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Full Length Original Research Paper

In-Vitro and In-Vivo evaluation of Transdermal prolonged release proniosomal gel formulations of Propranolol HCL

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Abstract:
Propranolol HCl, an antihypertensive drug was formulated as transdermal prolonged release proniosomal gel due its drawback of short life when given through oral route. Nine formulations of proniosomes were prepared using different polymers viz. Maltodextrin, span 40, span 60 and cholesterol by slurry, slow stirring method. All were in turn prepared as proniosomal gels by using carbapol 940 by simple stirring. Gels were evaluated for entrapment efficiency, *In-vitro* skin permeation studies, scanning electron microscopy (SEM) analysis, vesicle size analysis, drug excipient interaction studies. Different release models like zero order, first order, Higuchi, Korsmeyer-peppas etc were applied to *in-vitro* drug release data in order to evaluate the drug release mechanisms and kinetics. Among all proniosomal transdermal gel formulation, F5 produced from pronisomal powder containing 10mg of Propranolol HCl , 100mg of cholesterol, 100mg of maltodextin, Span 40 and Span 60 each of 250mg exhibited ideal diffusion characteristics of 20.25% release at 2nd hr followed by controlled release for 12 hrs with 80.5% release at 12th hr. In-vivo evaluation of pharmacokinetic evaluation exhibited remarkable enhancement of elimination half life up to 13.08 hrs.

Keywords: Propranolol HCl, prolonged release, proniosomes, proniosomal gel, Maltodextrin, cholesterol, carbopol 940, Scanning electron microscopy (SEM) analysis, *In-vitro* skin permeation studies, Half- life.

Introduction:

Propranolol HCL (PPLH) is an antihypertensive drug useful for treatment of angina pectoris. It is absorbed from GI tract completely but subjected to considerable hepatic tissue binding and first pass metabolism and possess short half life of 3-6 hrs. The daily dose of the drug is 40-80 mg twice daily and up to160-640mg and hence lead to more side effects[1]. Therefore the present research work is aimed to prepare transdermal controlled and prolonged release proniosomal gel containing 20 mg of PPLH for treatment of angina pectoris with enhanced half life, pharmacokinetic parameters[2] less side effects with one time comfortable application and.

Proniosomes are novel drug delivery systems for oral short acting drugs. They are the dry formulations of niosomes, a nonionic surfactant vescicles and are coated with surfactant. They get converted to noisome dispersions upon hydration and are more stable drug delivery systems for development of both highly lipopholic drugs[3]. In turn proniosomal gels are semisolid liquid crystal gels produced by dissolving proniosomes in minimal amount of suitable solvent like ethanol and hydration with minimum amount of water to form gel. They are liquid crystalline compact hybrids converted into niosomes immediately upon hydration. Use in topical/dermal delivery does not require hydration prior to application, but they can be applied as such or loaded on a base material of emulsion, gel, ointment, etc. prior to application[4].

MATERIALS AND METHODS:

Pure and certified sample of propranolol HCL was gifted by Aurobindo Pharma Ltd, Hyderabad. Span 60, span 40, cholesterol, maltodextrin were obtained from S.D. Fine Chemicals Pvt. Ltd Mumbai. All other chemicals and reagents were of analytical grade.

Preparation and evaluation of propranolol HCl transdermal proniosomal gel:

Proniosomes containing 20 mg of PPLH were prepared by slurry method. Nine formulations Viz., F1 to F9 as given in **Table 1** were prepared. PPLH, Span 40, Span 60 and cholesterol were taken in 100 ml beaker and chloroform: ethanol (2:1) solution was added in small quantities with mixing at each addition till smooth slurry is produced. The slurry was transferred to 100 ml round bottom flask containing the maltodextrin carrier. Additional chloroform: ethanol solution was added to form slurry in case of lower surfactant loading. The flask was attached to rotary flash evaporator maintained at temperature of 45 ± 2ºC and reduced pressure of 600 mm Hg. It was rotated at 60 to 70 rpm to evaporate solvent and continued to obtain dry and free flowing product. These materials were further dried overnight in a dessicator under vacuum at room temperature. This dry preparation referred as 'proniosomes' is stored in air tight container for further use.

All formulations, F1 to F9 were further prepared **as** proniosomal gels by using carbapol 934. The proniosomal powder was dispersed in 10 ml of 2% Carbapol 934 solution with stirring at 100 rpm for 1 hour on magnetic stirrer. This was further neutralized with few drops of 0.5% triethanolamin amine and few drops of 10% glycerine slowly with constant stirring to obtain proniosomal gel.

Table 1: Composition of Proniosomes of Propranolol HCL

Formulation Code	Drug (mg)	Span 60 (mg)	Span 40 (mg)	Cholesterol (mg)	Maltodextrin (mg)
F1	20	500	-	100	100
F2	20	400	100	100	100
F3	20	350	150	100	100
F4	20	300	200	100	100
F5	20	250	250	100	100
F6	20	200	300	100	100
F7	20	150	350	100	100
F8	20	100	400	100	100
F9	20	-	500	100	100

EVALUATION[5] :

Trial formulations of proniosomes were observed for spherical morphology and nine spherical shaped proniosomes were observed under SEM analysis an d further evaluated conerted in to gels evaluated for entrapment efficiency, FTIR analysis, *in vitro* skin permeation studies, vesicle size analysis and pharmacokinetic evaluation of

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS:

All formulations of proniosomes F1-F9 were coated uniformly with gold palladium by using Sputter coater under vacuum (0.1 mm Hg), after fixing the sample in individual stabs and was randomly examined for surface morphology (roundness, smoothness, and formation of aggregates).

