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Formulation and Evaluation of Capecitabine Proniosomal gel

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ABSTRACT

The aim of the present research was to formulate and evaluate proniosomal gel of capecitabine. Capecitabine proniosomal gel was prepared by the coacervation phase separation technique which is most widely adopted for the preparation of proniosomal gels. Preformulation studies like organoleptic characteristics, melting point etc. were carried out. The prepared proniosmal gels were evaluated for clarity, entrapment efficiency, drug content, *in vitro* drug release studies etc. Drug excipient compatibility studies were carried out by FTIR, DSC. Among the various formulations prepared, F8 was found to be the optimized formulation. By fitting the *in vitro* data into various models the best-fit model was found to be Peppas indicating drug release by non Fickian (anomalous) mechanism. From the present study it can be concluded that the prepared proniosmal gel can be a promising drug delivery system for Capecitabine.

Keywords: Capecitabine, Proniosomes, Drug Release, Coacervation Phase Separation

INTRODUCTION

In the past few decades considerable attention has been focused on the development of new drug delivery system (NDDS). The NDDS should ideally fulfil two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body [4] over the period of treatment. Secondly, it should channel the active entity to the site of action. Conventional dosage forms including prolonged release dosage forms are unable to meet none of these. At present, no available drug delivery system behaves ideally [2], but sincere attempts have been made to achieve them through various novel approaches in drug delivery [1]. Approaches are being adapted to achieve this goal, by paying considerable attention either to control the distribution of drug by incorporating it in a carrier system, or by altering the structure of the drug at the molecular level, or to control the input of the drug into the bio-environment to ensure an appropriate distribution profile [3].

Capecitabine is an orally administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. Chemically it is a prodrug of 5'-deoxy-5-fluorouridine, which is enzymatically converted to 5-fluorouracil in the tumour, where it inhibits DNA synthesis and slows growth of tumour tissue. Inspite of its strong therapeutic effect, oral administration is associated with several side effects such as gastrointestinal irritation, edema, dizziness and peptic ulceration when taken orally for a prolonged period.

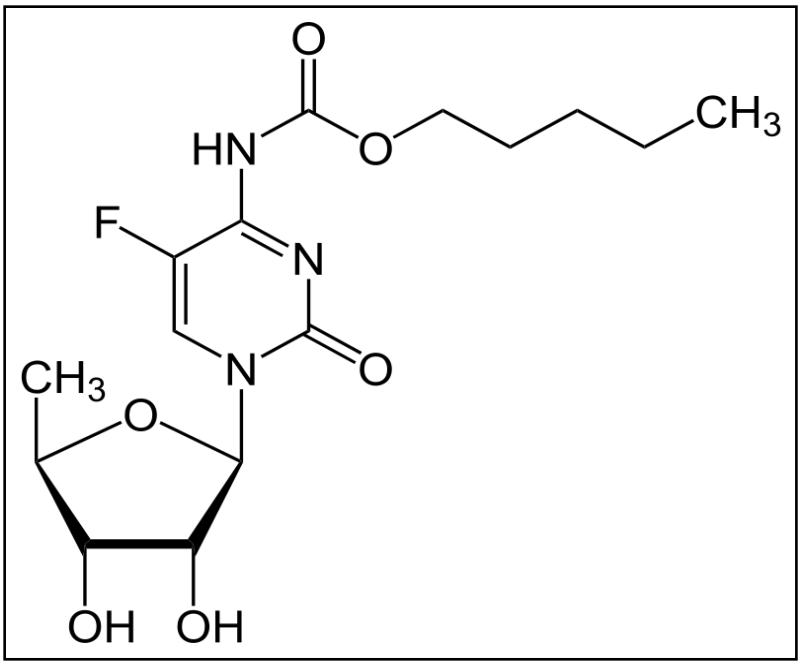

Figure 1: Chemical structure of capecita

TRANSDERMAL DELIVERY

Optimization of drug delivery through human skin is important in modern therapy. Recently the transdermal route compared with oral treatment is the most successful innovative research area in drug delivery [1]. Transdermal delivery of drugs offers advantages of a non-invasive parental route for drug therapy, avoidance of first pass gut and hepatic metabolism, decreased side effects and relative ease of drug input termination. Proniosomes offer a versatile vesicle drug delivery concept with potential for delivery of drugs via transdermal route. Provesicular approach has been proposed to enhance the stability of Proniosomes are provesicular approach which overcome the limitations of vesicular Niosomes. Proniosomes can be converted into the niosomes in-situ by absorbing water from the skin. Proniosomes are water-soluble carrier particles that are coated with surfactant and can be hydrated to form niosome dispersion immediately before use on brief agitation in hot aqueous media. Resulting niosomes are very similar to conventional niosomes and more uniform in size.

