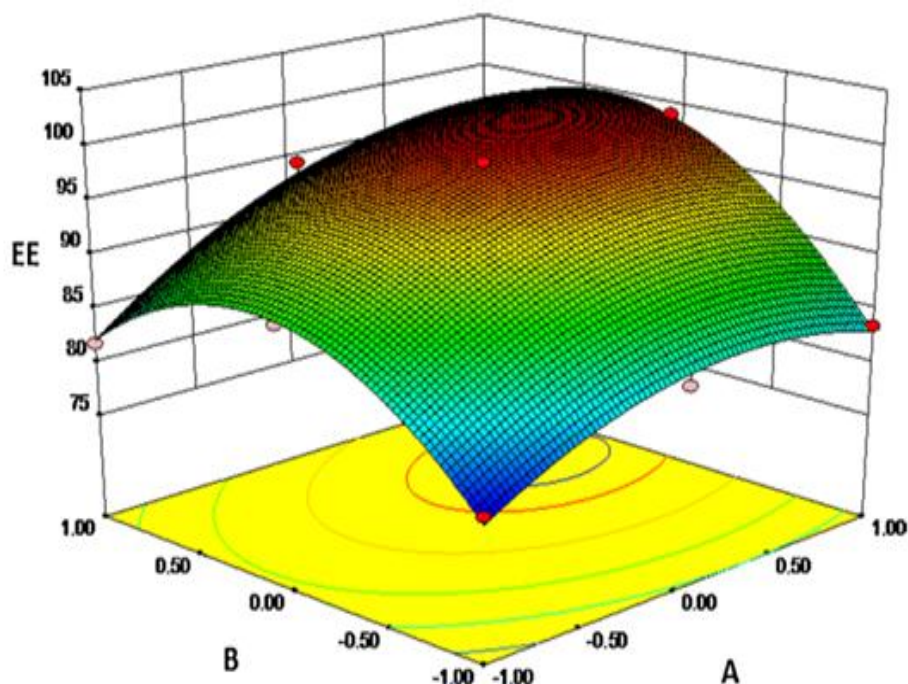


Fig. 4: Response surface plot for EE

Table 4: Records for the final optimized concentration with minimum % error

Conc. of CS	Conc. of HPMC K100M	Observations for PS			Observations for EE		
		Predicted	Experimental	% Error	Predicted	Experimental	% Error
0.05	0.06	185.235	181.4	2.072	98.81	99.84	-0.0104

The response surface plots, Figs. 3 and 4 reveal the concentration effect of CS (A) and HPMC K100M (B) on the PS (R1) and %EE (R2), respectively, in the alverine citrate-loaded polymeric NPs. Figs. 3 and 4 show an upward and downward curve-like relationship with PS and EE, respectively. This means an increase in polymer concentration first leads to a decrease in PS and an increase in EE up to a certain level, while further increases in concentration result in an increase in PS and a decrease in EE.

