FORMULATION AND CHARACTERIZATION OF EYE DROPS CONTAINING MICROSPHERE OF ACYCLOVIR AGAINST *HERPES SIMPLEX* INDUCED KERATITIS

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ABSTRACT

Novel Drug Delivery System promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with eye, that problems generally encountered in semisolid dosage forms. During the Preformulation studies it is found that the organoleptic properties of acyclovir comply as reported. White crystalline, tasteless, odorless, substance was sparingly soluble in water but dispersible. Five different formulations were prepared all the sodium alginate solutions were prepared by dissolving the respective amounts in distilled water. Dried microspheres were subjected for Evaluation were found yield between 83.68 to 92.02%, mean particle size between 583.34 to 816.21µm and % encapsulation between 78. 15 to 93.36% and swelling Index between 3.85 to 4.58 and shape was spherical with slightly elongated tips found in some microspheres. All microsphere formulations give more than 79 % drug release in steady way. All prepared microsphere formulations were tested In-vitro Drug Release and % drug release found as P-1(82.72), P-2(81.82), P-3(83.51), P-4(95.82) and P-5(89.15). On the basis of % drug release regression coefficient of P-4 was found greater (R^2 = 0.966), hence, P-4 is the best formulation having maximum % of drug release (95.82%) also selected for further studies. Formulated microsphere preparations were incorporated in eye drop suspension comprises of crystalline methyl cellulose as thickening agent, Boric acid as Buffering agent, Sodium chloride as Tonicity modifier, Sodium metabisulphate as Antioxidant, Benzalkonium chloride as Preservative and Sterile water as Vehicle. That eye drop suspensions were evaluated and found all formulations were clear, odorless, homogeneous and free from grittiness and also controlled pH between 7.1-7.8. Only batch F-5 was found irritant because of higher concentration of surfactant. In-vitro drug release data of Optimized formulation F-4 was subjected for kinetic modeling. Best fitted models are zero order, first order and Korsmeyer –Peppas model with R^2 of 0.998.

Keywords: *Novel Drug Delivery System, microsphere, Acyclovir, Herpes Simplex, Keratitis*

1. INTRODUCTION

Microspheres have been accepted as the formulation of controlled release and drug targeting[1]. Microspheres has been a topic of interest in the design of drug delivery system to prolong the residence time of the dosage form at the site of application or absorption and to facilitate absorption intimate contact of the dosage form with underlying surface to improve the bioavailability of drugs[2]. Several studies has been reported drug delivery system in the form of tablets, films, patches, and gels for oral, buccal, nasal, ocular, and topical routes[3]. The drug acyclovir has been

selected as suitable candidate for site specific delivery in eye by encapsulating in microsphere because it has narrow absorption window, its oral bioavailability is poor (10-20%), its $t_{1/2}$ is also less 2.5-3.3 hr[4]. Due to these reasons dose of conventional Acyclovir eye drops or solution 200mg/ml (5-8 times a day), it is a typical task to administered 5-8 times in a day, so there is need to develop to a suitable dosage form those increase the bioavailability of drug in body and reduce the dosing frequency[5].

When the microcapsules of Acyclovir administered adhere on eye surface, retain and release the drug continuously for prolong period of time in controlled release manner[6,7]. It can maintain the adequate concentration of drug on the site of action penetrates inner layer of eye and ultimately increase the bioavailability of drug and gave batter therapeutic action as compared to other conventional dosage forms[8,9].

There is a need to develop suitable formulation for commercial exploitation. Thus, the specific objective listed in the plan were achieved namely design, characterization and release studies of acyclovir Microsphere formulations.

2. MATERIALS AND METHODS

Acyclovir was obtained as gift from Mylan laboratories limited, Nashik. Chitosan, Sodium alginate and calcium chloride was used available in college lab and CMC purchased from Sigma Aldrich, Mumbai. All other reagents used in this experiment are belongs to laboratory grade.