The present research work is aimed to formulate and evaluate proniosmal gel for capecitabine which will have the potential of controlled drug release, increased drug stability and higher drug load.

MATERIALS AND METHODS

Materials used

All the materials and equipment's used in the formulation, evaluation and other experiments are given below.

Table no. 1 List of materials used with supplier details

Category	Chemical name	Supplier
Drug		Chandra Labs Hyderabad, India
Phospholipid	Soya Lecithin	Bright Laboratories
Surfactants	Span 80,tween 80,span 60 and tween60	Merck specialties pvt. limited (Mumbai)
Volatile solvents	Ethanol , Chloroform	S.D.Fine Chemicals, Mumbai
Gelling agent	Carbopol-940	Research lab fine chem. Industries(Mumbai)

INSTRUMENTS USED

Table no. 2 List of Equipment's used with Manufacturers details

INSTRUMENTS	SUPPLIER	MODEL
FT-IR spectrophotometer	BRUKER	ALPHA-T-1020
UV-Visible spectrophotometer	Lab India	UV 3200
Hot air oven	Universal	Q-5247
Electronic balance	Shimadzu	AX-200
Centrifuge	Remi	TROI
Probe sonicator	Heldolph	VCX750
PH meter	Labindia	SAB 5000
Magnetic stirrer	Remi	5MLH
Weighing balance	Shimadzu	ATX224
Homogenizer	Remi	RQT-124A

Pre-formulation study

Preformulation may be described as a phase of the research & development process where the formulation scientist characterizes the physical, chemical and mechanical properties of new drug substances, in order to develop stable, safe and effective dosage forms. Ideally the Preformulation phase begins early in the discovery process such the appropriate physical, chemical data is available to aid the selection of new chemical entities that enter the development process. During this evaluation possible interactions with various inert ingredients intended for use in final dosage form are also considered in the present study [5].

Organoleptic Properties of Capecitabine

Colour: white crystalline powder

Melting Point: The melting point was determined by using Thiele's tube apparatus method.

M.P Range: 110-121ºC

FT-IR studies for drug and excipients compatibilities

Prior to the development of the dosage forms the preformulation study was carried out. IR spectral

studies aid more in the qualitative identification of substances either in pure form or in combination with polymers and excipients and acts as a tool in establishing chemical interaction [6]. Since I.R. is related to covalent bonds, the spectra can provide detailed information about the structure of molecular compounds. In order to establish this point, comparisons were made between the spectrum of the substances and of the pure compound [7]. FTIR spectra were recorded with a Thermo Nicolet. Japan in the range 400–4000 cm−1 using a resolution of 4 cm−1 and 16 scans. Samples were diluted with KBr mixing Powder, and pressed to obtain self-supporting disks. Liquid samples formulations were analyzed to form a thin liquid film between two KBr disks [8].

Calibration curve of Capecitabine

100 mg of Capecitabine was dissolved in 10 ml of ethanol then volume was made up to 100ml with Phosphate buffer at pH of 6.8. Concentration of 1000 µg/ml of Capecitabine as stock solution. Adequate quantities of aliquots were sampled out from standard solution in 10 volumetric flasks to get concentration of 10 to 50 µg/ml of Capecitabine [9]. The absorbance of prepared solution of Capecitabine was measured at 304nm in Shimadzu UV/visible 1700 spectrophotometer against the blank [10]. The absorbance data for standard calibration curve are given in Table 6 and plotted graphically as shown in the Figure [6]. The standard calibration curve yields a straight line, which shows that drug obeys Beer's law in the concentration range of 10-50 µg/ml /ml [12].

Formulation procedure

- Proniosomal gel was prepared by phase separation coacervation technique.
- Precisely weighed amount of drug, surfactant, soya lecithin and cholesterol in a specified ratio were taken in a dry, clean, wide mouth small test tube.
- A measured amount of ethanol (absolute alcohol) was added to test tube to dissolve the ingredients.
- The open end of test tube was covered with a lid to prevent loss of solvent from it warmed over water bath at 67±3^{0}C for about 5 minute until the surfactant mixture was dissolved completely.
- Then the aqueous phase phosphate buffer saline (pH 6.8) was added and warmed on a water bath till a clear solution was formed.
- The clear solution formed was cooled to room temperature to convert it to a gel known as Proniosomal gel.
- The gel obtained was preserved in the same glass tube in dark for characterization.

