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FORMULATION AND EVALUATION OF PRONIOSOMAL GEL OF CAPECITABINE

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Abstract:

The aim of the study is to develop a proniosomal gel of Capecitabine, used for the treatment of metastatic breast cancer , that is capable of efficiently delivering entrapped drug over an extended period of time. The results showed that the type of lipid incorporated altered the entrapment efficiency of proniosomal gel and higher entrapment efficiency of 61.2 ±2.1 % was obtained with the proniosomal gel prepared from Span 40. Different formulations of Proniosomal gel using Span 40 as surfactant were prepared by changing the ratios of surfactant: lecithin and the optimized formulation was further characterized. SEM studies revealed uniform size and spherical shape of proniosomal gel, FTIR studies revealed that there was no interaction between the drug and excepients, in vitro experiments of the A1, A2, A3, A4, A5, and A6 formulations showed a release of 76.01, 55.4, 55.5, 80.5, 69.8, and 60.5 respectively. Hence formulation A4 was optimized as the drug release was found to be highest i.e. 80.5in 7 hours.

Keywords: *Capecitabine, Pronisomal gels, Anti-cancer, Breast cancer.*

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INTRODUCTION:

A number of novel drug delivery systems have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structures is one such system, which can be expected to prolong the duration of the drug in systemic circulation, and to reduce the toxicity by selective up taking.[1]

Proniosomes are dry formulations of surfactant-coated carrier, which can be measured out as needed and rehydrated by brief agitation in hot water. These "proniosomes" minimize problems of niosomes physical stability such as aggregation, fusion and leaking and provided additional convenience in transportation, distribution, storage and dosing. [2]

Transdermal therapeutic system has generated an interest as this system provides the considerable advantage of a non-invasive parentral route for drug therapy, avoidance of first pass gut and hepatic metabolism, decreased side effects and relative ease of drug input termination in problematic cases Proniosomes offer a versatile vesicle drug delivery concept with potential for delivery of drugs via transdermal route. This would be possible if proniosomes form niosomes upon hydration with water from skin following topical application under occlusive conditions.[3]

From early 1980s, proniosomes have gained wide attention by researchers for their use as drug targeting agents and drug carriers to have a variety of merits while avoiding demerits associated with the conventional form of drugs. Niosomes are water soluble carrier particles, and these are dried toform a niosomal dispersion on brief agitation in hot aqueous media. This dehydrated product is called proniosomes. [4]

The latest approach in the field of vesicular delivery is to combine the two previously mentioned techniques by extending the pro-vesicular approach to niosomes through the formation of "proniosomes" which are converted to niosomes upon hydration. [5]

Advantages of Niosomes are [6], [7]

1. They are osmotically stable. Drug molecules with a wide range of solubility can be accommodated in niosomes; they are able to entrap hydrophilic drug by partitioning of these molecules into their hydrophobic domain.
2. They can reduce drug toxicity because of their non-ionic nature.
3. Low cost of production as no special condition is required for handling and storage of niosomes.
4. Non-ionic surfactants are biodegradable, biocompatible and non-immunogenic.
5. They can be tailored according to the desired situation by modifying their structural characteristics (composition, fluidity and size).
6. They can enhance performance of drug by improving availability and controlled delivery at a particular site.
7. Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-ionic phase. Proniosomes are recent development in Novel drug delivery system. These are most advanced drug carrier in vesicular system which overcomes demerits of liposome sand niosomes as such.

Capecitabine is a fluoropyrimidine carbamate with antineoplastic activity indicated for the treatment of metastatic breast cancer and colon cancer. It is an orally administered systemic prodrug. This compound belongs to the class of organic compounds known as glycosyl amines. The vesicular systems have been receiving a lot of interest as a carrier for advanced drug delivery. Encapsulation of the drug in vesicular structures is one such system, which can be expected to prolong the duration of the drug in systemic circulation as drug controlled or sustain release drugs and to reduce the toxicity by selective up taking. In the ensuing years, great strides were made toward understanding the way in which vesicular systems interact with the biological membrane at the molecular and cellular level. [8]

MATERIALS AND METHODS:

MATERIALS: Capacitabine was a Gift sample from Dr.Reddy's Laboratories Ltd., Hyderabad, Cholesterol, Span 60, Carbopol 934 from S.D.Fine Chemicals, Mumbai. Methanol and chloroform from Merck Specialities Pvt Ltd, Mumbai, India. All other chemicals used were of analytical grade and were used without any chemical modifications.

