International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.14 | Issue 1 | Jan - Mar -2025

www.ijpar.com

ISSN: 2320-2831

DOI : https://doi.org/10.61096/ijpar.v14.iss1.2025.32-43

Research

Formulation and charecterization of didanisine micro beads

Dr. Nansri saha*

Associate Professor, Department of Pharmaceutics, SSJ College of Pharmacy, V.N Pally, Hyderabad, Telangana, India

*Author for Correspondence: Nansri saha
Email: nansrisaha@gmail.com

Published on: 27 Jan 2025

Published by:
DrSriram Publications

Abstract

From past few years Microbeads have been studied by many workers as a choice of novel drug delivery system to provide a better drug bioavailability considering, high penetration property of the Microbeads encapsulated agents through biological membrane and the stability of them. The present formulation study on Didanosine is an attempt to prepare Microbeads drug delivery system and evaluate its performance. The formulations were prepared, varying the ratios of polymer and sodium alginate/ sodium bicarbonate, by emulsification and ionic gelation method. An ideal or best formulation of Microbeads is the one which gives high entrapment efficiency along with good stability and drug release profile. In the present study entrapment efficiency is found to be drug and polymer ratio dependent. The release rate is found to be depended on polymer concentration and addition of the cations like Calcium chloride.

Keywords: Formulation, Evaluation, Didanosine, Microbeads.

INTRODUCTION

One antiretroviral drug is didanosine. mostly used to treat AIDS. A synthetic nucleoside counterpart of the naturally occurring nucleotide deoxyadenosine, didanosine has a hydrogen atom in lieu of the 3'-hydroxyl group. Cellular enzymes transform intracellular didanosine into dideoxyadenosine 5'-triphosphate, the active metabolite. HIV-1 reverse transcriptase activity is inhibited by dideoxyadenosine 5'-triphosphate.[1]

The cutting edge of today's established methodology, which incorporates several scientific techniques and serves for customized treatment, is the controlled medication delivery method[2]. The medication delivery method has several benefits over the current standard dose method, including increased efficacy, less toxicity, improved customer compliance, and ease of use[3,4]. Micromolecules are used in this kind of drug delivery method to care for medications. As new dosage forms are developed, such as microparticles and nanoparticles, their importance is growing[5,6]. An ideal and sophisticated oral drug delivery system is one that precisely regulates the medication's release site, speed, and

duration independently of normal physiological variables like the pH of the gastrointestinal tract, the state of the gastrointestinal tract's digestion, peristalsis movement, and circadian rhythm. Research and development effort in the design of drug delivery systems is accelerating due to advancements in polymer science and technology[7,8].
Microbeads range in size from 0.5 to 1000 μm and are almost spherical. A prolonged release or multiple release profiles of therapy with different active agents without significant adverse effects are made possible by the solid and free-flowing particulate carriers that contain dispersed drug particles in either crystalline or solution form. Furthermore, the microbeads can include medications to deliver locally at high concentrations, ensuring that therapeutic doses are achieved at the target location while minimizing adverse effects by maintaining a low systemic dosage. These microbeads can also continue to work under physiological settings.[9]

Methodology
Preparation of standard curve
Preparation of HCl pH 0.1
8.55 ml of Conc. HCl were taken and dissolved in 1000 ml of distilled water and then adjusted to pH 1.2 (0.1N) with Orthophosphoric acid.

Preparation of standard curve of Didanosine with HCl 1.2 pH
100 mg of was accurately weighed and dissolved in a small portion of 0.1N HCl in a 100 ml volumetric flask then the volume was made up to 100 ml with 0.1N HCl. This was primary stock solution, contained 1000 μg/ml. From this primary stock solution 10 ml was pipette out and transferred in to a 100 ml volumetric flask and volume was made up to 100 ml with buffer pH 1.2 which contained the concentration of 100 μg/ml. From the second stock solution again 10 ml was pipette out and diluted up to 100 ml with buffer pH 1.2 to get concentration of 10μg/ml. From third stock solution aliquots equivalent to 2,4,6,8 & 10 μg (2, 4, 6, 8, and 10ml) and from second stock solution aliquots equivalent to 12 & 16 (1.2 & 1.6 ml) were pipette out in to a series of 10 ml volumetric flask and volume was made up to 10 ml with buffer pH 1.2. The absorbances of these solutions were measured against the buffer pH 1.2 as blank at 254 nm using UV- Visible double beam spectrophotometer. Then a calibration curve was plotted taking concentration in μg/ml on X-axis and absorbance on Y-axis.

