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FORMULATION & EVALUATION OF ACYCLOVIR MICROBEADS

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ABSTRACT

The present study is concerned with the formulation and evaluation of acyclovir microbeads in the presence of polymer. Acyclovir drug is an antiviral drug which is preferentially taken up by the virus infected cells and thus it inhibits DNA synthesis and viral replication but only 20 percent of oral dose is absorbed as it is having less plasma protein bounding and having t ½ life is 2-3 hours. Microbeads are prepared to overcome its less oral absorbent to obtain prolonged controlled drug delivery, to improve bioavailability or stability of drug. This article contains different concentration of polymer (Chitosan and sodium alginate) and excipients to prepare Acyclovir microbeads which increase bioavailability by increasing t ½ life elimination i.e maximum up to 12 hours.

INTRODUCTION

Novel drug delivery technology has certainly infused new interests in seemingly traditional old drugs by providing trend new life specific therapeutically through their therapeutic targets. It is appreciated that, target oriented drug administration with improvements in therapeutic efficacy, reduction in side effects and optimize dosing regimen, shall be the leading trends in the area of therapeutics.[1]

Conventional dosage forms like tablets, capsules, pills, powders, parenteral preparations, emulsions, creams, ointments, solutions, suspensions and aerosols introduced to clinical medicine exert their effects by interactive interference with cell and cell membrane related to structure and function to obtain a desirable therapeutic response. The amount of drug should exactly reach to target site with subsequent control of drug input rate, the distribution of other tissues therefore unnecessary and a potential cause of toxicity.[2] It is apparent that most of disease treated by cytotoxic agents like Acyclovir drug which is an antiviral drug which is preferentially taken up by the virus infected cells and thus it inhibits DNA synthesis and viral

replication but only 20 percent of oral dose is absorbed as it is having less plasma protein bounding and having t ½ life is 2-3 hours[3] and thus so many cytotoxic drugs are not only demand for controlled drug delivery but also the pattern of drug delivery is directed to be specific, precise and defined at quantitative levels.

Microparticulate carrier systems can be administered through different routes such as intravenous, ocular, intra muscular, intra arterial, oral etc to achieve desired activity of either sustained action or targeting or both. Each route has its own biological significance, limitation and pharmaceutical feasibility. Biodegradable microparticulate carriers are of interest for oral delivery of drugs to improve the bioavailability to enhance drug absorption to target specific site and reduced toxicity, to improve gastric tolerance of stomach and as a carrier for antigen. It is also designed for controlled release of drug content depends on the size of microparticles and the drug contain within microspheres.[4]

In this study acyclovir microbeads are prepared by the method Ion gelation method using chitosan and sodium alginate polymer to compare the bioavailability with marketed drug by evaluating with Particle size & Characterization of the morphology of the microparticles, Percentage Yield, Drug Entrapment Efficiency, FTIR, in vitro studies etc.

MATERIALS AND METHODS

Acyclovir is an active ingredient drugs in our work which are purchased from Pharmacy of Hyderabad, Chitosan and Sodium alginate polymer and all other chemicals are obtained from Active Pharma Lab, Hyderabad.

I. Preparation of micro particles

Chitosan Microparticles were prepared by the ionic gelation of Chitosan with Calcium chloride and alginate anions in following steps i.e.

- Microparticles were prepared by using different drug concentration to polymer ratio into 9 different batches.
- Required quantity of drugs 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 which is used as cross-linking agents, these microspheres are very delicate or sensitive.
- To hardening the microparticles, microparticles were kept on ice water and then added into supersaturated dextrose solution. Then it was filtered and dried.

Table No. 1: Formulation of different batches of Acyclovir micro particles

INGREDIENTS	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9
Acyclovir	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 chloridesolution	5%	5%	5%	5%	5%	5%	5%	5%	6%

II. Iono-Trophic Gelation Method

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

Preformulation Study

It was carried out including of Identification of drug, Solubility study and Drug-excipients compatibility study**.**

Evaluation of Microparticles

1. Particle size & Characterization of the morphology of the microparticles

The particle size of all the batches of the formulated microparticles in a sample was measured with an optical micrometer fitted with a calibrated eye piece. Calibration of the microscope was done prior to particle size measurement of the microparticles. The mean of 100 particles was noted as particle size.

