Journal of Pharmacreations

PharmaCreations

Pharmacreations | Vol.4 | Issue 1 | Jan- Mar- 2017

Journal Home page: www.pharmacreations.com

Research article ***Open Access***

Formulation and evaluation of capecitabine proniosomes

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ABSTRACT

The objective of the study was to assess the bioequivalence of two tablet formulations of capecitabine and to explore the effect of age, gender, body surface area and creatinine clearance on the systemic exposure to capecitabine and its metabolites. *Methods*: The study was designed as an open, randomized two-way crossover trial. A single oral dose of 2000 mg capecitabine was administered on two separate days to 25 patients with solid tumors. On one day, the patients received four 500-mg tablets of formulation B (test formulation) and on the other day, four 500-mg tablets of formulation A (reference formulation). The washout period between the two administrations was between 2 and 8 days. After each administration, serial blood and urine samples were collected for up to 12 and 24 h, respectively. Unchanged capecitabine and its metabolites were determined in plasma using LC/MS-MS and in urine by NMRS. *Results*: Based on the primary pharmacokinetic parameter, $AUC_{0-\infty}$ of 5′-DFUR, equivalence was concluded for the two formulations, since the 90% confidence interval of the estimate of formulation B relative to formulation A of 97% to 107% was within the acceptance region 80% to 125%. There was no clinically significant difference between the t_{max} for the two formulations (median 2.1 versus 2.0 h). The estimate for C_{max} was 111% for formulation B compared to formulation A and the 90% confidence interval of 95% to 136% was within the reference region 70% to 143%. Overall, these results suggest no relevant difference between the two formulations regarding the extent to which 5′-DFUR reached the systemic circulation and the rate at which 5′-DFUR appeared in the systemic circulation. The overall urinary excretions were 86.0% and 86.5% of the dose, respectively, and the proportion recovered as each metabolite was similar for the two formulations. The majority of the dose was excreted as FBAL (61.5% and 60.3%), all other chemical species making a minor contribution. Univariate and multivariate regression analysis to explore the influence of age, gender, body surface area and creatinine clearance on the log-transformed pharmacokinetic parameters $AUC_{0-\infty}$ and C_{max} of capecitabine and its metabolites revealed no clinically significant effects. The only statistically significant results were obtained for $AUC_{0-\infty}$ and C_{max} of intact drug and for C_{max} of FBAL, which were higher in females than in males. *Conclusion*: The bioavailability of 5′-DFUR in the systemic circulation was practically identical after administration of the two tablet formulations. Therefore, the two formulations can be regarded as bioequivalent. The variables investigated (age, gender, body surface area, and creatinine clearance) had no clinically significant effect on the pharmacokinetics of capecitabine or its metabolites.