2.1 Preformulation study: To perform preformulation study drug sample was examined for its color, odor and appearance, Melting point, solubility and Partition Coefficient of Acyclovir were determined using standard methods. Determination of λ_{max} of Acyclovir in phosphate buffer (pH 7.4) was done in range of 200nm-400nm. The solutions of drug concentrations of range 4 to 20µg/ml were analyzed using UV spectrophotometer and a calibration curve was plotted between absorbance and concentration. FT-IR Spectroscopy was used to investigate and predict any physicochemical interactions between different components, in a formulation and therefore it can be applied to selection of suitable chemically compatible excipients.

2.2 Preparation of tear fluid: Simulated tear fluid (STF) was prepared by dissolving sodium chloride (0.67g), sodium bicarbonate (0.20g), calcium chloride dihydrate (0.008g) in distilled water q. s. 100 ml. prepared solution was stored in volumetric flask.

3. EXPERIMENTAL WORK

3.1 Formulation of Microsphere of Acyclovir: The microspheres of acyclovir were prepared according to the table. Briefly, all the sodium alginate solutions were prepared by dissolving the respective amounts in distilled water. To the sodium alginate solutions, 1% w/v of acyclovir was added under homogenization for 5 min to yield smooth dispersions of acyclovir in sodium alginate solutions. The chitosan solution (1% w/v) was prepared in 5% v/v aqueous acetic acid. To the chitosan solutions, respective amount of calcium chloride was added. The acyclovir–alginate dispersions were taken into syringe fitted with 0.45 mm needle and dropped at the rate of 1ml/min into chitosan – calcium chloride solutions stirred at 100 RPM at room temperature to yield opalescent beads. The microspheres were allowed to harden for another 2 h, filtered, washed thrice with distilled water and kept for drying at 40 ºC in an oven for 24 h. After 24 h, the size of the beads reduced and they were kept in labeled self-sealing plastic bags.

Table 1: Composition of Microapheres of Acyclovir

Code	Acyclovir (%)	Sodium alginate conc. (% w/v)	Calcium chloride conc. (% w/v)	Chitosan conc. (% w/v)
P-1	1	1	5	1
P-2	1	2	5	1
P-3	1	3	5	1
P-4	1	4	5	1
P-5	1	5	5	1

3.2 Methods of Characterization of the Acyclovir Microspheres

Calculation of % yield calculation: During this process, % yield was calculated as follows:

$$\% \textbf{ Yield } = \frac{\textbf{Practical yield}}{\textbf{Theoretical yield}} \textbf{ X 100}$$

Swelling index: 50 mg of microspheres were placed in water and set aside to swell over night. Microspheres were decanted using filter paper and weighed. The degree of swelling (α) was then calculated from the formula:

$$\textbf{Swelling Index } (\alpha) = \frac{\{\textbf{weight of swelled microspher } (\textbf{Wg}) - \textbf{ Initial weight ofmicrospherees } (\textbf{Wo})\}}{\textbf{Initial weight of microspheres } (\textbf{Wo})}$$

Particle size determination by optical microscopy: All the five batches prepared (P-1 to P-5) were analyzed for particle size by optical microscope. First the eye piece micrometer was calibrated using a stage micrometer and then on a clean glass slide a small quantity of microspheres were placed using a thin brush. Then they were covered carefully with a cover- slip and observed under 10X magnification. One hundred particles from each batch were counted and average particle diameter was found out by using the formula:

$$\textbf{Average particle diameter} = \sum \mathbf{n*d/ N}$$

Where,

n = total no. of particles in that size range
d = Diameter of the particles of that size range
N = total no. of particles.

Determination of %drug content & encapsulation efficiency: 20 mg of the microspheres from each batch were taken and digested in 100 ml of 0.1N HCl in a 100 ml volumetric flask and kept aside with intermittent shaking for 24 h. Then, the contents of the flask were filtered by using Whatman filter paper no.1. Then 1 ml of the filtrate was diluted with 50ml of dimethyl sulfoxide (DMSO) in a volumetric flask and sonicated for 10 min to extract ACV. This was again filtered by using Whatman filter paper no.1; one ml from this was further diluted with methanol up to 10 ml and absorbance measured at 252.0 nm using methanol as blank. After recording the absorbance the drug content and encapsulation efficiency were calculated. The readings were taken thrice and the average reading was taken for further calculation.