The surface morphology (roundness, smoothness and formation of aggregates) and the size of microparticles formulations were studied by scanning electron microscope (SEM). The data obtained after the observation were analyzed accordingly.[5]

2. Drug Entrapment Efficiency

The various formulations of the microparticles were subjected for drug content analysis. Suspension of the various formulations was prepared by suspending microparticles (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 microparticles. Concentrations of in the supernatant were determined by UV-visible spectrometry at 253 nm after suitable dilution. The amount of the drug incorporated in microparticles 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:

$$\textbf{The Entrapement Efficiency} = \frac{\textbf{Mass of drug in Microparticles}}{\textbf{Mass of drug used in formulation}} \times 100$$

Each determination was made in triplicate.[6,7]

3. 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 micro particles 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. IR absorption spectra of micro particles in the wavelength region of 450cm^{-1}to 3600cm^{-1} had been recorded. Resolution used in the scans was 4 cm^{-1} and the spectra had been scanned for 20 times (average taken).[8]

III. Drug release study

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 $\mathbf{Q_t = K_H . t^{1/2}}$ ($_t$= Amount of drug released in time t, K).[9,10]

IV. 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 acyclovir microparticles were tested for stability. The preparations were divided into 3 sets and were stored at 5-8^{0}C (refrigerator) 254C and at 40^{0}C. After 15, 30 and 60 days drug content of all the formulations was determined by the method discussed previously in entrapment efficiency section.[11]

RESULTS

Iontropic gelation and Emulsification and iontropic gelation methods are rapid and simple techniques for producing Acyclovir-loaded Chitosan microparticles with small size and good reproducibility from batch to batch. This production process is based on the solubility behavior of Chitosan, which is poorly soluble in water. Addition of an acid improves its solubility as a result of protonation of amino groups. Chitosan solubility is also affected by other anions present in the solution. In the presence of phosphate, polyphosphate and sulphate ions, Chitosan shows a decreased solubility. For this reason, sodium alginatewere chosen for microparticle formulations, leads to a poorly soluble Chitosan derivative, whereby microparticle formulation become possible.