Methodology:

Analytical Studies:

Determination of λ max. [9]

A solution containing the concentration 10 µg/ ml drug was prepared in distilled methanol; UV spectrum was taken using Double beam UV/VIS spectrophotometer. The solution was scanned in the range of 200 – 400.

Construction of standard graph.

For the spectrophotometric analysis stock solutions of CPTB was prepared by dissolving 10 mg of the drug in 10 ml distilled water to obtain a final concentration of 1 mg/ml. Serial dilutions were made to prepare diverse sample solutions of concentrations ranging from 2–20 µl/ml. The solutions were analyzed at an absorption maximum of 304 nm against the blank.

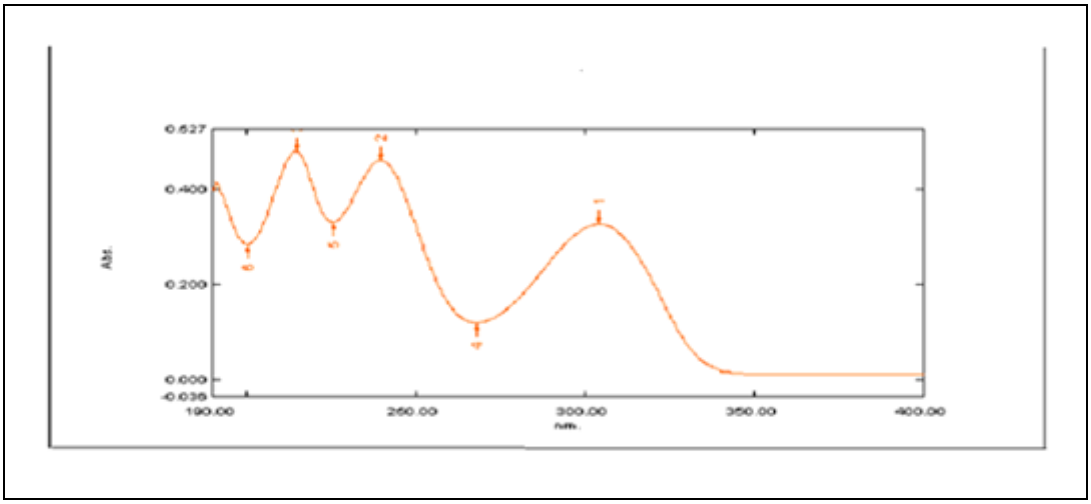


Fig 1: Lambda max of capecitabine.

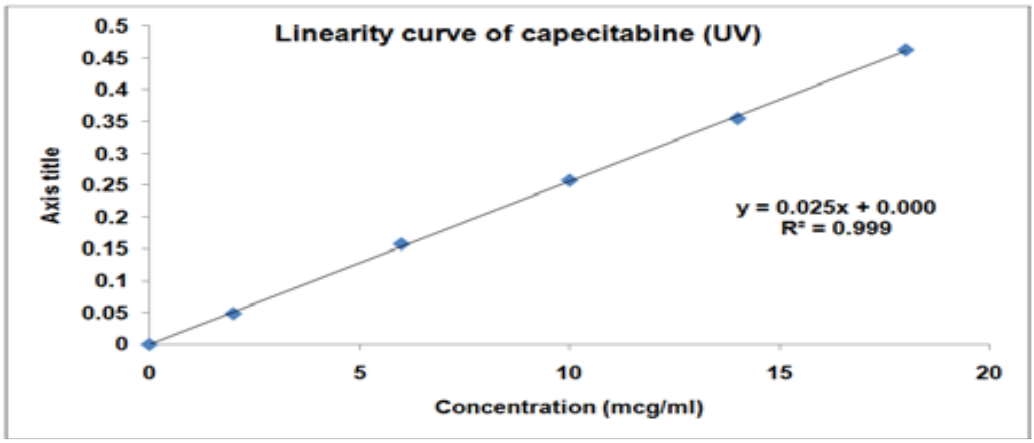


Fig 2: Standard graph of capecitabine.

Formulation of Proniosomes:

Proniosomes were prepared by using slurry method [10], [11] the composition of different proniosomal formulations as represented in table No1.