Preparation of Phosphate Buffer pH 7.4
Preparation of pH 7.4 Phosphate Buffer
50.0 ml of 0.2 M potassium di-hydrogen phosphate was placed in a 200 ml volumetric flask, added the specified volume

Potassium di-hydrogen phosphate, 0.2 M
27.218 g of potassium di-hydrogen phosphate was dissolved in distilled water and diluted to 1000 ml.

Sodium hydroxide solution 0.2 M
8 g of sodium hydroxide was dissolved in distilled water and diluted to 1000ml.

Preparation of standard curve of with Phosphate Buffer pH 7.4.
100 mg of was accurately weighed and dissolved in a small portion of phosphate buffer pH 7.4 in a 100 ml volumetric flask then the volume was made up to 100 ml with phosphate buffer pH 7.4.This was primary stock solution, contained 1000 μg/ml. From this primary stock solution 10 ml was pipette out and transferred in to a 100 ml volumetric flask and volume was made up to 100 ml with phosphate buffer pH 7.4 which contained the concentration of 100 μg/ml. From the second stock solution again 10 ml was pipette out and diluted up to 100 ml with phosphate buffer pH 7.4 to get concentration of 10 μg/ml. From third stock solution aliquots equivalent to 2-16 μg (5, 10, 15,20 and 25 ml) and from second stock solution aliquots equivalent to 12-16 μg(1.2 and 1.6 ml) were pipette out in to a series of 10 ml volumetric flask and volume was made up to 10 ml with phosphate buffer pH 7.4. The absorbances of these solutions were measured against the phosphate buffer pH 7.4 as blank at 254 nm using UV- Visible double beam spectrophotometer. Then a calibration curve was plotted taking concentration in μg/ml on X-axis and absorbance on Y-axis.

Preparation of Microbeads.
Chitosan Microbeads were prepared according to the procedure first reported by Calvo et al. (1997b) based on the ionic gelation of Chitosan with Calsium chloride and alginate anions. Microbeads were prepared by using different drug to polymer ratio. Required quantity of drug was dissolved in 10 ml of water, Polymer Chitosan is separately

dissolved in water in different ratios. Then Solution was injected drop by drop into different percentages of sodium alginate solution. Because of using sodium alginate. Here sodium alginate used as cross-linking agents. These microspheres are very delicate/ sensitive. To hardening the Microbeads, Microbeads were kept on ice and then added into supersaturated dextrose solution. Then filterate and dried the Microbeads. Then filterate and dried the Microbeads.

Formulation of different batches of Didanosine Microbeads

Table 1: Formulation table

Ingredients	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9
Didanosine	200	200	200	200	200	200	200	200	200
Na Alginate	1%	2%	3%	4%	5%	5%	5%	5%	5%
Chitosan	-	-	-	-	-	1%	2%	3%	2%
Water	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs
Calcium chloride solution	5%	5%	5%	5%	5%	5%	5%	5%	5%

Ionto trophic gelation method

Mix Polymer in water kept a side for soaking up to 24 hr. drug dissolved in pure water and mix it into the above polymer solution then this solution allow through 24guage syringe in to the 5% calcium chloride solution.

Evaluation of Microbeads

Particle size & Characterization of the morphology of the Microbeads

The particle size of all the batches of the formulated Microbeads in a sample was measured with an optical micrometer fitted with a calibrated eye piece. Calibration of the microscope was done piror to particle size measurement of the Microbeads. The mean of 100 particles was noted as particle size. The surface morphology (roundness, smoothness and formation of aggregates) and the size of Microbeads formulations were studied by scanning electron microscope (SEM). The data obtained after the observation were analyzed accordingly.

Percentage Yield

The percentage yield of different formulations was determined by weighing the Microbeads after freeze drying. The percentage yield was calculated as follows

$$\%\boldsymbol{Yield} = \frac{\boldsymbol{Total\ Weight\ of\ MicroParticles}}{\boldsymbol{Total\ weight\ of\ Drug\ and\ Polymer}}\boldsymbol{x100}$$

Each determination was made in triplicate.