ENTRAPMENT EFFICIENCY:

500 mg of the proniosomal gel was weighed and dispersed in distilled water phosphate buffer saline pH 7.4 and warmed for formation of niosomes. The niosome dispersion so obtained was centrifuged at 18,000 rpm for 30 min. The clear fraction obtained was used for the determination of free drug at 288 nm UV spectrophotometrically. (%EE = ((Ct-Cf)/Ct) X 100 where, C_t is the concentration of total drug and C_f is the concentration of free drug.

***In vitro* SKIN PERMEATION STUDIES**

The full-thickness male albino Wistar rat skin removed with a clipper was rinsed with with physiological saline and clamped between the donor and the receptor chamber of vertical diffusion cell with the stratum corneum surface facing donor compartment of vertical diffusion cell. The effective diffusion area of the cell was 2.0 cm^2 and had a receptor volume of 25 ml. The receptor chamber was filled with phosphate buffer saline pH 7.4. The diffusion cell was maintained at 37 ± 1°C and the solution in the receptor chambers was stirred continuously at 600 rpm with the help of magnetic bead. 1 gm of proniosomal gel of Propranolol HCL was gently placed in the donor chamber and spread evenly. 2 ml of the solution in the acceptor chamber was removed for drug content determination and replaced immediately with an equal volume of receptor media. Drug concentration was determined UV spectrophotometrically at 288 nm (y = 0.007x - 0.005, r = 0.997).

VESICLE SIZE ANALYSIS

Vesicle size of promising formulation F5 that exhibited highest drug release was determined. 100mg of proniosomal gel was hydrated by adding saline solution (0.9% solution) in a small glass vial with occasional shaking for 10 min. The dispersion was observed under optical microscope at 100X magnification. The size of 100 vescicles was measured using a calibrated ocular and stage micrometer fitted in optical microscope.

***In-vitro* DRUG RELEASE KINETICS**

In-vitro release kinetics were assessed using zero order (**$C = k_o t$**) , first order (**$LogC = LogC_o - kt / 2.303$**), 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).

DRUG EXCIPIENT INTERACTION STUDIES BY FTIR spectroscopy:

FTIR spectra of pure drug and the promising drug loaded proniosomal powder F5 were obtained on a Perkin-Elmer 841 model FTIR Spectrophotometer equipped with a DTSG detector. Samples were prepared by KBr pressed pellet technique. The scanning range was 4000-400 cm^{-1} and the resolution was 1 cm^{-1}.

PHARMACOKINETIC EVALUATION:

Pharmacokinetic studies were performed for promising proniosomal gel formulation, F5 exhibited good performance at ***In-vitro*** level. Male Wistar rats were stored under standard laboratory conditions (temperature25 ± 2°C and relative humidity of 55 ± 5%). The rats were kept in polypropylene cages (3/cage) with free access

to standard laboratory diet. About 2 cm^2 of skin was shaved on the abdominal side of rats. They were fasted for the period of 12 h for observations of any unwanted effects. Then they were applied with 1 g proniosomal gel in 2% carbopol.

Blood samples were withdrawn at time intervals of 1, 2, 4, 8, 12, 24hrs from retro-orbital venous plexus under ether anesthesia using glass capillaries into sodium citrate containing eppendorf micro-centrifuge tubes. Plasma was separated by centrifugation using Centrifuge and stored in vials at -70°C until further analysis. Propranolol HCL in plasma was determined by RP-HPLC method. Animal studies were approved by animals ethics committee, SPSP, Tirupati.

RESULTS AND DISCUSSION:

Propranolol HCl proniosomal formulations many were prepared using different non ionic surfactants ratio and were observed for spherical shape by SEM analysis for surface morphology. Nine formulations possessing spherical shape were selected as given in **Table 1.** SEM image of all formulations proniosomes have evidenced smooth spherical surface and the SEM image of F5 is shown in **Fig.1 and Fig.2. Hence a**ll nine pronisomal powders were converted to proniosomal gels and further subjected to evaluation parameters such as visualization of vesicles by optical microscope, encapsulation efficiency, *In-vitro* skin permeation studies, drug release kinetics and FTIR specrtoscipy, pharmacokinetic studies.

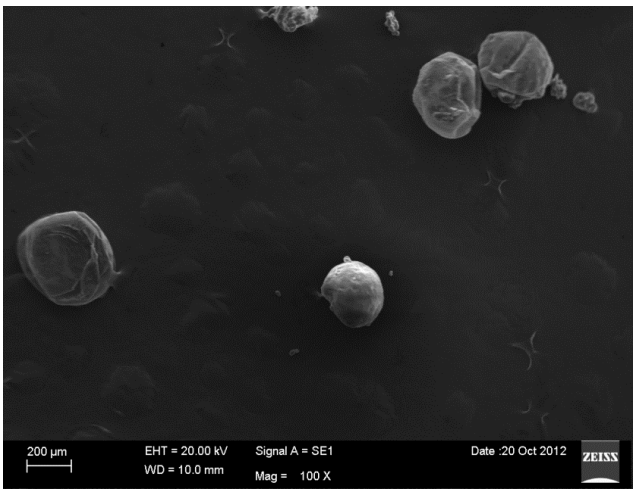

Fig.1: SEM Photography of Proniosomal Powder, (GROUP)

ENTRAPMENT EFFICIENCY:

Entrapment efficiency values for of F1to F9 are given in **Table 2**. The drug entrapment characteristics are good for Span 40 and Span 60 and produced least leaky niosomes that may be due to their highest phase transition temperature. It is indicated that, formulations F5 &F9 possess high EE values **of 85.47 and 88.71% respectively** which may be due to optimum ratio of surfactant to maltodextrin to provide a high entrapment of drug.