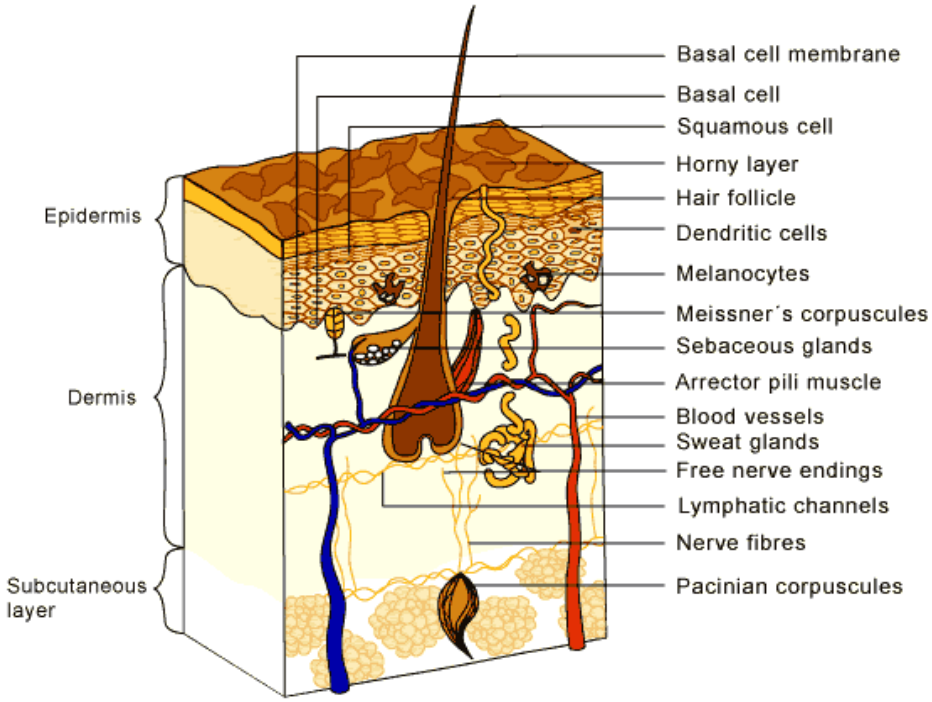
INTRODUCTION

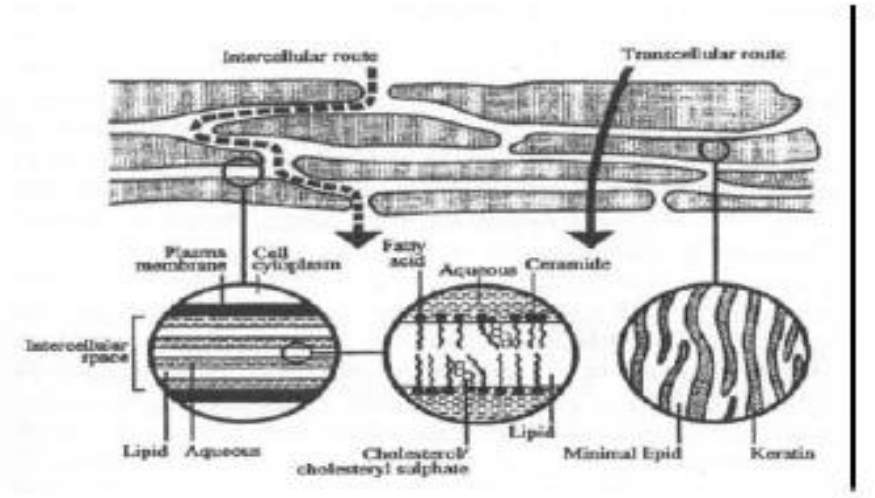
In the past few decades, considerable attention has been focused on the development of new drug delivery system (NDDS). The NDDS should ideally fulfill two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body [4],

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 profile of distribution [3].

TRANSDERMAL DELIVERY

Optimization of drug delivery through human skin is important in modern therapy. Recently, the transdermal route vied with oral treatment as the most successful innovative research area in drug delivery [1].





Simplified diagram of stratum corneum and two micro routes of drug penetration

MATERIALS AND METHODS

Materials used

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

List of materials used with supplier

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

List of Equipment's used with Manufacturers

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 interaction 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 lies more in the qualitative identification of substances either in pure form or in combination with polymers and excipients and acts as a tool in establishment of 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 the pure compound [7]. The above discussions imply that infrared data is helpful to confirm the identity of the drug and to detect the interaction of the drug with the carriers. 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 KBrdisks [8].

Calibration curve of Capecitabine

100 mg of Capecitabine was dissolved in 10 ml of ethanol then volumes make upto 100ml with Phosphate buffer P^H 6.8. Concentration 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 blank [10]. The absorbance data for standard calibration curve are given in Table and plotted graphically as shown in the Figure [11]. The standard calibration curve yields a straight line, which shows that drug obeys Beer's law in the concentration range of 10-50mcg/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 ± 3^0C 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 a dark for characterization.

Composition of different Proniosomal formulations by co servation 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 in dispersible 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 and 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 follows; turbid: +, clear: ++, very clear (glassy): +++.

