RESEARCH PAPER

Design And Characterization of Bilastine Nanoparticles to Treat Allergic Rhinitis and Chronic Urticaria

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ABSTRACT

Allergic rhinitis and chronic urticaria are two relatively prevalent clinical problems having unique and diverse features, cause severe deterioration quality of life (QoL) and loss of function. Bilastine, second-generation H1-antihistamine has a rapid onset and a long duration of action, and it is specific for H1 histamine receptor. It does, however, have a low oral bioavailability (40%) due to fast first-pass hepatic metabolism. As a result, there is a need for novel drug delivery systems.Bilastine loaded chitosan nanoparticles were prepared using Chitosan and EUDRAGIT®S 100 as polymers and polyvinyl alcohol as stabilizer. The characterization of prepared nanoparticles such as the zeta potential,SEM, polydispersity, particle size, entrapment efficiency, *in vitro* drug releasewas conducted. Among the formulations F1 to F6, F1 has exhibited the best results. In FTIR studies, there are no significant interactions between the drug's physical combination and MCC and Eudragit. SEM analysis results were within the limit of the nanorange, i.e., 250 nm. Drug loading capacity was between 13.56 and 20.22 percent. Encapsulation efficiency (%) of drug-polymer-containing nanoparticles in various ratios was in the range of 70.66 to 96.18 percent, respectively and increased with a rise in the amount of polymer. In *invitro* dissolution studies showed the drug release in between 88.93 and 96.54 percent for 10 hours. Compatibility,*invitro* studies and kinetic parameters have concluded that nanoparticles of Eudragit are superior toChitosanfor delivery of Bilastineand follow's zero-order kinetics. Among all formulations, F1 was found to be the best formulation.

Keywords: Bilastine, Micro Crystalline Cellulose, Eudragit, Double-emulsion Method**.**

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1.INTRODUCTION

Nanoparticles are invisible particles with a diameter between 1 and 100 nanometers (nm), in line with current research. The distinctive properties of nanoparticles, including their form, charge, size, high extent-to-mass ratio, and high reactivity, have led to potential applications in medical research and drug delivery(Guerrero-Garcia *et al.,* 2018).

Allergic rhinitis and chronic urticaria are two relatively prevalent clinical problems that cause patients to contact their medical practitioner. Patients do not achieve complete symptom control with single-medication therapy since histamine production is involved in all pathological aspects of allergic rhinitis save the inflammatory responses of the late phase. Bilastine is a second-generation H1-antihistamine that has just been licensed for the clinical treatment of allergic rhinitis and chronic urticaria in individuals over the age of 12 (Ridolo*et al.,* 2015).

It provides relief from an assortment of conditions such as runny nose, eye infections, watery eyes, conjunctivitis, and relief from itching, swelling in the eyes or nose, and seasonal fever. It also protects cells from infection. Bilastine has a rapid onset and a long duration of action, and it is specific for the H1 histamine receptor.Bilastine decreases the development of allergy symptoms caused by mast cell histamine release by binding to and blocking stimulation of the H1 receptor(Caldero *et al.,* 2011).It does, however, have a low oral bioavailability (40%) due to fast first-pass hepatic metabolism. As a result, there is a need for novel drug delivery systems (Flores-Mireles *et al.,* 2011; Yanai*et al.,* 2017). Eudragit possess optimum stability, size distribution, and positive surface charge and enhanced the oral bioavailability.

Another polymer, chitosan possesses has advantages such as biocompatible, biodegradable, nontoxic, and inexpensive(Soltani *et al.,* 2016; Wei *et al.,* 2016)

Hence, in this work it was attempted to design and characterize Bilastine nano particles using Eudragit and chitosan as polymers by double emulsion techniques and to study the invitro release and release kinetics.

2.MATERIALSANDMETHODS

2.1 Materials

Bilastine was a gift sample from Aurobindo Biotech Pvt. Ltd., Hyderabad. Microcrystalline cellulose from Research Lab Fine Chem. Ltd., Mumbai, and polyvinyl alcohol from Quali Chem Mumbai, methanol from Research Lab Fine Chem. Ltd. Mumbai, chloroform from Research Lab Fine Chem. Ltd. Mumbai, and all other analytical-grade chemicals were used for analysis.