I. Preformulation Study

1. Particle size & Characterization of the morphology of the microparticles

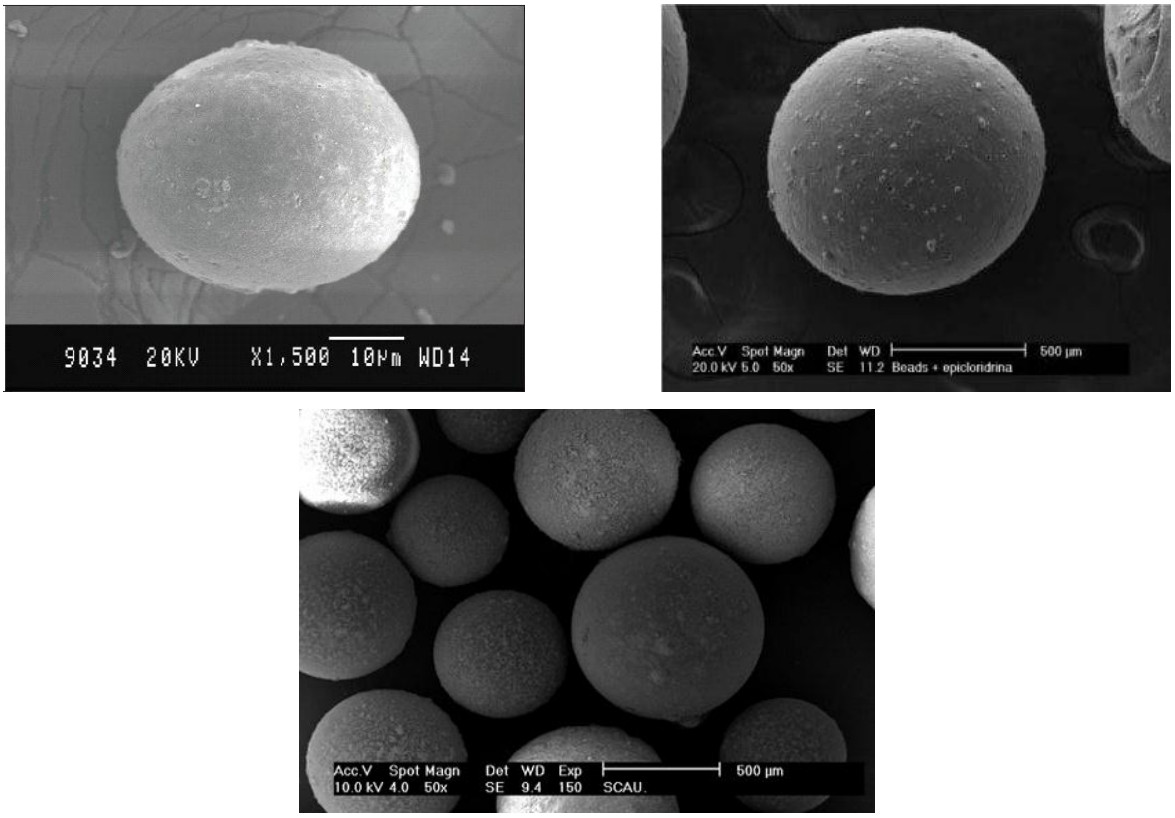


Fig 1- SEM images for particle size of optimized formulation

2. Drug Entrapment: (a) Standard drug

Table No. 2- Optimized Formula for standard drug

S. No.	Concentration(µg/mL)	Absorbance
1	0	0
2	2	0.06

3	4	0.11
4	6	0.162
5	8	0.228
6	10	0.296

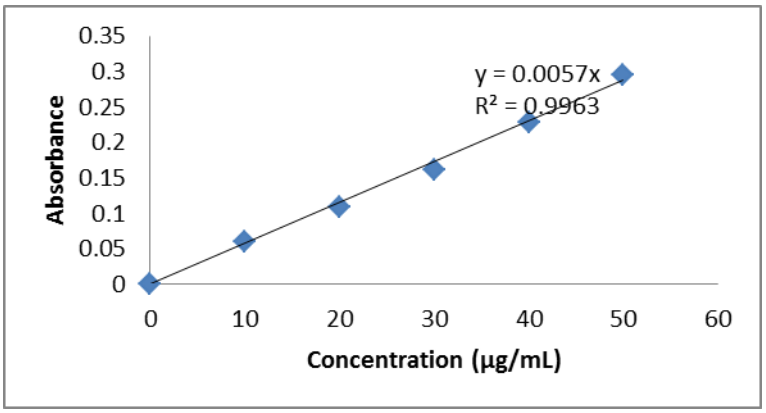


Fig No. 2 - Standard graph of Acyclovir 7.4 Buffer (253nm)