Measurement of ph

The pH of Capecitabine gel formulation was determined by using digtal 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 the gels have been stored in the container for their appearance and presence of any aggregate

Ph Determination

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

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 and 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

To 0.5 g of proniosomal gel weighed in a glass tube, 10 ml of the aqueous phase (phosphate buffer pH 6.8) were added; the aqueous suspension was then 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

LogQt = 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, K H = 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 ie. 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 DISCUSSIONS

Preformulation studies

Physical Evaluation Method of Drug

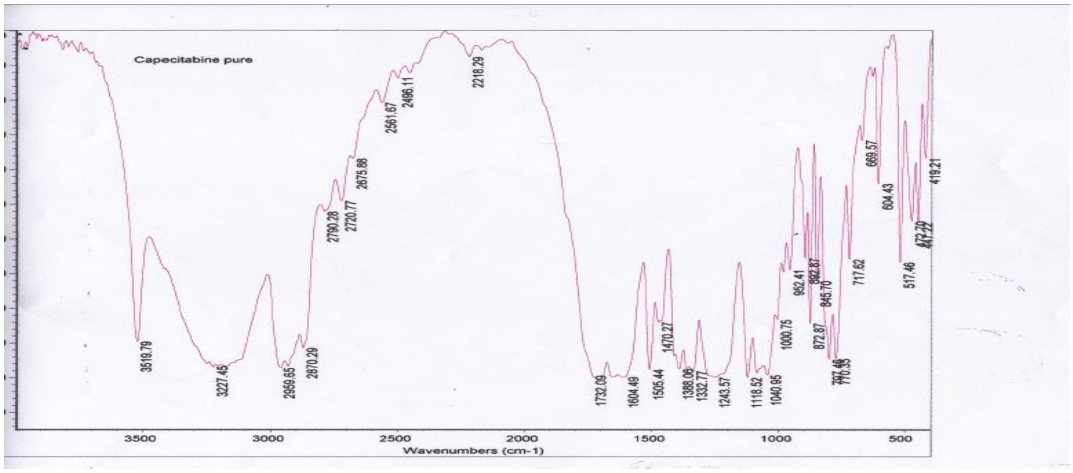
S. No	Description	Method Evaluated	0^{th} day	1^{st} week	2^{nd} week
1	Capecitabine	Physical Evaluation	White Crystalline powder	White Crystalline powder	White Crystalline powder