Table no. 3 composition of different Proniosomal formulations by co acervation method

Ingredients(mg)	F1	F2	F3	F4	F5	F6	F7	F8
Capecitabine	20	20	20	20	20	20	20	20
Soya lecithin	450	450	450	450	225	225	225	225
Span 80	450	--	--	--	450	--	--	--
Span 60	--	450	--	--	--	450	--	--
Tween 60	--	--	450	--	--	--	450	--

Tween 80	--	--	--	450	--	--	--	450
Cholesterol	50	50	50	50	100	100	100	100
Ethanol (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Observation	**Gel formed**	**Gel formed**	**Gel formed**	**Gel formed**	**Gel formed**	**Gel formed**	**Gel formed**	**Gel formed**

Preparation of topical Proniosomal gel

- As a vehicle for incorporation of Proniosomal gel for topical delivery, carbopol gels were prepared.
- Proniosomal aqueous dispersion was utilized for the formulation of topical gel. Gel polymer such as carbopol 940 was utilized to prepare Proniosomal gel.
- 1.5g of carbopol- 940 powder was dispersed into vigorously stirred (stirred by magnetic stirrer Remi 5MLH) distilled water (taking care to avoid the formation of indispersible lumps) and allowed to hydrate for 24 hrs.
- The dispersion was neutralized with tri ethanolamine to adjust the pH [6.8] by using pH meter (Lab India Sab 5000).
- Appropriate amount of proniosomes containing capecitabine was then incorporated into gel-base with continuous stirring until homogenous formulation was achieved.

Evaluation of Topical Gel

Formulated gel was evaluated for their physico-chemical properties, *in vitro* release studies, drug content and drug entrapment studies.

Clarity

The clarity of various formulations was determined by visual inspection under black and white background and it was graded as- turbid +, clear ++, very clear (glassy) +++.

Measurement of pH

The pH of Capecitabine gel formulation was determined by using digital pH meter. 1gram of gel was dissolved in 100ml of distilled water. The measurement of pH of each formulation was done in triplicate and average values were calculated.

Homogeneity

All developed gels were tested for homogeneity by visual inspection after storage in the container for their appearance and presence of any aggregate.

P^h Determination

The pH of the each formulation was measured by a digital pH meter standardized using pH 4.0 and 7.0 standard buffers.

Rheological characterization

The rheological studies of samples were carried out with Brookfield Digital viscometer (LV DV-E model) using S-18 spindle number. The developed formulations were poured into the small sample adaptor of the Brookfield viscometer and the angular velocity increased gradually from 0.5 to 100 rpm.

Drug content

Proniosomes equivalent to 20 mg were taken into a standard volumetric flask. They were lysed with 25 ml of methanol by shaking for 15 min. Then 10 ml of this solution was diluted to 100 ml with phosphate buffer 6.8. Aliquots were withdrawn. The absorbance was measured at 230 nm and drug content was calculated from the calibration curve.

The drug content was determined by using following equation

Drug content = (concentration× volume taken) × conversion factor

Determination of percentage entrapment efficiency

0.5 g of proniosomal gel was weighed in a glass tube and 10 ml of the aqueous phase (phosphate buffer pH 6.8) was added; the aqueous suspension was then ultra sonicated. Niosomes containing capecitabine were separated from un- entrapped drug by centrifugation at 9000 rpm for 45 min at 4^{o}C. The supernatant was recovered and assayed spectrophotometrically, using Shimadzu UV spectrophotometer (Japan) at 230 nm. The

encapsulation efficiency was calculated by the following equation.

% Encapsulation efficiency = {Total drug - (unencapsulated drug / total drug)} ×100

In vitro diffusion studies

The in vitro diffusion study of prepared gel was carried out in Franz diffusion cell using through an egg membrane. 18 ml of phosphate buffer was taken in as receptor compartment, and then 1 gm. capecitabine gel was spreaded uniformly on the membrane. The donor compartment was kept in contact with a receptor compartment and the temperature was maintained at 37±0.5^{0}C. The solution on the receptor side were stirred by externally driven Teflon coated magnetic bars at predetermined time intervals, pipette out 5 ml of solution from the receptor compartment at specified time intervals for up to 24hrs and immediately replaced with the fresh 5 ml phosphate buffer. The cumulative % release of drug was calculated against time.

Kinetic studies

The results of *in-vitro* release profile obtained for all formulations were plotted in modes of data treatment as follows:

1. Cumulative percent drug release V/s. Time (Zero-order).
2. Cumulative percent drug release V/s. Square root of Time (Higuchi Matrix Model).
3. Log Cumulative percent drug retained V/s. Time (First-order).
4. Log Cumulative percent drug release in V/s. log Time (Korsmeyer-Peppas Model).

Zero order kinetics

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation.

Qt = Qo + Ko t

Where Qt = amount of drug dissolved in time t, Qo = initial amount of drug in the solution and Ko = zero order release constant.

First order kinetics

To study the first order release kinetics the release rate data were fitted to following equation

Log Qt = log Qo + Kt / 2.303

Where Qt is the amount of drug released in time t, Qo is the initial amount of drug in the solution and K1 is the first order release constant.

