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RESEARCH ARTICLE

Development of proniosomes gel as a drug carrier for transdermal delivery of acyclovir

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ABSTRACT

Non-ionic surfactant vesicles of acyclovir, an antiviral, were prepared by coacervation phase separation method. The prepared systems were characterised for encapsulation efficiency, shape, size and in vitro drug release scanning electron microscopy (SEM). Proniosomal prepared by using different polymers span 40, span 60, span 20, tween 20, tween 80 and cholesterol and soya lecithin. Among all proniosomal transdermal gel formulation, The results showed that acyclovir in all the formulations was successfully entrapped and a substantial change in release rate and an alteration in the encapsulation efficiency of acyclovir from proniosomes were observed upon varying the type of surfactant and cholesterol content The encapsulation efficiency of proniosomes prepared with Span 60,cholesterol and lecithin gave maximum encapsulation efficiency (89.70%) as compared to other compositions. , the niosomal gel formulation could be a promising transdermal delivery system acyclovir with prolonged drug release profiles.

Key words: Acyclovir, proniosomes, proniosomal gel, cholesterol, Scanning electron microscopy (SEM) analysis, *In-vitro* skin permeation studies, Half- life.

INTRODUCTION:

Proniosomes are dry formulation of water-soluble carrier particles that are coated with surfactant and can be measured out as needed and dehydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media within minutes. The proniosomal approach minimizes the above mentioned problems, as it involves a dry product or a liquid crystalline gel that can be hydrated immediately before use[5-7]. Proniosomes are water soluble carrier particals that are coated with surfactants and can be hydrated to form niosomal dispersion immediately before use in hot aqueous media. Proniosomes offer a versatile drug delivery concept with potential for delivery of drugs via transdermal route. This would be possible if proniosome form niosomes upon hydration with water from skin following topical application under occlusive conditions[9] Proniosomes minimizes the problems of niosomes such as physical stability like leaking, fusion, aggregation, sedimentation. It Avoid hydrolysis of encapsulated drugs. Liposomes and niosomes require special storage and handling while proniosomes not required[1-4]. Acyclovir, 9-(2-hydroxy) ethoxymethylguanine, is used as an antiviral agent and is especially active against herpes simplex virus and zoster herpes. Acyclovir is administered intravenously, locally, and perorally. For peroral administration, acyclovir is as tablets (200 mg) that are recommended to be taken five times per day. It has been noted that not more than 20% of the acyclovir is absorbed. The daily therapeutic dose is attained by taking 400-mg tablets 12 times or 800-mg tablets 5 times. According to the FDA, the time for reaching the maximum concentration of acyclovir in blood plasma is 1.5 – 1.75 h. The biological availability of acyclovir is only 10 – 20% and decreases with increasing dose. The half-elimination period for constant kidney function is 2.5 – 3.3 hr Blood plasma proteins bind 9 – 33% of acyclovir[11-18].

The objective of the present study was to prepare and characterize proniosomes of acyclovir, the encapsulation of acyclovir in that vesicular structure may be expected to the problem like gastric side effect, short half life and low bioavailability etc of acyclovir can be solved by developing the formulation of acyclovir as proniosome gel.

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MATERIALS & METHODS:

Materials used for the study:

Table 1: List of chemicals used for the study

Sr. No.	Materials	Source
1	Acyclovir	Cipla,Mumbai
2	Tween 20	Ases chemicals,jodhpur
3	Span 80	Ases chemicals,jodhpur
4	Cholesterol	Ases chemicals,jodhpur
5	Soya Lecithin	Ases chemicals,jodhpur
6	Span 20	Ases chemicals,jodhpur
7	Span 40	Ases chemicals,jodhpur
8	Span 60	Ases chemicals,jodhpur
9	Methanol	Central drug house,gujrat
10	Ethanol	Changshu
11	Potassium dihydrogen orthophosphate	S.D Fine chemicals
12	Sodium hydroxide	Qualigens

Methods of Preparation of Acyclovir Proniosomal Gel:

Coacervation-Phase Separation Technique[12-16]:

Proniosomal gel was prepared by a coacervation-phase separation technique. Precisely weighed amounts of surfactant, lecithin, cholesterol and drug were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol (1.0 ml) was added to it. After warming, all the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture was dissolved completely. Then the aqueous phase (phosphate buffer pH 7.4) was added and warmed on a water bath till a clear solution was formed which was converted into Proniosomal gel on cooling. The gel so obtained was preserved in the same glass bottle in dark conditions for characterization