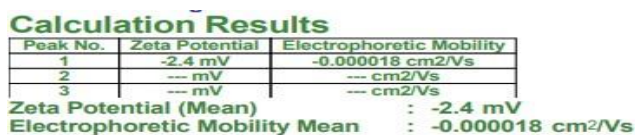
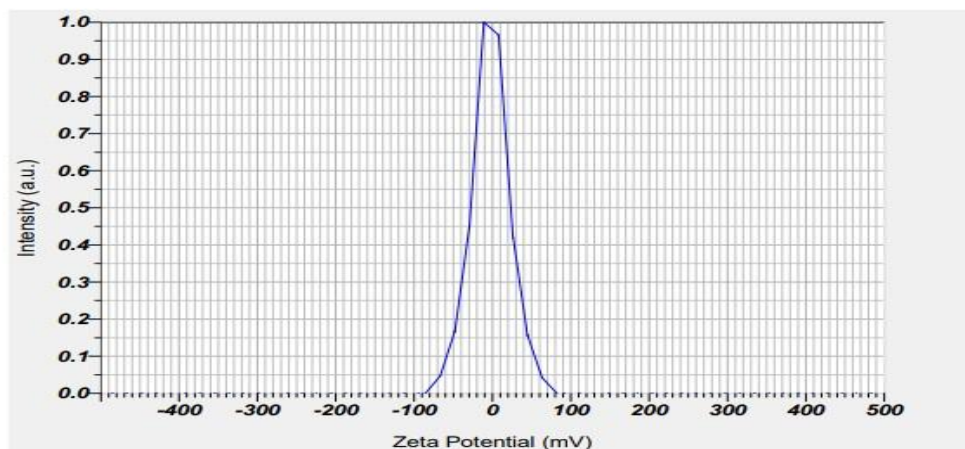
After ANOVA analysis, numerical optimization was employed to obtain the final optimized concentration of the formulation with desired responses like minimum PS (R1) and maximum EE (R2). The results for the final optimized concentration are tabulated in Table 4.

4.3. Zeta Potential:

Zeta Potential was determined using the Horiba SZ-100 zeta-sizer instrument. Zeta potential analysis is carried out to find the surface charge of the particles to determine their stability during storage. The magnitude of the zeta potential is predictive of the colloidal stability. Nanoparticles with zeta potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. If all the particles in suspension have a large negative or positive zeta potential, then they will tend to repel each other, and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values, then there will be no force to prevent the particles from coming together and flocculating.

For Alverine Citrate polymeric nanoparticles, the zeta potential of formulation F5 was found to be -2.4 mV. Zeta potential distribution of formulation F5 is depicted in Figure 5.

Fig.5: Zeta Potential of Polymeric nanoparticles formulation (F5)



4.4. FT-IR Spectroscopy Analysis:

Fourier Transform Infrared (FT-IR) spectra of the samples were obtained using a FTIR Jasco 4500 Spectrometer by the KBr disc method. The spectra were recorded for the pure drug, polymers, and a physical mixture of the drug and polymers.