Amount of drug = Abs−intercept/ slope (∗10∗100) /1000

%Drug content = Calculated amount of drug total /amount of microspheres X 100

%Encapsulation efficiency = Calculated drug content /theoretical drug content X 100

***In-vitro* drug release studies:** The in vitro dissolution studies were carried using USP- 34 paddle type dissolution apparatus. 50mg ACV loaded microspheres were placed in a dialysis bag and introduced into100ml dissolution medium of buffer solution pH 7.4 maintained at 37± 0.5 ºC at a rotation speed of 50 RPM. 1 ml of aliquots was withdrawn at predetermined time intervals and an equivalent volume of fresh medium was replaced to maintain sink condition. The aliquots were diluted and analyzed spectrophotometrically at 252.0 nm to determine the concentration of drug present. The readings were taken thrice and the average reading was taken for further calculation.

Accelerated stability studies: Above prepared samples were kept in sealed vials for 7 days at 40 ^{o}C and 75% RH.

3.3 Method of formulation of microsphere eye drop suspension of Acyclovir

Accurately weighed amount of CMC (thickening agent) was dissolved in sterile water were mixed together using a magnetic stirrer, at 25 rpm. Added prepared acyclovir microspheres equivalent to 1% of acyclovir. Then banzalkonium chloride as preservative, sodium chloride as tonicity enhancer and boric acid as viscolyser were added until a homogenous suspension was prepared. After preparation of suspension, the pH was adjusted and the formulated suspension was taken for further analysis.

Sterilization: After the preparation of eye drop suspension of acyclovir was sterilized by vacuum filtration using 0.45µm membrane filter then UV light.

3.4 Method of Evaluation of Microsphere eye drop solution of Acyclovir

The pH of accurately 2.5 ml of suspension formulation of each batches were measured by using digital pH meter. Viscosity of the eye drop suspension was determined using Brook field Viscometer, Spindle No. 2 (Brookfield Engineering Labs., USA). Clarity test was done against dark and white background board apparatus, for the presence of foreign particles.

3.5 Stability studies

Stability of drug is a great importance factor that must be considered for its efficacy. Stability studies were done according to ICH guidelines for drug and formulation stability. Optimized formulation was kept in the stability chamber at specified temperature and humidity (40°± 5°C and 75 % RH), ambient condition and (4°± 2°C and 15 % RH) for one month. The chemical stability was assessed by the estimation of % drug remaining in the formulation, pH and physical stability was evaluated by monitoring any change in pH, viscosity and appearance.

3.6 Data Analysis and Statistics

Data are expressed as mean ± SD. Statistical analysis was performed by Student's test using MS Excel Significance was defined at p values <0.05.

4. RESULT AND DISCUSSION

4.1 Preformulation Study: During the Preformulation studies it is found that the organoleptic properties of acyclovir comply as reported. White crystalline, tasteless, odorless, substance was sparingly soluble in water but dispersible in these solution, soluble in 0.1N HCl, 0.1 N NaOH, Phosphate buffer (pH 7.4) and freely soluble in ethanol and methanol. Acyclovir was observed melting point at 256-258^{0}C. λ_{max} was determined in Phosphate buffer (pH 7.4) solvent at 252.0 nm. Standard calibration curve was prepared using concentration range 05-25 µg/ml and linearity equation as y = 0.052x+0.003 with R^2 = 0.996. Drug Acyclovir was also compatible with used excipients. It is physically stable and chemically stable as observed by FT-IR spectra.