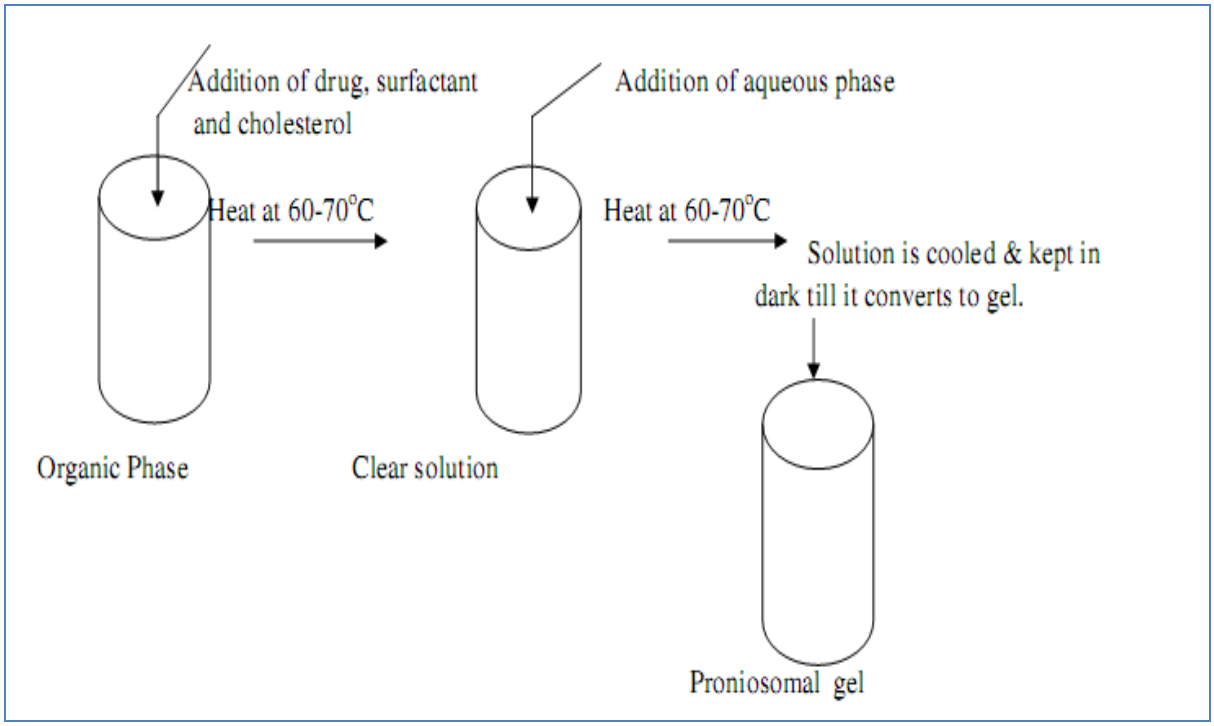

Figure 1: Method of Preparation of Proniosomal Gel

CHARACTERIZATION OF PRONIOSOMAL GEL:

Morphological Evaluation:

Physical Appearance[17-19]:

The prepared gel was viewed by naked eye to characterize color and physical state of gel. The appearance for each formula was checked such as color, consistency and fluidity and comparison of each one with the other.

Microscopy[15, 20-22]:

The prepared gel was viewed by naked eye to characterize color and physical state of gel. The appearance for each formula was checked such as color, consistency and fluidity and comparison of each one with the other.

Vesicular size distribution and average particle size determination[23-25]:

Particle size analysis was carried out using an optical microscope with a calibrated eyepiece micrometer. A standard stage micrometer was used for calibration. Each division value on stage is 10µ. The eye piece micrometer consists of 100 divisions. Calibration was undertaken to find out the measure of each division using the standard stage micrometer. After calibration, the eye piece micrometer was used for particle size determination. A suspension of proniosomes was prepared in buffer solution pH 7.4. A drop of the suspension was mounted on a slide and observed under the microscope. The sizes of 150-200 vesicles were measured using a calibrated ocular and stage micrometer fitted in the optical microscope.

Determination of Ph[26, 27]:

The Ph of the Proniosomal gels was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

Entrapment Efficiency[28, 29]:

Free acyclovir was separated from noisome entrapped Acyclovir by centrifugation. A 1-ml aliquot of niosome dispersion was centrifuged at 20,000 rpm at 0°C for 1 h. The supernatant was separated, and the niosomal residue was resuspended in phosphate buffer (pH 7.4) and centrifuged again. This washing procedure was repeated two times to ensure that the free drug was no longer present in the voids between the niosomes. The collected supernatant fractions were diluted to 10 ml with phosphate buffer (pH 7.4) and were used for determination of the free Acyclovir spectrophotometrically at 254 nm as this wavelength represents the maximum absorbance of acyclovir in phosphate buffer (pH 7.4) Acyclovir concentration was expressed as % entrapment efficiency which can be defined as the percent fraction of the total input drug encapsulated in the surfactant bilayers and or aqueous compartments in the niosomes and obtained by subtracting the amount of free drug from the total drug incorporated in 1 ml niosomal dispersion according to the equation

$$\text{Entrapment efficiency (\%)} = \frac{\text{amount of drug entrapped}}{\text{total amount of drug}} \times 100$$

Infrared spectroscopy[26-29]:

IR spectroscopy was conducted using a Perkin Elmer spectrum RXIFT-IR system and the spectrum was recorded in the wavelength region of 4000– 500cm−1. The procedure consisted of dispersing a sample (acyclovir, Span 60, Span 20, Tween 20, Tween 80, cholesterol, Soya Lecithin, and four selected proniosome gel formulations) in KBr and compressing into discs by applying a pressure of 5 ton for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was recorded.

