



DESIGN AND DEVELOPMENT OF ACYCLOVIR MICROSOMAL GEL

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ABSTRACT

The present study focuses on the formulation and evaluation of mucoadhesive microcapsules containing acyclovir using different concentration of the naturally occurring natural polymer gelatin (1% to 6%); cross-linked with glutaraldehyde, using sodium alginate designed for oral controlled release. The drug acyclovir has been selected as suitable candidate for site specific delivery in stomach by encapsulating it in microcapsules 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. Due to these reasons dose of conventional ACV tablet 200mg (5 times a day), it is a typical task to administered tablet 5 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. When the mucoadhesive microcapsules of ACV administered orally they adhere on stomach mucosa and will retain inside the stomach and release the drug continuously for prolong period of time in controlled release manner. It can maintain the adequate concentration of drug in body and ultimately increase the bioavailability of drug and gave better therapeutic action as compared to other conventional dosage forms.

KEYWORDS- Microcapsules, Acyclovir, Gel, controlled release, glutaraldehyde, sodium alginate.

INTRODUCTION

Microcapsule is one of the micro particulate systems and is prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites. Microcapsule have been used for pharmaceutical and medical purpose, in cosmetic and food product, agriculture formulation as well as in the chemical textile and construction material, biotechnology, electronics and waste treatment Microencapsulation process helps for converting the liquid to solids, changing the colloidal and surface properties, providing environmental protection and controlling their characteristic of different coated materials. Novel drug delivery system which were initiate with the course of optimization the bioavailability by the modification of the bioavailability of the drug concentration in blood.^[5,6] with the sustained and controlled release products, drug therapy can be improved that is the common goal achieved over with their non-sustained and controlled release with the same drug.

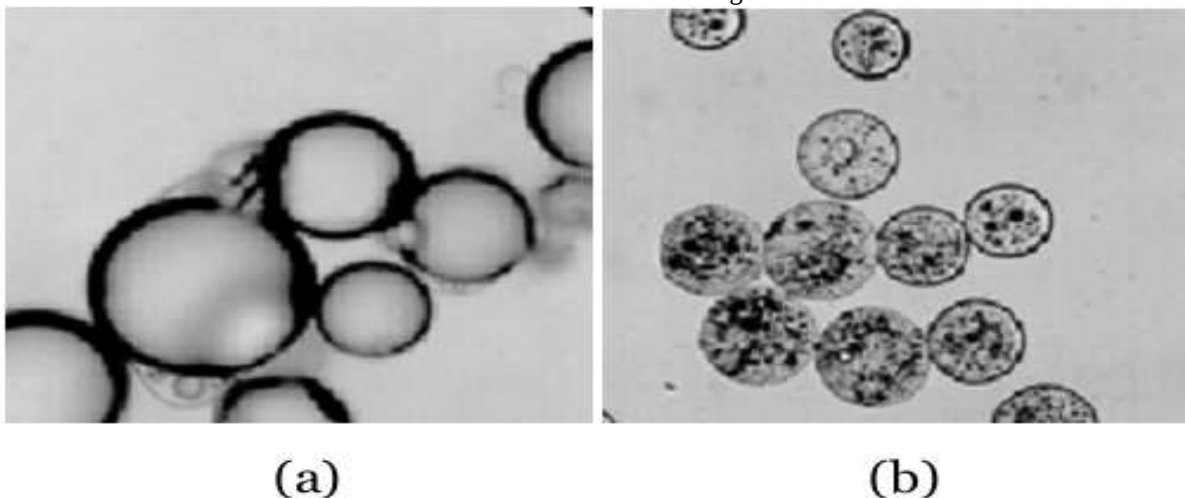


Figure 1: Scanning electron micrograph (a) mononuclear (b) multinuclear microcapsule



Table 1 Examples of some microencapsulated drugs ^[13]

Active moiety	Characteristic Property	Purpose of encapsulation	Final Product
Aspirin	Slightly soluble in water	Taste masking, sustained release, reduced in gastric irritation	Tablet, or capsule
Paracetamol	Slightly soluble in water	Taste masking	Tablet
Islet of Langerhans	Viable cells	Sustained normalization of diabetic condition	Injection
Isosorbide dinitrate	Water Soluble	Sustained release	Capsule
Progesterone	Slightly Soluble in Water	Sustained release	Varied
Menthol	Volatile solution	Sustained Release Reduction in Volatility	Lotion
Potassium Chloride	Highly Soluble in Water	Reduction in gastric irritation	Capsule
Urease	Water soluble enzyme	Perm selectivity of enzyme, substrate and reaction	Dispersion
Vit. A Palmitate	Nonvolatile Liquid	Stabilization to Oxidation	Dry Powder
Nifedipine	Practically insoluble in Water	Prevention from photoinstability	Dry Powder