2.2 Method

Formulation of nanoparticles of Bilastine

Polymeric nano particles of Bilastine were prepared by the double emulsion method through the oil-in-water-in-oil emulsion method (Dongyi *et al.,* 2023). As drugs have lower solubility, high rpm is required in the double emulsion method. About 0.5gm of Bilastine (the drug) is dissolved in 10 ml of methanol for 30 minutes. The polymer was dissolved in the oil phase at 400 rpm. About 0.5gm of microcrystalline cellulose and EUDRAGIT®S 100is dissolved in 10 ml of chloroform for 30 minutes separately. Addition of the oil phase into the aqueous phase is done with continuous stirring allowing W/O phase to form. The secondary aqueous phase of polyvinyl alcohol (PVA) in methanol (CH_3-OH) is added in this step, where polyvinyl alcohol serves as a stabilizer to form double emulsion in oil-in-water-in-oil phase (Mohamad *et al.,* 2023). The composition of Bilastine nanoparticles is shown in Table 1.

Calibration curve of Bilastine

100 mg of Bilastine was accurately weighed and dissolved in 10% glacial acetic acid and made up to 100 ml with 10% glacial acetic acid. The standard stock solution was further diluted with 10% glacial acetic acid to get a 10 µg/ml concentration. The solution was scanned between 200 and 400 nm range using 10% glacial acetic acid as blank in UV spectrophotometer. From the UV Spectra 207nm was selected as λ max for analysis of Bilastine. The absorbance of different concentration solutions was measured at 207nm against blank. The samples were found to be linear from 0-10 µg /ml. The calibration curve was plotted using concentration Vs. absorbance.

2.3 Characterization of Bilastine nanoparticles

***Particle Size*:** The particle sizeof nano emulsions isanalyzed employing photon correlation spectroscopy(PCS) using Malvern Zetasizer, which monitors the variation in light scattering because ofBrownianmotionofparticles asfunctionoftime Guang-Zhen *et al.,* 2015).

Zeta Potential: The Zeta potential of nanoparticles was adjudicated by using a Malvern Zeta sizer. All the studies were performed at 25^0C (Fornaguera*et al.,* 2016).

Scanning Electron Microscopy: Nanoparticles surface morphology was observed using SEM. The nanoparticles were placed on gold coating metal stubs and the images were taken by the Jeolscanning electron microscope (Sanjesh kumar*et al.,* 2021).

***FTIR studies*:** The FTIR studies are performed to study the compatibility between drug and the polymer.

***DSC studies*:** The DSC studies are performed to study the compatibility between drug and the polymer.

% Encapsulation Efficiency and Drug Loading

The quantity of the drug encapsulated can be calculated by a known quantity of nanoparticles (50 mg) added into ethanol and dichloromethane (50 ml each) in order to fully concentrate the drug. The filtrate (1ml) is mixed with pH 6.4 phosphate buffer (50 ml). This solution was assayed by a UV spectrophotometer at 207 nm for drug content (Anselmo *et al.,* 2019).

EE (%) = [Actual Drug Content/Theoretical Drug Content] x 100

Drug Loading (DL) was calculated as:

DL (%) = (Actual Drug content Content/Weight of Nanoparticles) x 100

***In-vitro dissolution study*:**Bilastine nanoparticles (500 mg) were taken and kept in a dialysis bag made up of cellulose. The dialysis bag is dipped into the receptor compartment that possesses the dissolution medium at 100 rpm at 37°C (dissolution medium as a blank). At selected time intervals, 2 ml of sample was withdrawn and analyzed at 207nm in a double-beam UV spectrophotometer (Rodriguez Amado *et al.,* 2017).

Kinetics of in-vitro dissolution studies: Drug release kinetics is the application of mathematical models of drug release processes that calculate drug release values using the following mathematical models: the zero-order kinetic model, the first-order kinetic model, the Higuchi model, and the Korsmeyer-Peppas model. The significance of kinetic models for the stability and release kinetics of drugs is analyzed(Fattal *et al.,* 2012).