Drug Entrapment Efficiency

The various formulations of the Microbeads were subjected for drug content analysis. Suspension of the various formulations was prepared by suspending Microbeads (equivalent to 150 mg of pure) in aqueous solution. Each suspension was sonicated for 30 min to separate the free drug in the supernatant from the drug incorporated in the Microbeads. Concentrations of in the supernatant were determined by UV-visible spectrometry at 254 nm after suitable dilution. The amount of the drug incorporated in Microbeads was calculated from the difference in drug concentrations between the supernatant and the original given concentrations. The entrapment efficiency was calculated according to the following equation:

$$\boldsymbol{Entrapment\ efficiency} = \frac{\boldsymbol{Mass\ of\ Drug\ in\ Microparticles}}{\boldsymbol{Mass\ of\ Drug\ used\ in\ formulation}}\boldsymbol{x100}$$

Fourier Transform Infra-red Spectroscopy (FT-IR) Analysis

The Fourier transform infra-red analysis was conducted for the analysis of drug polymer interaction and stability of drug during formulation process. Fourier transform infra-red spectrum of pure and formulated Microbeads were recorded. The formulation was kept for stability study before going for the FT-IR study. After the completion of the stability study formulation is used for the FT-IR study and the peaks of were observed. Infrared absorption spectra of and Microbeads in the wavelength region of 450cm-1 to 3600cm-1 were recorded using FT-IR (SHIMADZU, JAPAN). Resolution used in the scans was 4 cm-1 and the spectra were averaged over 20 scans.

In-vitro Release Studies

Drug release studies on the loaded HPMC Microbeads were carried out using a USPXXI dissolution rate test apparatus for 30 h at a stirring speed of 100 rpm. An amount of Microbeads equivalent to 150 mg of was placed in the dissolution medium Citric acid buffer pH 0.1 for 2 h. Then, after 2 h replaced phosphate buffer pH 7.4 maintained for 30 h at a temperature of 37± 0.5ºC. A 5 ml of sample aliquot of the dissolution medium was withdrawn at different time intervals and fresh dissolution medium was simultaneously used to replace the quantity withdrawn. The samples were then filtered using a Whatmann No. 1 qualitative filter paper and assayed spectrophotometrically (Varian Carry 50 Bio, USA) at 254 nm to estimate the drug concentration. All experiments were performed intriplicate.

Kinetic modelling

Zero order kinetics

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation

$$\mathbf{Q\ t = Q\ o + K\ o\ t}$$

Where Q t = amount of drug dissolved in time t, Q o = initial amount of drug in the solution and K o = zero order release constant.

First Order Model

The first order equation describes the release from systems where the dissolution rate is dependent upon the concentration of the dissolving species.

Release behaviour generally follows the following first order equation:

$$\mathbf{Log\ C = Log\ Co\text{-}kt/2.303}$$

Where C is the amount of drug dissolved at time t, Co is the amount of drug dissolved at t=0 and k is the first order rate constant. A graph of log cumulative of % drug remaining vs time yields a straight line.

Higuchi model

Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. And the equation is

$$\mathbf{Q\ t = KH.\ t\ 1/2}$$

t= Amount of drug released in time t, K

Krosmeyer and Peppas release model

To study this model the release rate data are fitted to the following equation

$$\mathbf{Mt\ /\ M\ \infty = K.t\ n}$$

Where Mt / M ∞ is the fraction of drug release, K is the release constant, t is the release time and n is the Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form.

If the exponent n = 0.5 or near, then the drug release mechanism is Fickian diffusion, and if n have value near 1.0 then it is non-Fickian diffusion

Stability Study

Samples from each batch were withdrawn after the definite time intervals and

the residual amount of drug in the vesicles was determined. Stability data of three formulations were further analyzed for significant difference by paired t-test. All the batches of Didanosine Microbeads were tested for stability. The preparations were divided into 3 sets and were stored at 5-80C (refrigerator) 254C and at 400C. After 15, 30 and 60 days drug content of all the formulations was determined by the method discussed previously in entrapment efficiency section.

RESULTS AND DISCUSSION

Preformulation Study: The FT-IR spectra of the pure Didanosine and formulation were recorded to check interaction between drug and polymers. Before FT-IR examination formulation is kept for the stability testing. The characteristic peak due to pure Didanosine has appeared in the spectra without any markable change in the position. It indicates that there was no chemical interaction between Didanosine and CHITOSAN.