Reasons for Microencapsulation

- The main reason for microencapsulation is for sustained or prolonged release of the drug.
- This technique has been widely used for masking the organoleptic properties like taste and odor of many drugs and thus improves patient compliance e.g. paracetamol, nitrofurantoin for masking the bitter taste.
- By using microencapsulation technique the liquid drugs can be converted into a free flowing powder.
- The drug can be protected by microencapsulation which is sensitive to moisture and oxygen, such as nifedipine is protected from photoinstability.
- Microencapsulation techniques are also helpful to prevent the incompatibility between drugs.
- The drug which is volatile in nature may vaporize at room temperature like aspirin and peppermint oil can be prevented by microencapsulation.
- Reduction in toxicity and GI irritation including with KCL and ferrous sulphate can be achieved by microencapsulation.
- Microencapsulation has also been employed to change the site of absorption. This application has been used for those drugs which have toxicity at lower pH.
- Bakan and Anderson were reported that microencapsulated vitamin A palmitate had enhanced stability, as prevent from oxidation.
- Microencapsulation method has also been employed to prepare intrauterine contraceptive devices.^[14]

2. MATERIALS AND METHODS

2.1 Materials

Table 2: List of Drugs and Excipients and their source

S. No.	Name of drug / Excipients	Manufactured by
1.	Acyclovir	Mylan laboratories limited, Nashik
2.	Chitosan	RRL, Bhopal
3.	Sodium alginate	RRL, Bhopal
4.	Calcium chloride	RRL, Bhopal
5.	Nerolidol	Sigma Aldrich, Mumbai
6.	Methyl paraben	MCW Industries Indore
7.	Propyl paraben	MCW Industries Indore
8.	Carbapol	MCW Industries Indore
9.	<i>Aloe vera</i>	Health Secure (India) Pvt. Ltd. Taloja, Dist. Raigad

Table 3: List of Equipment/Instruments and their manufacturers



S. no.	Instrument	Manufacturer
1.	Single Pan Electronic Balance	Scale-tec
2.	UV Visible Spectrophotometer	ELCO BL 198
3.	FT-IR Spectrometer	Shimadzu 84005
4.	Melting Point Apparatus	Kumar VMP-D
5.	Hot air oven	Bio Technics India BTI 11
6.	Digital pH meter	Simtronic model SE 962
7.	Viscometer	Brookfield DV-II + PRO
8.	Magnetic stirrer	Remi 2 MLH
9.	Ultrasonicator	Selec DTC 503
10.	Stability chamber	Thermo lab TH 200S

2.2 Methods-

2.2.1 Preformulation study

Determination of $\lambda_{\max}$ of Acyclovir in phosphate buffer

Accurately weighed 10 mg of acyclovir was dissolved in 10ml of solvent (1000 $\mu\text{g/ml}$) called A stock solution of the drug sample. was prepared in water and phosphate buffer of pH 7.4 by dissolving accurately weighed quantity of drug sample in 100 ml volumetric flask, it was then suitably diluted scanning was done in range of 200nm-400nm. The $\lambda_{\max}$ found was checked, whether it complies with the reported ones or not.

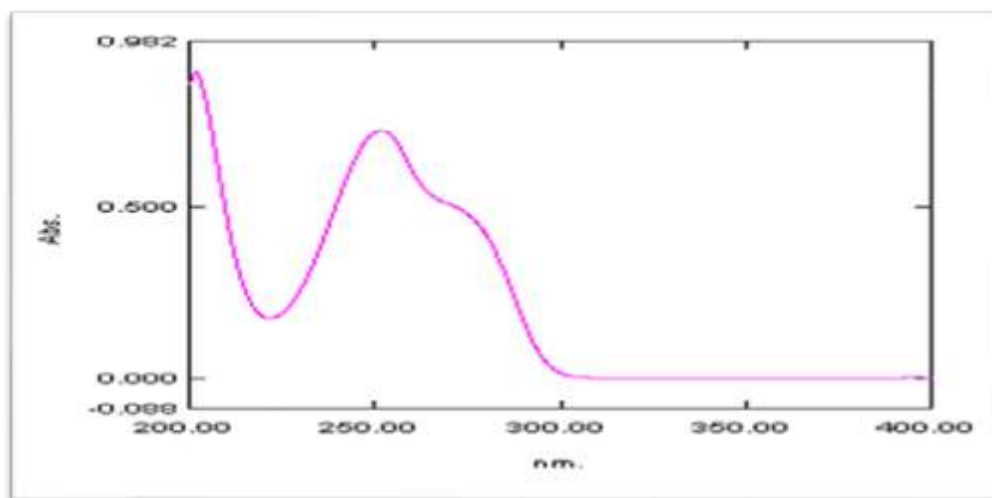


Figure 2: UV spectrum of Acyclovir in phosphate buffer of pH 7.4

2.2.2 Calibration Curve of Acyclovir in phosphate buffer pH 7.4

Accurately weighed quantity of Acyclovir (10 mg) was dissolved in little quantity of phosphate buffer of pH 7.4 and volume was made up to 10ml (1000 $\mu\text{g/ml}$). Appropriate aliquots from above stock solution were and final volume was made up to 10 ml by phosphate buffer solution, so as to get solutions with drug concentrations of range 4 to 20 $\mu\text{g/ml}$. These solutions were then analyzed using UV spectrophotometer and a plot of absorbance v/s concentration was plotted.