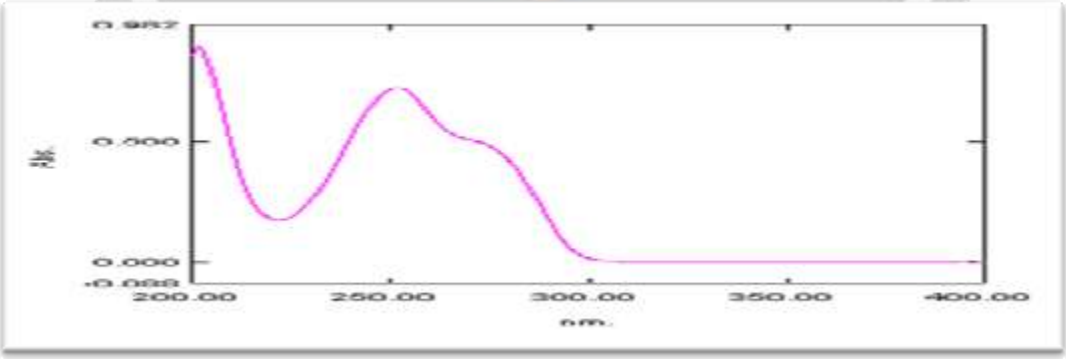

Figure 1: UV spectrum of Acyclovir in phosphate buffer of pH 7.4

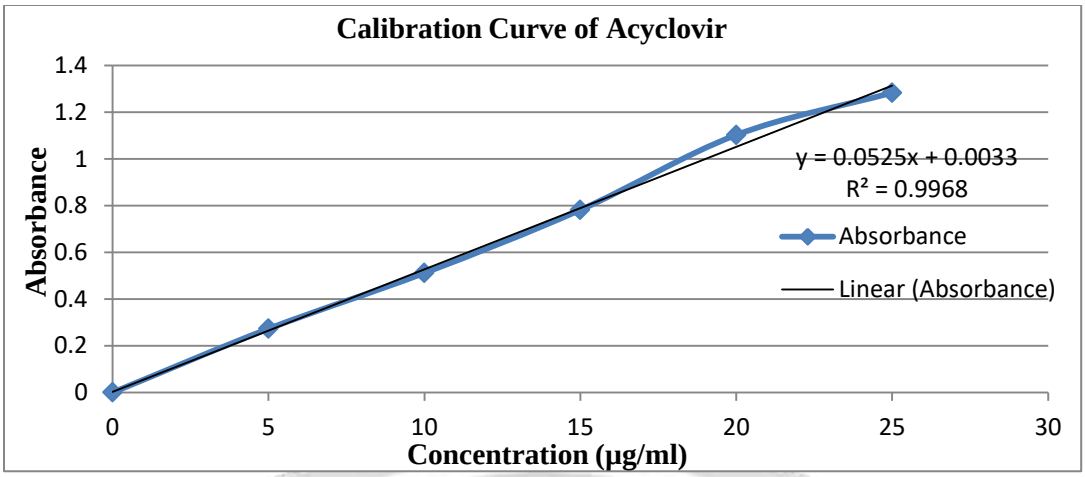

Figure 2: Calibration curve of Acyclovir in phosphate buffer (pH 7.4)