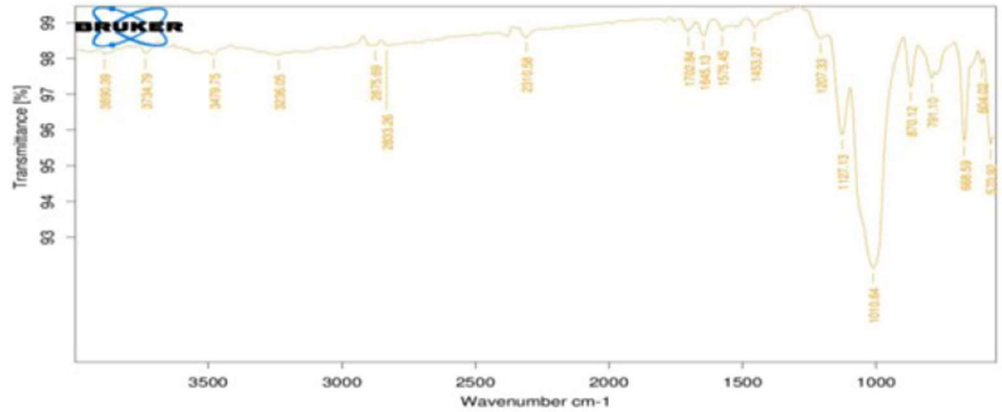

Fig 1: FTIR of Didanosine

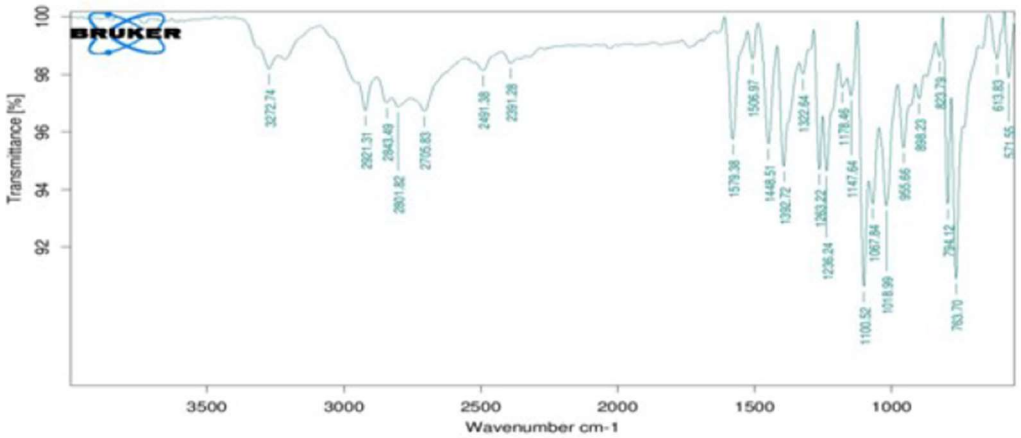

Fig 2: FTIR of Didanosine with Excipients

Optimised Formulation

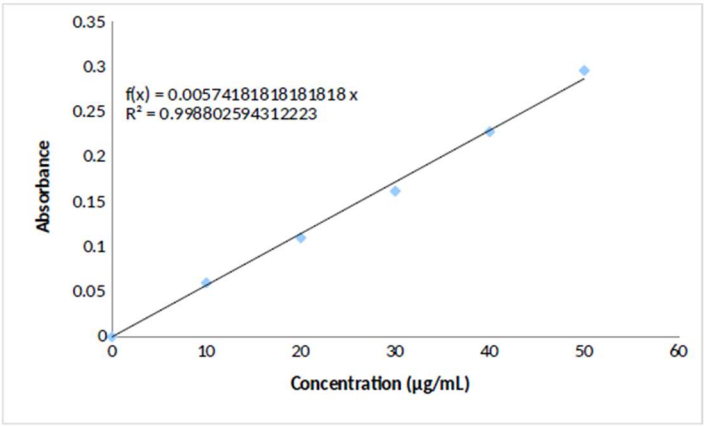

Fig 3: Standard graph of Didanosine in 0.1 N HCl (lamda max 254nm)

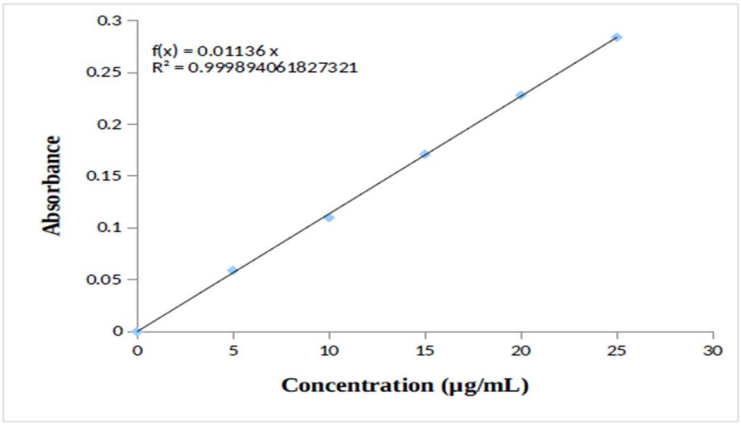

Fig 4: Standard graph of Didanosine 7.4 Buffer(lamda max 254nm)