In brief, accurately weighed amounts of lipid mixture comprising of span 60 and cholesterol as per formulation ratios were dissolved in 20ml of solvent mixture containing chloroform and methanol (2:1). The resultant solvent solution was transferred into a 250ml round bottom flask. The flask was attached to a rotary flash evaporator and the organic solvent was evaporated under reduced pressure at a temperature of 45±2^{0}C. The obtained proniosomes were stored in a tightly closed container for further evaluation.

Table 1: composition of proniosomal gel of capecitabine of various formulations

Formulation code	Drug (Capecitabine)	Surfactant	Cholesterol	Solvent (2:1)
PNG1	150	Span 20	100	Chloroform and methanol
PNG2	150	Span 40	100	Chloroform and methanol
PNG3	150	Span 60	100	Chloroform and methanol
PNG4	150	Tween 40	50	Chloroform and methanol
PNG5	150	Tween 80	50	Chloroform and methanol

Preparation of Gel: Carbopol 934 was taken as polymer for the formation of Gel. 1% Carbopol was chosen for preparing gel. Weighed amount of Carbopol 934 was taken in a dry beaker and to this water was added and kept aside for swelling of carbopol for 3 hours. After complete swelling of carbopol , slowly stir with glass rod and to this add few drops of Tri ethanol amine (TEA) and this turns the preparation into gel. To this gel the prepared proniosomes were added.

Evaluation:

Morphological evaluation of prepared proniosomal powders by Scanning Electron Microscopy. [12]

The surface morphology of the pro-niosomes was evaluated by scanning electron microscopy. The proniosomal gel was placed on a cavity glass slide and water was added drop wise along the side of the cover slip. The formation of vesicles was monitored through a microscope and photomicrograph was taken.

Percentage Drug Entrapment. [13]

The PDE of Capecitabine proniosomes was calculated after determining the amount of unentrapped drug by dialysis. The dialysis was performed by adding the niosomal dispersion to a dialysis tube (donor compartment) and then dipping the tube into a beaker containing 200 mL of PBS pH 7.4 with 0.05% SLS (receptor compartment) on a magnetic stirrer, rotated at a speed of 80 to 120 rpm for 3 hours. After 3 hours, the solution in the receptor compartment was estimated for unentrapped drug at 304 nm by using a UV spectrophotometer.

$$Percent\ Entrapment = Total\ drug - Diffused\ drug / total drug X100$$

***In vitro* diffusion study.** [14], [15]

In vitro dissolution study of proniosomal gel was performed by using franz diffusion cells by taking phosphate buffer 6.8 pH. The volume of diffusion medium used was 20 ml and maintained at a temperature of 37 ± 0.5ºC with paddle speed set at 50 rpm throughout the experiment. An aliquot of 5 ml was collected at predetermined time intervals 30 min, 1, 2, 3, 4, 5, 6, 7 hrs respectively and replaced with fresh buffer to maintain constant volume. Samples were analysed for Capecitabine using UV-Visible spectrophotometer at 304 nm.

Application of Release Rate Kinetics to Dissolution Data. [16]

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi, Hixson-Crowell release model and Korsmeyer-Peppas release model.

Drug: Polymer Interactions. [17]

Fourier Transform Infrared Spectroscopy:

Infrared spectra of pure drug, and optimized proniosomal formulation were obtained using FT-IR spectrophotometer (Bruker, Alpha-T, Lab India) by the conventional KBr pellet method

Stability Studies of Optimized Formulation.

[18], [19]

Stability study was carried out to investigate the degradation of drug from proniosomal gel and powder formulation during storage. The stability study of all prepared formulation were performed by storing 4ºc, 25ºc and 45ºc for a period of 45 days. Throughout the study, proniosomal formulation was stored in aluminium foil sealed glass vials. The formulation was analyzed for the drug content spectrophotometrically.

RESULT AND DISCUSSION:

Determination lamda max:

Drug showed maximum absorbance in methanol and hence methanol was used as solvent. Drug solution of 10µg/ml was scanned over the range of 200-400nm in UV region. It was observed that the drug showed maximum absorbance at 304nm and hence 304nm was selected as the detection wavelength. (fig.1)

Preparation of calibration curve:

The standard curve was prepared in the concentration range of 2–20 µl/ml. Different volumes of standard stock solutions, containing 2-20 µg mL^{-1} of drug were transferred to 10ml volumetric flasks and volume was made up with methanol. The absorbance was measured at 304 nm against the corresponding reagent blank. The drug concentrations of CPTB were analyzed by UV-Spectrophotometer at 304 nm (fig.2)

Formulation of proniosomes:

Five formulations have been prepared using various concentrations of surfactant and cholesterol shown table no,1

Morphological evaluations:

particle size analysis by scanning electron microscope of prepared niosomal gel showed that niosomes are spherical but slightly heterogenous with sharp boundaries. Although the size distributions were same and average size of niosomes were within range shown (fig.3)

Percentage drug entrapment:

The entrapment efficiency of prepared niosomal gel was found to be in the range of 17%-60%

The entrapment efficiency of formulation prepared from span 40 was found to be maximum compared to other grades of surfactants. This was attributed to the fact that span 40 was solid at room

temperature, showed higher phase transition temperature and lower permeability. (Table No: 2) Furthermore span 40 formulation was optimised based on encapsulation efficiency by taking different ratios of surfactant and lecithin. The figure shows the effect of various ratios of sorbitan fatty acid esters and lecithin on the encapsulation of Capecitabine in pro-niosomal gel. (fig.4)

Table 2: Encapsulation percentage of various Pro-Niosomal Gel Formulations

Sl. No	Niosomal code	Encapsulation percentage (%)
1.	PNG A1	23.84 ±1.4
2.	PNG A2	59.50 ±2.3
3.	PNG A3	23.84 ±1.6
4.	PNG A4	28.84 ±2.0
5.	PNG F5	17.24 ±1.9

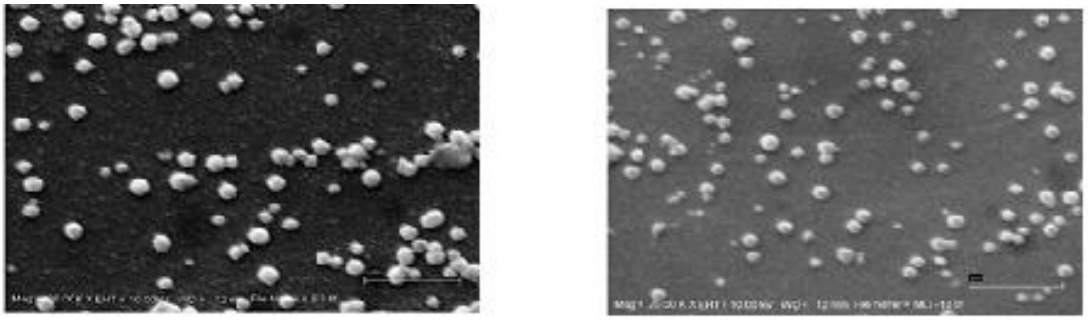


Fig 3: Images of scanning electron microscopy of optimised formulation.

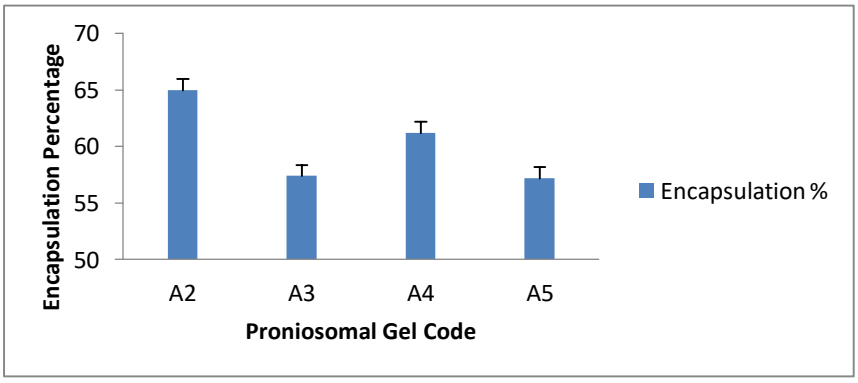
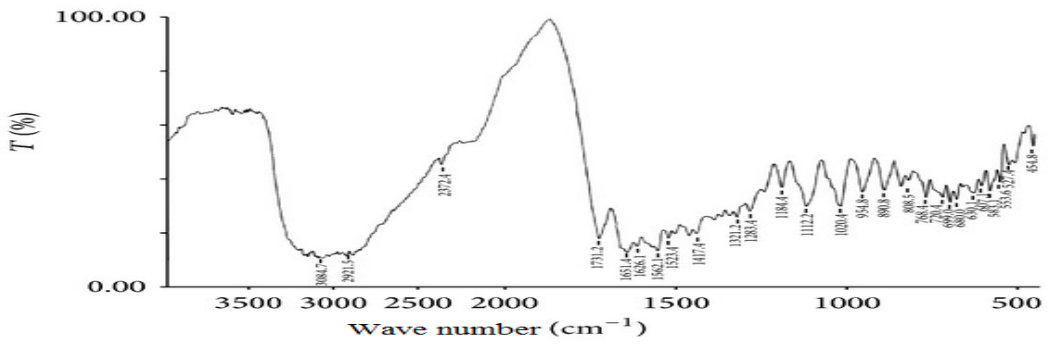


Fig 4: Encapsulation percentage of optimised formulations.