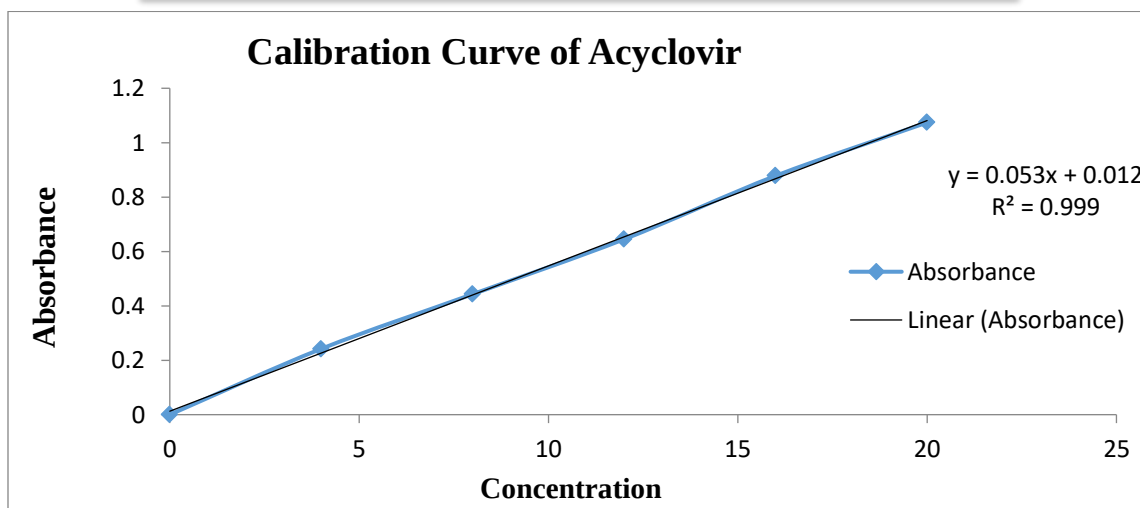


Figure 3: Calibration curve of Acyclovir in phosphate buffer (pH 7.4)

2.2.3 Fourier Transform Infrared (FTIR) analysis

Acyclovir was dried in oven at 50°C for 2 hour. The samples were prepared by mixed it thoroughly with potassium bromide. This physical mixture was compressed under pressure of 10ton/nm² & converted in a circular disc. This disc was then placed in the scanning slot of FTIR & scanned at range from 400 to 4000 cm⁻¹ to obtain FTIR of spectrum with reference spectrum.

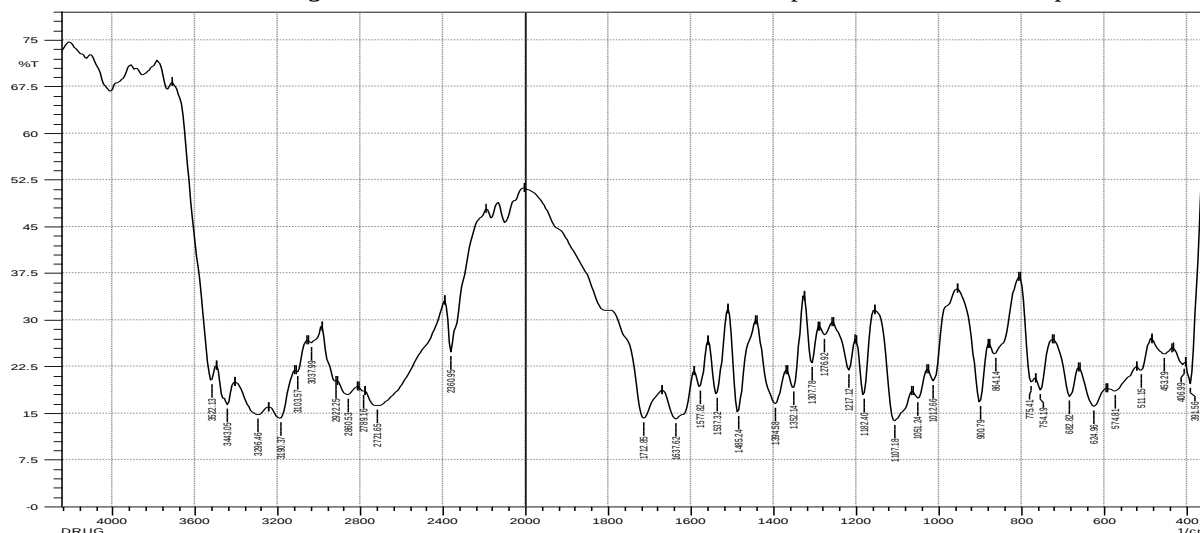


Figure 4: IR spectra of Acyclovir

3. FORMULATION OF MICROSOMES OF ACYCLOVIR

The microsomes 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 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. The microspheres were allowed to harden for another 2 h, filtered, washed thrice with distilled water and kept for drying at 40 °C 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 4: Composition of Microsomes of Acyclovir**