SEM studies

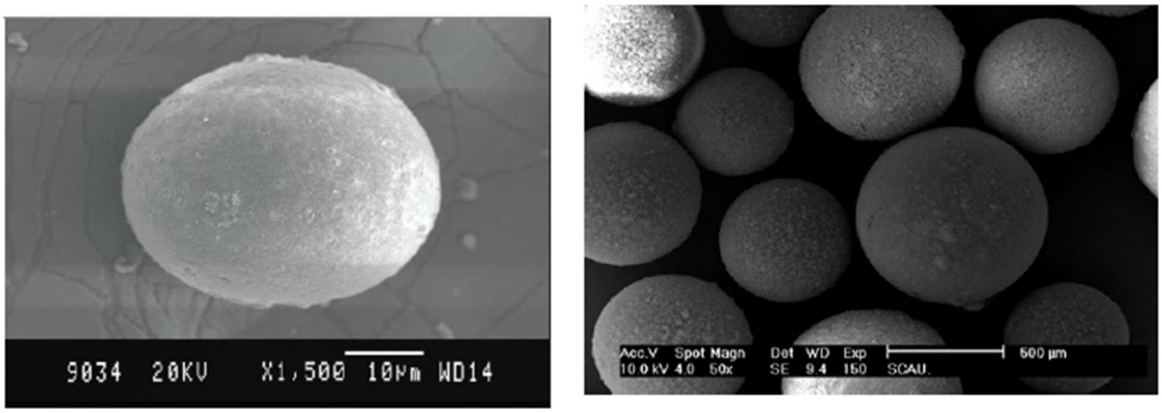

Fig 5: SEM images and particle size of optimised formulation

In vitro Drug Release

Release studies were carried out by using two different release media. HCL pH 0.1 and Phosphate buffer at pH 7.4 were used in order to evaluate the influence of the pH inside gastric and intestine on Didanosine release from CHITOSAN Microbeads. In Figure, Didanosine release profiles from Didanosine-loaded CHITOSAN Microbeads at pH 0.1 and 7.4 buffer solutions respectively, are shown. As can be seen from the figures, an initial burst effect was observed from all CHITOSAN Microbeads (between 13 and 22% of loaded Didanosine). After this initial burst, all studied microspheres released Didanosine at a lower rate. Didanosine release from the was pH dependent (faster release at pH 0.1 than at pH 7,4). This is attributed to the higher solubility of the polymer at lower pH. In fact, as proposed earlier, CHITOSAN Microbeads can also provide pH responsive release profile by swelling in acidic environment of the gastric fluid. When comparing the release profiles from cross-linked (with SS/SC) and with & without adding calcium chloride. By addition of the cations like calcium chloride, the drug release was diminished hence it was more controlled. we see that at pH 7.4 the release of Didanosine is substantially decreased in the crosslinked particles. It has been proposed before that addition in CHITOSAN particles can be used as a method to modulate release kinetics of drugs, as demonstrated for theophylline. However, the difference between the release kinetics of Didanosine from the two types of CHITOSAN particles is more or less diminished (or is a lot smaller) at pH 3.0, possibly due to the rapid swelling and increased solubility of this polymer a low pH, which results in a very fast release of particle- loaded Didanosine from all CHITOSAN Microbeads during the first 4-5 hours of incubation.

Table 2. % Drug release for F-1 to F-4

Time(hr)	% Drug release			
	F-1	F-2	F-3	F-4
0	0	0	0	0
1	30.72	28.57	26.42	22.49
2	52.6	50.5	48.6	47.9
4	67.8	64.7	62.46	61.4
6	81.6	80.8	78.24	76.9
8	90.6	88.9	84.6	84.2
10			94.8	92.56
12				