Table 2: Entrapment Efficiency of Proniosomal gels (mean±s.d. n=3)

S. No	Formulation Code	Percent Drug Loading
1	F1	62.48±0.015
2	F2	68.63±0.121
3	F3	68.18±0.008
4	F4	83.90±0.012
5	F5	85.47±0.140
6	F6	72.58±0.038
7	F7	84.18±0.116
8	F8	74.37±0.023
9	F9	88.71±0.010

n=3±s.d

In vitro Skin permeation studies:

Drug diffusion data indicating skin permeation is shown in Table 3, 4 and in Fig.2 and 3. Diffusion profiles for all formulations were found to be

linear within a period of 12 hours. Data in terms of %drug release isd shown in Table5. All formulations exhibited similar diffusion characteristics, but F5 was considered as promising formulation because 20% amount of drug released in 2hrs which is deemed to be required for providing initial loading dose with in 2hrs for controlled drug delivery system and also . Formulation F5 evidenced 81.26% of drug release and it is considered as promising formulation with ideal prolonged release for 12 hours.

Table 3: Cumulative amount of drug release for formulations (F1-F5)

S. No	Time (hrs)	Cumulative amount of drug release				
		F1	F2	F3	F4	F5
1	0	0	0	0	0	0
2	1	0.038±0.017	0.040±0.009	0.041±0.011	0.051±0.006	0.117±0.01
3	2	0.069±0.002	0.094±0.018	0.112±0.003	0.123±0.011	0.218±0.009
4	3	0.130±0.008	0.165±0.04	0.182±0.008	0.173±0.01	0.300±0.03
5	4	0.205±0.01	0.213±0.05	0.222±0.03	0.234±0.012	0.351±0.08
6	5	0.296±0.005	0.325±0.012	0.342±0.007	0.325±0.019	0.422±0.011
7	6	0.334±0.010	0.357±0.08	0.368±0.016	0.376±0.002	0.490±0.08
8	7	0.423±0.06	0.440±0.01	0.465±0.02	0.480±0.05	0.559±0.012
9	8	0.537±0.012	0.550±0.012	0.567±0.018	0.571±0.01	0.621±0.017
10	9	0.622±0.009	0.630±0.02	0.668±0.009	0.651±0.08	0.69±0.018
11	10	0.675±0.011	0.670±0.08	0.685±0.03	0.694±0.02	0.759±0.07
12	11	0.706±0.005	0.694±0.05	0.717±0.017	0.734±0.06	0.798±0.01
13	12	0.738±0.008	0.725±0.013	0.749±0.012	0.785±0.01	0.812±0.012

Table 4: Cumulative Amount of Drug Release for Formulations (F6-F9)

S. No	Time (hrs)	Cumulative amount of drug release			
		F6	F7	F8	F9
1	0	0	0	0	0
2	1	0.048±0.01	0.042±0.014	0.038±0.01	0.035±0.02
3	2	0.118±0.012	0.101±0.017	0.099±0.013	0.074±0.01
4	3	0.156±0.008	0.148±0.01	0.139±0.018	0.124±0.017
5	4	0.226±0.011	0.201±0.06	0.194±0.07	0.165±0.015
6	5	0.308±0.09	0.300±0.08	0.289±0.09	0.245±0.08
7	6	0.368±0.05	0.359±0.012	0.357±0.017	0.328±0.011
8	7	0.482±0.01	0.452±0.016	0.448±0.014	0.400±0.02
9	8	0.554±0.05	0.537±0.09	0.518±0.01	0.452±0.05
10	9	0.609±0.07	0.596±0.015	0.552±0.06	0.524±0.014
11	10	0.660±0.016	0.641±0.002	0.613±0.08	0.586±0.019
12	11	0.681±0.08	0.685±0.011	0.664±0.03	0.639±0.002
13	12	0.700±0.015	0.734±0.01	0.717±0.09	0.689±0.004