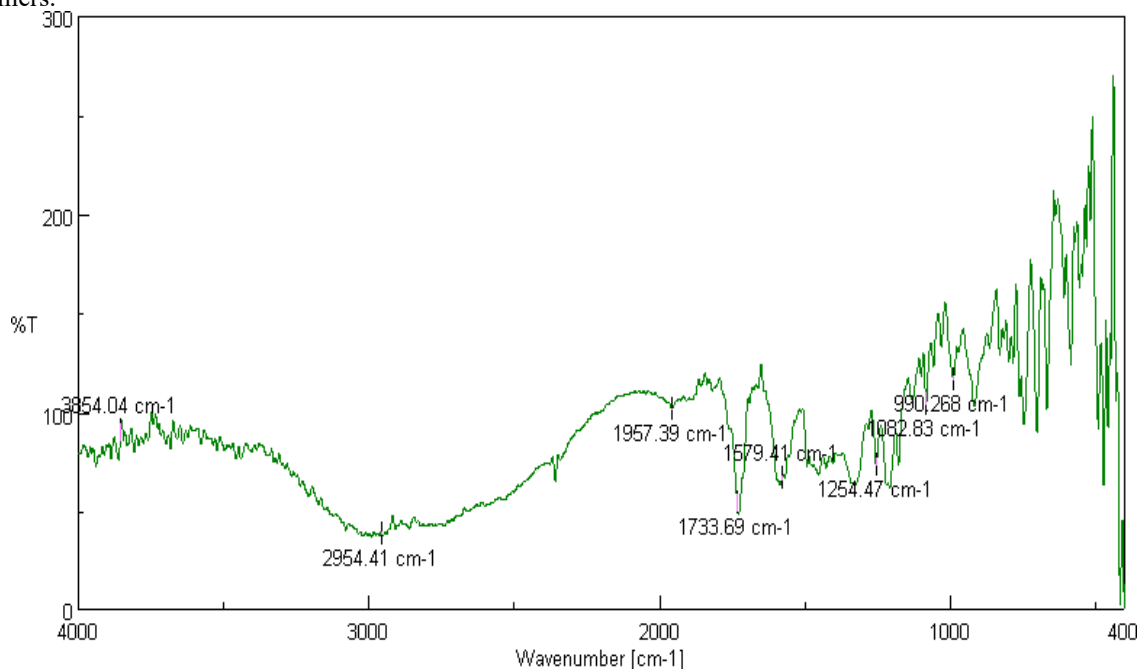
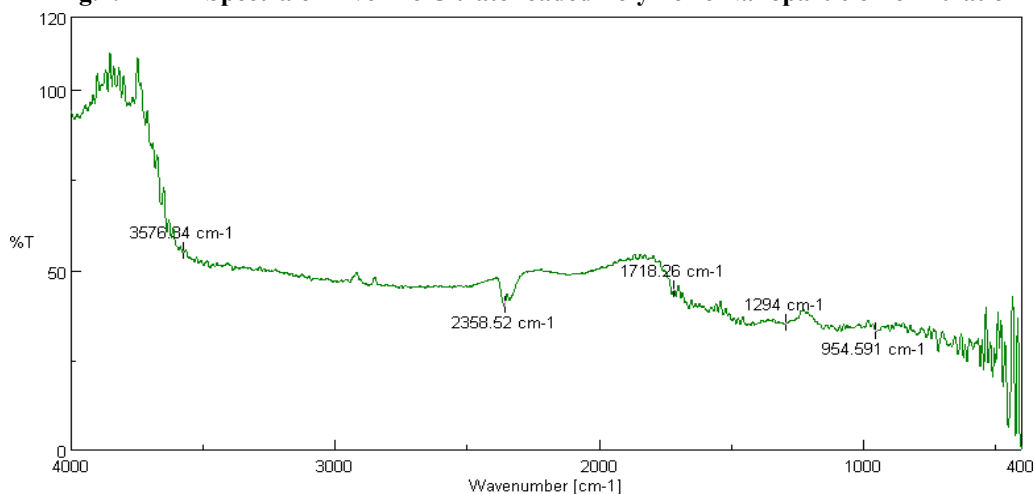


Fig.6: FT-IR Spectra of Alverine Citrate

Figure 6 shows the FT-IR spectra of pure Alverine Citrate. Characteristic IR absorption peaks of Alverine Citrate, C-OH stretch (3854.04 cm⁻¹), C-H stretch (2954.41 cm⁻¹), C=O stretch (1957.39 cm⁻¹), and C-N stretch (1254.47 cm⁻¹), were present in the IR spectrum of Alverine Citrate. The FT-IR spectra of Alverine Citrate indicated that the positions of characteristic peaks of Alverine Citrate were not altered.

Fig.7: FT-IR Spectra of Alverine Citrate-loaded Polymeric Nanoparticle Formulation



The FT-IR spectra of the Alverine Citrate-loaded polymeric nanoparticle formulation are reported in Figure 7. Characteristic IR absorption peaks of the Alverine Citrate-loaded polymeric nanoparticle formulation, O-H stretch (3576.84 cm^{-1}), C=O stretch (2358.52 cm^{-1}), C-H bend (1718.26 cm^{-1}), C-N stretch (1294 cm^{-1}), and O-H bend (954.591 cm^{-1}), were present in the IR spectrum of the Alverine Citrate-loaded polymeric nanoparticle formulation. The FT-IR spectra of the Alverine Citrate-loaded polymeric nanoparticle formulation indicated that the positions of the characteristic peaks of the Alverine Citrate-loaded polymeric nanoparticle formulation were not altered, suggesting the absence of chemical interactions between the drug Alverine Citrate and the polymers chitosan and HPMC K100M.

