Advance Research in Pharmaceuticals and Biologicals
(A Peer Reviewed International Journal for Pharmaceutical and Allied Research)
ISSN 2250-0774 USA CODEN: ARPBGZ

BIOSTATISTICAL OPTIMIZATION OF SUSTAINED RELEASE TABLET OF ACYCLOVIR USING LINEAR REGRESSION MODEL AND DETERMINATION OF ITS RELEASE MECHANISM

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Received on 09/12/2013 **Revised on 19/12/2013** **Accepted on 23/12/2013**

ABSTRACT:
Acyclovir is an antiviral agent which is active in vitro against Herpes simplex (HSV) types I and II and Varicella zoster virus (VZV). Fluctuating biological short half life, low bioavailability, distorted therapeutic drug regimen are some of the characteristics that bestow upon acyclovir to be an ideal Sustained release candidate. The present research paper focuses on designing sustained release tablets of acyclovir to ensure time-dependent, sustained release formulation with optimizing the process variables and perform pre-formulation studies. The results obtain show the linear regression analysis of all the fabricated tablets shown as R2 values . When the data were plotted according to the first-order equation, for all formulations (ACL1 to ACL8) showed a fair linearity, with regression (R^2) values between (0.685 - 0.915) clearly indicate that drug was not release as per first order mechanism. These values suggested that more than one mechanism may be involved in release kinetic. In the case of formulation ACL3 with Xanthan gum and sodium alginate shows non-fickian diffusion mechanism with n value as (0.925) therefore diffusion with erosion mechanism play role release from natural gum. So ACL3 was taken as a best formulation to achieve a prolonged maintenance of effective concentrations of drug for a period of 12hrs.

Keywords: : Biological short half life, Time-dependent, Sustained Release formulation, Non-Fickian diffusion

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INTRODUCTION

Acyclovir is an antiviral agent which is active in vitro against Herpes simplex (HSV) types I and II and Varicella zoster virus (VZV). However, the relationship between in vitro sensitivity of herpes viruses to Aciclovir and clinical response to therapy has yet to be established. Acyclovir needs to be phosphorylated to the active compound, aciclovir triphosphate, in order to become active against the virus. Such conversion is very limited in normal cells and in addition cellular DNA polymerase is not very sensitive to the active compound[1-3].

However, in infected cells HSV or VZV coded thymidine kinases facilitates the conversion of acyclovir to acyclovir monophosphate which is then converted to acyclovir triphosphate by cellular enzymes. Acyclovir triphosphate acts as an inhibitor of, and substrate for, the herpes specified DNA polymerase, preventing further viral DNA synthesis. Animal studies indicate that at high doses aciclovir is cytotoxic[4-5].

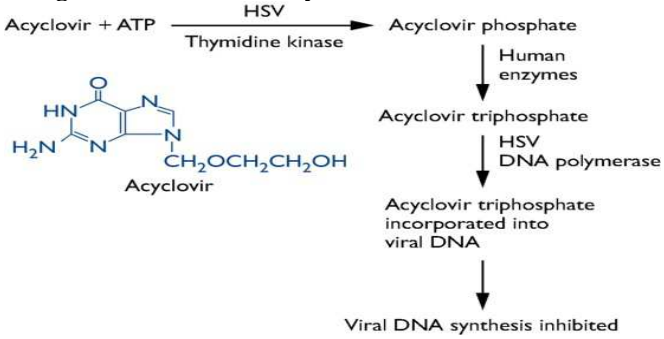

Fig. 1: Mechanism of Action of Acyclovir

Table 1: Dose administration of Acyclovir

Indications	Immune Status	Status
Herpes Simplex Virus (HSV)	Normal or ICP	5mg/kg every 8 hours
Very Severe Herpes Zoster infection (shingles)	Normal	5mg/kg every 8 hours
Varicella zoster infection	ICP	10mg/kg every 8 hours
Herpes simplex encephalitis	Normal or ICP	10mg/kg every 8 hours

Short-term Administration: The most frequent adverse events reported during clinical trials of treatment of genital herpes with acyclovir 200 mg administered orally 5 times daily every 4 hours for 10 days were nausea and/or vomiting in 8 of 298 patient treatments (2.7%). Nausea and/or vomiting occurred in 2 of 287 (0.7%) patients who received placebo.[6]

Long-term Administration: The most frequent adverse events reported in a clinical trial for the prevention of recurrences with continuous administration of 400 mg (two 200 mg capsules) 2 times daily for one year in 586 patients treated with acyclovir were nausea (4.8%) and diarrhea (2.4%). The 589 control patients receiving intermittent treatment of recurrences with acyclovir for one year reported diarrhea (2.7%), nausea (2.4%), and headache (2.2%).[7]

Acyclovir pharmacokinetic parameters: Ideal SR Candidate[8-13]

1. **Low oral Bioavailability** Sustained release SR tablets dosage forms are preferred for drugs with low oral bioavailability. As mentioned in the data discussed earlier, The preparation of a time dependent release pattern of the drug results in:
   - Uniform drug delivery at the site of action owing to lower fluctuations in the dissolution and bioavailability during the therapeutic regime of the drug.
   - The fact that time dependent release and drug-excipient ratios would be optimized, bioavailability determination becomes more accurate.
   - The Area under the curve and the pharmacokinetic data produced now would be in accordance to dose optimization and drug excipient ratios.