Higuchi model

Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media, the equation is

Q t = KH. t 1/2

Where Q t = Amount of drug released in time t, KH = Higuchi dissolution constant

Korsmeyer and Peppas release model

To study this model the release rate data are fitted to the following equation

Mt / M = K.tn

Where Mt / M is the fraction of drug release, K is the release constant, t is the release time and n is the Diffusion exponent for the drug release that is dependent on the shape of the matrix dosage form.(Paulo C et al., 2001)

A plot of log drug release verses log time will be linear with slope of n and intercept gives the value of log k

- n = 0.5 indicating pure fickian diffusion.
- n = 0.5-1 or 0.45-0.89 indicating non- fickian diffusion i.e. the rate of solvent penetration and drug release are in the same range.
- n = 0.89 or 1 indicate zero order release which can be achieved when drug diffusion is rapid compared to the constant rate of solvent induced relaxation.(Grassi.M., 2005)

RESULTS AND DISCUSSION

Preformulation studies

Table no. 4 Physical Evaluation

S. No	Description	Method Evaluated	0^{th} day	1^{st} week	2^{nd} week
1	Capecitabine	Physical Evaluation	White Crystalline powder	White Crystalline Powder	White Crystalline Powder

Table no. 5 Melting Point

Range	Result
$110\text{-}123^0$ C	117^0 C

The melting point of Capecitabine was found to be of 117 ^{0} C which is in the range of reported values.

Drug –excepient Compatibility studies

The drug and polymers were characterized by FTIR spectral analysis for any physical as well as chemical alteration of the drug characteristics. From the results, it was concluded that there was no interference in the functional groups as the principle peaks of the Capecitabine were found to be unaltered in the spectra of the drug-polymer mixture.