***In vitro* release studies[30-32]:**

The release of acyclovir from niosomal formulations was determined using membrane diffusion technique. The niosomal formulation equivalent to 50mg of acyclovir was placed in a glass tube having a diameter 2.5cm with an effective length of 8cm that was previously covered with soaked osmosis cellulose membrane, which acts as a donor compartment. The glass tube was placed in a beaker containing 100ml of phosphate buffer pH 7.4, which acts as receptor compartment. The whole assembly was fixed in such a way that the lower end of the tube containing suspension was just touched (1-2mm deep) the surface of diffusion medium. The temperature of receptor medium maintained at 37±100C and the medium was agitated at 100rpm speed using magnetic stirrer. Aliquots of 5ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analysed at 254nm in Double beam UV-VIS spectrophotometer using Phosphate buffer 7.4 as blank.

Kinetics study[33-36]:

In-vitro release kinetics were assessed using zero order [$C = k_o t$], first order [$\text{LogC} = \text{LogC}_o$ kt / 2.303], Fickian diffusion [$Q = Kt^{1/2}$], Hixson-Crowell cube root law [$Q_0^{1/3} - Qt^{1/3} = K_{HC} t$]. *Mechanism of drug release* was assessed by Korsmeyer eq. that describes drug release from a polymeric system [$M_t M_\infty = Kt^n$]. The plots were made by

considering : cumulative % drug release vs. time (zero order kinetic model); log cumulative of % drug remaining vs. time (first order kinetic model); cumulative % drug release vs. square root of time (higuchi model) log cumulative % drug release vs. log time (korsmeyer model) and cube root of drug % remaining in matrix vs. time (hixson-crowell cube root law).

RESULT AND DISCUSSION:

Compositions of Preliminary formulations:

Table 1: Different formulations of acyclovir proniosome

Formulation Code	Drug (mg)	Span 60 (mg)	Span 40 (mg)	Span 20 (mg)	Tween (20)	Tween (80)	Cholesterol (mg)	Soya Lecithin (mg)	Ethanol (ml)
PA1	50	1000	-	-	-	-	100	0.5	0.5
PA2	50	-	1000	-	-	-	100	0.5	0.5
PA3	50	-	-	1000	-	-	100	0.5	0.5
PA4	50	-	-	-	1000	-	100	0.5	0.5
PA5	50	-	-	-	-	1000	100	0.5	0.5
PA6	50	500	500	-	-	-	100	0.5	0.5
PA7	50	500	-	500	-	-	100	0.5	0.5
PA8	50	-	500	500	-	-	100	0.5	0.5
PA9	50				500	500	100	0.5	0.5

Physical appearance of various formulations:

Table 2: Physical appearance of various formulations

Formulation code	Color	Observations
PA1	Brown	Brown Semisolid
PA2	White	White Semisolid
PA3	White	White Semisolid
PA4	Brown	Brownish gel
PA5	Yellow	Yellowish gel
PA6	Yellow	**Yellowish semisolid**
PA7	White	White Semi-solid
PA8	Brown	Brown Liquid
PA9	Yellow	Yellowish gel

Entrapment Efficiency of various formulations:

Entrapment efficiency values for of PA1 to PA9 are given in Table 4 The drug entrapment characteristics are good for Span 60 and tween 20 and produced least leaky niosomes that may be due to their highest phase transition temperature. It is indicated that, formulations PA1 possess high EE values of 89.70 respectively. Results were showed in the table:

Table 3: Entrapment Efficiency of Proniosomal gels

Formulation code	Absorbance	Concentrate (ug/ml)	Dil. Factor (1000 ml)	Conc.*dil. Factor(ug/ml)	%EE
PA1	0.43	0.48	1000	4.48	89.70
PA2	0.41	4.28	1000	4.28	85.74
PA3	0.32	3.39	1000	3.39	67.92
PA4	0.29	3.09	1000	3.09	61.98
PA5	0.42	4.38	1000	4.38	87.72
PA6	0.21	2.30	1000	2.30	46.13
PA7	0.36	3.79	1000	3.79	75.84
PA8	0.38	3.99	1000	3.99	79.80
PA9	0.29	0.09	1000	3.09	61.98

pH of various formulations:

pH determination of all the formulations was found in range of pH5.6- pH5.8. This results showed slightly acidic property of the formulation which can be easily orally administered. Results were showed in the table:

Figure 4: PH of various formulations

Formulation Code	pH
PA1	7.2
PA2	6.8
PA3	6.2
PA4	6.4
PA5	7.4
PA6	6.2
PA7	6.9
PA8	7.0
PA9	5.4

7.1. Vesicle size analysis:

Vesicular size, shape and size distribution: The size of the The average vesicular size of niosomes of all the batches was measured in the range of **4.04±0.470 to 4.18±0.350 µm**. The size distribution was in the range of **0.5 to 12.5 µm**. The result suggests that niosomes prepared were of uniform size and spherical in shape. The microphotographs of all niosomal formulations revealed that the niosomes were spherical in their shape and were shown in figures 2.

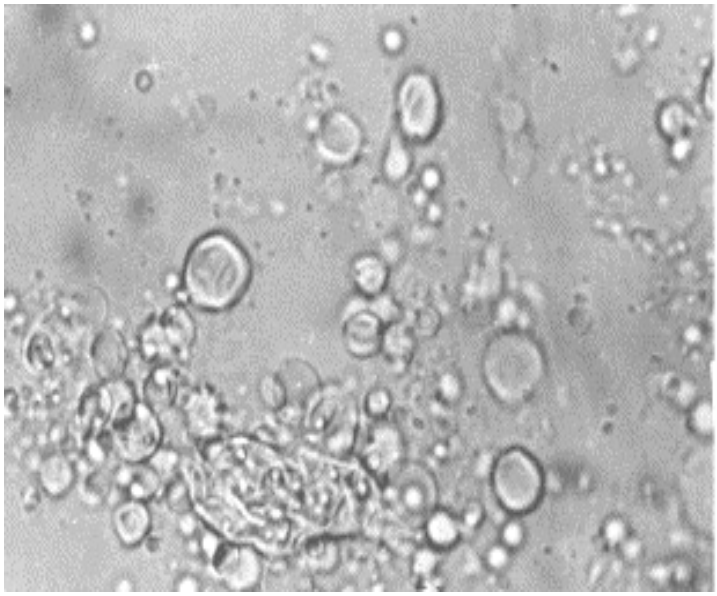

Figure 2: Vesicles under Optical Microscopy

7.2.

7.3. *In vitro* Skin permeation studies:

Drug diffusion data indicating skin permeation is shown in Table 5,6 and figure no.3 Diffusion profiles for all formulations were found to be linear within a period of 6 hours. Data in terms of %drug release its shown in Table 5, 6. All formulations exhibited similar diffusion characteristics. Formulation PA1show higher drug content.

Table 5: Cumulative amount of drug release for formulations (PA1-PA6)

Time	% Cumulative drug release					
	PA1	PA2	PA3	PA4	PA5	PA6
0.25	15.53	15.72	11.35	16.31	11.97	11.94
0.5	27.86	23.31	24.25	25.28	21.95	21.95
1	38.92	33.27	36.31	38.83	31.566	31.56
2	54.74	44.45	51.24	52.71	44.26	44.22
3	66.99	54.26	64.70	65.15	57.34	57.34
4	80.69	64.47	74.44	75.47	68.30	68.30
5	89.40	71.17	81.21	85.58	77.44	77.46
6	93.80	75.16	85.35	89.71	82.71	82.71

Table 6: Cumulative Amount of Drug Release for Formulations (PA7-PA9)

Time (hrs)	% Cumulative drug release		
	PA7	PA8	PA9
0.25	14.36	16.50	8.73
0.5	22.36	31.15	14.61
1	31.89	39.13	26.09
2	36.89	54.56	42.19
3	47.31	63.59	55.19
4	61.17	70.02	65.75
5	72.52	79.19	74.52
6	81.48	86.09	79.21