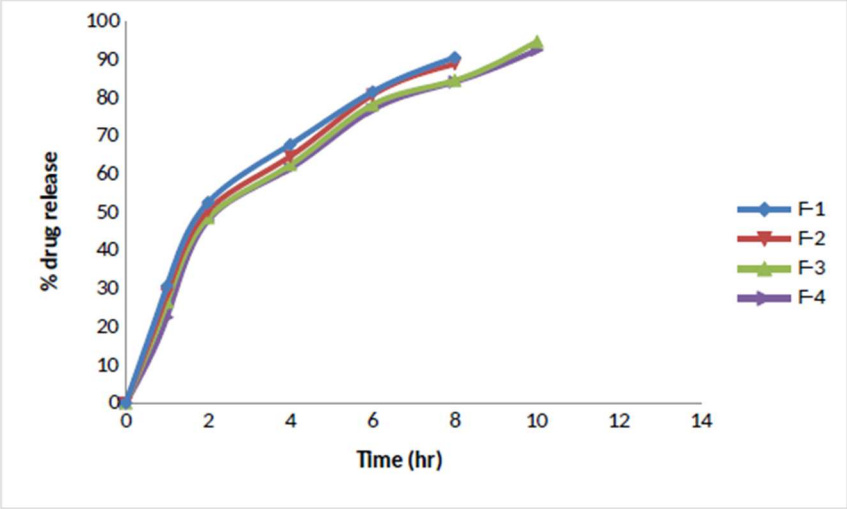

Fig 6: %Drug release of formulations F-1 to F-4

Table 3. % Drug release for F-5 to F-8

Time(hr)	% Drug release			
	F-5	F-6	F-7	F-8
0	0	0	0	0
1	14.6	12.8	11.45	8.34
2	28.32	27.53	25.3	20.6
4	48.4	46.9	44.8	41.9
6	64.2	61.8	58.5	56.36
8	82.6	79.6	78.2	74.7
10	94.6	91.3	90.3	894
12				97.4

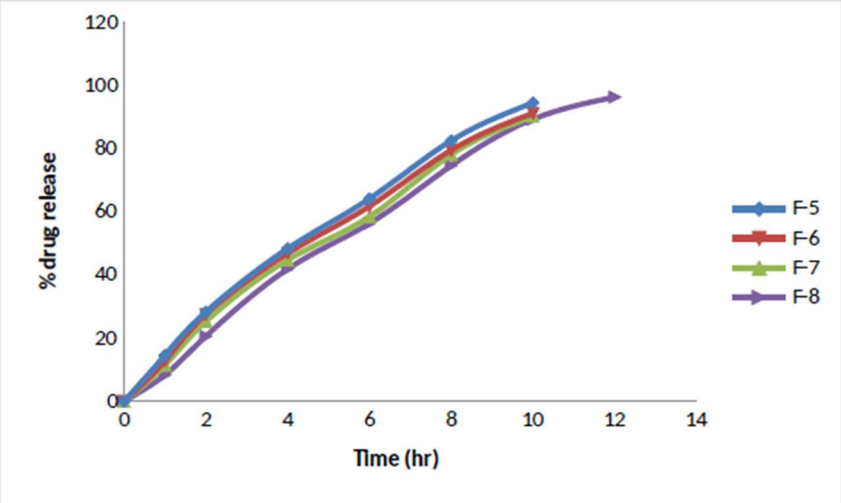

Figure 7: %Drug release of formulations F-5 to F-8

Table 4. % Drug release for F-9

Time(hr)	%Drug release
	F-9
0	0
1	15.3
2	32.72
4	48.3
6	54.34
8	62.27
10	70.46
12	82.8

Kinetic modeling

The various kinetic models were applied to in vitro release data for prediction of the drug release kinetic mechanism. The release constants were calculated from the slope of appropriate plots, and the regression coefficient (r2) was determined. It was found that the in vitro drug release of Microbeads was best explained by First order kinetics as the plots shows highest linearity. The correlation coefficient (r2) was 0.9877 for f8 formulation as shown in Table .For formulation correlation coefficient (r2) is found to be 0.9566, indicating that the drug release was nearly dependent of concentration, followed by Higuchi's (r2 = 0.9161). In the current study, drug release kinetic according to korsmeyer-

peppa's model is also followed. The values of release rate exponent (n), calculated as per the equation proposed by peppa's, and all the slope values range 0.9945 revealed the fact that the drug release follows a super case II transport.