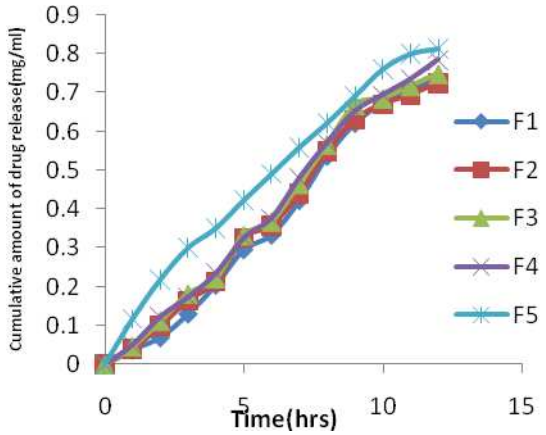

Fig. 2: Cumulative Amount of Drug Release, F1-F5

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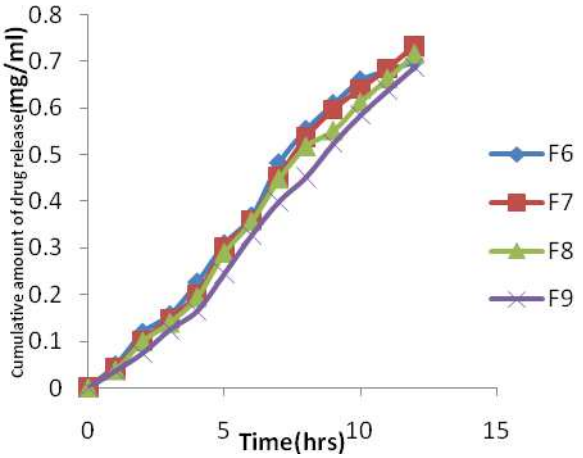


Fig. 3: Cumulative Amount of Drug Release, F6-F9

Vesicle size analysis:

Vesicle size of promising formulation F5 examined by optical microscopy was found to be 4.16 ± 2.1 µm.

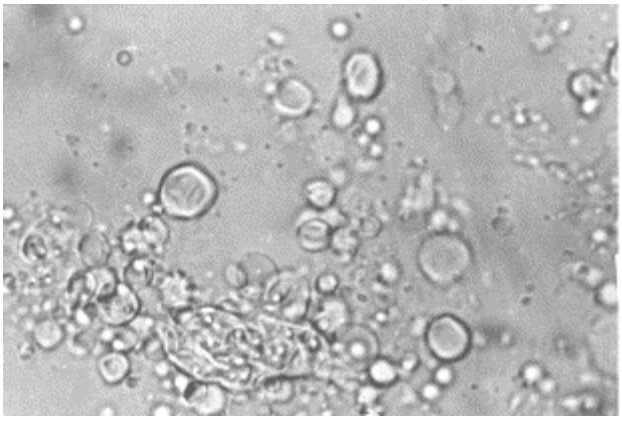
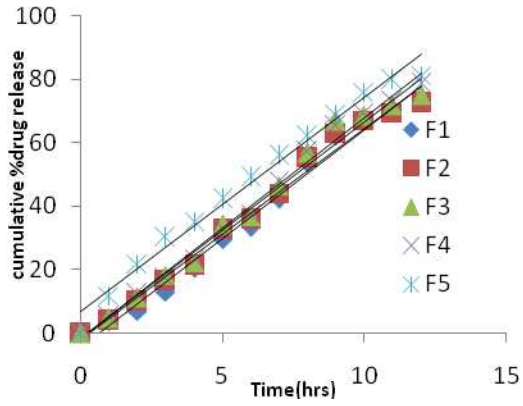


Fig. 5: Niosomal Vesicles under Optical Microscopy

IN-VITRO DRUG RELEASE KINETICS

The *in-vitro* release profiles for all formulations were assessed by zero order, first order, Higuchi and Korsmeyer equation and were shown in table5. Relevant plots are shown in fig 6 to 9.

Fig. 6: ZERO ORDER PLOTS



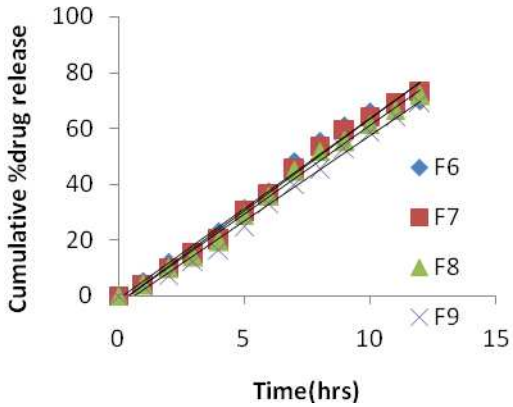
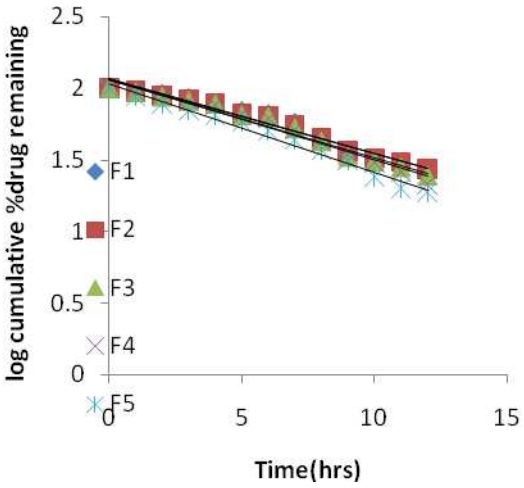