(b) Microbeads

Table No. 3- Optimized Formula for Micro beads

S. No.	Concentration (µg/mL)	Absorbance
0	0	0
1	5	0.059
2	10	0.110
3	15	0.171
4	20	0.228
5	25	0.284

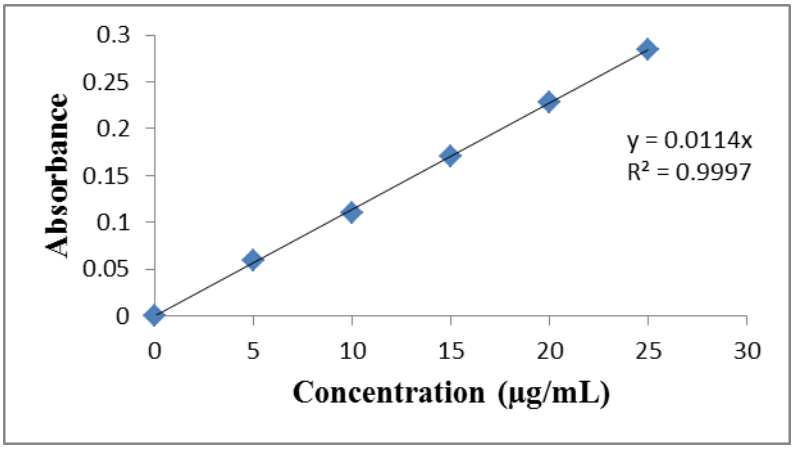


Fig No. 3 - Standard graph of Microbeads

- **FTIR**

The FT-IR spectra of the pure Acyclovir 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 Acyclovir has appeared in the spectra without any makeable change in the position. It indicates that there was no chemical interaction between Acyclovir and chitosan.

- **Standard drug**

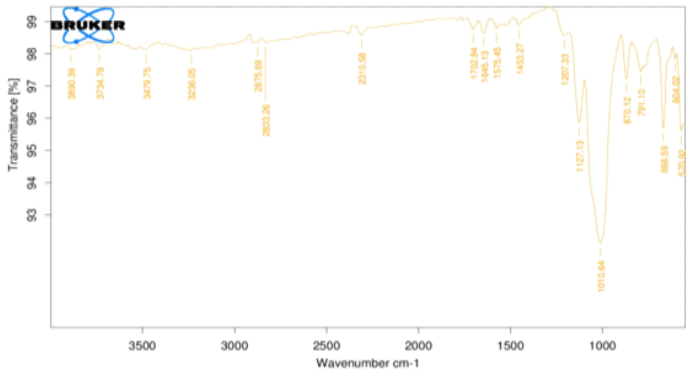


Fig 4 - FTIR OF Acyclovir (Standard drug)

(b) Microbeads

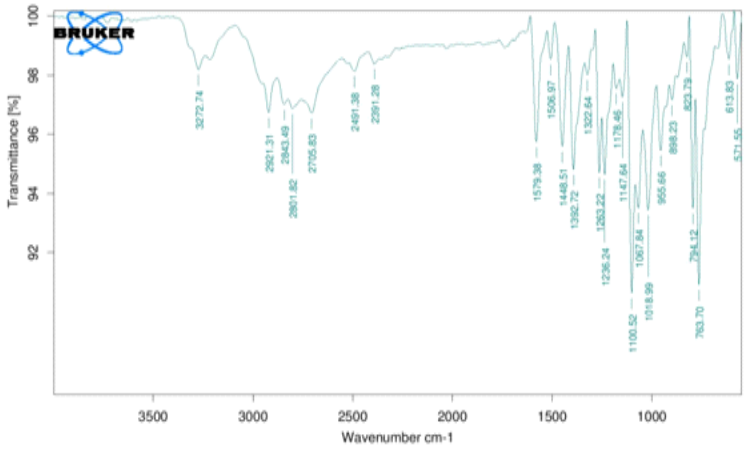


Fig 5- FTIR OF Acyclovir Micro Beads

II. 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 Acyclovir release from Chitosan micro particles. In Figure, Acyclovir release profiles from Acyclovir-loaded Chitosan microparticles at pH 0.1 and 7.4 buffer solutions respectively, as shown below;

Table 4- Invitro Drug release for Micro bead formulation from F1-F4

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	61.54
6	81.6	80.8	78.24	76.9
8	90.6	88.9	84.6	84.2
10			94.8	92.56
12				