Zero Order

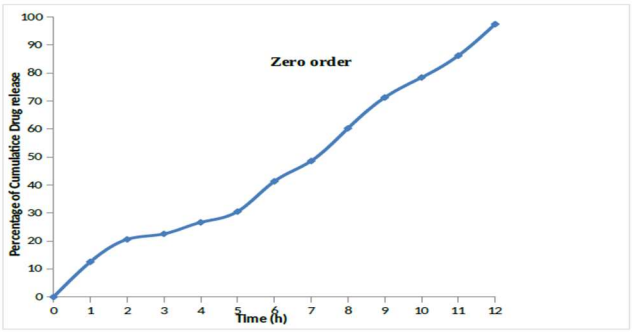

Fig 8: Zero order

First Order

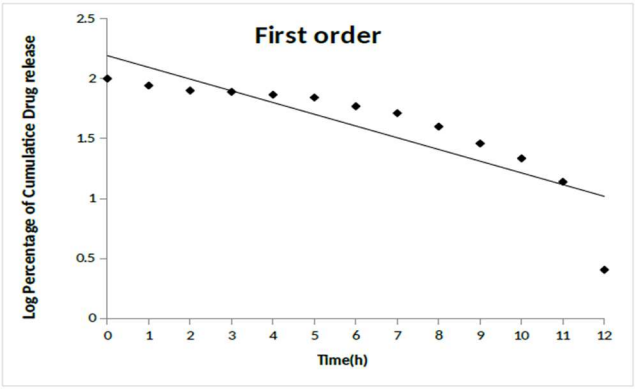

Fig 9: First order

Higuchi Plot

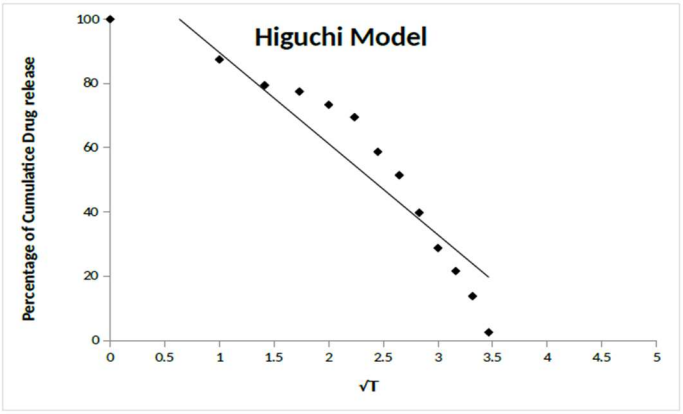

Fig8: Higuchi order

Peppas Plot

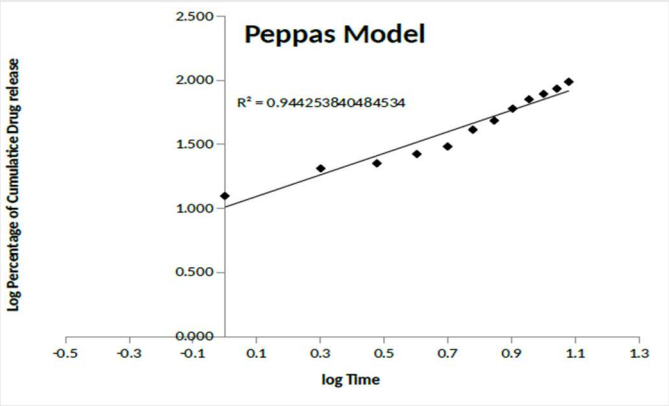

Fig 8: Peppas order

Table 5. Drug release kinetics

Code	EE	Zero	Higuchi	First
	%	R^2		
F-1	69.11	0.9859	0.826	0.9811
F-2	71.55	0.9865	0.8568	0.9919
F-3	72.67	0.9844	0.9145	0.9813
F-4	84.45	0.9835	0.9283	0.9816
F-5	67.91	0.9872	0.7941	0.9816
F-6	71.6	0.9858	0.7919	0.9879
F-7	86.64	0.9829	0.9161	0.9891
F-8	90.18	0.9877	0.9161	0.9887
F-9	56.56	0.9923	0.8612	0.9812
F-10	59.81	0.9918	0.8913	0.9823
F-11	74.62	0.9928	0.8913	0.9823
F-12	76.5	0.9898	0.9121	0.9719

Short term Stability Study

Stability study is the important part of the study for any pharmaceutical formulation. There are procedures given for the stability study in ICH guidelines.