Fig.7: **FIRST ORDER PLOTS**



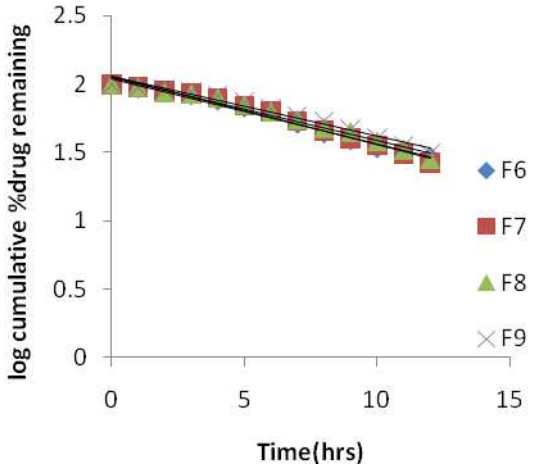
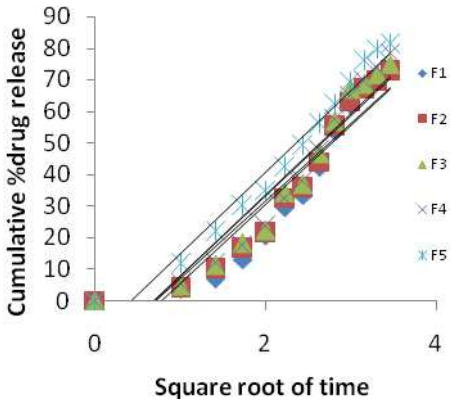
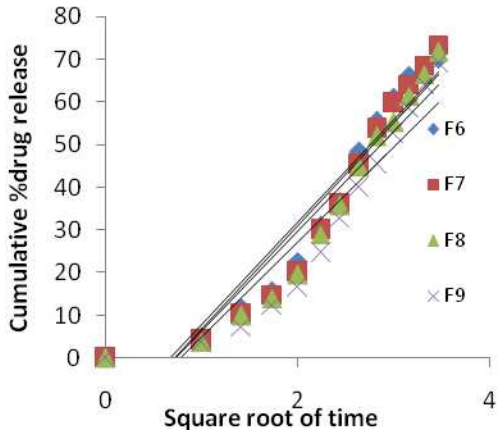


Fig. 8: HIGUCHI PLOTS

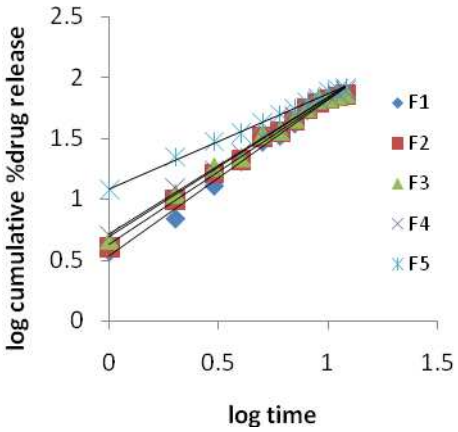


F1-F5



F6-F9

Fig. 9: KORSMEYER-PEPPAS: (F1-F5) **KORSMEYER-PEPPAS: (F6-F9)**



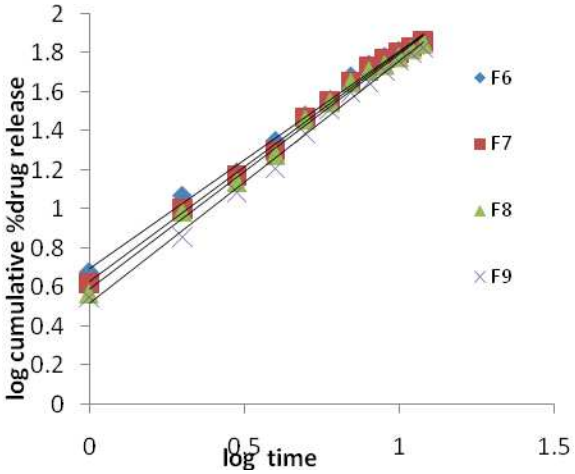


Table 5: Correlation coefficients of different equations of F1-F5 formulations

Formulation code	Zero order		First order		Higuchi	Korsmeyer- Peppas		
	r	K	R	K	r	R	K	N
F1	0.994	15.549	0.979	0.121	0.939	0.991	2.953	1.28
F2	0.993	15.305	0.973	0.117	0.950	0.994	2.747	1.19
F3	0.995	15.712	0.984	0.124	0.952	0.992	2.634	1.14
F4	0.991	16.036	0.984	0.130	0.952	0.995	2.586	1.12
F5	0.994	15.770	0.981	0.142	0.990	0.997	2.609	0.78
F6	0.992	14.833	0.990	0.110	0.955	0.992	2.554	1.10
F7	0.996	15.125	0.987	0.115	0.949	0.995	2.710	1.18
F8	0.997	14.600	0.988	0.106	0.950	0.994	2.764	1.20
F9	0.996	14.089	0.984	0.099	0.939	0.995	2.855	1.24

Linear plots in case of zero order with correlation coefficient values nearing one in **Fig. 6** indicated that the drug release is zero order kinetics. Correlation values shown in **Table 5** of Higuchi's plot revealed that the mechanism of drug release was diffusion. The *in vitro* kinetic data subjected to log time vs log drug release transformation plot (peppa's model),for the best formulation the value lies were found to be0.78 which lies between 0.45 and 0.89, this revealed that the drug release follows non fickian diffusion.

Drug excipient interaction studies (FTIR-ATIR STUDIES):

FTIR spectra of pure PPHL (FIG.) main functional groups in drug as N-H(3323.99), AROMATIC C=C(2967.37), c-n(1323.78) and C-O(1267.51and f5 and in formulation N-H(3382.51), AROMATIC C=C(2921.34), c-n(1359.98) and C-O(1027.62) (Fig.) revealed that there is no appreciable change characteristic absorption peaks of puxtrin, span30 etc when produced as proniosomes.