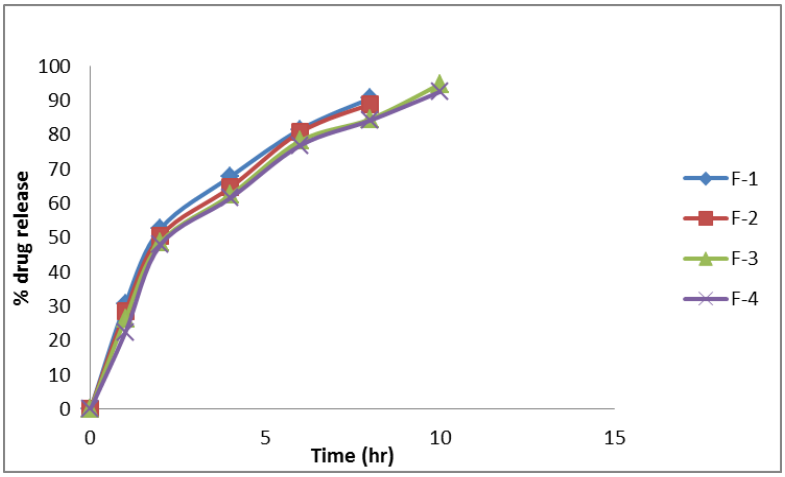


Fig 6 Optimized formula Graphs (F1-F4)

Table 5- Invitro Drug release for Micro bead formulation from F5-F9

Time (hr)	% Drug release				
	F-5	**F-6**	**F-7**	**F-8**	**F-9**
0	0	0	0	0	0
1	14.6	12.8	**8.34**	2.34	20.2
2	28.32	27.53	**20.6**	14.6	42.68
4	48.4	46.9	**41.9**	38.5	58.4
6	64.2	61.8	**56.36**	56.6	66.46
8	82.6	79.6	**74.7**	73.8	72.8
10	94.6	91.3	**89.4**	81.5	78.4
12			**97.4**	90.1	84.8

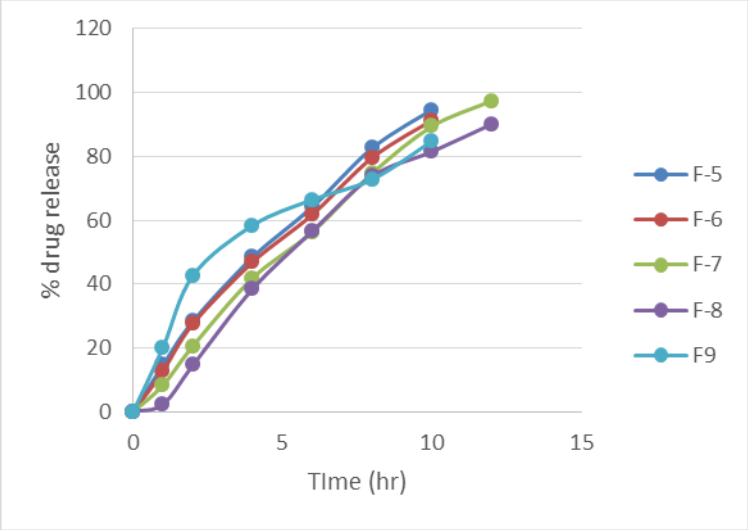


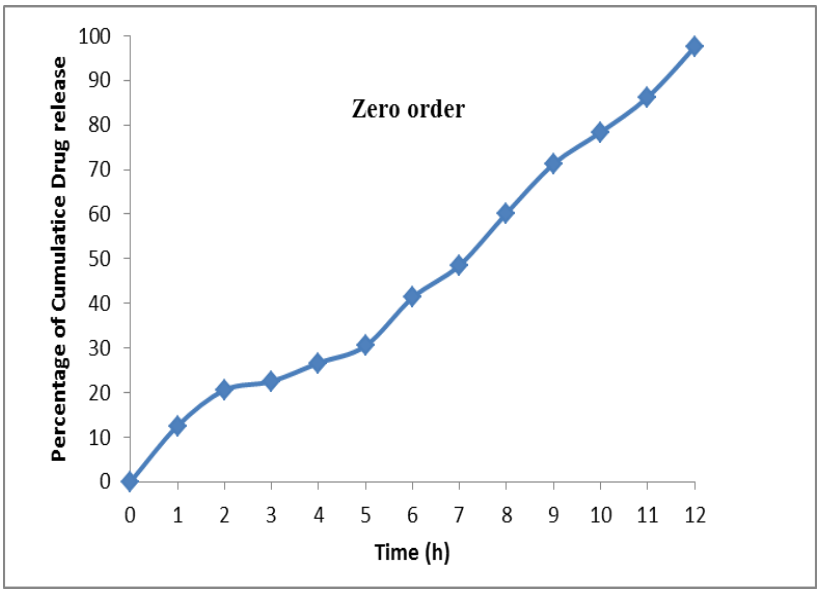
Fig 7 Optimized formula Graphs (F5-F9)