2. Plasma and Elimination Half life

The ideal half-life for preparing the sustained release dosage form should range from 3 to 8 hours. Unstable plasma half life depending upon absorption is 2.9 to 3.3 hrs makes it an ideal candidate for Sustained release tablets.

3. Dosing frequency

The oral doses of Acyclovir for adults range from 200 mg every 4 h (while awake) to 800 mg three times a day for 5–10 days. For chronic suppression of recurrent infections, the dose is 400 mg twice a day. The oral dose for treatment of chickenpox and herpes zoster is 800 mg acyclovir every 4 h for 5–10 days. The dosing is non patient compliant.

4. Toxic IV Doses

Although Intravenous route is preferred for the drug owing to its low bioavailability (15-30%), but owing to its toxicities there occurs a need of Sustained release or extended release dosage form to overcome hurdles of plasma profile fluctuations.

5. Pharmacokinetic Data

Mean steady state peak plasma concentrations (Cssmax) following a one hour infusion of 5mg/kg or 10mg/kg were 9.8 $\pm$ 2.6 SD and 20.7 $\pm$10.2 SD g/ml respectively. The trough plasma concentrations (Cssmin) were 0.7 $\pm$0.3 SD and 2.0 $\pm$0.1 SD g/ml respectively. In children over 1 year of age similar mean peak (Cssmax) and trough (Cssmin) levels were observed when a dose of 250mg/m2 was substituted for 5mg/kg and a dose of 500mg/m2 was substituted for 10mg/kg.

In children aged 0-3 months the terminal plasma half-life is approximately 4 hours. However, experience is insufficient at present to recommend therapy for this age group.

6. Oral administration and Patient Compliance

Hydrophilic polymer Sustained release is widely used for formulating an SR dosage form. The role of ideal drug delivery system is to provide proper amount of drug at regular time interval & at right site of action to maintain therapeutic range of drug in blood plasma.

MATERIALS

The research was carried out Nishka Labs in Hyderabad under stringent lab conditions using instruments like Friability Test Apparatus, DSC 200F3, Dissolution Test Apparatus (8 basket), UV Spectrophotometer (Shimadzu). Acyclovir (Pure drug) and excipient polymers (Xanthan gum, Micro crystalline cellulose Analytical grade, Sodium alginate, Talc and HPMC K15 grade) were provided by Nishka labs. Mannitol, Sodium chloride and Potassium Dihydrogen OrthoPhosphate were bought from SD Fine, Mumbai.

METHODS

The research methodology adopted in the study included Preformulation, formulation and evaluation of Sustained release matrix tablets using linear regression model. The present study tries to optimize the polymeric ratios and variables which are required not

only for drug dissolution but also determine the mechanism of drug release. The formulations are designed in arithmetic progressions of MCC and Xanthan Gum, whereas sodium alginate and HPMC are proportional to the dose. The effects of concentration on mechanism of drug release are studied and the best formulation is understood.

The first step before formulating a tablet is the preformulation. Preformulation is defined as the phase of research and development process where physics, chemical and mechanical properties of a new drug substance are characterized alone and when combined with excipients in order to develop stable, safe and effective dosage form. A thorough understanding of physicochemical properties may ultimately provide a rationale for formulation design or support the need for molecular modification or merely confirm that there are no significant barriers to the compound development. Hence, preformulation studies were performed on the obtained sample of drug for solubility analysis, identification and compatibility studies.

a) Solubility analysis

Preformulation solubility analysis was done, which include the selection of suitable solvent, to dissolve the respective drug as well as various excipients. The solubility was performed visually by dissolving in suitable solvents and water. The available literature on solubility profile of Acyclovir indicated that the drug is very soluble in methanol, DMSO, dioxane and ethanol and practically insoluble in water.

b) Melting point determination:

Melting point is the temperature at which the pure liquid and solid exist in equilibrium. In practice, it is taken as equilibrium mixture at an external pressure of 1 atmosphere; this is sometimes known as normal melting points. The Thiel's tube method of melting point determination in liquid paraffin was used in the present study.