Ex-vivo Permeation studies

The fresh mucosa of the nose was extracted from agoat's nasal cavity which was procured from a nearbyslaughterhouse.The franzdiffusion cell was

used tostudy the drug's ex-vivo drug permeation profile. The acceptor chamber was filled with 11 mL ofPhosphate Buffer, pH 5.5, at 37°C. After a 20-min pre-incubation period, 3 mlof Bilastine - NP(F1) formulation was placed in the donorchamber. After each sampling, 0.5ml of sample wastaken from the acceptor compartment and refilled withPhosphate buffer pH 5.5 at predefined time intervals. Phosphate Buffer was used to dilute the samples to10ml, filter them, and use them for analysis. The amountof permeated drug was calculated by using UV-visiblespectrophotometer. The sampling was carried out intriplicates. The same is repeated for Bilastine solution to compare the results

Statistical analysis

The results were evaluated using a one-way ANOVA or t-test at the 0.05 level of probability.

3.RESULTS AND DISCUSSION

3.1 Results

Preparation of standard calibration curve

The absorbance was estimated in a UV spectrophotometer at 207nm against 10% glacial acetic acid. The absorbance so obtained was tabulated as in Table 2. The calibration curve was plotted and shown in figure 1, and a regression value of R^2 of 0.999 was obtained.

FTIR spectralanalysis

The peak of the IR spectrum for Bilastine (a pure drug) shows up in 2160. 27cm^{-1},1994. 96 cm^{-1}, 1751. 36 cm^{-1}, 1597.06 cm^{-1}. On the other hand, the physical mixture exhibits peaks at 2181. 56 cm^{-1}, 1980. 96 cm^{-1}, 1708. 99 cm^{-1}, 1786; 14 cm^{-1}, 1579.75 cm^{-1}. Thus, it may be inferred that there are no significant interactions between the drug's physical combination and its formulation ingredients, such as Chitosan and Eudragit as shown in figures 2, 3, and 4.

Differential scanning calorimetry

The best formulation of Bilastine nanoparticles was evaluated for DSC. The pure Bilastine shows a sharp endothermic peak at 244.54°C, and some similar changes in the endothermic peak were observed at similar temperatures in prepared formulations at 252.52°C. Thus, it is concluded that there are no drug-excipient interactions, as shown in figures 5 and 6.

ParticleSize

The particle sizes of the nanoparticles, which range from 100 nm to 300 nm, substantially decreased when the polymer concentration decreased, as shown in Table 3.

ZetaPotential

The Zeta potential of nanoparticles was evaluated using laser Doppler anemometry and a Malvern Zetasizer. The zeta potential of Bilastine nano particles was in-between -20.65mV to -30.25 mV as shown in Table 3 and Figure 7.

Percentdrugloading and encapsulationefficiencyofnanoparticles

Percent loading of drugs and percent encapsulation efficiency of drug-polymer-containingnanoparticlesindifferentratioswasfoundtobebetween13.56to20.22%and70.66to 96.18% respectively,increasedwithariseintheamountofpolymer. Theamountof drugencapsulatedmaybeestimatedby dissolvingaknownamountofnanoparticles(50mg)in 50 mlofethanolanddichloromethanetofullyextractthemedication.The solution was then filtered, and the pH 6.4 phosphate was added.1ml of this solution wasusedtodilute thebuffersolutionto50ml as shown in Table 3.

Scanning electronmicroscopyanalysis

Bilastine nanoparticles are in good spherical shape with a smooth surface and some rough space in their morphology, and the particles were uniformly distributed without forming any lumps. The particle sizes observed in SEM photo microscopy are in the same range as those determined by the optical microscopy method. However, SEM photographs of the optimized formulation are shown in Figure 8. The surface smoothness of Bilastine nanoparticles was discrete, large, and showed less aggregation with spherical and uniform free flow, which was confirmed by SEM.

***In-vitro* release studies**

The *in-vitro* release studies of nanoparticles were carried out for a period of 10 hours in phosphate buffer at pH 7.4 as a dissolution mediumusing dialysis membranemethod. The percentage drug release for F1, F2, F3, F4, F5, and F6 was found to be 96.54%, 92.13%, 91.95%, 93.56%, 93.56%, and 88.93% at the end of the 10th hour as shown on table no. 4.