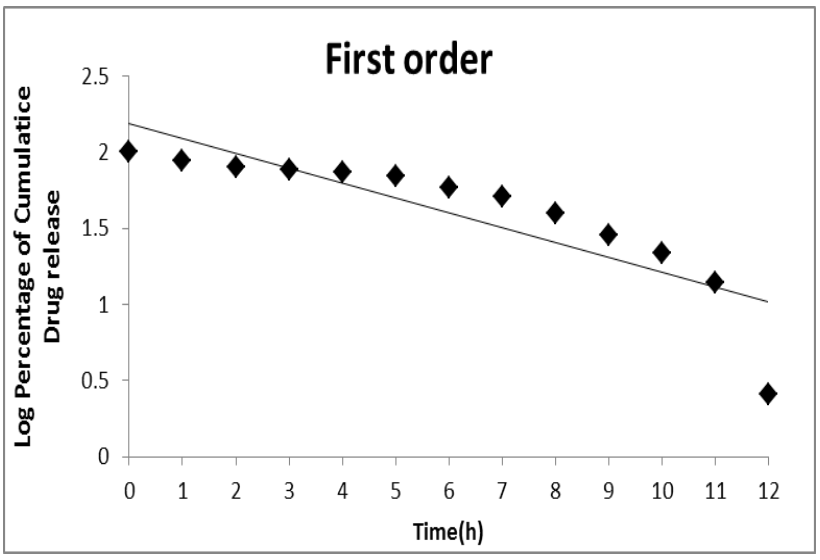
Kinetic modeling

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 (r^2) was determined. It was found that the *in vitro* drug release of microparticles was best explained by First order kinetics as the plots shows highest linearity. The correlation coefficient (r^2) was 0.9877 for f8 formulation as shown in Table. For formulation correlation coefficient (r^2) is found to be 0.9566, indicating that the drug release was nearly dependent of concentration, followed by Higuchi's (r^2 = 0.9161).

Table 6 - kinetic modeling of Microbeads

Code	Entrapment Efficiency	Zero	Higuchi	First
	%	R^2		
F-1	69.11	0.9859	0.8269	0.9811
F-2	71.55	0.9865	0.8568	0.9919
F-3	72.67	0.9844	0.9045	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	**96.64**	**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





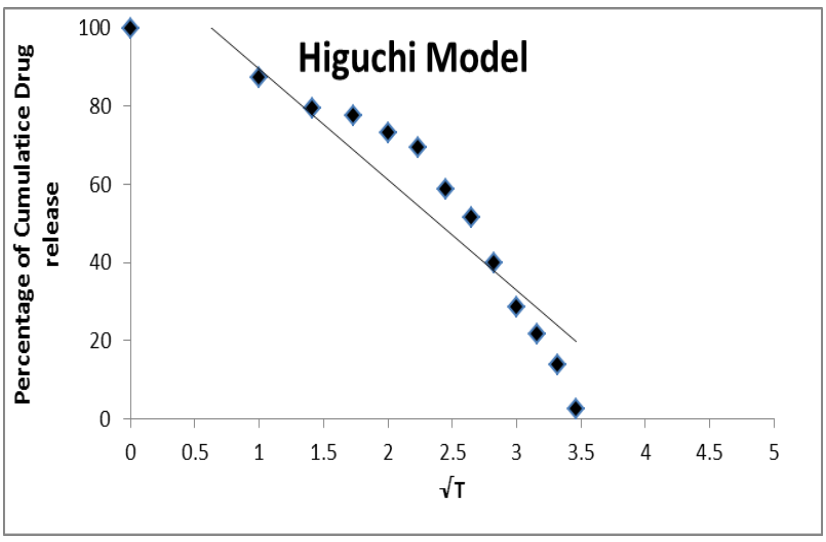


Fig 8- Kinetic modeling of drug

III. 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 7 - Short term stability study data of optimized formulation

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 Acyclovir -loaded CHITOSAN micro particles for a period of 3 months. The microparticles 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 Acyclovir-loaded micro particles.