Drug-Excipients Compatibility studies

Compatibility of drug (Acyclovir) and polymers which are used to prepare tablets was established by infrared absorption spectral analysis

a) FTIR Spectral analysis

IR spectral analysis of pure drug Acyclovir, and excepients was carried out and observation was made whether changes in chemical constitution of drug after combining it with the polymers occurred. The samples were crushed with KBr to get pellets by applying pressure of 600 Kg/cm^2. Infra Red spectroscopy is one of the most powerful analytical techniques to identify functional groups of a drug.

b) Differential scanning calorimetry (DSC) studies

Thermograms were obtained by using scanning colorimeter (Netzsch, 200F) ata heating rate 10°c/min. over a temperature range of 35-500°c. The sample washermetically sealed in an aluminum crucible. Nitrogen gas was purged at rate of 10 ml/min. for maintaining inert atmosphere.

Calibration curve for Acyclovir and determination of λ_{max} of Acyclovir

The calibration curve for Acyclovir was prepared by using PBS 6.8 pH. The λ_{max} of pure Acyclovir drug was determined using the UV Spectrophotometer and solvent as water.

Evaluation of Pre Compression Parameters of Acyclovir Sr Tablets

For a drug substance to formulate into a dosage form, it is necessary to study the physicochemical properties of the bulk drug.

Determination of bulk density and tapped density

An accurately weighed quantity of the powder (W), was carefully poured into the graduated cylinder and the volume (V_o) was measured, then the graduated cylinder was closed with lid, set into the density determination apparatus. The density apparatus was set for 500 taps and after that, the volume (V_f) was measured and continued operation till the two consecutive readings were equal. The bulk density, and tapped density were calculated using the following formulas:

Bulk density $= W / V_o$

Tapped density$= W / V_F$

Where, W = weight of the powder, V_0 = initial volume, V_F = final volume

Flow Properties

Angle of repose of different formulations was measured by fixed funnel standing method. Granules were weighed and passed through the funnel, which was kept at a certain height from horizontal surface. The passed microspheres formed a pile of height 'h' above the horizontal surface and the pile was measured.[6] The angle of repose was determined by

$\tan(\theta) = h / r$

Angle of repose $(\theta) = \tan^{-1}(h / r)$

Where h is the height of pile and r is radius

Compressibility Index and Hausners Ratio

Compressibility is indirectly related to the relative flow rate, cohesiveness and particle size of a powder. The compressibility of a material can be estimated from the tap and bulk density measurements. It is represented as percentage. In theory, the less compressible material is more flowable. Compressibility index were calculated using the formula

$$\text{Compressibilty}[\%] = \frac{\text{Tapped density} - \text{Bulk density}}{\textit{Tapped density}} \times 100$$

The objective of the proposed work was to design and develop sustained release tablets of the given drug and to ensure time-dependent, sustained release formulation with optimizing the process variables and perform pre-formulation studies. To evaluate the drug content, in vitro drug release kinetics of the drug as per the design of the experiment. Different drug solubility's is considered, as highly soluble drugs will dissolve immediately after administration. Reduced drug solubility increases the tendency of the tablet to erode due to particle displacement. The aim of the formulation is that:

1. The drug should have a short half-life. If a drug has a long half-life then there is a risk of accumulation as it will be eliminated at a slower rate compared to its absorption.
2. A drug that is tested in-vitro needs to be able to provide similar release characteristics once administered and is under pathophysiological or in-vivo conditions.
3. A direct correlation of in-vitro data with in-vivo release is not possible without thorough and careful analysis. For example, there is a difference in the availability of water in different parts of the gastrointestinal tract and such factors need to be considered when designing tablets for extended release.
4. The dissolution characteristics should allow for drug to be released in a controlled manner, highlighting the importance for the correct selection of polymers according to their physical, mechanical and pharmacokinetic properties.

Table 2: Formulation of Acyclovir Sustained release tablets

Formulation Code	Drug ACY	SA	XAN	HPMC K 15	MCC	Talc	MAN	Total
ACY1	400	20	20	15	20	10	QS	550
ACY2	400	20	40	15	21	10	QS	570
ACY3	400	20	80	15	22	10	QS	590
ACY4	400	20	120	15	23	10	QS	610
ACY5	600	30	30	20	24	10	QS	750
ACY6	600	30	60	20	25	10	QS	780
ACY7	600	30	90	20	26	10	QS	810
ACY8	600	30	120	20	27	10	QS	840

Evaluation of post compression parameters for Acyclovir Sustained Release Tablets

a) Thickness

Thickness of the Acyclovir was important for the uniformity of tablet. Thickness was measured using the Vernier callipers.

b) Hardness

Tablets require a certain amount of strength, or hardness and resistance tofriability, to withstand mechanical shocks of handling in manufacture, packaging and shipping. The hardness of the tablets was determined using Monsanto Hardness tester.

c) Friability

It is the phenomenon whereby tablet surfaces are damaged and/or show evidence of lamination or breakage when subjected to mechanical shock or attrition. The friability of tablet was determined by using Veego Friabilator as per IP procedure of friability. It is expressed in percentage (%).