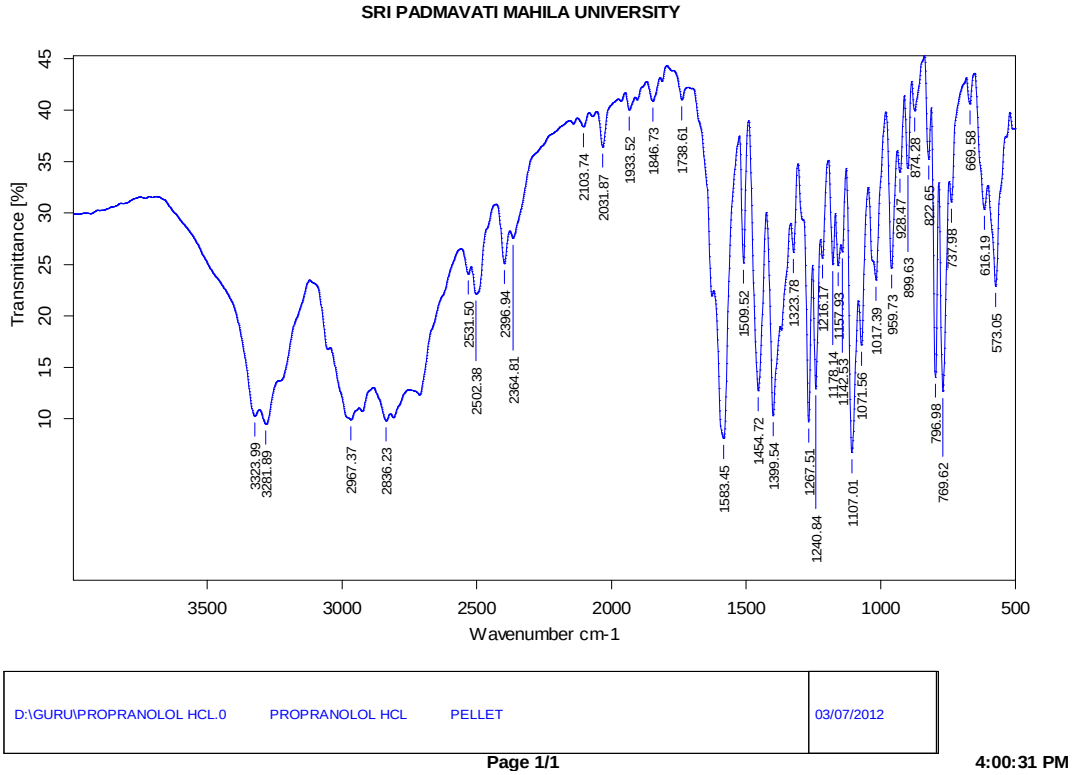


Fig.10: FTIR of Pure drug Propranolol HCL

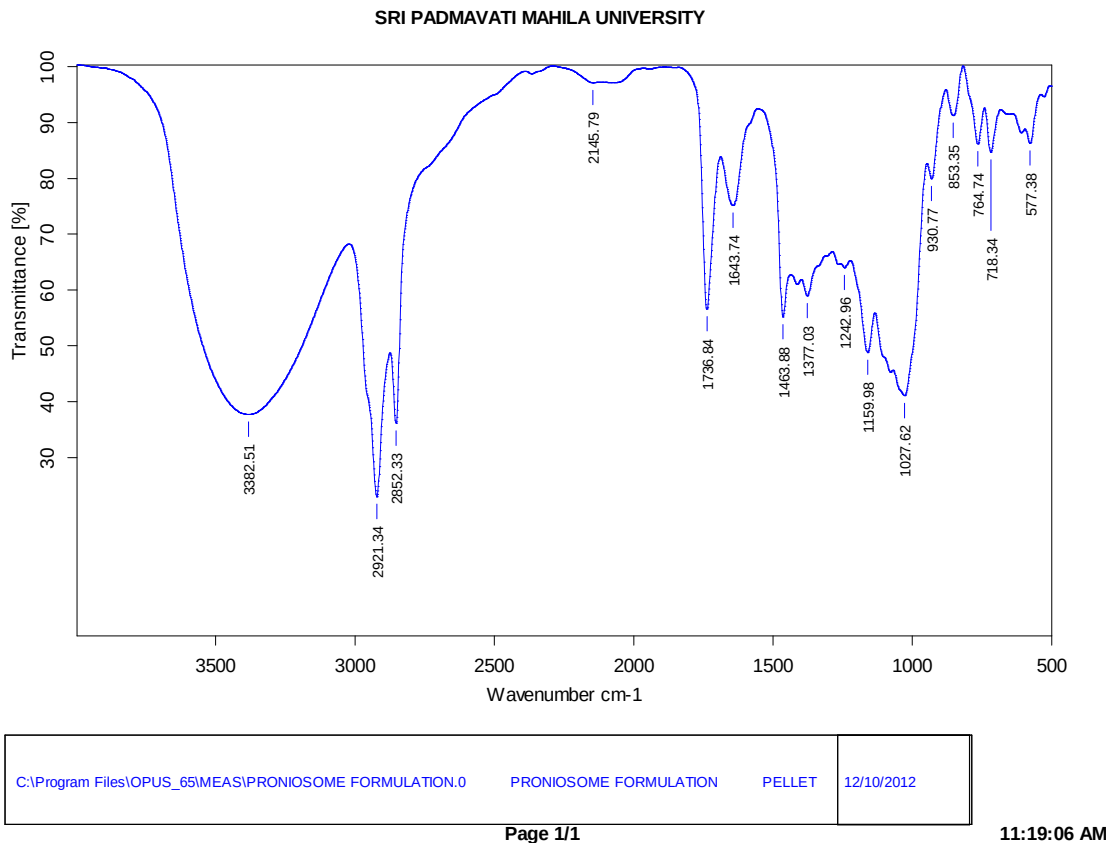