Code	Sodium alginate concentration (% w/v)	Calcium chloride concentration (% w/v)	Chitosan concentration (% w/v)
P1	2	5	1
P2	4	5	2
P3	4	2.5	1
P4	2	2.5	2
P5	3	2.5	1

3.1 CHARACTERIZATION OF THE ACV MICROSOMES-**3.1.1 Calculation of % yield calculation**

During this process, % yield was calculated as follows:

$$\% \text{ Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

$$\text{Swelling Index } (\alpha) = \frac{\{\text{weight of swelled microsomes (Wg)} - \text{Initial weight of microsomes (Wo)}\}}{\text{Initial weight of microsomes (Wo)}}$$

3.1.2 Particle Size Determination by Optical Microscopy

All the four batches prepared (P1 to P4) 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:

$$\text{Average particle diameter} = \sum n \cdot d / N$$

Where,

n = total no. of particles in that size range

d = Diameter of the particles of that size range

N = total no. of particles.

3.1.3 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 251.3 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.

$$\text{Amount of drug} = \text{Abs-intercept/ slope} (*10*100) / 1000$$

$$\% \text{Drug content} = \text{Calculated amount of drug total / amount of microspheres} \times 100$$

$$\text{Encapsulation efficiency} = \text{Calculated drug content / theoretical drug content} \times 100$$

3.1.5 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 into 100 ml dissolution medium of buffer solution pH 7.4 maintained at 37 ± 0.5 °C 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 251.3 nm to determine the concentration of drug present. The readings were taken thrice and the average reading was taken for further calculation.

3.1.6 Accelerated stability studies

The above prepared samples were kept in sealed vials for 7 days at 40 °C and 75% RH.

3.2 FORMULATION OF GEL**3.2.1 Preparation of gel base from Aloe Vera**

The mucilage or pulp of *A. vera* leaf, which is free from any resinous content (the dark red resin has to be drain out by holding the leaf upside for several seconds until the resin drips out), has to be taken to prepare *A. vera* gel. Then the mucilage was washed repeatedly with pure water, since it is highly acidic finally washings with 0.1N sodium hydroxide (NaOH) solution increase the pH of



Aloe pulp. By using a blender, the pulp is to be blended to obtain the juice. Then the juice is prefiltered for many times by using a cotton bed to remove any adhered rind. Then repeated subjection of the juice to the vacuum filtration produces a clear fluid. The Carbopol 934 (1%) is mixed with aloe juice to prepare *A. vera* gel by dispersion technique, where lump free mixture will be formed, and it allows free entrapped air upon standing. During the dispersion of juice to carbopol (jellifies under alkaline conditions), 0.5%w/w methyl paraben was added. Then 0.5 N NaOH solutions was added drop wise until to form a gel. After that required quantity of Nerolidol was added in *aleo-vera* gel and mixed properly in mortar pestle. Finally, the obtained gel was stored in air tight container to prevent any reactions.

3.2.2 Incorporation of microsomes containing drugs into the gel

Under certain atmospheric conditions, at 25 rpm, Microsomes containing drugs were mixed into the prepared *A. vera* gel by using an electrical mixer.

The formulations are varied for the concentration of the penetration enhancer (Nerolidol) from 1-5% as it is found to be safe within this range. Above 5% Nerolidol can be irritating to the skin.

Table 5: Design of formulations

Formulation Code	Acylovir Microsphere	Nerolidol	Methyl paraben	Propyl paraben	Aloe vera gel
P-1	2.5g	1% w/w	0.15% w/w	0.08% w/w	q.s. 50 g
P-2	2.5g	2% w/w	0.15% w/w	0.08% w/w	q.s. 50 g
P-3	2.5g	3% w/w	0.15% w/w	0.08% w/w	q.s. 50 g
P-4	2.5g	4% w/w	0.15% w/w	0.08% w/w	q.s. 50 g
P-5	2.5g	5% w/w	0.15% w/w	0.08% w/w	q.s. 50 g