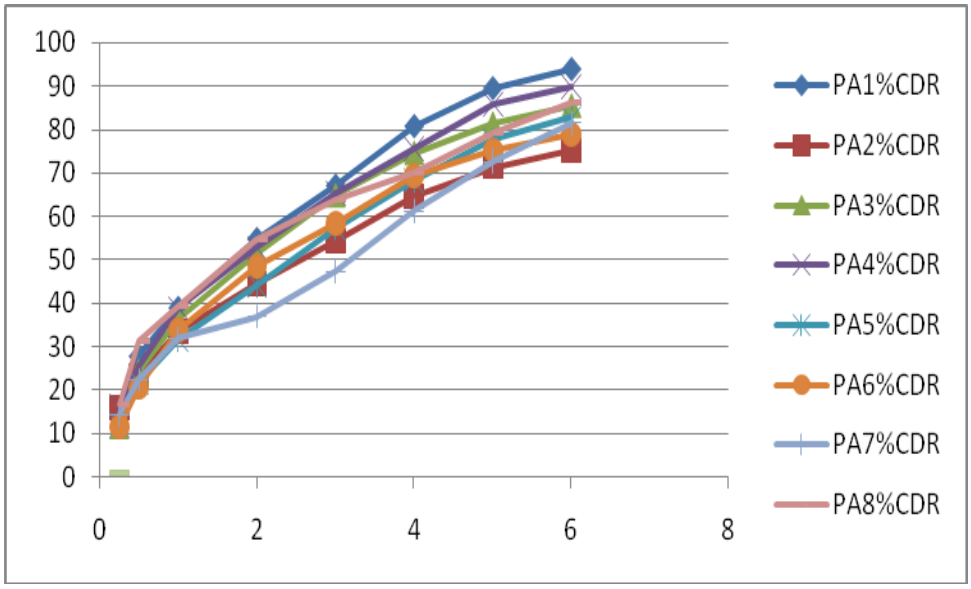

Figure 3: Comparative *in vitro* release study of different formulation

Release Kinetics:

The results obtained from *in vitro* drug release studies were plotted adopting five different mathematical models of data treatment as follows:

Table 7: Release kinetics data of PA1

Time (Hrs)	Log T	SQRT	%CDR	Log %CDR	Log % drug retained	(% drug retained)$^{1/3}$
0.25	-0.602	0.500	15.53	1.191	1.926	4.519
0.5	-0.301	0.707	27.86	1.445	1.858	4.369
1.00	0	1.000	38.92	1.590	1.785	4.102
2	0.176	1.224	54.74	1.738	1.655	3.487
3	0.301	1.414	66.99	1.826	1.518	3.068
4	0.477	1.732	80.69	1.906	1.285	2.796
5	0.602	2.000	89.40	1.951	1.025	2.573
6	0.698	2.236	93.80	1.972	0.792	2.550

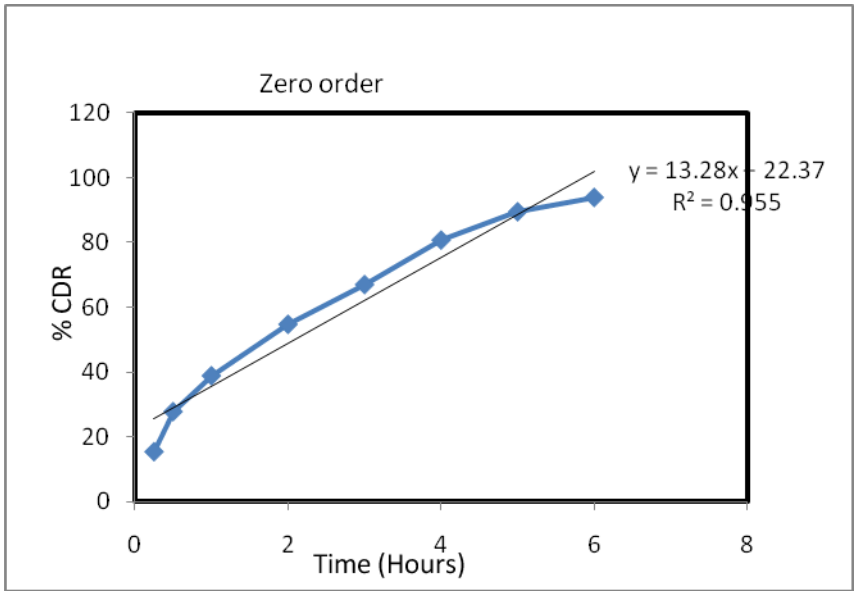

Figure 4: % Cum. Drug Release Vs. Time (Zero order rate kinetics)

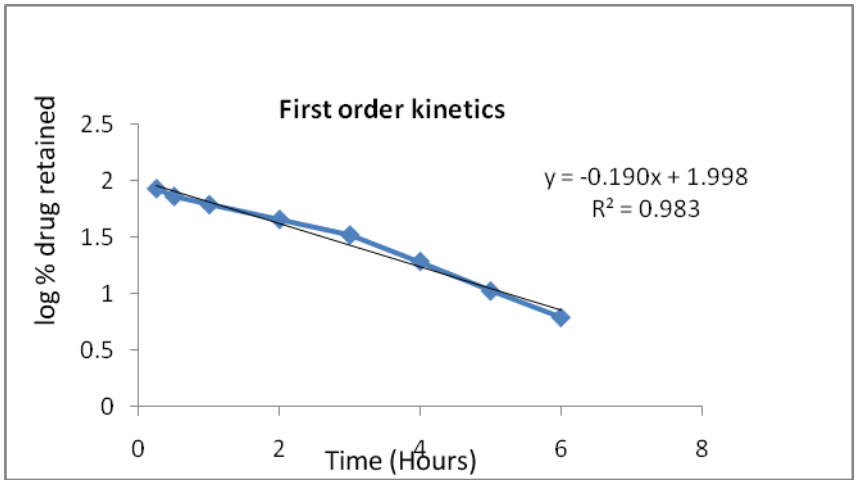

Figure 5: Log % Cum. Drug Retained Vs. Time (First order rate kinetics)