d) Swelling Index

The diameter of tablets was taken at intervals of five minutes until maximum diameter was attained with a digital Vernier caliper. Thereafter the swelling indices (SI) were calculated from initial diameter of tablet (D1) and maximum diameter on swelling in water (D2) as expressed below:

$$SI\ (\%) = D2/D1 \times 100$$

e) In-Vitro Drug Release Studies

In-vitro dissolution studies of Acyclovir tablets were performed using USP type-II (Paddle) Type dissolution test apparatus. 600ml of buffer is used as a dissolution medium. The medium was maintained at 37±0.5ºC at a speed of 50rpm. The *in vitro* dissolution studies were performed at two different pH in 0.01N HCl for 2 hrs. An accurately weighed sample was responded in dissolution medium consisting 900ml of buffer and dissolution was done up to 12hrs. At prefixed time intervals (every 1 hour); 5ml of sample was withdrawn and filtered through 0.4 µm membrane filter. The volume of the dissolution medium was adjusted to 900ml at every sampling time by replace same 5ml of dissolution medium. Then the samples were analyzed Spectrophotometrically at 451nm.

RESULTS AND DISCUSSION

1. Preformulation Studies

a) Organoleptic Characteristics of Acyclovir

Table 3:

Properties	Result
Odour	Odorless
Colour	white
Form	Crystalline

b) **Melting point of Acyclovir:**
The melting point of drug was found to be 255.9˚c similar to reported.

c) **Solubility analysis:** Acyclovir was found to be soluble in methanol, ethanol and DMSO. The study was carried out to select suitable dissolution medium for *in-vitro* release studies.

ISSN 2250-0774

d) **Standard curve of Acyclovir in PBS pH6.8**

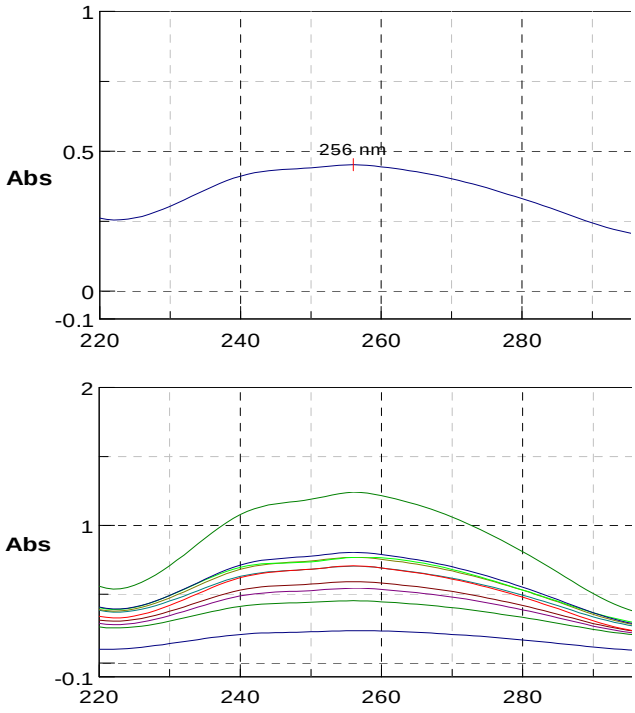

Fig. 2: λmax and overlay spectrum of acyclovir in pH 6.8 at256nm

Table 4 : Obesrvations for Standard graph of Acyclovir in pH 6.8 at 256nm

Concentration (µg/ml)	Absorbance (236nm) in pH 6.8
2	0.185
4	0.312
6	0.403
8	0.531
10	0.604
12	0.705
14	0.769
16	0.874
18	0.978
20	1.18

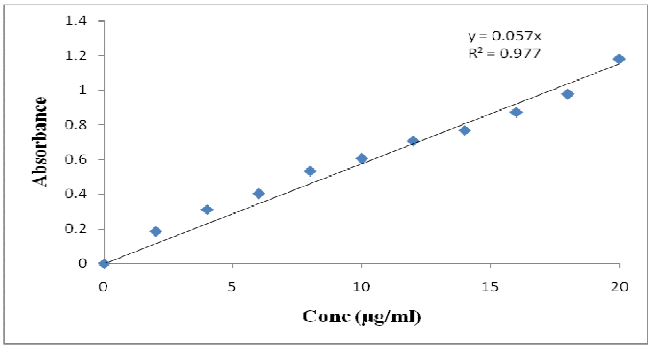

Fig. 3 : Calibration curve of Acyclovir in pH 6.8 at 256nm

e) **Drug-excipient compatibility study (FT-IR spectral analysis)**

The development of a successful formulation depends only on suitable selection of excipients. Hence the physical state of the drug Acyclovir and the excipients, Xanthan Gum, HPMC, Sodium Alginate, MCC, Talc and Mannitol individually and the combination of drug and polymers used for tablets preparation were studied by FTIR (Fourier transform infra red spectroscopy) to know the drug – polymer compatibility. The FTIR Spectrum of Pure acyclovir showed peaks at 3563.81 cm-1(O-H stretching), 1608.63cm-1 (O-H deformation), 3444.24 cm-1 (10 N-H stretching), 2927.94 cm-1 (aliphatic C-H stretching anti symmetric), 2856.06 cm-1 (aliphatic C-H stretching symmetric), 1482.99 cm-1 (aliphatic C-H deformation), 1714.41 cm-1 (C=O stretching) and 1143.8 cm-1 (C-O stretching). Therefore, there was no alteration and no interaction was observed between excipients and drug in combination. All the characteristic peaks of Acyclovir were present in combination, thus indicating compatibility between drug and excipients and finally confirm that there was no chemical modification of drug.