4. RESULT AND DISCUSSION

4.1 Optical microscopy

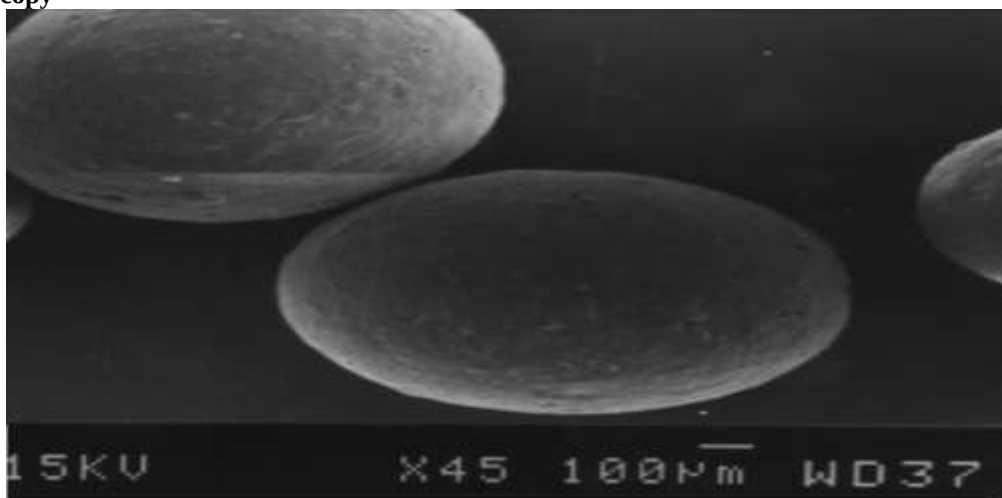


Figure 5: Surface morphology of optimized formulation

4.2 Characterization of ACV Microsomes

Table 6: Results of characterization of ACV Microsomes

Formulation	% Yield	Microsome Size (µm)	(%)Encapsulation	Swelling Index
P-1	89.73	568.65 ± 0.461	80.21 ± 0.25	3.52
P-2	86.49	715.57 ± 0.154	82.46 ± 0.45	3.04
P-3	94.22	636.12 ± 0.506	97.01 ± 0.85	3.64
P-4	83.54	751.82 ± 0.297	93.62 ± 0.75	3.14
P-5	87.34	585.65 ± 0.898	84.36 ± 0.45	3.28

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



4.3 In-vitro Drug Release Studies

Table 7: in-vitro cumulative % drug release from Microsomes

Time (hrs)	P-1	P-2	P-3	P-4	P-5
0	0	0	0	0	0
1	07.44	09.00	10.56	09.60	07.20
2	14.14	18.45	17.12	19.77	14.49
4	21.91	27.89	25.98	28.49	22.63
6	29.33	36.99	34.23	39.02	29.44
8	36.86	45.48	41.29	49.31	37.46
10	44.88	55.41	48.46	58.88	44.75
12	51.69	67.25	57.20	67.61	51.57
16	60.31	77.66	67.97	77.29	58.15
18	66.76	87.10	75.97	87.82	65.57
20	75.50	89.71	84.83	88.99	73.34
24	81.23	92.82	94.39	90.90	79.79