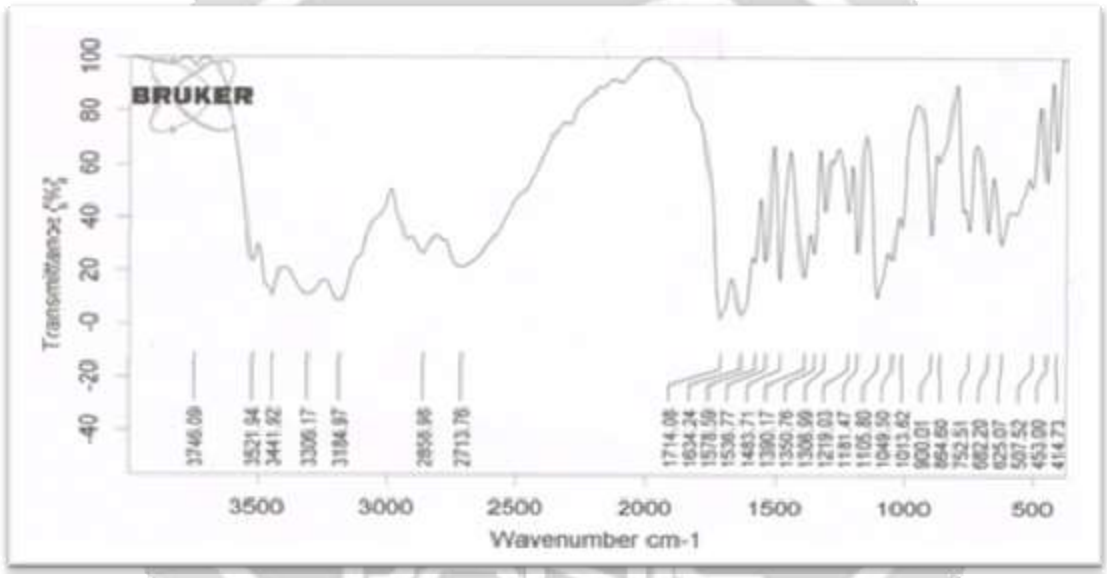

Figure 3: IR spectra of Acyclovir

Table 2: Interpretation of IR

Standard Peaks(Cm^{-1})	Peak Assigned	Observed Peaks (Cm^{-1}) Acyclovir	Observed Peaks (Cm^{-1}) Formulation
3700-3000	N-H str.	3521	3446
3400-3000	O-H	3306	3311
3040-3000	C-H str. methyl group	2858	2974, 2876
1900- 1600	C=N str.	1714	1712
1650-1600	C=O str.	1634	1631
1500-1300	C – H band methylene group	1483	1481

Formulation of Micrspheres of Acyclovir

The Acyclovir microspheres were formed due to the ionotropic gelation of the monovalent sodium alginate, an anionic polymer by the divalent Ca++ ions emerging from calcium chloride. The gelling was further enhanced due to the formation of an interpolymer polyelectrolyte complex between the anionic alginate and cationic chitosan polymer. Thus the chitosan alginate polyelectrolyte complex retards the diffusion of the drug.

Table 3: Composition of ingredients Micrspheres of Acyclovir

Code	Acyclovir (%)	Sodium alginate conc. (% w/v)	Calcium chloride conc. (% w/v)	Chitosan conc. (% w/v)
P-1	1	1	5	1
P-2	1	2	5	1
P-3	1	3	5	1
P-4	1	4	5	1
P-5	1	5	5	1

Characterization of the Acyclovir Microspheres

Surface morphology by SEM Analysis of P-4: The shape was spherical with slightly elongated tips found in some and microspheres, smooth surface observed.

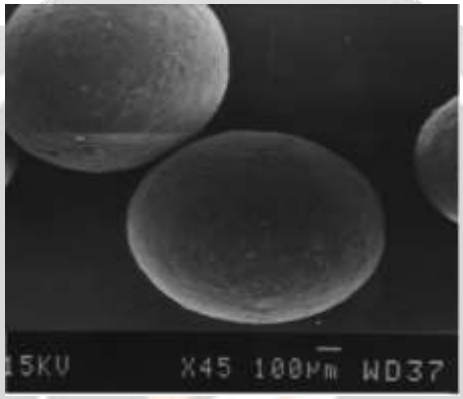

Figure 4: Surface morphology of optimized formulation

Characterization of Acyclovir Microspheres

Table 4: Results of characterization of Acyclovir Microspheres

Formulation	% Yield	Microspheres Size (µm)	(%)Encapsulation	Swelling Index
P-1	88.21	816.21 ± 1.931	78.15 ± 1.21	4.12 (burst)
P-2	83.68	812.46 ± 1.152	82.26 ± 1.45	4.01(burst)
P-3	89.50	713.16 ± 1.621	83.73 ± 2.88	3.85
P-4	91.12	623.83 ± 0.638	93.36 ± 1.73	4.58
P-5	92.02	583.34 ± 0.932	81.24 ± 0.46	3.98

Statistically significant difference among the values ($P < 0.0001$)

***In-vitro* Drug Release Studies**

Table 5: *in-vitro* cumulative % drug release from Microspheres

Time (hrs)	P-1	P-2	P-3	P-4	P-5
0	0	0	0	0	0
1	06.36	06.61	06.95	07.24	06.35
2	12.52	10.42	11.82	15.23	14.49
3	17.93	15.24	17.27	20.91	22.63
4	24.21	19.29	23.81	26.11	29.44
6	36.72	27.83	31.89	39.18	37.46
8	45.13	39.91	42.72	43.75	44.75
10	55.72	48.93	50.29	56.19	51.57
12	64.12	57.93	58.23	65.12	58.15
16	69.93	64.10	67.91	76.63	65.57
20	76.26	71.71	71.73	89.23	73.34
24	82.72	81.82	83.51	95.82	89.15