Melting Point

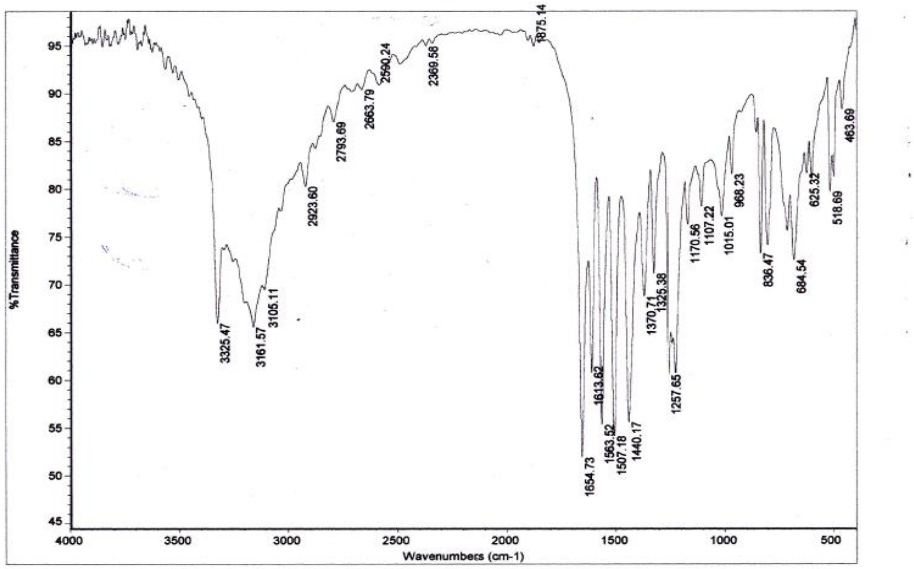
Range	Result
110-123^0 C	**117^0 C**

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

Compatibility studies



FTIR of Capecitabine pure drug



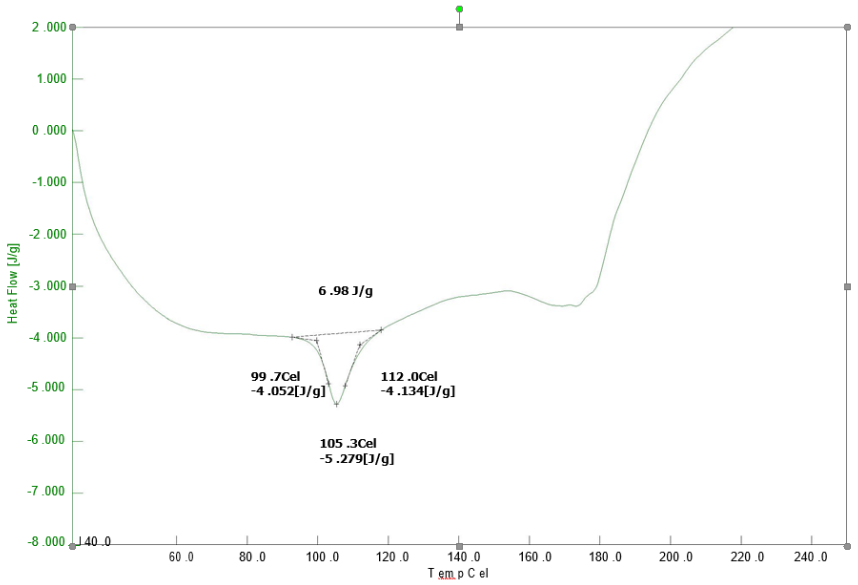
FTIR of Capecitabine optimized formulation

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.

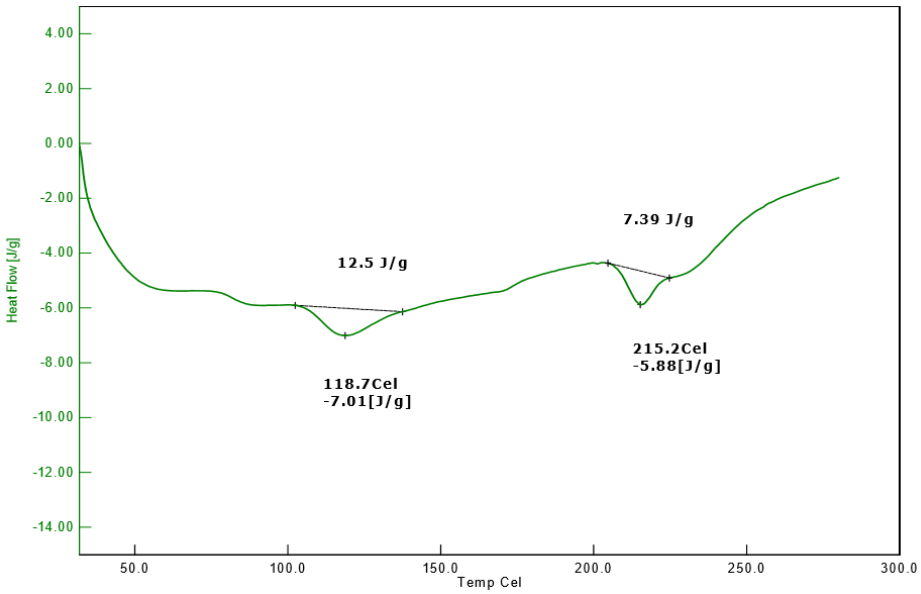
Dsc studies

Module: DSC

Data Name: SUNIL-16
Measurement Date:/ / 201
Sample Name: capcetabine pure
Sample Weight: 5.170 mg
Reference Name: AL
Reference Weight: 0.000 mg
Comment:
Operator: EXSTAR
Gas1: Nitrogen
Pan: Aluminium