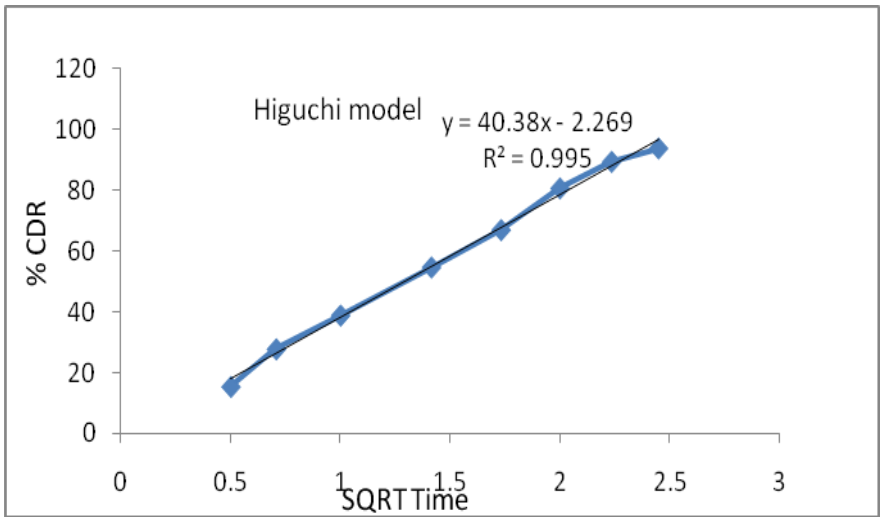

Figure 6: % Cum. Drug release vs SQRT (root time). (Higuchi model)

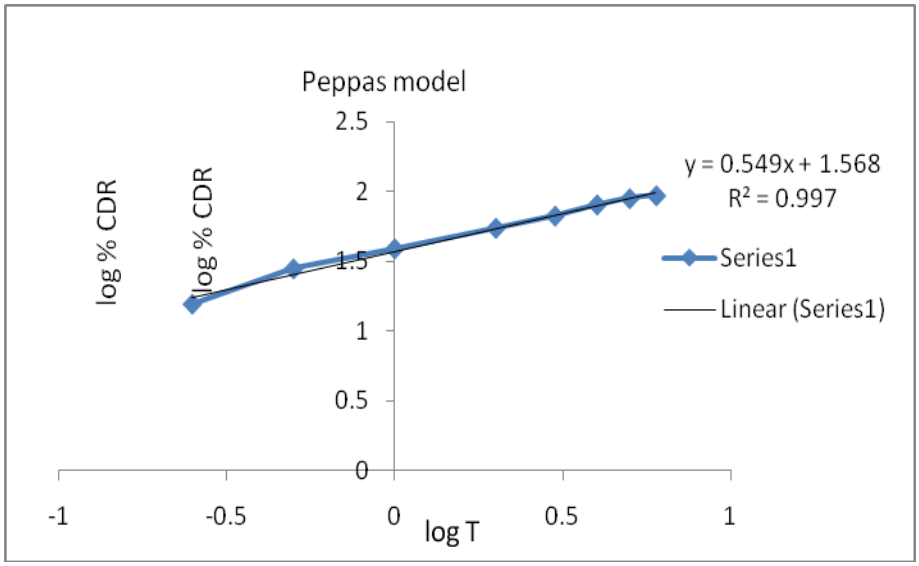

Figure 7: Log % Cum. Drug Release Vs. Log Time (Peppas exponential equation)