It was clear from the dissolution data that there is a proportional increase in the drug release with the rise in the mucoadhesive polymer concentration. The greater release of drug from the Chitosan nanoparticles than Eudragit nanoparticles can be accredited to their higher degree of swelling which allows water penetration in to nanoparticles as shown in graphical representation in figure 9.

Kinetics of In-vitro dissolution studies

The kinetic parameters of studies on *invitro*dissolution are shown in Table 5, figures 10, 11, 12, and 13. The implications of the analysis are that the Bilastine nanoparticles could release the drug under Zero's order.

Ex vivo permeation studies

Ex vivo permeation studies were performed for Bilastine-NP(F1) to show that it has better permeation than Bilastine solution. Figure 5 shows the ex-vivo release study for Bilastine-NP(F1) and Bilastine solution. In a time period of 10hr 96.46%

ofthe drug was permeated through the nasal mucosa inthe Franz Diffusion cell apparatus. Thus, it is clearlydemonstrated that permeation of XYL is much moreenhanced as compared to the drug solution.

3.2 Discussion

Eudragit and Chitosan loaded polymeric nano particles of Bilastine were prepared by double emulsification method (Tirumala Devi *et al.,* 2022). Bilastine in glacial acetic acid has shown maximum absorption at -207nm. However, the UV–visible spectrum of the synthesized Bilastine nano particles exhibited absorption peak at 207 nm corresponds to absorption maxima of Bilastine which indicates loading of Bilastine within nanoparticles. Bilastine nano particles were synthesized using Eudragit ®S 100 and Chitosan. Further characterization of nano particles has shown uniform size distribution and zeta potential with both polymers.

The particle size was increased when Eudragit was used rather than Chitosan. Percentage drug loading was found to more with Eudragit ®S 100 nano particles when compared with chitosan nano particles. This may be due to the higher swelling capacity of Eudragit ®S 100As the polymer concentrationincreased drug entrapment efficiency was also increased**.** *Invitro* dissolution studies confirmed more than 96.54%of drug release from Eudragit nano particles over the period of 10 hours. The drug release follows zero order kinetics

4. CONCLUSION

Second generation antihistamines may be an appealing therapeutic choice for the treatment of allergic rhinitis and chronic urticaria allergy due to their longer period of action, effectiveness, fewer sedation effects, and much less performance impairment.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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Table1: Formulation design of Bilastine nanoparticles

S. No	Ingredients	F1	F2	F3	F4	F5	F6
1	Bilastine(mg)	500	500	500	500	500	500
2	Chitosan (mg)	-	-	-	500	450	420
3	EUDRAGIT®S 100(mg)	500	450	420	-	-	-
3	Polyvinylalcohol(µl)	q.s	q.s	q.s	q.s	q.s	q.s
4	Methanol(µl)	100	90	80	100	90	80
5	Chloroform(µl)	100	100	100	100	100	100

Table 2: Calibration curve value

S. No	Concentration µg /ml	Absorbance
1	0	0
2	2.0	0.061
3	4.0	0.119
4	6.0	0.179
5	8.0	0.240
6	10	0.290

Table3:Particlesize,zetapotential, percentdrugloading and encapsulation efficiency ofnanoparticles

Formulation	ParticleSize (nm)	Zeta Potential (mV)	%DrugLoading	Encapsulation Efficiency (%)
F1	270.85	-30.25	20.22	96.18
F2	177.15	-29.86	16.23	81.26
F3	129.22	-22.91	13.56	75.34
F4	183.19	-28.22	15.99	83.46
F5	205.36	-22.99	18.26	72.14
F6	106.64	-20.65	17.40	70.66

Table 4: Percentage drug release of Bilastine nanoparticles inphosphatebufferof pH7.4