FTIR of Optimized formulation

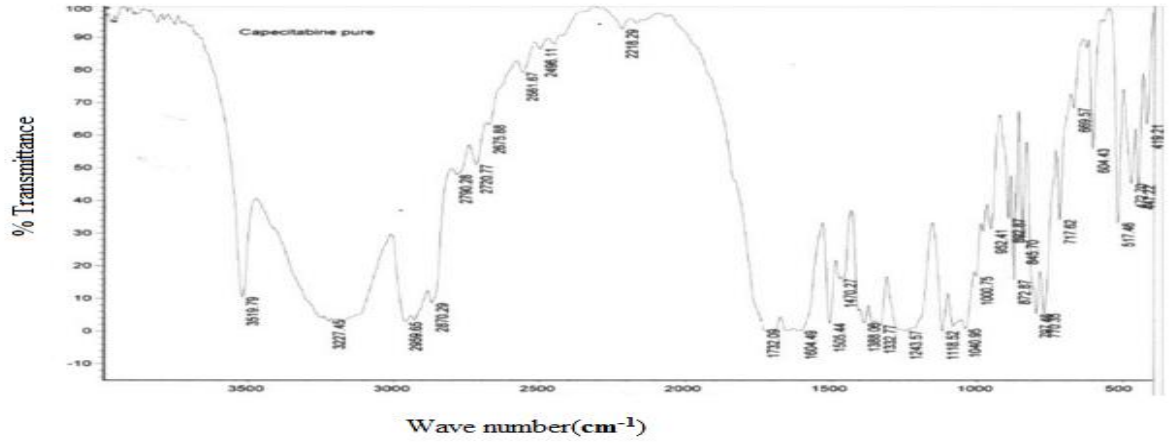


Fig 5: FTIR graphs of pure and optimised formulation.

In *vitro* studies of different formulations:

The figure 4 shows the in *vitro* release profile from various formulation of capecitabine proniosomes From the in *vitro* release data it was clear that the formulations prepared with span 40 and lecithin ratio 1:3 show 80% of drug release, where as the formulation containing ratios 1:2 and 2:1 span 40 and lecithin shows only 55% of drug release. This was significant evidence that more concentration of surfactant led to leakier niosomal membrane. In addition lecithin acted as penetration enhancer.

The percentage of drug release at the end 7h was found 76.01%, 55.4%, 55.5%, 80.05% and 69.8% for F1, F2, F3, F4 and F5 respectively. (fig.5)

Table 3: In *vitro* release of capacitabine from different formulations

Time(Hr)	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	25.84	24.04	21.47	22.89	20.06
2	34.33	28.72	30.72	38.17	39.28
3	40.1	31.96	34.98	45.3	45.63
4	47.22	39.72	43.14	52.64	50.36
5	54.01	42.69	48.94	60.05	58.87
6	60.34	49.4	52.74	67.92	64.77
7	76.01	55.4	55.5	80.5	69.8

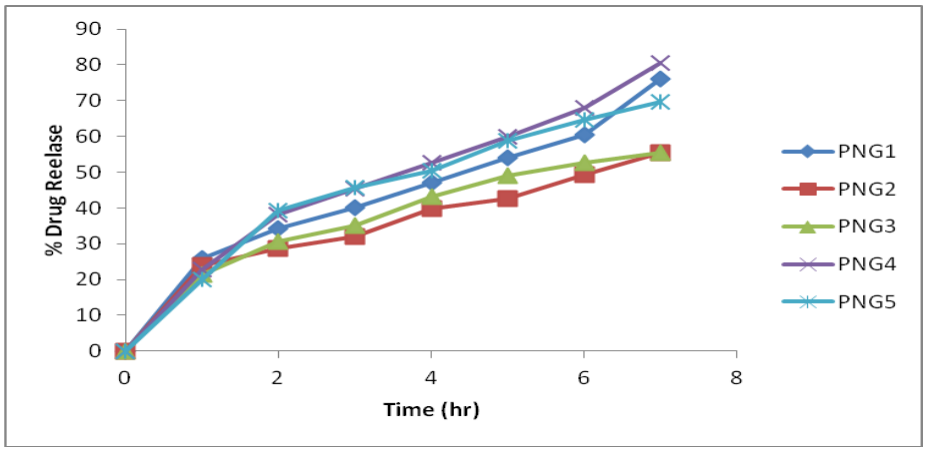


Fig 6: In *vitro* drug release of capecitabine from proniosomal gel.