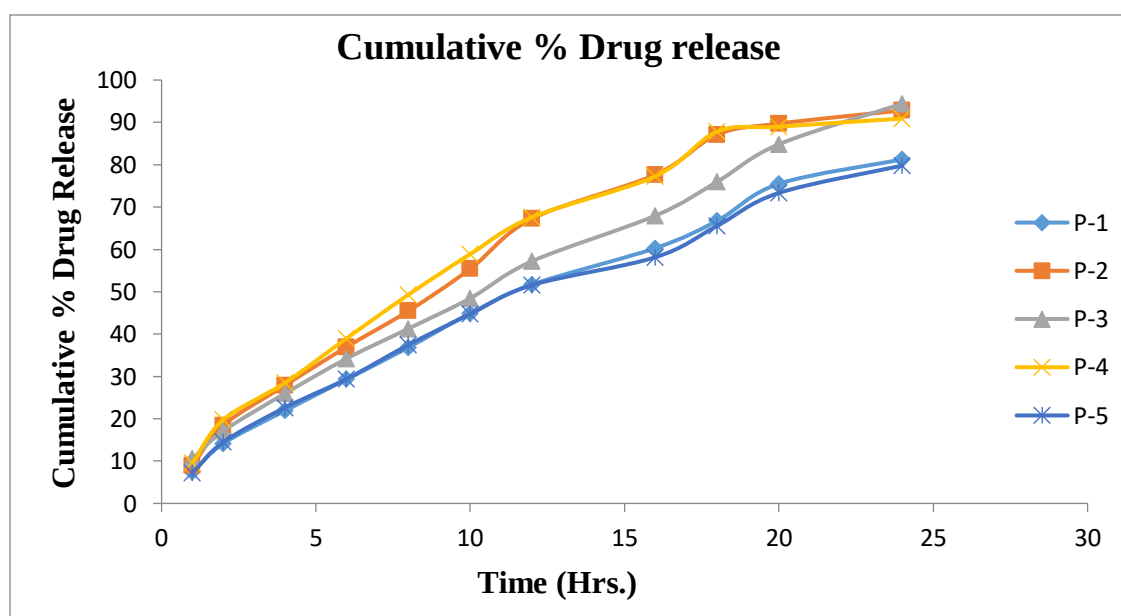


Figure 6: Cumulative % drug release of prepared Microsomes

Table 8: R² values of all microsome formulations

Model	Equation	R ²
P-1	$y = 3.350x + 7.057$	$R^2 = 0.980$
P-2	$y = 4.006x + 10.24$	$R^2 = 0.959$
P-3	$y = 3.774x + 8.436$	$R^2 = 0.986$
P-4	$y = 3.912x + 12.02$	$R^2 = 0.944$
P-5	$y = 3.249x + 7.601$	$R^2 = 0.976$

4.4 Preparation of aloe-vera gel base using penetration Enhancer Nerolidol

Aloe-vera gel base using penetration Enhancer Nerolidol was prepared as designed formula described in method section.



4.5 Evaluation of Microsomal gel preparation

4.5.1 Determination of pH, viscosity, % Drug content, Spreadability

Table 9: pH of formulations

Formulation code	pH	Viscosity (cps)	Drug content (%)	Spreadability (gm.cm/sec)
P-1	6.63	54703	98.92	12.66
P-2	6.63	52779	100.32	11.30
P-3	6.63	58767	99.40	11.23
P-4	6.61	54587	98.69	12.34
P-5	6.52	47770	100.35	12.69

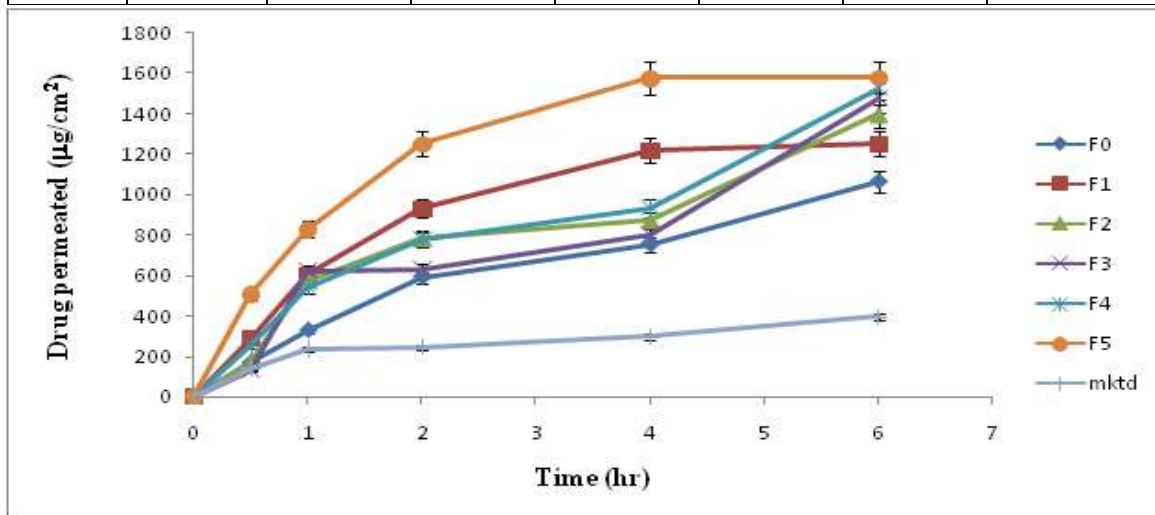
Viscosity is an expression of the resistance of the fluid to flow; the higher the viscosity, the greater the resistance. It is observed in reports that the viscosity of the formulations goes on decreasing as the rpm increases i.e an inverse relationship exists between the viscosity and the shear rate. From the figures it can be said that the formulations follow pseudo plastic behavior.

4.5.2 Diffusion study

Diffusion study using cellophane membrane

Table 10: Cumulative amount of drug released Q ($\mu\text{g}/\text{cm}^2$) at different time intervals across cellophane membrane

Time (hr)	Q ($\mu\text{g}/\text{cm}^2$)						Marketed
	P-0	P-1	P-2	P-3	P-4	P-5	
0.5	170.3	288.49	173.56	129.04	253.94	504.00	142.01
1	329.42	601.25	573.00	619.05	540.69	828.03	235.90
2	590.58	931.99	780.50	628.04	777.79	1251.04	248.79
4	754.62	1221.0	871.13	801.00	929.70	1577.02	296.00
6	1065.80	1251.01	1395.01	1474.92	1522.89	1579.45	398.05

**Figure 7: Plot of cumulative amount of drug release v/s time for different formulations of Acyclovir gel across cellophane membrane**

The release profiles were constructed by plotting the cumulative amount of Acyclovir diffused per unit membrane surface area (Q , $\mu\text{g}/\text{cm}^2$) versus time (hr). The steady state flux (J_{ss} , $\mu\text{g}/\text{cm}^2.\text{hr}$) of Acyclovir was calculated from the slope of the plot using linear regression analysis.