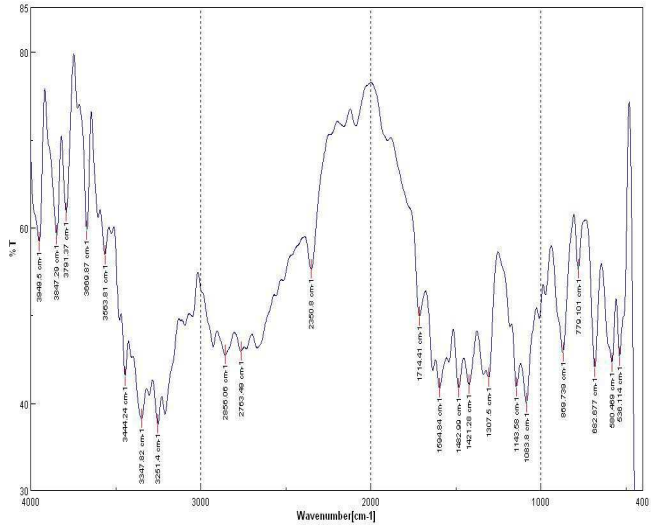

Fig. 4: FTIR Spectrum of Pure Acyclovir

f) **DSC studies**

DSC thermo grams of Acyclovir along with their combination of drug excipients have been shown. In case of Acyclovir two endothermic peaks were observed one at 197^{0}C, which corresponds to melting process and the other at 236^{0}C due to thermal decomposition. Combination of drug and excipients showed endothermic peak at 169.2^{0}C, it may be concluded that the drug has not shown any interaction with different excipients used in preparing the different formulations.

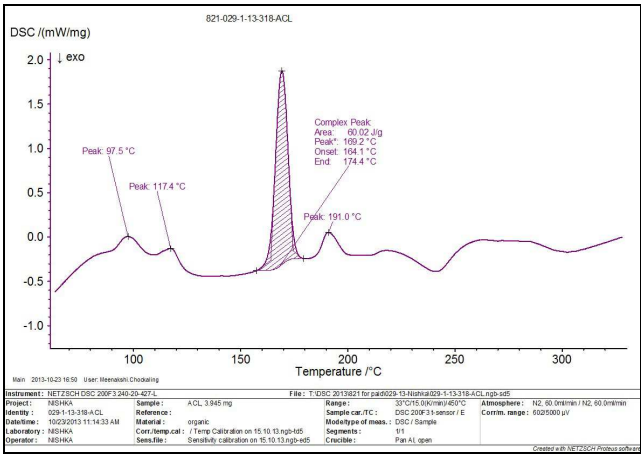

Fig.5 DSC of Drug + mixture

Pre Compression Parameters of Acyclovir Sustained release tablets

The bulk density, tapped density, Hausner's ratio, Compressability index and angle of repose for the blend was performed and reported in the Table. Bulk density and Tapped densities showed good packability of the granules. The ACL7 is having lowest hausner's ratio indicating good flow property. Angle of repose for Acyclovir blend ranges from 21.16±0.921 to 28.49±0.572 respectively. These represents that the blend flows freely through the hopper.(Table 5)

Table 5: Pre-compression parameters of Acyclovir Sustained release Tablets

S. No	F code	Bulk density ± S.D	Tapped density ± S.D	Hausner's ratio ±S.D	Carr's index± S.D	Angle of repose ± S.D
1	ACL1	4.57±0.015	5.097±0.058	1.116±0.01	10.393±0.784	24.83±0.865
2	ACL2	4.58±0.015	5.097±0.061	1.114±0.013	10.194±1.08	22.98±1.06
3	ACL3	4.56±0.006	5.653±0.011	1.24±0.002	19.28±0.144	22.813±0.949
4	ACL4	4.57±0.006	5.003±0.047	1.094±0.009	8.589±0.794	19.77±0.782
5	ACL5	4.58±0.011	4.99±0.066	1.088±0.011	8.079±1.0	24.37±0.754
6	ACL6	5.56±0.006	6.67±0.052	1.199±0.009	16.588±0.69	28.06±0.398
7	ACL7	5.58±0.01	6.06±0.053	1.086±0.011	7.915±0.93	23.617±0.647
8	ACL8	5.57±0.006	6.67±0.011	1.196±0.0008	16.4±0.058	26.49±0.65

Post compression parameters for Acyclovir Sustained Release Tablets

Thickness of the Acyclovir was important for the uniformity of tablet. Thickness was measured using the Vernier callipers. The thickness of Acyclovir tablet for all the formulations were in the range of 2.46±0.312 to 4.347±0.189. The hardness of all the tablets prepared by Direct Compression method for Acyclovir tablets was within the range of 6.17±0.289 kg/cm^2 to 7.33±0.289 kg/cm^2. Tablet hardness was increased as increasing the compression force. This ensures good handling characteristics of all batches. The friability of all the prepared formulations was within the I.P limit. The % of Friability for tablet ranges from 0.44±0.036 to 0.713±0.127 respectively. The results were tabulated.