4.5. Differential Scanning Calorimetry (DSC):

DSC is a useful tool to monitor the effect of additives on the thermal behavior of the material. The DSC thermogram of Alverine Citrate showed a sharp exothermic peak at 106.50°C . The obtained value is much closer to the reported value of the Alverine Citrate Melting Point. Alverine Citrate-loaded polymeric nanoparticles Formulation exhibited a double, sharp endothermic peak at 86.27°C .

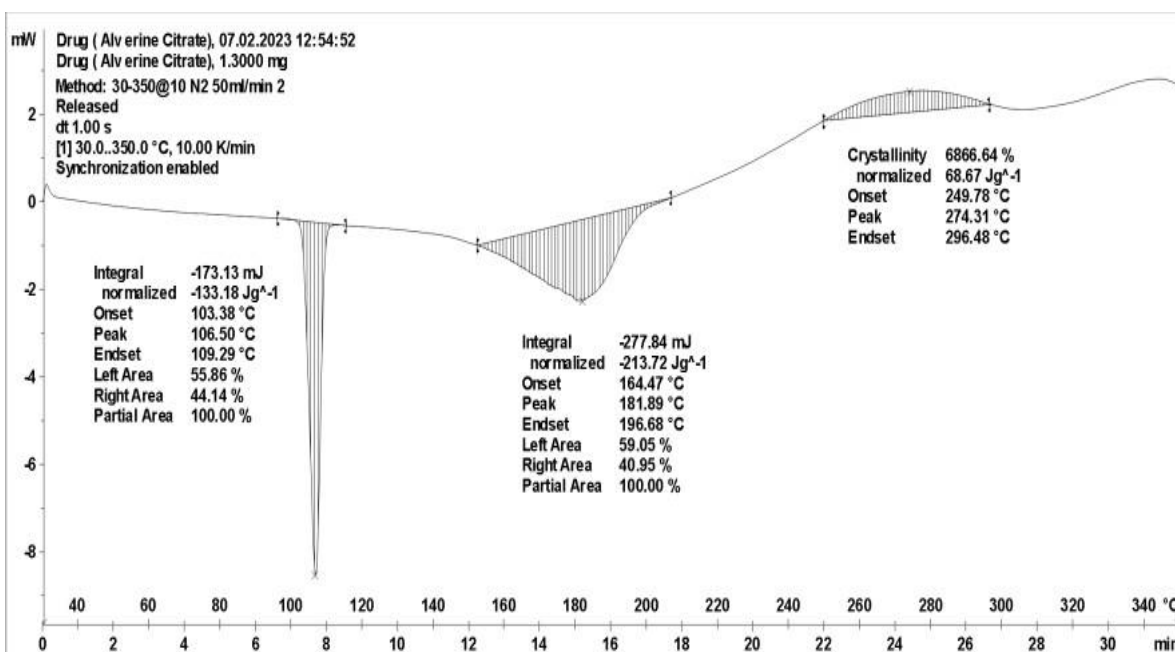


Fig.8: DSC Thermogram of Alverine Citrate

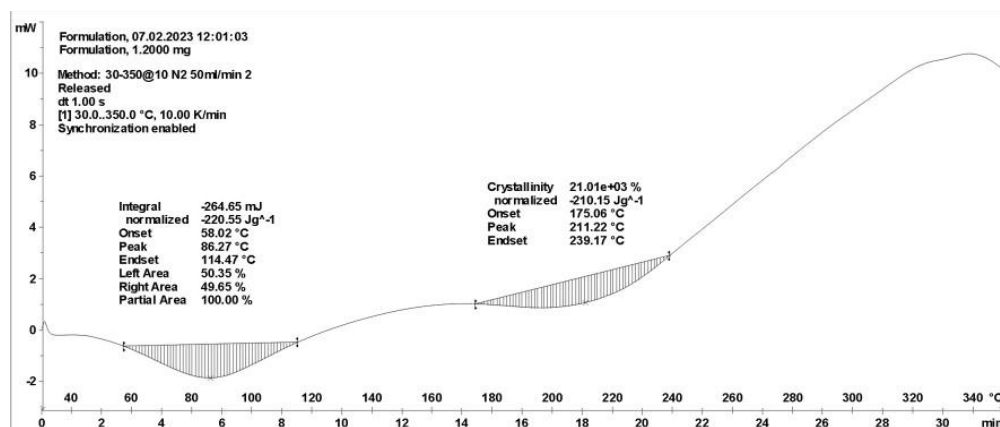


Fig.9: DSC Thermogram of Alverine Citrate-loaded polymeric nanoparticle Formulation.

4.6. *In vitro* Drug Release:

In vitro, a drug release study of the prepared Alverine Citrate polymeric nanoparticles was carried out using the dialysis bag diffusion method. The amount of drug released in different time intervals was observed. *In vitro*, drug release profile data obtained from Alverine Citrate polymeric nanoparticles are given in Table 5.