**Table 11: Results of drug diffusion parameters across cellophane membrane**

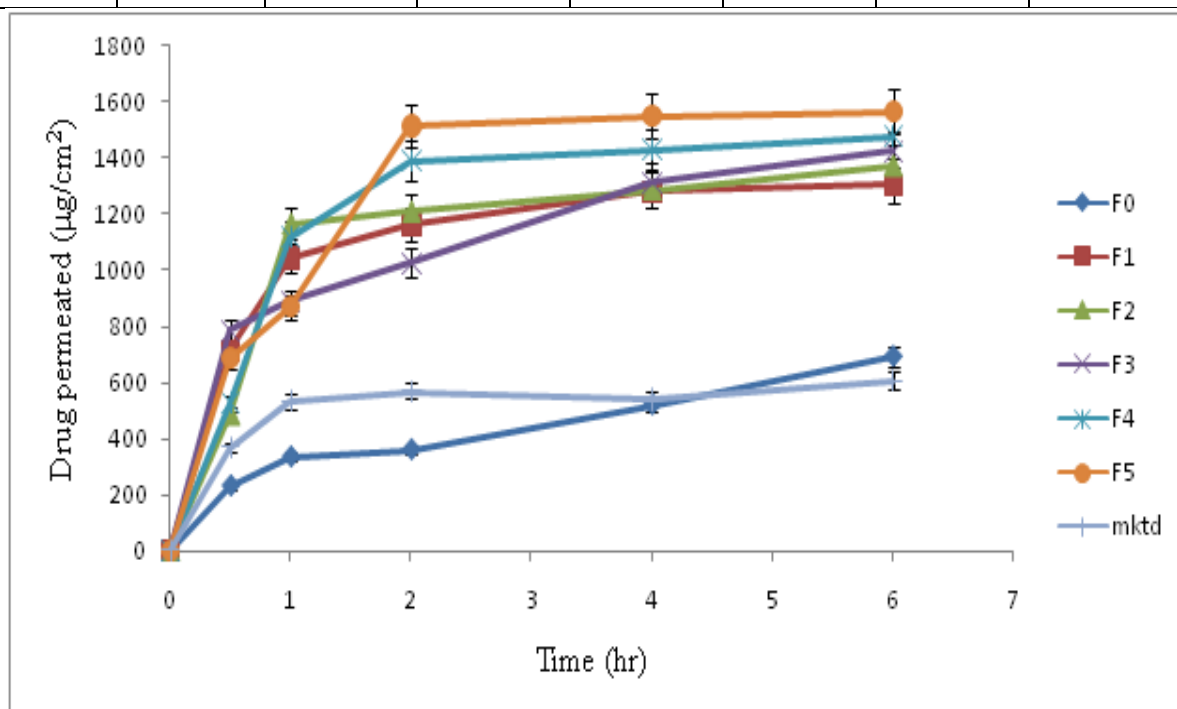
Formulation code	Q ₆ ($\mu\text{g}/\text{cm}^2$)	Jss ($\mu\text{g}/\text{cm}^2 \cdot \text{hr}$)
P-0	1065.80	177.05
P-1	1251.01	198.40
P-2	1395.01	202.00
P-3	1474.92	214.50
P-4	1522.89	225.10
P-5	1579.45	240.9
Marketed	398.05	166.3

It is observed that the flux and permeability of Acyclovir goes on increasing as the concentration of Nerolidol increases.

4.5.3 Using egg membrane

Table 12: Cumulative amount of drug released (Q) $\mu\text{g}/\text{cm}^2$ at different time intervals across egg membrane

Time (hr)	Q ($\mu\text{g}/\text{cm}^2$)						Marketed
	P-0	P-1	P-2	P-3	P-4	P-5	
0.5	231	711.9	485.8	786	523.97	685.44	369.37
1	336.1	1046	1166.08	877.99	1119.01	869.99	534.28
2	363.01	1164.98	1210	1027.03	1391.06	1512.96	571.02
4	522.9	1286	1298.04	1314.88	1428.01	1552.08	539.78
6	695.2	1303.24	1376	1425.22	1474.98	1564.42	609.07

**Figure 8: Plot of cumulative amount of drug release v/s time for different formulations of Acyclovir gel across egg membrane**

The release profiles were constructed by plotting the cumulative amount of Acyclovir diffused per unit membrane surface area (Q, $\mu\text{g}/\text{cm}^2$) versus time (hr). The steady state flux (Jss, $\mu\text{g}/\text{cm}^2 \cdot \text{hr}$) of Acyclovir was calculated from the slope of the plot using linear regression analysis. The permeability coefficient (Kp) and enhancement ratio (ER) are calculated using following equations



$$Kp = \frac{J_{ss}}{C}$$

Where, J_{ss} is flux at steady state and C is the initial concentration of Acyclovir in the donor compartment.

$$ER = \frac{Kp \text{ with pretreatment}}{Kp \text{ without pretreatment}}$$

Table 13: Results of drug diffusion parameters across egg membrane.