Module: DSC Data
Name: SUNIL-02
Measurement Date: 03/9/2016
Sample Name: Capcitabine opt.
Sample Weight: 3.990 mg
Reference Name: Aluminium
Reference Weight: 0.000 mg
Comment:
Operator: EXSTAR
Gas1: Nitrogen
Gas2:
Pan: Aluminium



CONCLUSION

A micro emulsion system for the ORAL DELIVERY OF VINCRISTINE was prepared using a series of oils, surfactants and co-surfactants. Initially 8 BLANK formulations were prepared and tested for their transparency, density, viscosity, DISPERSIBILITY and conductivity. Based on these parameters, 3 formulations were selected and further continued for solubilizing capacity and *invitro* dissolution, evaluation. From the obtained data's from dissolution evaluation, one formulation which had the capacity of releasing the drug in sustained manner was selected. The formulation was ME3 and it had the components NIGELLA SATIVA OIL, Tween 80, TWEEN 20. The *exvivo* evaluation was done and the penetration rate of VINCRISTINE in

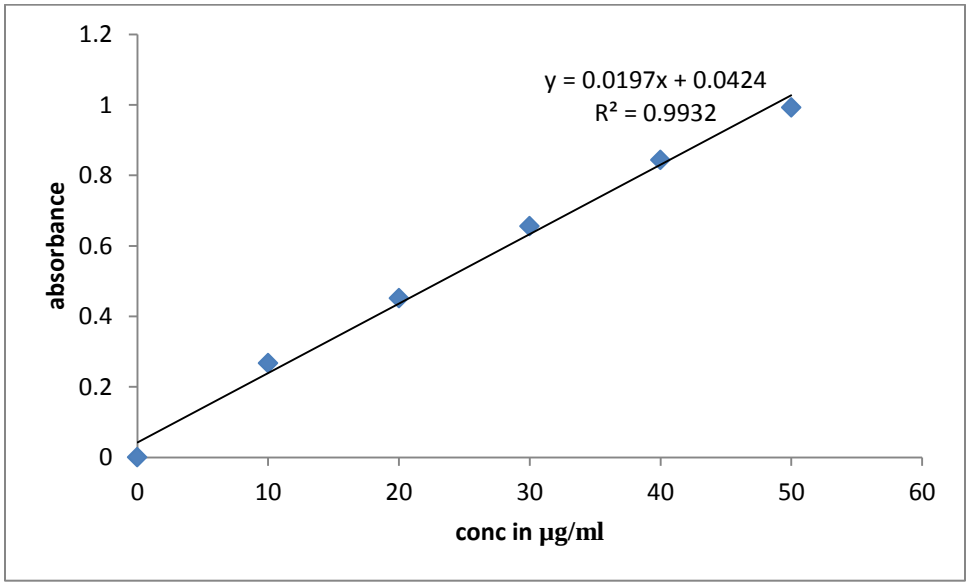
the formulation was analyzed in the Franz Diffusion cell. The result obtained shows a drug solubility of 70mg/ml in N. sativa oil and its water solubility is 0.03mg/ml. The *in vitro* permeation studies showed a 93.7%. The *ex vivo* evaluation proved that after 6 hours the release percentage vincristine were 86.7% respectively. Hence ME3 may be the most optimized preparation for the oral delivery of and the developed O/W micro emulsion formulation was expected to be potential vehicles for the oral delivery of the drugs having low solubility, poor bioavailability. Finally, it can be concluded from the results of present study that Micro emulsions improve the drug delivery, prolong the release, and improve the site specificity of the drug Vincristine. Micro emulsions creates a new opportunity for the well-controlled drug delivery of a number of drugs that have a problem of administration by other routes

Calibration curve

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

Standard calibration curve 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



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

Ph

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 behavior 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 content of formulations were found satisfactory. Hence methods adopted for gels formulations were found suitable