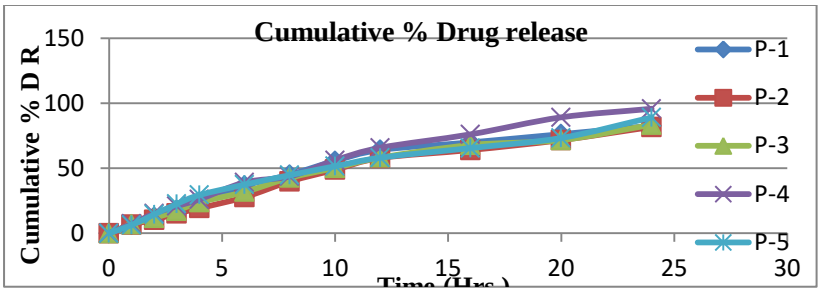

Figure 5: Cumulative % drug release of prepared Microspheres

On the basis of highest R^2 value, P-4 is the best formulation having maximum % of drug release (95.82%) also selected for further studies.

Formulation of eye drop suspension incorporated Microspheres batches

Table 6: Formulation of eye drop solution

S. No.	Name of ingredients	Role	Quantities
1	Crystalline methyl cellulose (CMC)	Thickening agent	0.1 %
2	Boric acid	Buffering agent	0.1 %
3	Sodium chloride	Tonicity modifier	5 %
4	Sodium metabisulphate	Antioxidant	0.1 %
5	Benzalkonium chloride	Preservative	0.01 %
6	Sterile water	Vehicle	q. s.
7	Microspheres equivalent to acyclovir	Active Ingredient	3 %

Evaluation of Microspheres eye drop solution of Acyclovir

Table 7: Evaluation of Acyclovir Microspheres eye drop solution

Formulation code	pH	Clarity	Irritation Test	Viscosity(cps)
F-1	7.3	Clear	No irritation	5.3 ± 1.8
F-2	7.1	Clear	No irritation	5.2 ± 2.0
F-3	7.7	Clear	No irritation	5.5 ± 1.2
F-4	7.4	Clear	No irritation	5.9 ± 0.8
F-5	7.8	Clear	Irritating	5.2 ± 1.2

Stability Studies

All formulations were kept in the stability chamber at specified temperature and humidity for one month. The chemical stability was assessed by the estimation of % drug remaining in the formulation; pH and physical stability was evaluated by monitoring any change.

Table 8: Stability of acyclovir Microspheres eye drop solutions at different conditions

Conditions	Evaluation Parameter	F-1	F-2	F-3	F-4	F-5
4° ± 2°C and 15 % RH	% Drug remaining	90.23 %	91.20 %	88.13 %	97.81%	82.61 %
	pH	7.2	7.2	7.5	7.3	7.7
	Physical change	No	No	No	No	No
Ambient Temperature	% Drug remaining	91.32 %	99.13 %	88.61 %	98.53 %	91.83
	pH	7.1	7.3	7.6	7.2	7.3
	Physical change	No	No	No	No	No
40° ± 5°C and 75 % RH	% Drug remaining	91.21 %	98.99 %	86.32 %	93.53 %	90.37%
	pH	7.1	7.3	7.3	7.2	7.3
	Physical change	No	No	No	No	No

Kinetics modeling of optimized formulation (F-4)