Time (hrs.)	PercentageCumulativeDrugRelease					
	F1	F2	F3	F4	F5	F6

0	0	0	0	0	0	0
1	25.50±02	23.92±01	21.09±03	24.01±02	21.25±04	20.52±01
2	33.96±03	31.33±04	23.54±01	30.96±03	27.83±02	25.88±02
3	41.89±01	36.84±02	34.18±02	32.99±01	34.76±02	35.04±04
4	46.54±04	42.12±05	42.06±03	42.98±05	40.01±03	37.71±03
5	54.43±02	53.38±03	52.21±06	50.01±04	47.95±01	48.59±01
6	66.66±05	60.54±02	60.01±05	60.65±02	55.06±02	54.32±03
7	76.23±01	70.14±02	74.06±04	72.11±03	70.92±01	66.52±04
8	84.43±03	81.18±01	77.67±02	80.04±03	79.01±01	73.21±04
9	90.09±06	86.63±02	85.00±02	85.43±02	84.14±03	78.89±03
10	96.54±02	92.13±03	91.95±04	93.56±01	93.56±03	88.93±03

Table 5: kinetics of *in-vitro* dissolution studies

Cumulative (%) release q	Time (T)	Root (T)	LOG CM (%) RELEASE	LOG (T)	LOG CM (%) Remain
0	0	0	0	0	0
25.50	1	1.000	1.406	0.000	0.147
33.96	2	1.414	1.530	0.301	0.184
41.89	3	1.732	1.622	0.477	0.210
46.54	4	2	1.667	0.602	0.221
54.43	5	2.236	1.735	0.698	0.239
66.66	6	2.449	1.823	0.778	0.260
76.23	7	2.645	1.882	0.845	0.274
84.43	8	2.828	1.926	0.903	0.284
90.09	9	3	1.954	0.954	0.290
96.54	10	3.16	1.984	1	0.297

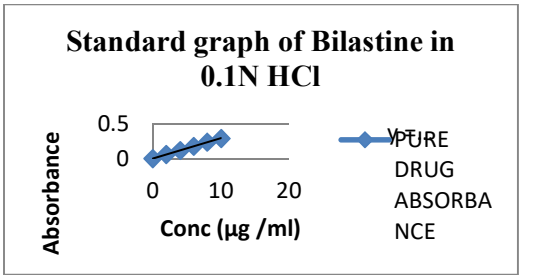


Figure.1 Standard graph of Bilastine in 0.1N HCl

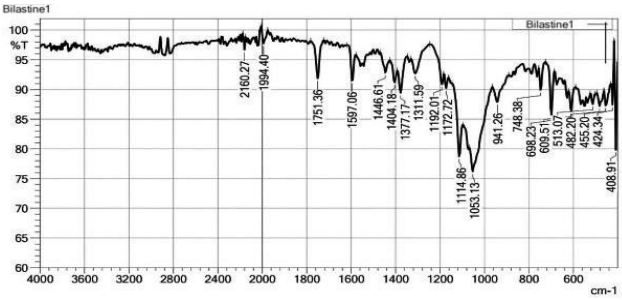


Figure.2FTIRSpectrum ofPureBilastine(Standard)