Table: 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
F9	+++	6.5	Good	1575	99.2	89.5
F10	+++	6.3	Good	1651	99.2	90.2
F11	+++	6.7	Good	1784	97.2	90.7
F12	+++	6.6	Good	1674	98.4	89.6
F13	+++	6.5	Good	1581	99.5	91.9
F14	+++	6.5	Good	1794	99.6	93.1
F15	+++	6.5	Good	1664	99.4	89.2
F16	+++	6.7	Good	1685	96.1	90.5
F17	+++	6.6	Good	1551	97.2	91.5
F18	+++	6.5	Good	1641	97.8	90.8
F19	+++	6.5	Good	1785	97.1	91.6
F20	+++	6.6	Good	1664	98.2	90.2
F21	+++	6.7	Good	1585	96.8	88.5
F22	+++	6.6	Good	1791	97.5	87.4
F23	+++	6.5	Good	1674	98.4	88.9
F24	+++	6.8	Good	1654	98.6	90.2

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.

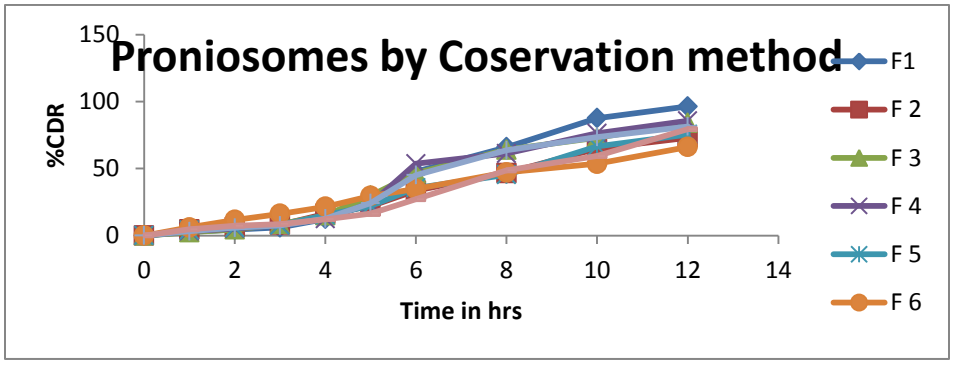
The maximum entrapment efficiency of Niosomal vesicles as determined by ultracentrifugation was 93.1% for Niosomal formulation F14.

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:

Table no: *In-Vitro* drug release of Proniosomal gel formulations (Co-Servation 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

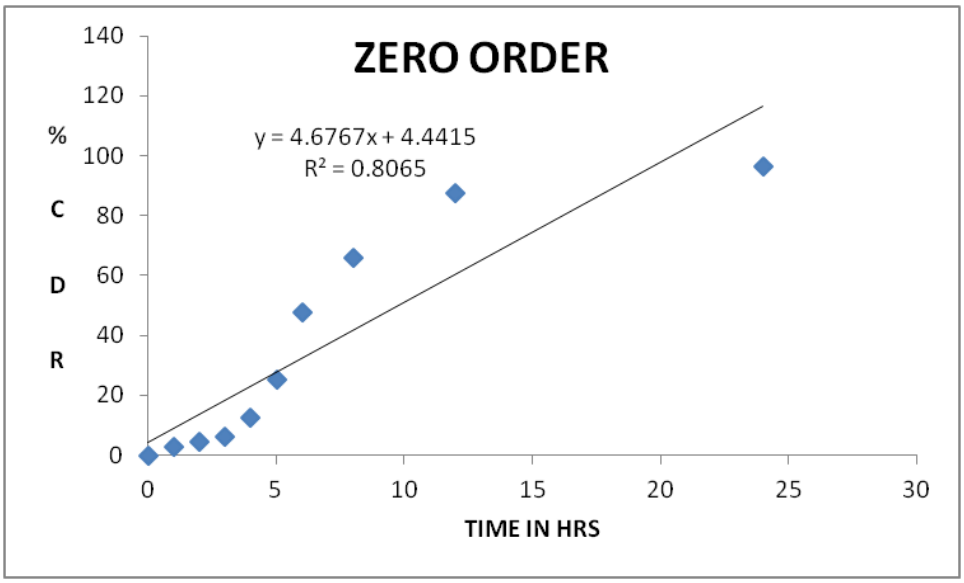


Graph showing in vitro drug release for proniosomal formulations by conservation method