DISCUSSION

From past few years microparticles 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 microparticles encapsulated agents through biological membrane and the stability of them.

The present formulation study on Acyclovir is an attempt to prepare microparticles drug delivery system and evaluate its performance. The formulations were prepared, varying the ratios of polymer and sodium alginate, by ionic Gelation method. An ideal or best formulation of microparticles 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.

The mean particle size of all formulations were increased when the drug to polymer concentration is decreased, mean particle size was decreased. Addition of Calcium chloride to the Sodium alginate polymer, it was increases the particle size. Sodium bicarbonate

formulations showed small particle size than the sodium sulfate formulations. This increased mean particle size could be due to the fact as polymer concentration increases. Scanning Electron Microscopy images showed that the microparticles were rounded oval but not spherical in shape.

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 Acyclovir release from CHITOSAN microparticles. As can be seen from the figures, an initial burst effect was observed from all CHITOSAN microparticles. 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.

CONCLUSIONS

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 **F7** which showed higher entrapment efficiency 98.6 provides desired drug release rate.

Entrapment efficiency % was decreased with increasing the Chitosan concentration by increasing the viscosity leads to low drug entrapment capacity. More Entrapment efficiency % was with the addition of Calcium chloride of Acyclovir- loaded Chitosan microparticles.

Based on the above results we can conclude that formulation **F7** was the best optimized formulation of all remaining formulations that it has greater entrapment efficiency so it can give maximum drug release ultimately it will give maximum drug response.

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REFERENCES

1. Vyas S.P., Khar R.K., Targeted & Controlled Drug Delivery: as Novel Carrier System, CBS Publishers & Distributors, New Delhi, 1st Edition 2002, Reprint 2011, 417.

2. Muller.C.C., et. al., Physicochemical characterization of colloidal drug delivery systems such as reverse micelles, vesicles, liquid crystals and nanoparticles for topical administration, European Journal of Pharmaceutics and Biopharmaceutics. 2004; 58(2): 343-356.
3. Tripathi KD, Essentials of Medical Pharmacology, Jaypee Brothers Medical Publishers ltd, 6th Edition, 2008; 768.
4. Alagusundaram.M et. al., Microspheres As A Novel Drug Delivery System- A Review, IJCRGG, ISSN, 0974-4290, Vol.1, pp 526-534, 2009.
5. Angeles Heras, Niuris Acos, Evaluation of Brain targeting for Nasal delivery of Midazolam. International journal of Pharmaceutical Sciences. 2012; (12): 55-75.
6. Mishra B., Rajnikanth P. S, Preparation and Evaluation of Ornidazole insitu Gelling system for Gastro retentive drug delivery system. International journal of Pharmacy. 2013; 3(2): 62-69.
7. Frederic Lagarce, Baclofen-loaded Micro spheres preparation and efficiency testing in a new rabbit method. Europian journal of Pharmaceutics and Bio-Pharmaceutics. 2005; 59: 449-459.
8. Ida Gentaa, Influence of glutarldelyde on drug release and muco-adhesive properties of Chitosan Micro spheres & Carbohydrade polymer. 1998; 36: 81-88.
9. J. Kreuter, Micro capsule Nano capsules in Neveripane drug delivery system. Journal of Pharma, 1996; 91: 192-199.
10. Chung, Marphology, Drug distribution an invitro release profiles bio degradable polymeric Micro spheres. Carbohydrade polymers, 2011; 2: 231-241.
11. S. Freiberg, Marphological Characterization of Polymeric Micro spheres. Carbohydrade polymers. 2004; 8: 10-15.