Table 6: Short term stability study data of optimized

Duration (month)	Parameter Studied	Formulation code
0	Drug content	82.11
	% Drug release	62.16
1	Drug content	80.54
	% Drug release	61.54
2	Drug content	79.83
	% Drug release	60.59
3	Drug content	79.05
	% Drug release	59.74

The short term stability study was performed as per ICH guidelines using selected Didanosine -loaded CHITOSAN micro particles for a period of 3 months. The Microbeads were periodically evaluated for drug content and in vitro

drug release. The evaluated parameters did not show any significant change during the time course of storage confirmed that the prepared Didanosine-loaded CHITOSAN Microbeads

SUMMARY& CONCLUSION

From past few years Microbeads have been studied by many workers as a choice of novel drug delivery system to provide a better drug bioavailability considering, high penetration property of the Microbeads encapsulated agents through biological membrane and the stability of them. The present formulation study on Didanosine is an attempt to prepare Microbeads drug delivery system and evaluate its performance. The formulations were prepared, varying the ratios of polymer and sodium alginate/ sodium bicarbonate, by emulsificaion and ionic gelation method. An ideal or best formulation of Microbeads is the one which gives high entrapment efficiency along with good stability and drug release profile. In the present study entrapment efficiency is found to be drug and polymer ratio dependent. The release rate is found to be depended on polymer concentration and addition of the cations like Calcium chloride. In the present study entrapment efficiency is found to be dependent on drug and polymer ratio. The drug entrapment efficiency of different formulation is in the range. The formulation F8 which showed higher entrapment efficiency 98.6 provides desired drug release rate. The in-vivo results of Microbeads have emerged as an efficient means of enhancing the bioavailability and sustained delivery of Didanosine. Different batches of Didanosine microparticle formulations were developed using Chitosan polymer at various ratios and addition of Calcium chloride as cation. The Microbeads were prepared by emulsification and ionotropic gelation technique. Pre formulation studies for compatability were carried out by infrared spectroscopy and differential scanning calorimetric analysis. All the batches of the formulated Didanosine loaded CHITOSAN Microbeads of all formulations were evaluated for various physicochemical parameters like mean particle size distribution , % yield and drug entrapment efficiency. % yield of all formulations calculated and showed in table . Percentage yield was Increased with the increasing the polymer concentration found to be in the range.

Entrapment efficient of all formulations were calculated and showed in table . E% was decreased with increasing the CHITOSAN concentration by increasing the viscosity leads to low drug entrapment capacity. More E% was with the adition of Calcium chloride of Didanosine- loaded CHITOSAN Microbeads. Release studies were carried out by using two different release media. Bicarbonate acid buffer pH 0.1 and Phosphate buffer at pH 7.4 were used in order to evaluate the influence of the pH inside gastric and intestine on Didanosine release from CHITOSAN Microbeads. As can be seen from the figures, an initial burst effect was observed from all CHITOSAN Microbeads. After this initial burst, all studied microspheres released SAQ at a lower rate. % drug release of all formulations as indicated in table . Addition of Calcium chloride to the Sodium alginate polymer, swelling property of Sodium alginate was decreased hence % drug release was decreased. After all the discussions, finally I concluded that **F8** formulation was the best and optimized formulation of all remaining formulations.

REFERENCES

1. K. L. Senthil Kumar, S. Ashokkumar, R. P. Ezhilmuthu, Formulation and Evaluation of Didanosine Enteric Coated Sustained Release Tablet, J Biomed Sci and Res., Vol 2 (3), 2010,126-131.
2. Petersen K, SchmutzlerW. Proton pump inhibitors. Active substance release from different preparations. Deutsche Apotheker Zeitung, 1999; 139:64–65.
3. Pillay V, Fassihi R. In-vitro release modulation from cross linked pellets for site-specific drug delivery to the gastrointestinal tract: I. Comparison of pHresponsive drug release and associated kinetics. Journal of Controlled Release, 1999; 59(2):229-242.
4. Nagata K, Takagi E, Tsuda M. Inhibitory action of Lansoprazole and its analogs against helicobacter pylori: inhibition of growth is not related to inhibition of urease. AntimicrobialAgents and Chemotherapy, 1995; 39:567-70.
5. Florence A. The oralabsorption of micro-and nanoparticulates: neither exceptional nor unusual. Pharmaceutical Research, 1997; 14:259–266.
6. Ulrich K, Matthias S. Topical delivery of therapeutic agents in the treatment of inflammatory bowel disease. Advanced Drug Delivery Review. 2005; 57(2):267-279.
7. Roy S, Das S.. Design and in-vitro evaluation of dapsone-loaded micropellets of ethyl cellulose. Pharmaceutical Research, 1989; 6(11):945-948.
8. DashevskyA, Kolter K, Bodmeier R. Compression of pellets coated with various aqueouspolymer dispersions. International Journal of Pharmaceutics, 2004; 279:19–26.

9. Mullaicharam Bhupathyraaj * , Alka Ahuja, Jayasekher and Sushama Pole, FORMULATION OF MICRO BEADS: A REVIEW, IJPSR, 2021; Vol. 12(1): 95-103.