Table 4: Release kinetics of optimized formulations in in-vitro drug release

Sl.No	Formula	Zero Order	First Order	Hixson-Crowell (R^2)	Higuchi (R^2)	Korsmeyer-Peppas	
						(R^2)	N
1	A2	0.9764	0.6821	0.9153	0.7948	0.9762	0.6643
2	A3	0.9737	0.7518	0.9286	0.7878	0.9734	0.5341
3	A4	0.9822	0.6938	0.9262	0.8073	0.9823	0.6572
4	A5	0.9672	0.7736	0.9245	0.7756	0.9677	0.5758

Stability studies:
Stability study was carried out in term of % drug release. Results showed that proniosomal gel formulation was quite stable at refrigeration and room temperature. In this condition not much leakage of drug was found at their temperature. Percent drug retained at 45ºC might have decreased due to the melting of surfactant and lipid present in the formulation to the proniosomal gel formulation can be stored at refrigeration and room temperature. Result of stability studies for proniosomal powder formulation was more promising than the proniosomal gel formulation. As at all sampling points significantly higher drug retention was observed in case of proniosomal powder. Thus it can be concluded that the shelf life of proniosomal powder formulation is more than the proniosomal gel formulation.

Drug polymer compatibility studies:
The drug excipients compatibility study done by FTIR indicated that partial amorphisation and solubilisation of capacitabine due to the processing and absence of any additional peak indicated that there was no interaction between the drug and excipients used in the formulation. FTIR spectra of major peaks 1731 cm^{-1},1651 cm^{-1}, 1652 cm^{-1}, 1417 cm^{-1}, 1283 cm^{-1}, 3084 cm^{-1},1112 cm^{-1}, 2921 cm^{-1}, and 2372 cm^{-1} indicating the presence C=O stretching, R-C=O-R stretching, C=C aromatic stretching, N-H bending, C-C stretching, -OH stretching, C-N stretching, =C-H stretching, C-H stretching, clearly indicated principle peaks of capacitabine were retained in physical mixture with other excipients. (fig. 6)

Release Kinetics:
The release study was conducted for all the optimized formulation (formulation showing better entrapment efficiency, optimum vesicle size, Spherical surface morphology). Thus it is evident from the study that A3 and A5 formulation of Capecitabine proniosomal gel showed good stability characteristics, prolonged release of entrapped Capecitabine with enhanced penetration and retention of drug in vesicles. To ascertain the drug release mechanism and release rate data of the various formulations, the data's were model fitted by Drug Kinetic Models. The models selected were Zero order, First order, Higuchi Matrix, Weibull, Korsemayer Peppas, Hixon-Crowell. The release pattern was found to be Zero Order and the best fit model was found to be Korsemayer-Peppas with 'n' value between 0.45 to 0.89 suggesting that the drug was released by non-fickian release mechanism

CONCLUSION:
From the entrapment studies it is clear that capacitabine was effectively incorporated in to the proniosomal gel. The in *vitro* release permeation showed that drug was able to sustain over a period of 7h from the proniosomes with 80% release. Thus it is concluded that the proniosomal gel approach is said to be one of promising drug delivery system for cytotoxic drugs. On the basis of stability studies it was concluded that proniosomal powder posses more shelf life than proniosomal gel.