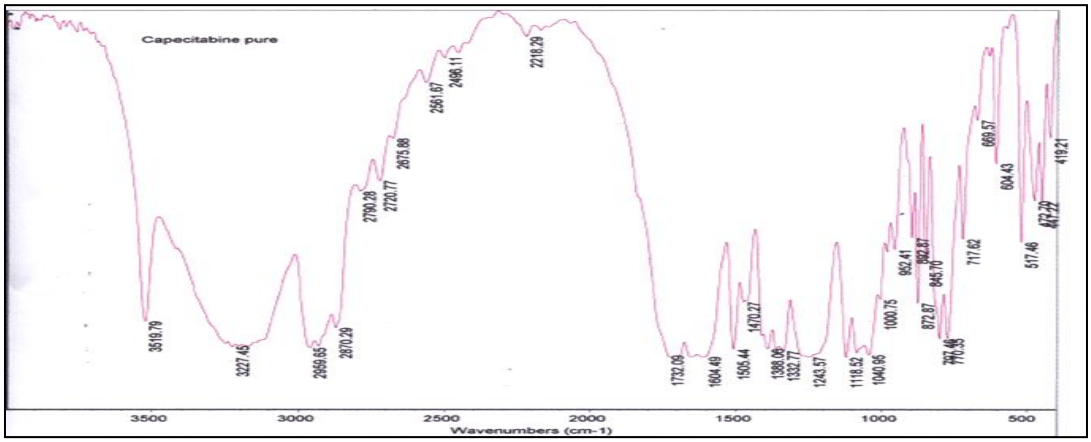

Figure 2: FTIR of Capecitabine pure drug

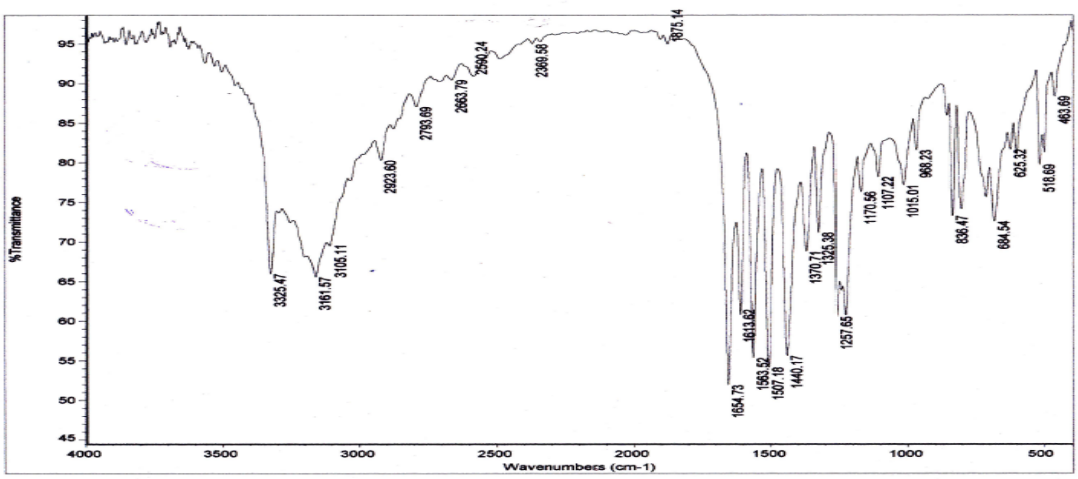

Figure 3: FTIR of Capecitabine optimized formulation

DSC studies

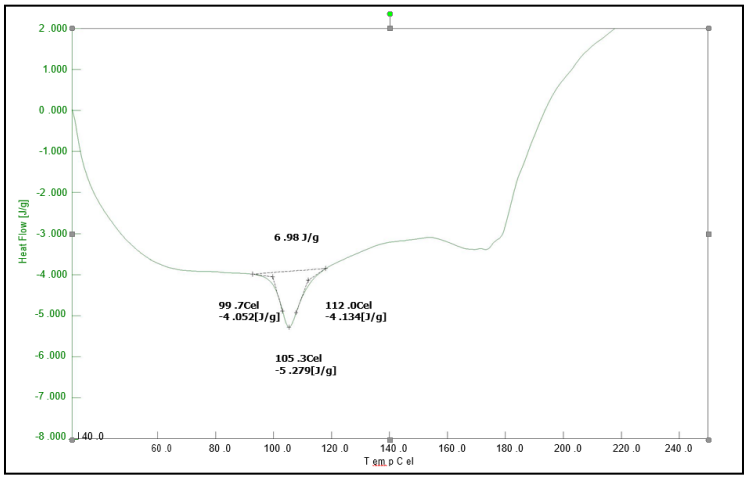

Figure 4: DSC of pure drug

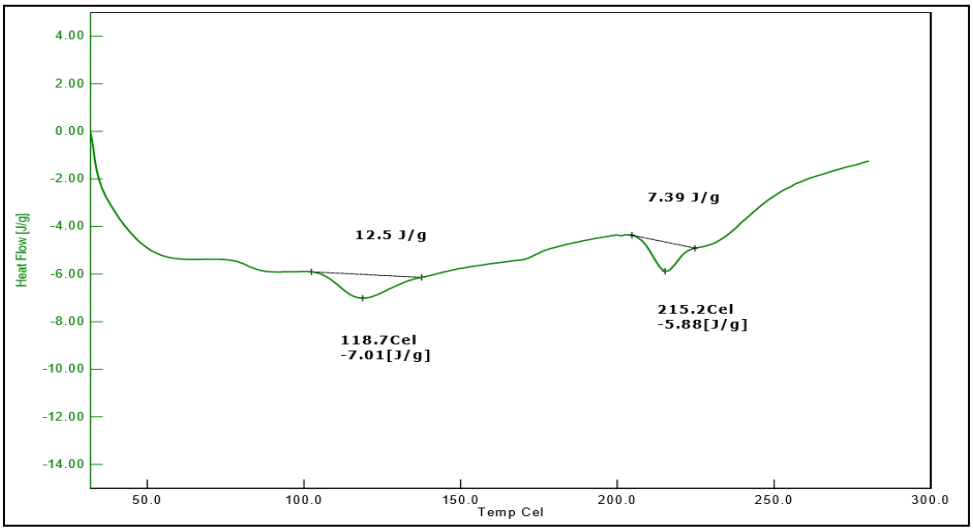

Figure 5: DSC of mixture

Calibration curve

λMax of Capecitabine 304 nm, it obeys beers law in the concentration range 10-50µg/ml.

Table no. 6 Calibration curve data of Capecitabine

S.No	Concentration (µg/ml)	Absorbance at 304nm
1	0	0
2	10	0.267
3	20	0.451
4	30	0.655
5	40	0.843
6	50	0.992

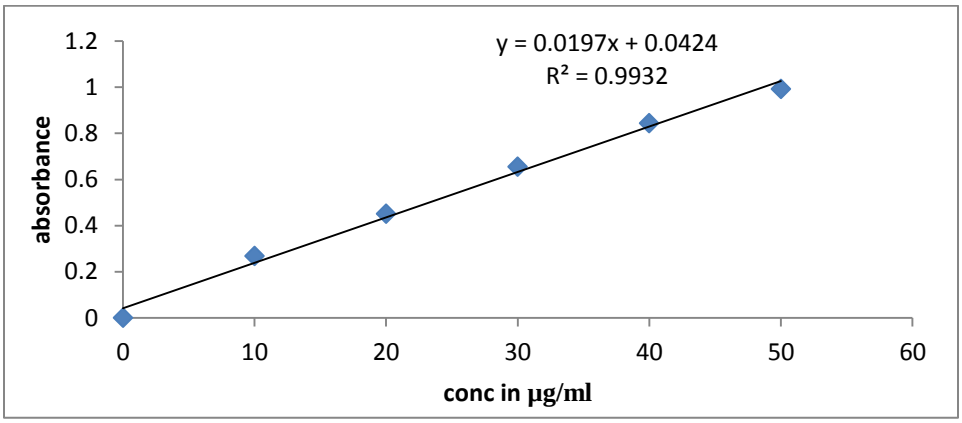

Figure 6.Calibration curve of Capecitabine

Evaluation of Gels

Clarity

Proniosomes containing gels were found to be sparkling and transparent were found to be translucent and white viscous. All gels were free from presence of particles