Table 5: Cumulative percentage drug release (%)

Time in min	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
60	18.5±0.40	20.2±0.11	15.8±0.33	13.5±0.13	16.4±0.15	14.8±0.21	19.3±0.16	17.7±0.5	20.6±0.18
120	26.2±0.68	30.0±0.18	21.4±0.15	26.3±0.32	27.1±0.24	28.1±0.34	27.4±0.32	29.5±0.13	35.3±0.35
180	37.8±0.72	42.5±0.24	27.8±0.55	33.2±0.48	36.6±0.32	38.5±0.18	41.8±0.15	39.8±0.17	47.0±0.27
240	45.1±0.70	50.4±0.51	35.6±0.58	40.5±0.85	45.9±0.45	49.8±0.11	53.9±0.71	48.1±0.76	52.7±0.96
300	52.2±0.32	55.1±0.53	46.7±0.51	53.7±0.75	54.7±0.56	53.6±0.73	62.5±0.58	61.2±0.62	63.2±0.54
360	63.3±0.54	64.8±0.83	56.5±0.30	58.2±0.21	68.5±0.63	67.5±0.86	73.1±0.45	66.9±0.93	69.9±0.37
420	77.8±0.72	75.4±0.79	69.3±0.24	61.9±0.84	79.1±0.51	72.8±0.91	82.2±0.39	77.8±0.29	78.1±0.22
480	85.4±0.84	87.9±0.91	81.2±0.95	72.8±0.51	95.8±0.25	88.7±0.64	90.4±0.26	85.2±0.38	90.9±0.15