The % Friability was NMT 1% in all formulations ensuring that the all the tablets were mechanically stable. The weight variation in tablet formulations was in the range of 800.49±1.3 to 1500.48±1.578 mg. All the prepared tablets passed the weight variation test. The weights of all the tablets were found to be uniform with low standard deviation values. (Table 6)

Table 6: Evaluation of Hardness and Thickness of Acyclovir Sustained Release Tablets

S. No	F Code	Hardness (kg/cm^2)	Thickness (mm)	Avg.wt. of Tablet ±S.D (mg)	Friability ±S.D (%)	Drug Content ±S.D (%)
1	ACL1	6.5±0.5	2.46±0.312	551.86±1.94	0.557±0.275	98.89±0.801
2	ACL2	6.5±0.866	2.69±0.015	571.18±1.47	0.713±0.127	98.78±0.322
3	ACL3	6.83±0.764	2.68±0.036	590.8±1.13	0.623±0.03	98.67±0.61
4	ACL4	6.67±0.289	2.69±0.061	610.49±1.3	0.577±0.042	98.81±0.501
5	ACL5	6.83±0.764	2.68±0.075	751.47±1.08	0.617±0.11	99.52±0.395
6	ACL6	7.17±0.764	3.19±0.045	782.03±1.305	0.577±0.042	99.33±0.45
7	ACL7	6.5±0.5	3.137±0.064	810.39±1.32	0.677±0.06	99.05±0.615
8	ACL8	7.67±0.289	3.167±0.021	841.5±0.947	0.57±0.06	98.43±0.255

In Vitro Dissolution and Drug release Kinetics

The initial release of drug from these matrices occurs by the drug dissolution in the water penetrated into the matrix. The overall drug release from these matrices is governed by hydration, gel layer formation and drug diffusion into the gel layer and to the dissolution media. Polymer erosion also plays a major role in releasing drug from these matrices. These considerations indicate that hydrophilic polymers have the potential to sustain the release of drug from matrix tablets. The release of drug is retarded as concentration of gum increases in all formulation. In order to investigate the effect of polymer type and percentage on drug release profile, different formulations containing various percentages of Xanthan gum and Sodium alginate was used. All these natural gum is hydrophilic cellulose ether, which is used as a retarding release of drug in controllable manners up to 12 hrs. The formulations ACL1 to ACL5 are containing 200mg of drug with a combination of different excipients. The drug release showed in ACL1 was 95.78%, for only 10hrs and ACL2 showed 90.66% within 11hrs because there was less presence of Xanthan Gum. The Acyclovir tablets of ACL3 showed 91.35% in 12hrs, ACL4 and ACL5 showed drug release of 95.33% for 8hrs and 95.85% for 9hrs. From these five formulations it was concluded that increase in concentration of Xanthan Gum there was a decrease in drug release. In further formulations the dose of Acyclovir was increased to 400mg ACL6 to ACL10 that are containing combination of excipients. The drug release for the formulations ACL6 showed a drug release of 97.44% for 10hrs and ACL7 showed a drug release 92.4% for 11hrs.

ISSN 2250-0774

Table 7: Regression and Slope Data of Release Kinetics of Acyclovir SR Tablets

Formulation code	Mathematical models (release kinetics)				
	Zero order kinetics	Firstorder kinetics	Higuchi's	Peppa's	
	r^2	r^2	r^2	r^2	n
ACL1	0.997	0.871	0.926	0.996	1.02
ACL2	0.998	0.915	0.928	0.997	0.939
ACL3	0.998	0.905	0.934	0.997	0.925
ACL4	0.995	0.797	0.877	0.999	1.12
ACL5	0.996	0.788	0.903	0.998	1.0
ACL6	0.996	0.847	0.932	0.996	1.02
ACL7	0.998	0.912	0.931	0.998	0.954
ACL8	0.997	0.88	0.94	0.995	0.916