P^H

The pH value of all developed formulations of gels were in the range of 6.2– 6.7.

Homogeneity

All developed showed good homogeneity with absence of lumps. The developed preparations were much clear and transparent.

Viscosity measurement

The viscosity of various formulated Capecitabine gels was measured using a Brookfield viscometer. The rheological behaviour of all formulated gels systems was studied. In gel system, consistency depends on the ratio of solid fraction, which produces the structure to liquid fraction. Viscosity of various formulated gels was found in range of 1560 to 1791 centipoises.

Drug content

The percentage drug content of all prepared gel formulations were found to be in the range of 96.00–99.6 %. The percentage drug contents of formulations were found satisfactory. Hence methods adopted for gels formulations was suitable.

Table no. 7 Values of evaluation parameters of developed gel

Formulation code	Clarity	pH	Homogeneity	Viscosity (cps)	%Drug Content	%drug entrapped
F1	+++	6.4	Good	1560	96.7	92.8
F2	+++	6.5	Good	1625	95.3	83.3
F3	+++	6.4	Good	1763	96.0	87.3
F4	++	6.5	Good	1656	96.8	89.1
F5	+++	6.2	Good	1577	96.3	82.4
F6	++	6.7	Good	1770	97.4	84.6
F7	+++	6.6	Good	1654	97.1	81.2
F8	++	6.5	Good	1653	97.1	80.1

Entrapment efficiency

Once the presence of vesicles was confirmed in the Niosomal system, the ability of vesicles for entrapment of drug was investigated by ultra-centrifugation. Ultra-centrifugation was the method used to separate the Niosomal vesicles containing drug and un-entrapped or free drug, to find out the entrapment efficiency.

The maximum entrapment efficiency of Niosomal vesicles as determined by ultracentrifugation was 93.1% for Niosomal formulation F14.

In vitro drug diffusion studies

In vitro drug release studies were carried out on dissolution test apparatus Franz diffusion cell. These release studies revealed that, the order of release was found to be first order.

Table no. 8 *In-Vitro* drug release of Proniosomal gel formulations (Coacervation method)

Time(hr)	F1	F 2	F 3	F 4	F 5	F 6	F 7	F 8
0	0	0	0	0	0	0	0	0
1	4.7	4.5	2.5	3.4	4.1	6.1	3.2	2.9
2	7.3	6.2	4.6	5.2	5.8	11.67	5.8	4.4
3	8.6	8.1	8.6	7.9	7.8	16.23	7.5	6.1
4	12.2	16.3	15.0	12.6	15.3	21.48	12.7	12.4
5	16.4	21.8	29.8	23.8	22.1	29.64	24.3	25.2
6	27.1	33.5	46.4	53.6	35.6	34.79	45.1	47.6
8	48.6	46.7	64.1	61.2	45.4	47.16	63.6	65.9
10	59.3	64.78	72.9	76.3	66.5	53.65	73.5	87.6
12	79.4	73.10	84.1	85.6	75.5	66.10	81.4	96.3

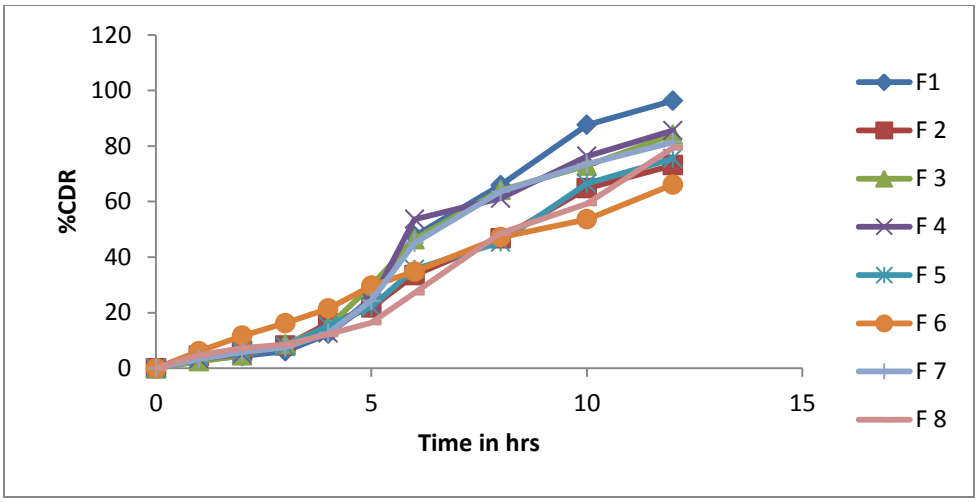

Figure 7: Graph showing in vitro drug release for proniosomal formulations by coacervation method