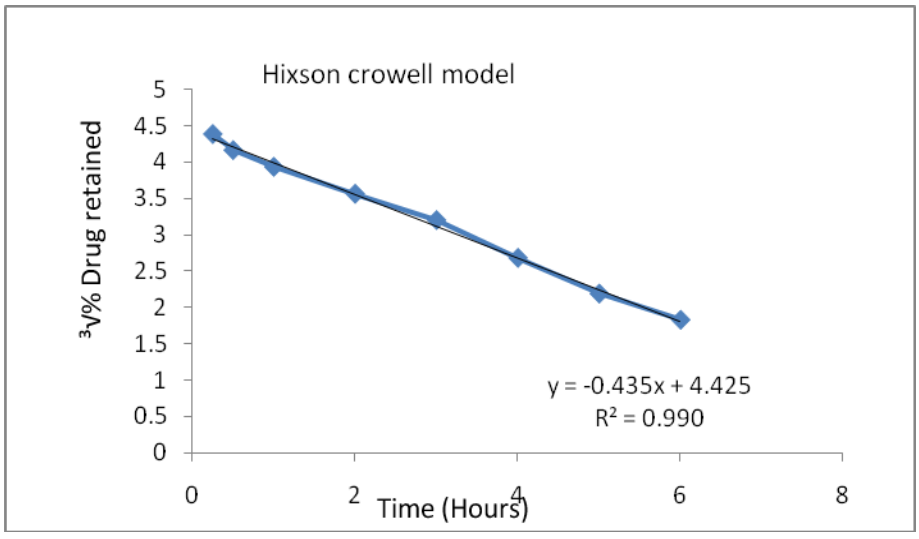

Figure 8: (% Cum. Drug Retained)$^{1/3}$ Vs. Time (Hixon-Crowell's erosion equation)

The curve fitting results of the release rate profile of the designed formulation are shown in the Figures 5-8 which gave an idea on the release rate and the mechanism of release. The values were compared with each other for model and drug equation based on the highest regression values (r), fitting of the release rate data to the various models revealed that the formulation code PA1 was best fitted with peppas exponential equation.

The Peppas model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved. 'n' value could be used to characterize different release mechanisms.

Peppas model equation is given as: -

$\% R = K t^n$

Or $\text{Log } \% R = \log K + \log t$

Where R = drug release, k =constant, n= slope, t=time.

'n'	**Mechanism**
< 0.5	Fickian Diffusion (Higuchi matrix)
0.5 < n < 1	Non Fickian Diffusion or anomalous release
> 1	Case II Transport

In the case of the Fickian release mechanism, the rate of drug release is much less than that of polymer relaxation (erosion). So the drug release is chiefly dependent on the diffusion through the matrix. In the non-Fickian (anomalous) case, the rate of drug release is due to the combined effect of drug diffusion and polymer relaxation.

SEM analysis:

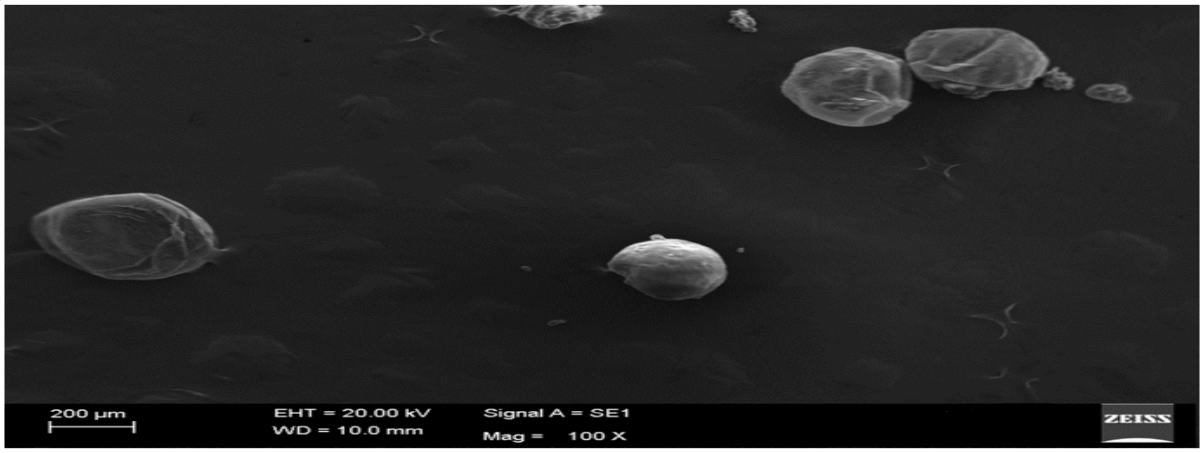

Figure 9: SEM of formulation code PA1 of proniosome gel

FTIR Studies of various Formulations:

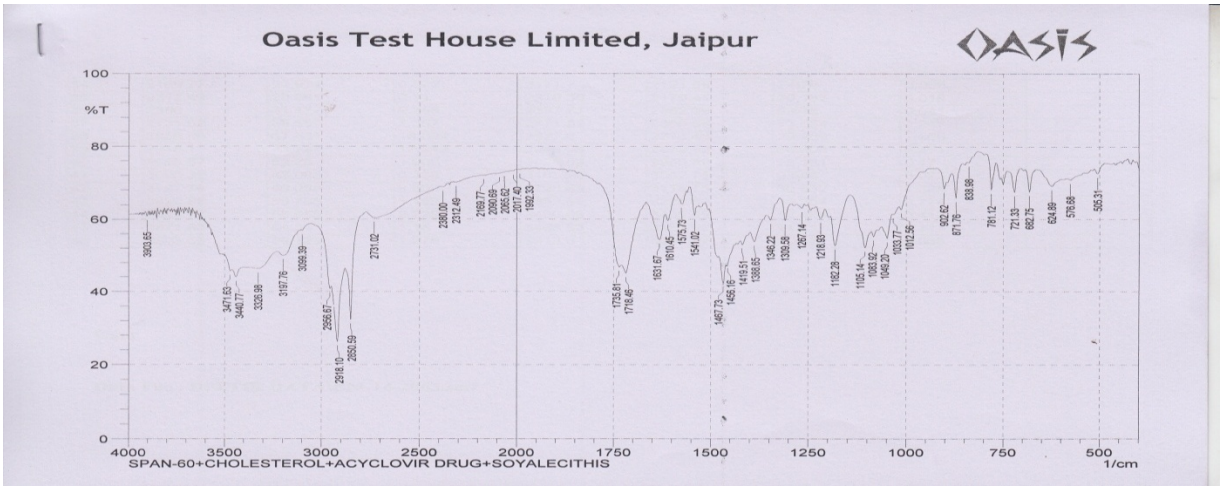

Figure 10: FTIR of Formulation Code PA1

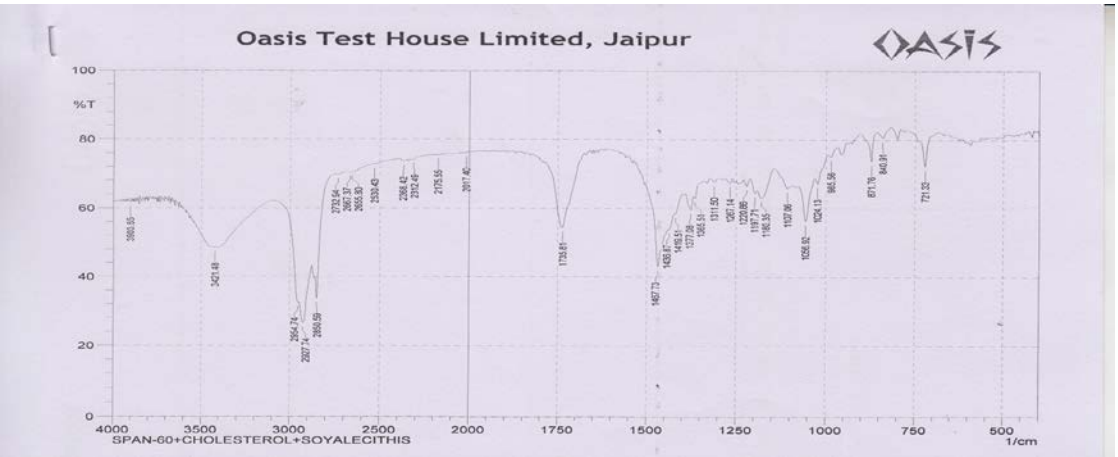

Figure 11: FTIR of Formulation without Drug

DISCUSSION:

Using coacervation phase separation method, acyclovir has been successfully incorporated in proniosomal formulations which can be potentially useful for delivery of this drug. Results of the present work have shown that surfactant type and content of cholesterol and lecithin affect the encapsulation efficiency and drug release rate from proniosomes. A maximum encapsulation efficiency of 89.70% and drug release of 93.80% after 6 hr have been attained. Encapsulation efficiency of proniosomes formed by Span 60 was observed to be higher compared to that obtained with other formulation. Acyclovir proniosomes were also found to be quite stable at 4-8^{o}C over a one-month period. This work has established the foundation for future study on the potential of acyclovir-loaded niosomes for a transdermal delivery system.

CONCLUSIONS:

Proniosomal formulations can be conveniently prepared by coacervation-phase separation technique non-ionic surfactants (span 60, span 40,span 20, tween 20, tween 80), cholesterol and lecithin at different concentrations. The entrapment efficiency of proniosomal formulations with span 60 show highest entrapment efficiency, but in case of proniosomal formulations combination with span 60 and apan 40 show lowest entrapment efficiency. The average vesicular size of niosomes of all the batches was measured in the range of **4.16 µm to 4.18µm**. The size distribution was in the range of **0.5 to 12.5 µm**. The result suggests niosomes prepared were of uniform size and spherical in shape. The microphotographs of all proniosomal formulations revealed that the niosomes were uniform size and spherical in shape. The IR spectral analysis suggested that there is no interaction between the drug and formulation additive, the drug exists in original form and available for the biological action. The drug release from vesicles is dependent on cholesterol and lecithin in proniosomal formulations with span 60 highest in their concentrations. For all the proniosomal formulations the release was extended up to 6 hours Better dissolution profiles were found with proniosomal formulations prepared with span 60 highest drug release. The best fit model is peppas with 'n' value between 0.549 suggesting release by fickian mechanism i.e., the drug was released by diffusion controlled.

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