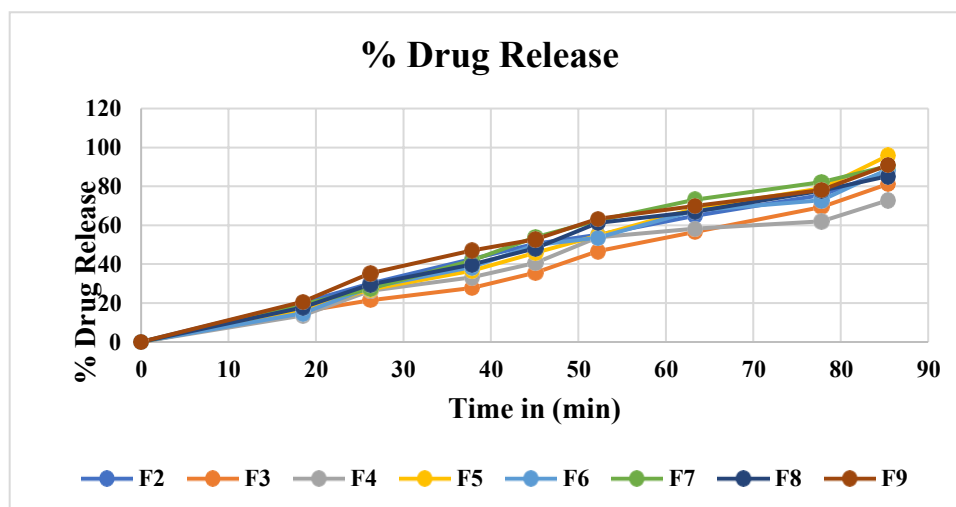
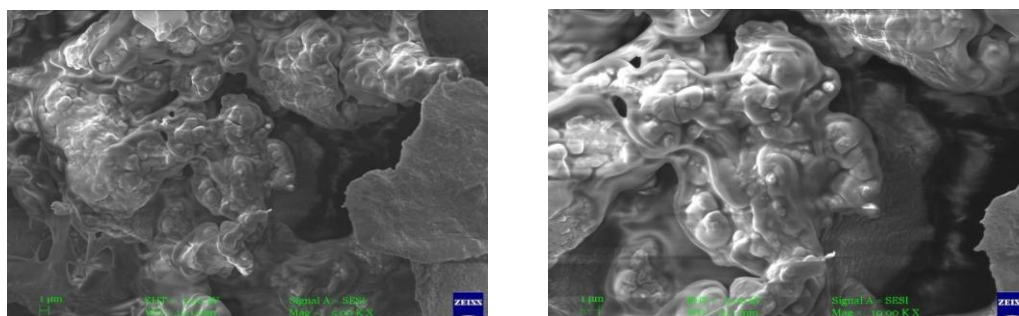


Fig.10: Cumulative percentage drug release

4.7. Scanning Electron Microscopy (SEM):

SEM analyses of the formulated Alverine Citrate polymeric nanoparticles were performed to evaluate the surface morphology of the nanoparticles. SEM images showed that the nanoparticles had a smooth surface morphology and were spherical in shape. The SEM images of formulation F5 are shown in Figure 11.

Fig.11: SEM Images of Alverine Citrate polymeric nanoparticle formulation



4.8. Transmission Electron Microscopy (TEM):

TEM analyses of the formulated Alverine Citrate polymeric nanoparticles were performed to evaluate the surface morphology of the nanoparticles. TEM images showed that the nanoparticles had a smooth surface morphology and were spherical in shape. The TEM images of formulation F5 are shown in Figure 12.

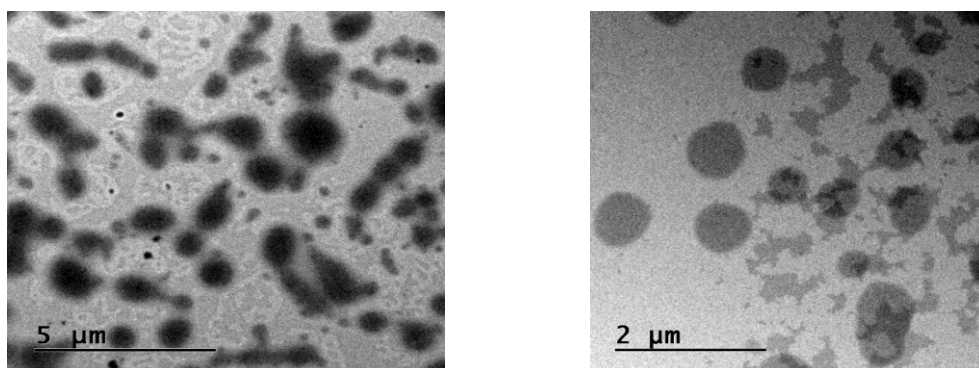


Fig.12: TEM Images of Alverine Citrate polymeric nanoparticle formulation

4.9. *In vitro* Drug Release Kinetics:

The data obtained from the *in vitro* release study were used to fit into kinetic models. This was done to find out the mechanism of drug release from Alverine Citrate polymeric nanoparticles. In order to determine the release model, the *in vitro* release data were analyzed according to zero-order, first-order, matrix, Peppas, and Higuchi-Crowell models. The preference for a certain mechanism was based on the coefficient of determination (r^2) for the parameters studied, where the highest coefficient of determination is preferred for the selection of the order of release. The kinetic parameters of Alverine Citrate polymeric nanoparticles are shown in Table 6.