Fig.7: FTIR of Pure drug Propranolol HCL

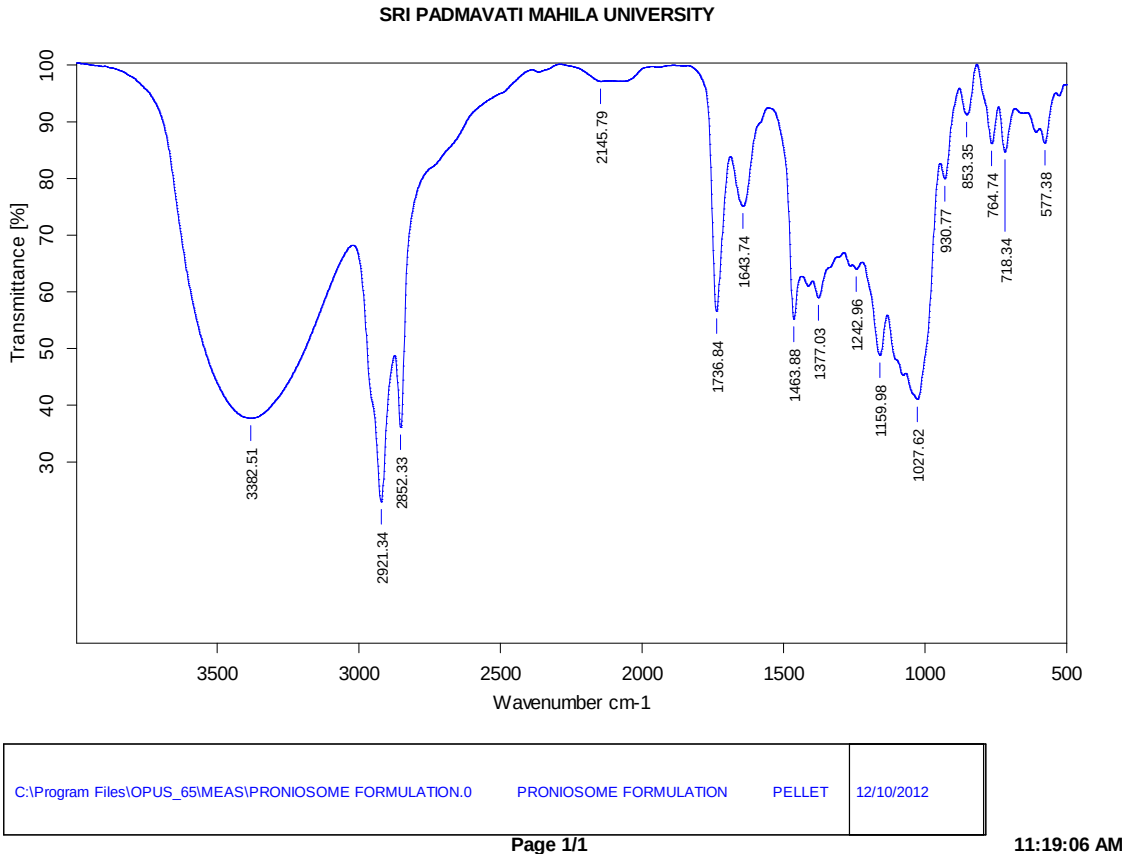
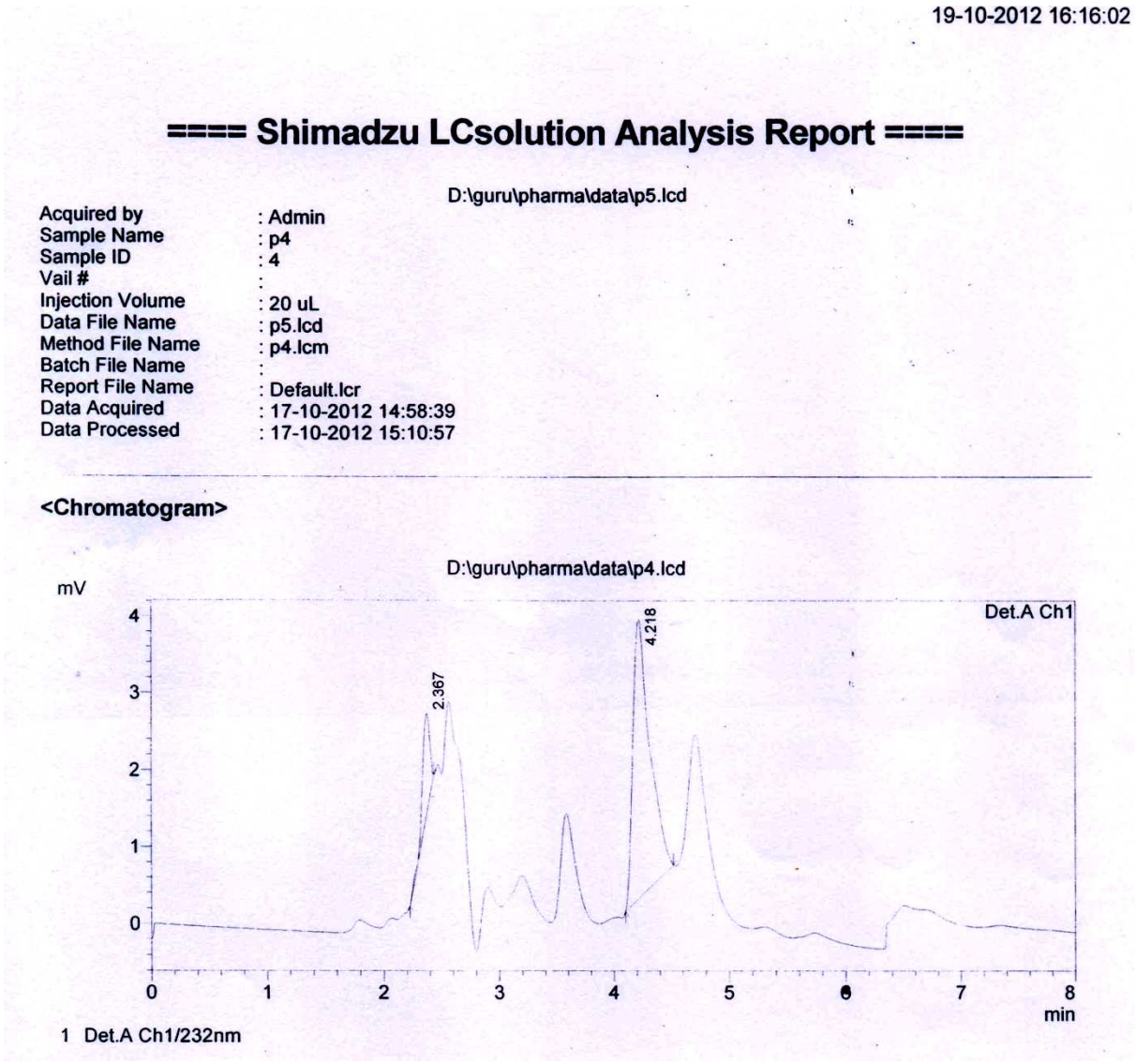