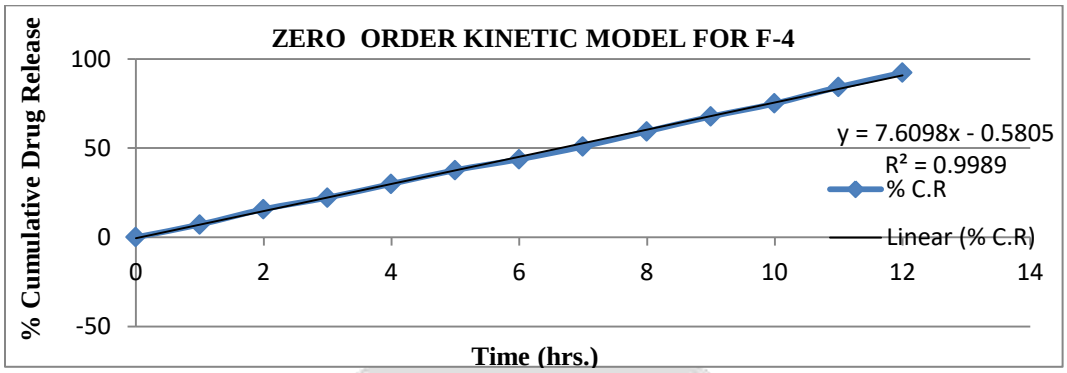

Figure 6: Zero Order kinetics for F-4

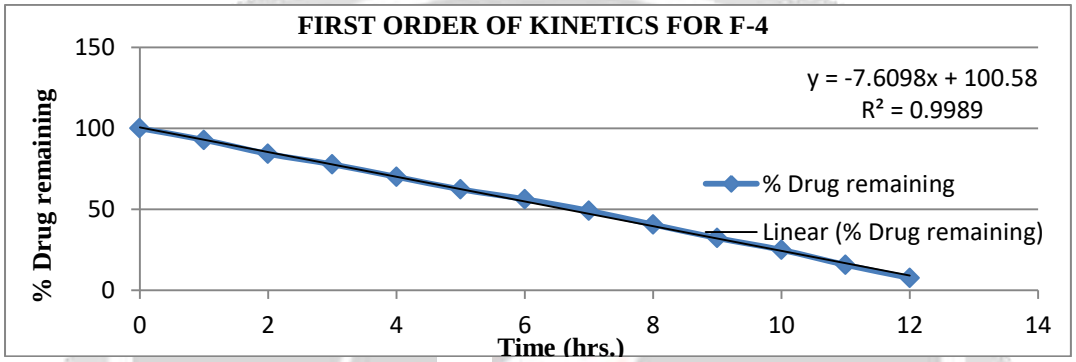

Figure 7: First Order kinetics for F-4

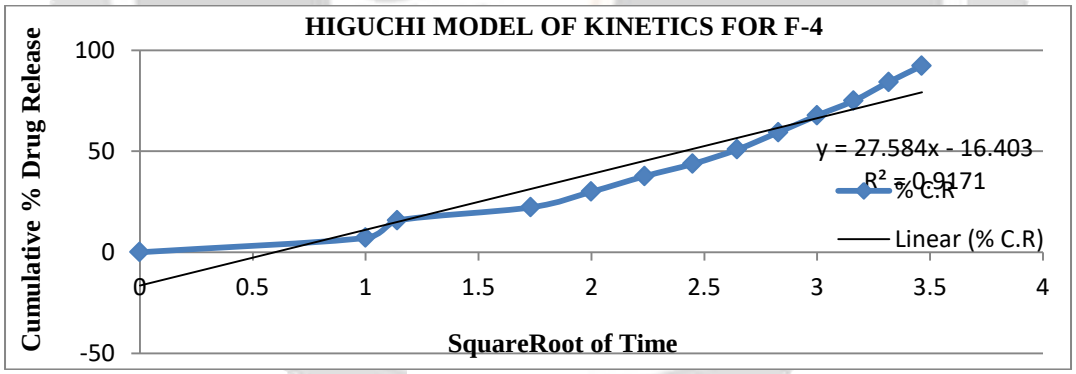

Figure 8: Higuchi model of kinetics for F-4

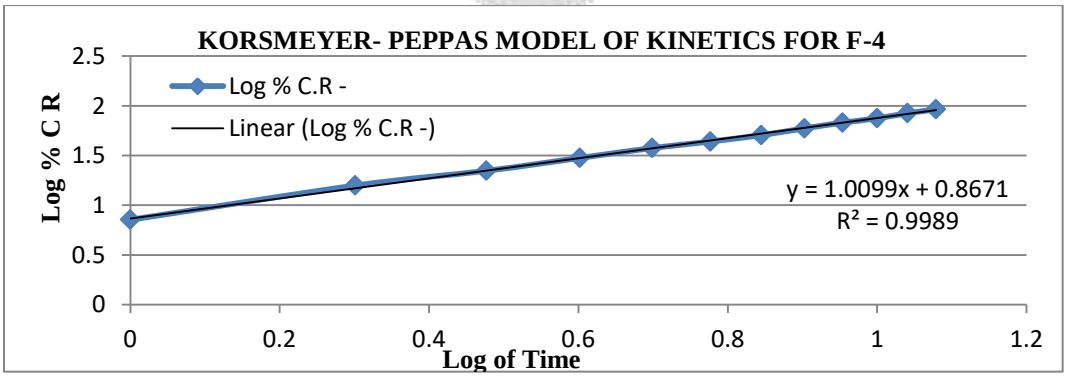

Figure 9: Korsmeyer- Peppas model of kinetics for F-4

DISCUSSION

This novel drug delivery system promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with eye, that problems generally encountered in semisolid dosage forms. During the Preformulation studies it is found that the organoleptic properties of acyclovir comply as reported. White crystalline, tasteless, odorless, substance was sparingly soluble in water but dispersible in these solution, soluble in 0.1N HCl, 0.1 N NaOH, Phosphate buffer (pH 7.4) and freely soluble in ethanol and methanol. Acyclovir was observed melting point at 256-258^{0}C. λ_{max} was determined in Phosphate buffer (pH 7.4) solvent at 252.0 nm. Standard calibration curve was prepared using concentration range 05-25 µg/ml and linearity equation as y = 0.052x+0.003 with R^2 = 0.996. Drug Acyclovir was also compatible with used excipients. It is physically stable and chemically stable as observed by FT-IR spectra.

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Drug stability concerns about drug product safety, efficacy, and quality, found it to appropriate. The percentage drug loss from the formulations was used as a measure of storage stability. The initial entrapped drug in vesicular system was considered as 100%. The leakage of drug from microsphere vesicles was not more significant at refrigerated condition. This fact can be justified by drug was remaining in microsphere after 1 months at different conditions. The drug leakage at elevated temperatures may be related to the degradation of lipid in the layers resulting in defects in membrane packing them leaky. So there is a requirement of improve stability of microsphere dispersion by incorporating them in eye drop suspension form. These properties help in breaking or bursting of vesicles