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REFERENCES:
1. D.Akhilesh*, G.Faishal, Dr. P.Prabhu And Dr. J.V Kamath Development and optimization of proniosomes for oral delivery of glipizide international journal of pharmacy and pharmaceutical sciences vol 4, issue 3, 2012
2. Kapil Kumar*,A.K Rai Development and evaluation of proniosomes as promising drug carrier to improve transdermal drug derivery international research journal of pharmacy2, 11, 71-74
3. Ankur Gupta*, Sunil Kumar Prajapati, M Balamurugan, Mamta Singh, Daksh Bhatia Design and development of a proniosomal transdermal drug delivery system for captopril tropical journal of pharmaceutical research, june 2007; 6 (2): 687-693
4. G. V. Radha, T. Sudha Rani, B. Sarvania review on proniosomal drug delivery system for targeted drug actionjournal of basic and clinical pharmacyvol. 4 | issue 2 | march-may 2013
5. Sonam Ajay Singh*, Yogesh Chaudhari, Ranbahadur R Singh, Abhijeet Kunwarpuriya Proniosomes: a recent advancement in vesicular drug delivery system world journal of

pharmaceutical research volume 4, issue 4 1671-1689
6. N,.Bharti*, S. Loona And M.M.U.Khan Proniosomes: a recent advancement in nanotechnology as a drug carrier international *journal of pharmaceutical sciences review and research* volume 12, issue 1, january – february 2012; article-011
7. Ramandeep Kaur*, Meenu Nagpal, Rupinder Sidhu, Sukhdev Singh, Upendra Kumar Jain A review on proniosome, a promising carrier for delivery of drug across membranes world journal of pharmacy and pharmaceutical sciences volume 3, issue 9, 714-727
8. Rishikesh Gupta1, Santosh Kumar*2, Nikhil Gupta 2, Anurag Kumar 3 The new advancement nanotechnology: proniosomes as a promising and Potential drug carrier *international journal of research and development in pharmacy and life sciences*october - november, 2014, vol. 3, no.6, pp 1258-1265
9. U. Harini And A. K. M. Pawar*Validated uv and visible spectrophotometric method for the estimation of capecitabine – a cancer drug scholars research library Der pharmacia lettre, 2016, 8 (13):11-16
10. Ajay B Solanki*, Jolly R Parikh[1], And Rajesh H Parikh Preparation, optimization and characterization of ketoprofen proniosomes for transdermal delivery international journal of pharmaceutical sciences and nanotechnology. volume 2 issue 1 April-june 2009
11. Salwa Abdel Rahman1, Nevine Shawky Abdelmalak2, Alia Badawi2, Tahany Elbayoumy1, Nermeen Sabry3, And Amany El Raml. Formulation of tretinoin-loaded topical proniosomes for treatment of acne: in-vitro characterization, skin irritation test and comparative clinical study Drug Delivery Early online 1-9
12. Ibrahim A. Alsarra*, A.A. Bosela, S.M. Ahmed, G.M. MahrousProniosomes as a drug carrier for transdermal delivery of ketorolac European Journal of Pharmaceutics and Biopharmaceutics 59 (2005) 485–490
13. Rishu Kakkar , Rao Rekha 1,*, Dahiya Navin Kumar 1 And Nanda Sanju Formulation and characterisation of valsartan proniosomes *Maejo International Journal of Science and Technology*2011, *5*(01), 146-158
14. Sudhamani.T*, Priyadarisini.N, Radhakrishnan.M Proniosomes – A Promising Drug Carriers International Journal of PharmTech Research Vol.2, No.2, pp 1446-1454, April-June 2010
15. Anindita De and Amandeep Kaur Gill Proniosomal Gel of Tretinoin for the Treatment of Acne Vulgaris Journal of Applied Pharmaceutical Science Vol. 3 (07), pp. 081-086, July, 2013
16. Thulasi Chowdary G, Harini Chowdary V*, Vandana Kr, Jayasri V And Prasanna Raju Y *In Vitro* Dynamics Of Ibuprofen Incorporated Proniosomal Gel Indian Journal Of Pharmaceutical Education And Research,2013 | Vol 47 | Issue 4 .
17. Hemant N. Patil*, Sharwaree R. Hardikar, Ashok V. Bhosale Formulation Development & Evaluation Of Proniosomal Gel Of Carvedilol International Journal Of Pharmacy And Pharmaceutical Sciences ,2012;Vol 4(1):
18.Sunil Kumar*, Pushpendra Kumar Jain, Nalini Pandey And Gyanendra Saxena Comparative Study Of Proniosomal Drug Delivery System Of Flurbiprofen Journal Of Chemical And Pharmaceutical Research, 2016;8(5):222-228
19. Nirav N. Patel*1*, Vikran*1*, Komal Roopchandani*1*, Arvind Gupta*2*, Amit Gupta Proniosomes For Improved Transdermal Drug Delivery – A Review journal of pharmacy research 62-82.