Fig.11: FTIR of Proniosomal gel, F5

6.7. PHARMACOKINETIC EVALUATION:

The pharmacokinetic parameters were calculated from the plasma concentrations of the drug and Peak plasma concentration, C_{max} was found to be 526.24 ng/ml and t_{max} was 12 h. Area under the plasma concentration-time curve , $AUC_{0\rightarrow24}$ was found to be 9403.58 ng-h/ml and$AUC_{0\rightarrow\omega}$ was 14581.86 ng-h/ml. Elimination rate constant (K_e) calculated from semi logarithmic plot was found to be 0.053/h. Elimination half life ($T_{1/2}$) was found to be 13.08 h.



Peak#	Ret. Time	Area	Height	Area%	Height%
1	2.367	5880	1332	17.152	26.746
2	4.218	28401	3647	82.848	73.254
Total		34281	4979	100.000	100.000

Fig. 12: Chromatogram of a rat plasma sample 24 hours after application of propranolol HCL proniosomal gel

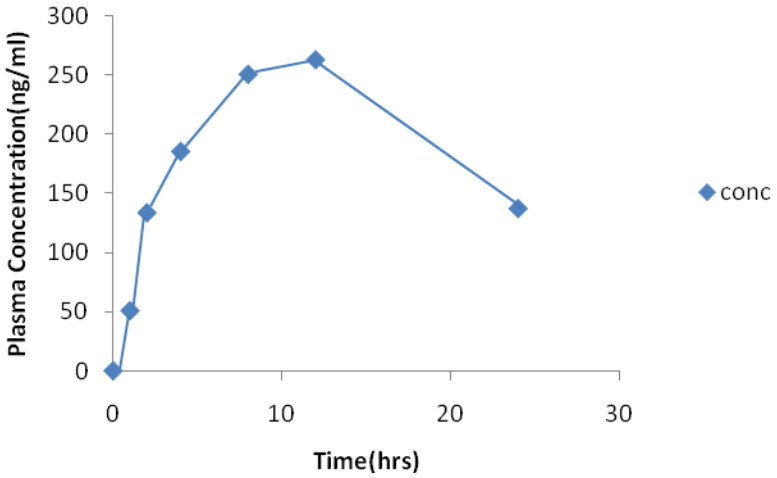


Fig.13: Mean plasma concentration-time curve of Propranolol HCL in rat plasma

Conclusion:

Propranolol HCl was developed successfully as prolonged release transdermal gel using pronisomal powder containing 10mg of Propranolol HCl , 100mg of cholesterol, 100mg of maltodextin, Span 40 and Span 60 each of 250mg optimized after several trials. The prepared gel exhibited of ideal prolonged drug release characteristics of 20.25% release at 2nd hr continued up to 12 hrs. The prepared gel exhibited remarkable enhancement of elimination half life up to 13.08hrs.

References

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