Kinetic studies

The *in vitro* drug release data of optimized formulation was fit into various models like zero order first order, higuchi, koresmayer peppas model

Table no. 9 Release kinetics for optimized F8 formulation

	ZERO	FIRST	HIGUCHI	PEPPAS
	% CDR Vs T	Log% Remain Vs T	%CDR Vs √T	Log C Vs Log T
Slope	4.676685083	-0.06633457	24.9592609	1.47576491
Intercept	4.441546961	2.098220458	-20.1286047	0.262964082
Correlation	0.89804441	-0.97818203	0.91514192	0.953581567
R 2	0.806483762	0.9568401	0.837484733	0.909317805

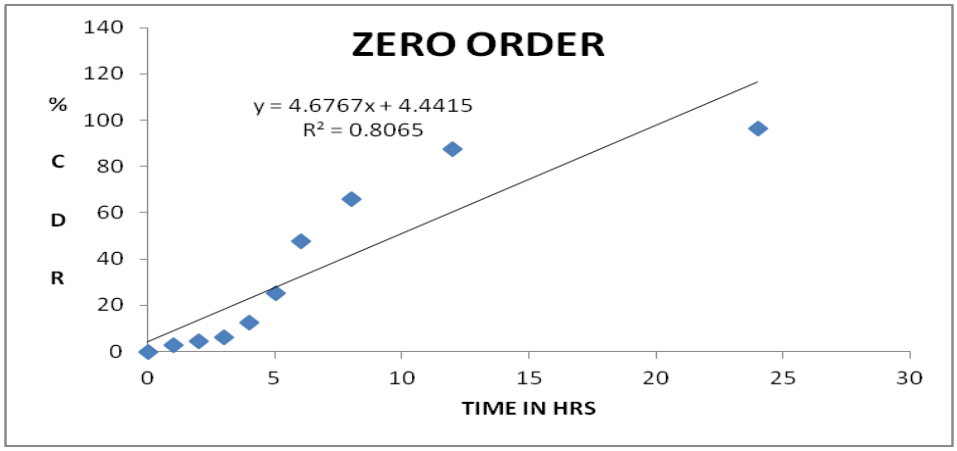

Figure 8: Zero order graph of optimized formulation

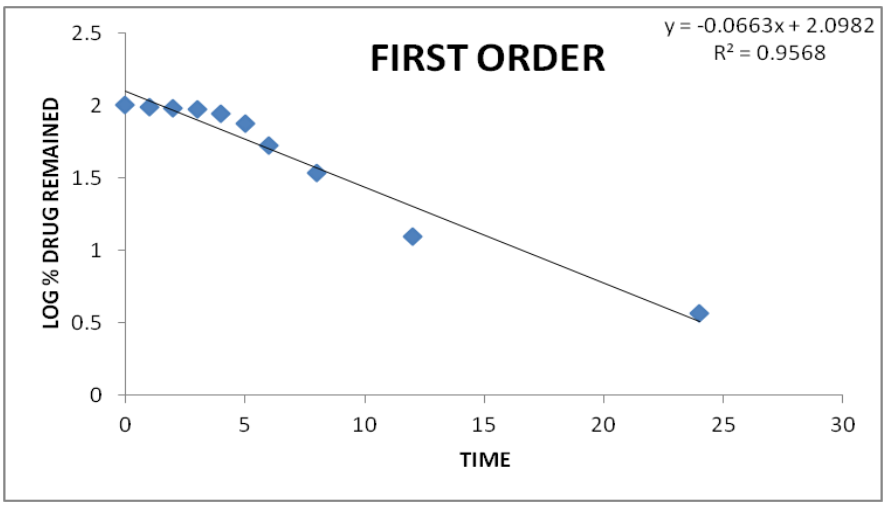

Figure: 9 First order graph of optimized formulation

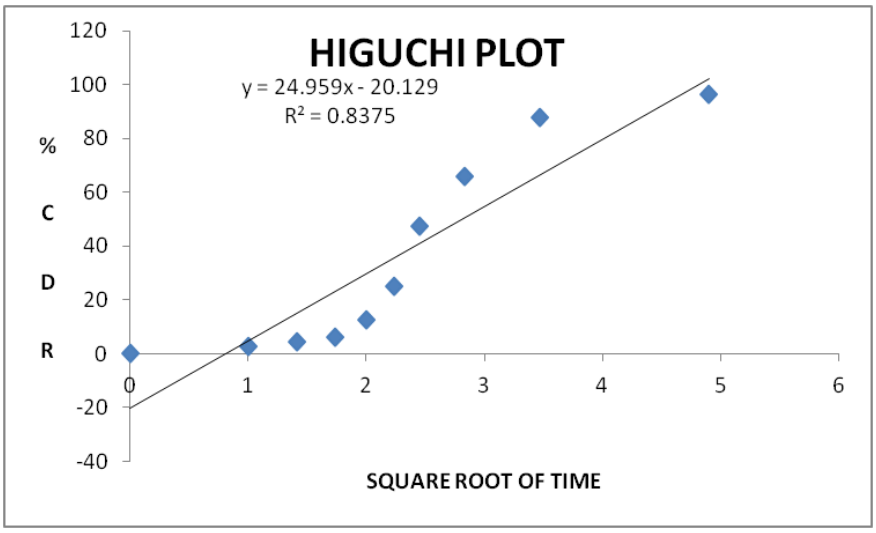

Figure 10: Higuchi graph of optimized formulation

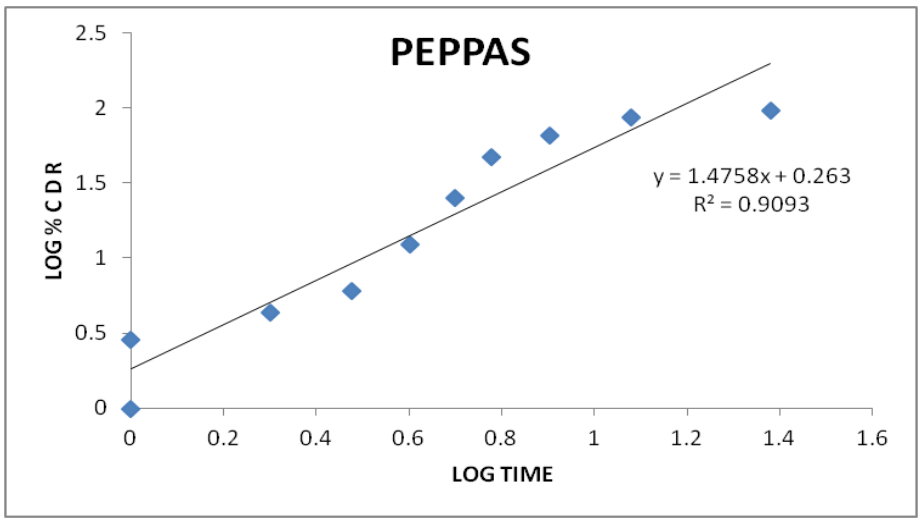

Figure 11: Peppas graph of optimized formulation

CONCLUSION

It is well known that if drug molecules presenting any difficulties in its solubility and bioavailability along the GI tract, are candidates for other routes of administration and if the site of action for drug candidate is sub dermal, an effective penetration enhancers are required to provide the drug molecule deeper into skin tissue for optimized therapeutic delivery of drug. It is generally agreed that classic liposomes are of little or no value as carriers for transdermal drug delivery because they do not penetrate the skin.