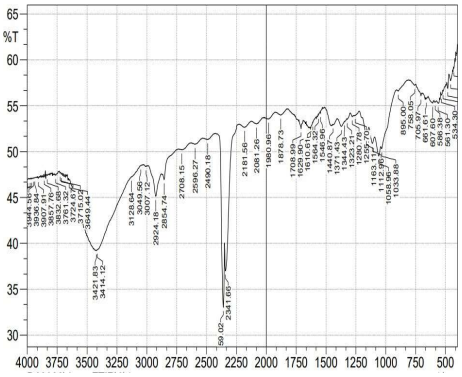


Figure.3FTIRSpectrumofBilastine + Chitosan

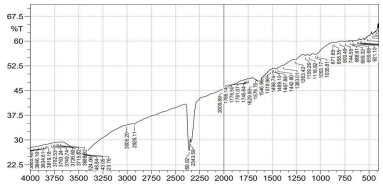


Figure.4FTIRSpectrumof Bilastine + Eudragit ®S 100

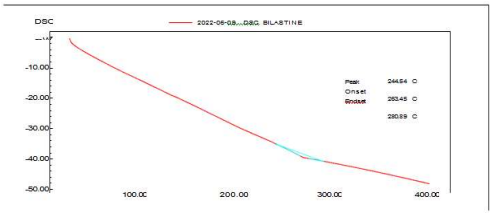


Figure.5 DSC of Bilastine

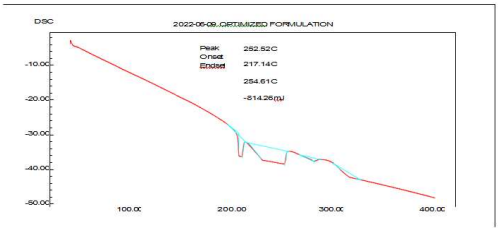


Figure.6 DSC of Optimized formulation

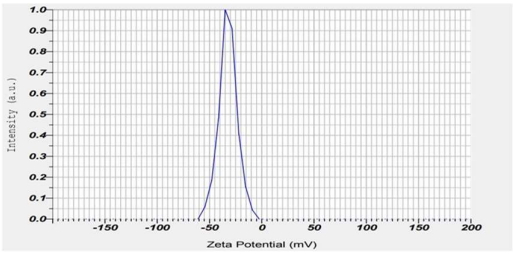
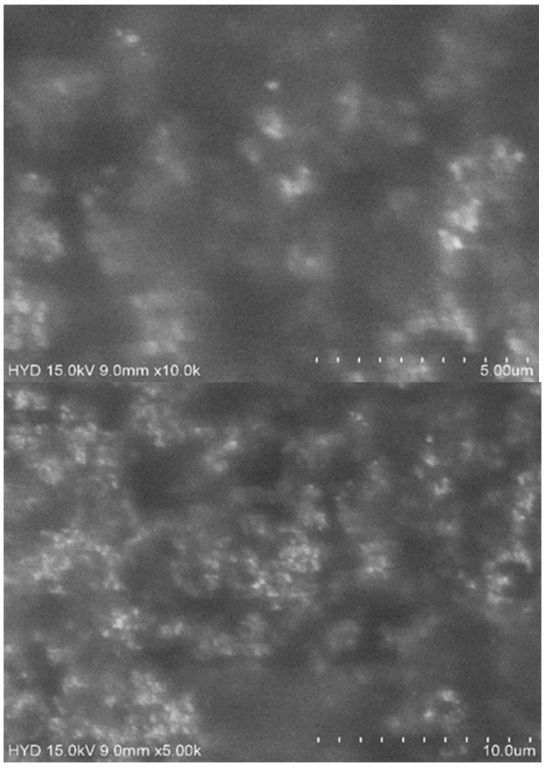


Figure.7 Zeta potential of optimized formulation



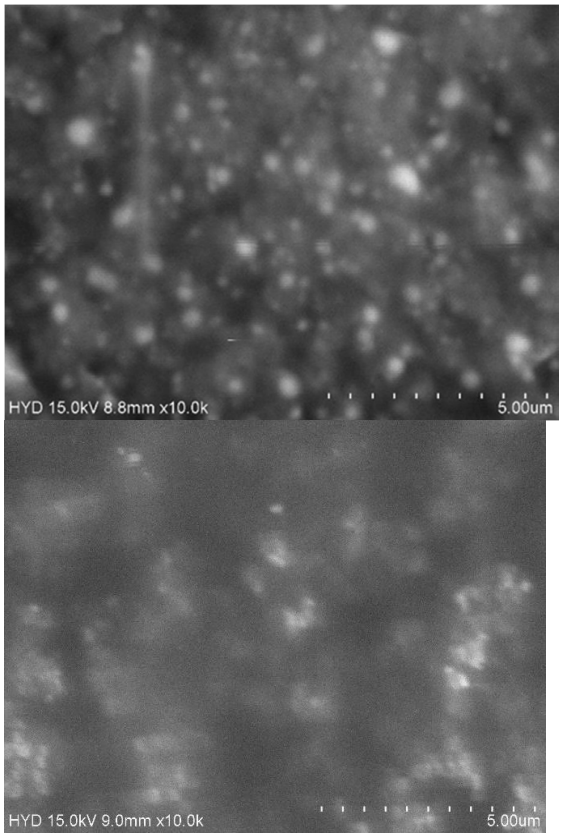


Figure.8SEMAnalysis of BilastineNanoparticles

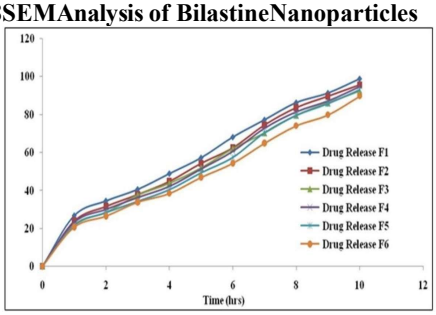


Figure.9Percentage drugreleaseofBilastine nanoparticlesin phosphatebufferofpH7.