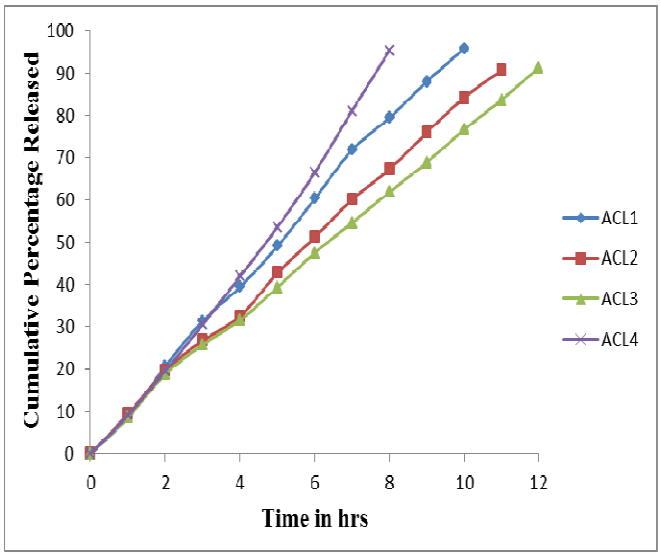

Fig. 6: Zero order Release Plot for ACL1 to ACL4 SR Tablets

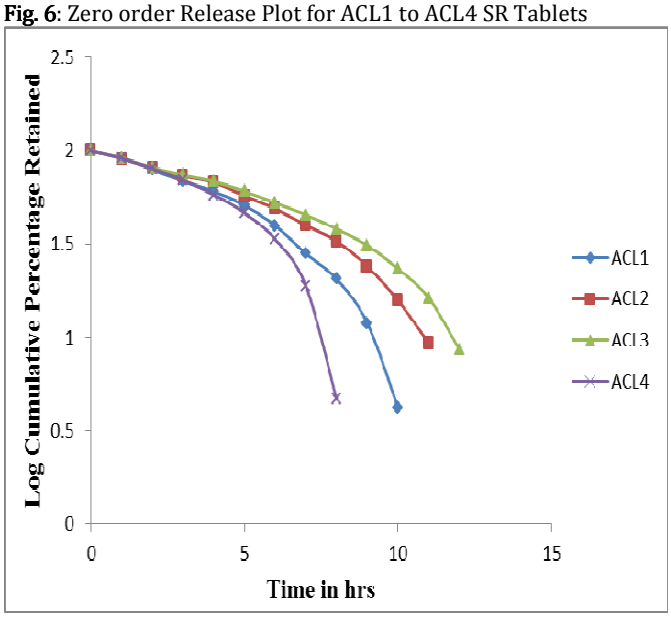

Fig. 7: First order Release Plot for ACL1 to ACL4 SR Tablets

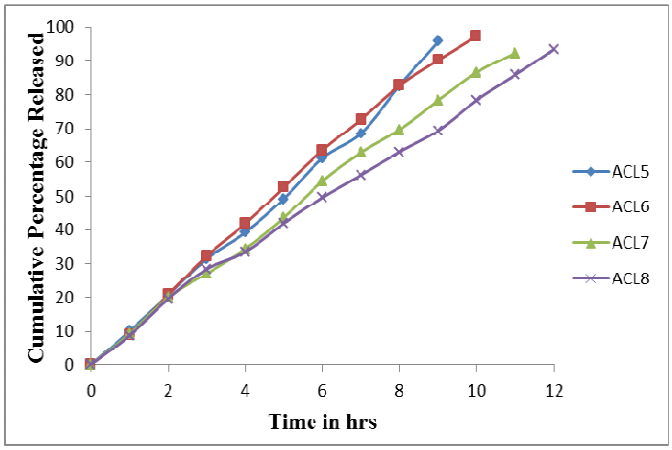

Fig.8: Zero order Release Plot for ACL5 to ACL8 SR Tablets

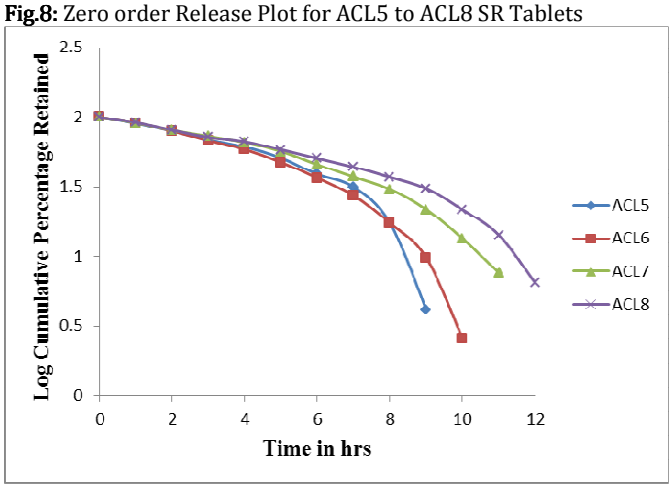

Fig.9: First order Release Plot for ACL5 to ACL8 SR Tablets

SUMMARY AND CONCLUSION

From the above results it was clearly concluded that the drug release was governed with the combination of these natural polymers. When more quantity of sodium alginate was used rather than Xanthan gum there was a less sustaining effect and faster drug release as compared to combination of Xanthan gum because when matrices containing swellable polymers are exposed to dissolution medium, tablet surface becomes wet and hydrated to form a gel layer. It was also concluded that increase in dose with decrease in concentration of polymers or keeping constant in polymers ratio also affected the release of drug.