Since the r^2 value is higher for the Peppas model, it is selected as the best-fitted model. This was confirmed by plotting the percentage cumulative drug release against the square root of time and r^2 value ranges between 0.9087 and 0.9936. In this, the Korsmeyer–Peppas model was used to analyze the release kinetics.

Table 6: *In vitro* Drug Release Kinetics

Zero Order	First Order	Matrix	Peppas	Hixson-Crowell	Best fitting model
0.9930	0.9859	0.9087	0.9936	0.9892	Peppas

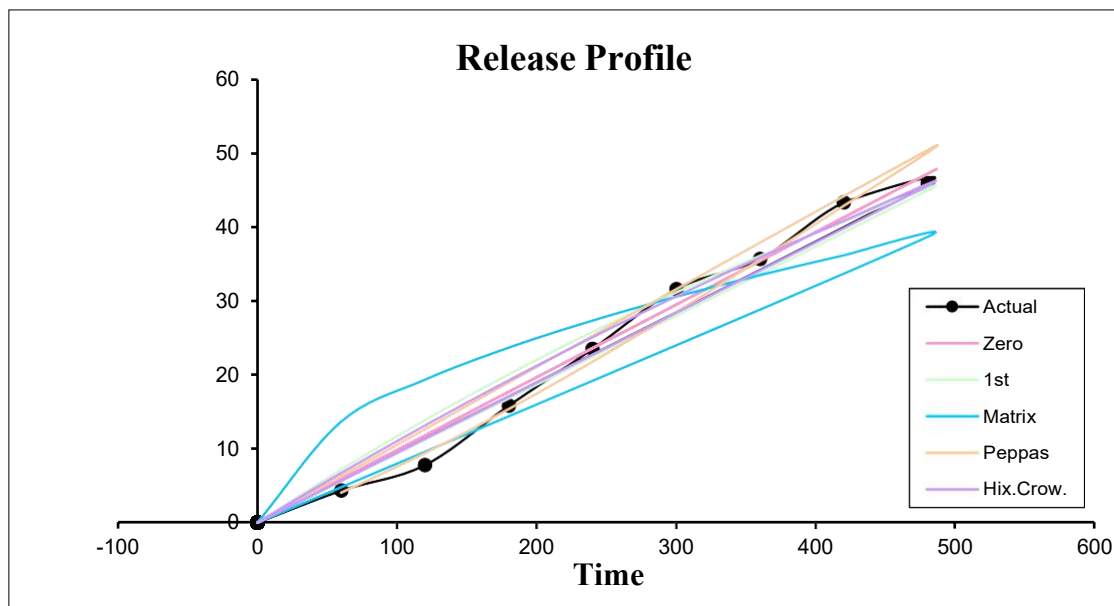


Fig.13: *In vitro* Drug Release Kinetics of Formulation (F5)

CONCLUSION

The nanoprecipitation method was used for preparation of alverine citrate-loaded polymeric NPs and further optimized using 3 level factorial design. From the observations, it is concluded that minimum PS and maximum EE were achieved near the 0-level of chitosan and HPMC K100M. Fourier transform infrared analysis did not reveal any chemical interaction between the drug and polymers used for the nano-formulation. A release study conducted *in vitro* over a period of 24 h indicated primarily burst release after that sustained release of Alverine Citrate from the formulation. The release kinetics studies revealed that drug release from the nanoparticles follows the Korsmeyer–Peppas model. Further, the optimized alverine citrate-loaded polymeric NPs were found to be spherical in shape, as confirmed by TEM. In addition, the nanoparticulate formulation has high encapsulation efficiency; showing sustained release of alverine citrate.

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