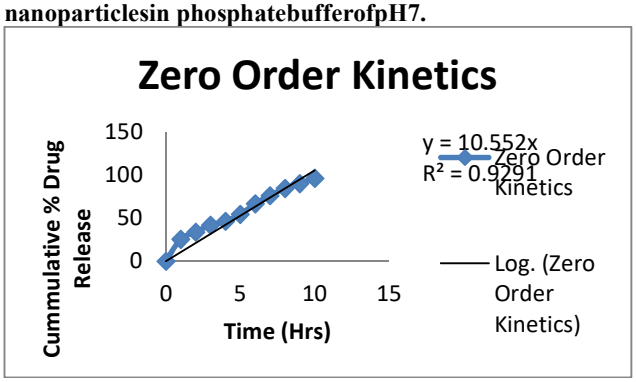


Figure.10 Graphical Representation of Zero Order Kinetics

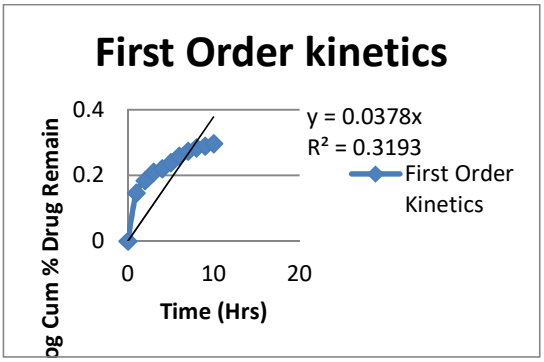


Figure.11 Graphical Representation of First Order Kinetics

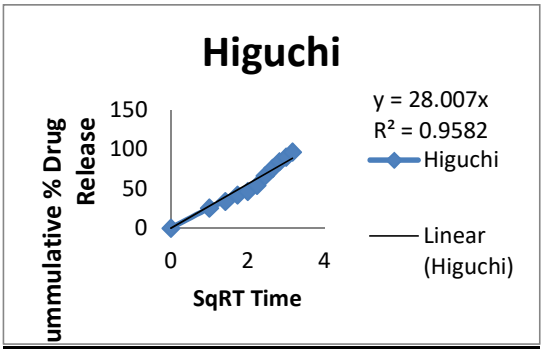


Figure.12 Graphical Representation of Higuchi Plot

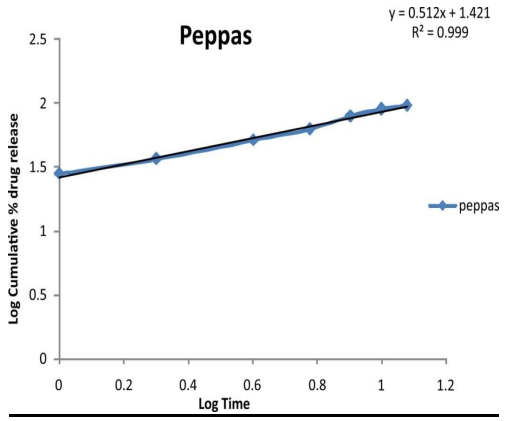


Figure.13 Graphical Representation of Korsmeyer-Peppas Plot

0	0	0
1	25.50±0.2	15.4±0.1
2	33.96±0.3	20.12±0.4
3	41.89±0.1	28.07±0.2
4	46.54±0.4	32.06+0.1
5	54.43±0.2	38.03±0.2
6	66.66±0.5	45.23±0.5
7	76.23±0.1	51.09±0.2
8	84.43±0.3	58.34±0.3
9	90.09±0.6	64.34±0.4
10	96.54±0.2	71.34±0.3