So ACL3 was taken as a best formulation to achieve a prolonged maintenance of effective concentrations of drug for a period of 12hrs.

The linear regression analysis of all the fabricated tablets shown as R2 values. When the data were plotted according to the first-order equation, for all formulations (ACL1 to ACL8) showed a fair linearity, with regression (R^2) values between (0.685 to 0.915)

clearly indicate that drug was not release as per first order mechanism.

1. All the formulation expressed by Higuchi classical diffusion equation as the plot shows linearity with regression coefficient (R2) value as (0.88 to 0.948) also not close to infinity indicate drug release process is not as per Higuchis plot.
2. The zero-order plots of all formulations were found to be highly linear, and close to infinity as indicated by their high regression (R^2) values as (0.994 to 0.998).
3. Therefore it was ascertained that the drug permeation from these formulations could follow either near zero or zero order kinetics. Hence release mechanism was shifted from zero order to Higuchis followed by first order kinetics.
4. Further, to understand the drug release mechanism, the data were fitted to Peppas equation. In the present study also it was observed, that n value was obtained between (0.91 to 1.12) for all formulation.
5. These values suggesting that more than one mechanism may be involved in release kinetic. In the case of formulation ACL3 with Xanthan gum and sodium alginate shows non-fickian diffusion mechanism with n value as (0.925) therefore diffusion with erosion mechanism play role release from natural gum.

REFERENCE

1) P. Chetoni, S. Rossi, S. Burgalassi, D. Monti, S. Mariotti, M. F. Saettone. Comparison of liposome-encapsulated acyclovir with acyclovir ointment: ocular pharmacokinetics in rabbits, J Ocul Pharmacol Ther. 20(2): 169-77 (2004).
2) De Clercq, Erik, Field, J. Hugh. Antiviral prodrugs – the development of successful prodrug strategies for antiviral chemotherapy, British Journal of Pharmacology Wiley-Blackwell 147 (1): 1–11 (2006).
3) H. Meulen, P. Nauwynck, P. Deprez, De Backer and S. B. Garré, K. Shebany, A. Gryspeerdt, K. Baert, K. van der Croubels, *Antimicrob. Agents Chemother,* 51(12): 4308 (2007).
4) G. P. Allen, J. T. Bryans. Molecular epizootiology, pathogenesis and prophylaxis of equine herpesvirus-1 infections, Prog. Vet. Microbiol. Immunol. 2: 78–144 (1986).
5) Anonymous. Antiviral agents, *In* J. E. F. Reynolds (ed.), Martindale, the Extra Pharmacopoeia, The Pharmaceutical Press, London, United Kingdom, 1993, 13th ed., pp. 536–563.
6) A. Barger, C. Fuhst, and B. Wiedemann. Pharmacological indices in antibiotic therapy, J. Antimicrob. Chemother. 52:893–898 (2003).
7) B. G. Bentz, L. K. Maxwell, R. S. Erkert, C. M. Royer, M. S. Davis, C. G. MacAllister, and C. R. Clarke. Pharmacokinetics of acyclovir after single intravenous and oral administration to adult horses, J. Vet. Intern. Med. 20: 589–594 (2006).
8) J. T. Bryans, and G. P. Allen. Herpesviral diseases of the horse, *In* G. Wittman (ed.), Herpesviral diseases of cattle, horses and pigs. Kluwer, Boston, MA, 1989, pp. 176–229.
9) C. Fletcher, and B. Bean. Evaluation of oral acyclovir therapy. Drug, Intell. Clin. Pharm. 19: 518–524 (1985).
10) K. Yamaoka, T. Nakagawa, and T. Uno. Application of Akaike's information criteria (AIC) in the evaluation of linear pharmacokinetics, J. Pharmacokinet. Biopharm. 6: 165–175 (1978).
11) S. Tanna, C. Wood, and M. J. Lawrence. Competition studies to elucidate the mechanisms of acyclovir uptake in the small intestine, J. Pharm. Pharmacol. 44(Suppl. 1): 1047 (1992).
12) M. Tod, F. Lokiec, R. Bidault, F. De Bony, O. Petitjean, Y. Aujard, and the Acyclovir Pediatric French Group. Pharmacokinetics of oral acyclovir in neonate and infants: a population analysis, Antimicrob. Agents Chemother. 45:150–157 (2001).
13) G. Stagni, M. E. Ali, D. Weng. Pharmacokinetics of acyclovir in rabbit skin after i.v.-bolus, ointment, and iontophoretic administrations, Int. J. Pharm. 274: 201–211 (2004).