Formulation code	Q_6 ($\mu\text{g}/\text{cm}^2$)	J_{ss} ($\mu\text{g}/\text{cm}^2 \cdot \text{hr}$)	Kp ($\times 10^{-3}$)	ER
P-0	695.20	97.05	6.44	
P-1	1303.24	159.56	10.13	1.68
P-2	1376.00	178.39	10.99	1.89
P-3	1425.22	182.32	12.15	1.90
P-4	1474.98	199.17	13.30	2.02
P-5	1564.42	220.66	14.48	2.29
Marketed	609.07	67.42	4.3	

Q_6 = Cumulative amount permeated at 6 hr; J_{ss} = Steady state flux; Kp = Permeability coefficient; ER= Enhancement ratio
It is observed that the flux and permeability of Acyclovir goes on increasing as the concentration of Nerolidol increases.

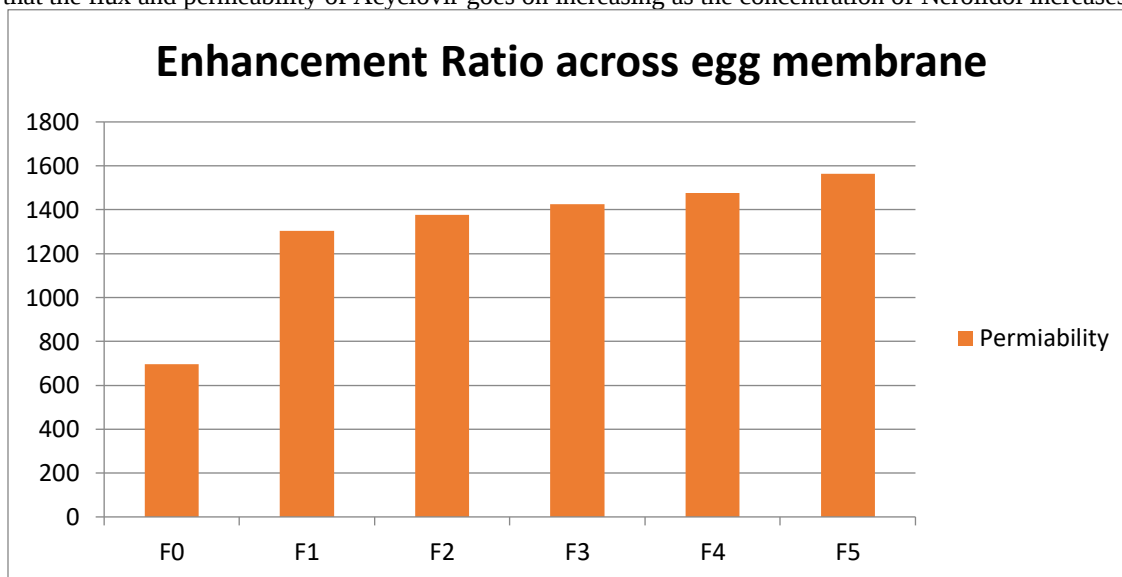


Figure 9: Effect of Nerolidol in terms of Enhancement Ratio across egg membrane

4.6 Stability Study

4.6.1 Physical examination

No visible change in the formulation was observed as compared to control formulation.

6.6.2 Determination of drug content

Table 14: Drug content for stability testing

Drug content after stability	Drug content before stability
98.01	100.33



4.6.3 Diffusion study

Table 15: Results of drug diffusion parameters across egg membrane for stability testing

Time (hr)	Q ($\mu\text{g}/\text{cm}^2$)	Jss ($\mu\text{g}/\text{cm}^2 \cdot \text{hr}$)
0.5	658.11	220.33
1	849.02	
2	1495.56	
4	1545.15	
6	1551.65	

The cumulative amount of drug permeated at 6 hour (Q₆) and the steady state flux (Jss) for the formulation were found to be 1551.65 $\mu\text{g}/\text{cm}^2$ and 220.33 $\mu\text{g}/\text{cm}^2 \cdot \text{hr}$ respectively. No significant changes were observed in the formulation after its stability testing.

CONCLUSION

Microsomes 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. Nerolidol was used as permeability enhancer of Acyclovir. Microsomes 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. Acyclovir also causes irritation on skin, *aloe-vera* was used in preparations which reduced irritation. Major advantages of the system include ease of preparation, good enhanced solubility, high % encapsulation efficiency and sustained drug release. From this study, it was concluded that microsomes of Acyclovir were offers enhanced solubility and continuous release of the medicament and bioavailability of the drug and subsequent efficacy is improved.

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