Kinetic studies

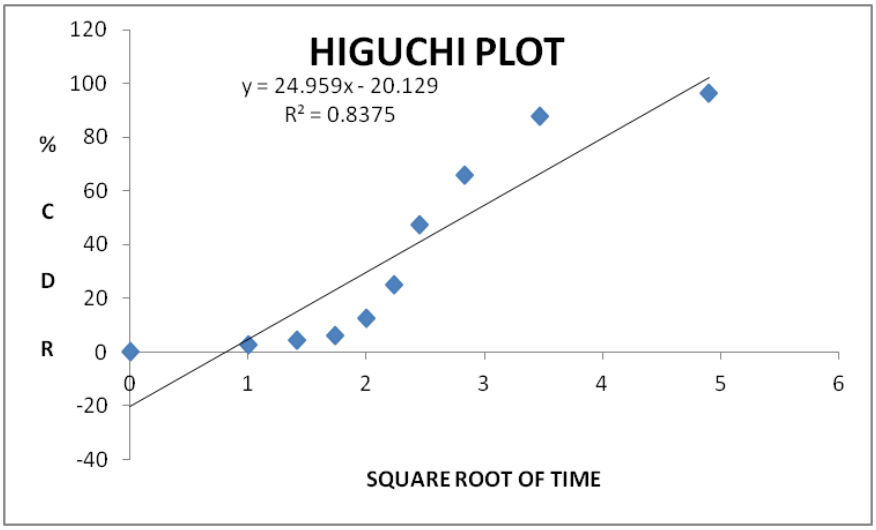
Table no: Release kinetics for optimized F8 formulation

	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

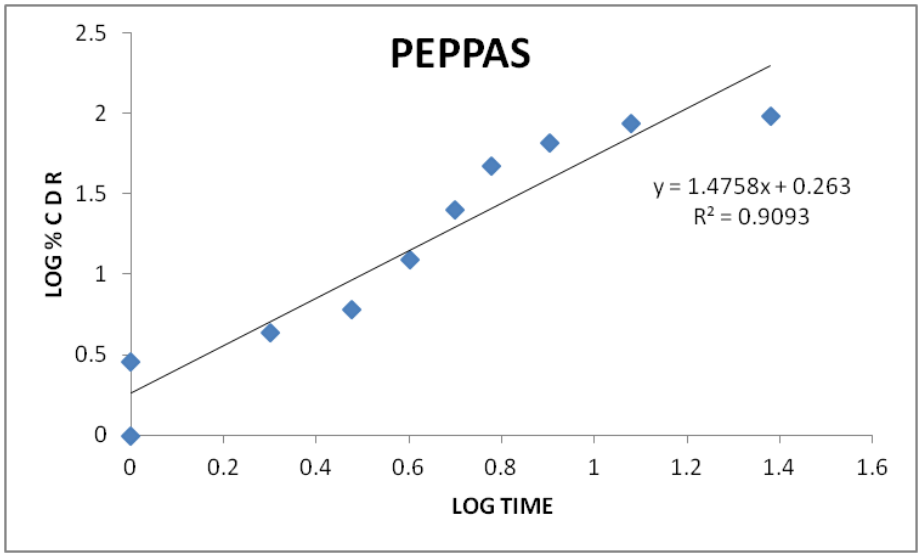


Zero order graph of optimized formulation

First order graph of optimized formulation



Higuchi graph of optimized formulation



Peppas graph of optimized formulation

CONCLUSION

It is well known that if drug molecules presenting any difficulties in it's solubility and bioavailability along the GI tract, are candidates for other routes of administration and if the site of action for drug candidate is subdermal, 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 invitro 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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