Recently derived Niosomal system can deliver drug molecules into and through the skin. In the present study an attempt was made to formulate and evaluate Niosomal system of Capecitabine. Estimation of Capecitabine was done in Phosphate buffer 6.8 spectrophotometrically at 304nm. The preformulation studies include identification, melting point, pH calibration and drug excipient compatibility studies were carried out.

All the gels were evaluated for their appearance, pH, drug content, rheological properties, drug entrapments study and in-vitro release (Franz diffusion cell using through an egg membrane). Visually gels were sparkling & transparent.

The following conclusions are drawn from the result and discussion described in the previous chapter. Promising results were obtained with F8 formulation containing tween 80 with Soya Lecithin because of the highest entrapment efficiency and high localization in the stratum corneum than the span with Soya Lecithin. However Niosomes prepared by phase separation coacervation method were more uniform and small in size when compared to that of the other methods, which is essential for skin penetration. The proniosomes on hydration with phosphate buffer produced niosomal dispersions. The *in vitro* drug release revealed the formulations followed by slow sustained release of the drug for 24 h.

These findings are very encouraging and confirm that proniosomes are a very promising carrier for the topical administration due to the enhanced delivery of drugs through the skin thus prompting various opportunities for the development of suitable therapeutic strategies through the topical route. The formulation is easy to scale up as the procedure is simple and do not involve lengthy procedure and unnecessary use of pharmaceutically unacceptable additives.

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