after penetration to skin. It can be concluded that for better stability, the formulations should be stored at low temperature in refrigerator. *In-vitro* drug release data of Optimized formulation F-4 was subjected for kinetic modeling. Best fitted models are zero order, first order and Korsmeyer –Peppas model with R^2 of 0.998.

CONCLUSION

Microspheres of acyclovir were prepared using the sodium alginate solutions, 1% w/v of acyclovir; the chitosan solutions (1% w/v or 2% w/v) were prepared in 5% v/v aqueous acetic acid. To the chitosan solutions, respective amount of calcium chloride was added. The acyclovir–alginate dispersions were taken into syringe fitted with 0.45 mm needle and dropped at the rate of 1ml/min into chitosan-calcium chloride solutions stirred at 100 RPM at room temperature to yield opalescent beads. Because of acyclovir was sparingly soluble in water, so, the drug requires a novel drug delivery system which can provide enhanced solubility, an extended period of time and improve absorption via skin. Microspheres were characterized for compatibility study, particle size and shape, % entrapment, *in-vitro* drug release. Due to their matrix character, these drug delivery systems showed good enhanced solubility

and sustained release, required for bioavailability and therapeutic activity. From characterization parameters and stability study, it was concluded that the formulation has acceptable morphology and particle size, no any chemical interaction and was stable at refrigerated condition respectively.

An extensive investigation is needed with reference to depth of penetration into the eye, determination of zeta potential and confirmation of configuration of phospholipids in lipid layer. There is a need to develop suitable formulation for commercial exploitation. Thus, the specific objective listed in the plan of work of this thesis were achieved namely design, characterization and release studies of acyclovir Microsphere formulations.

CONFLICTS OF INTERESTS

There are no conflicts of interests.

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