FORMULATION OF ZIDOVUDINE PRONIOSOMES FOR ORAL DRUG DELIVERY SYSTEM

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ABSTRACT:

Drugs regularly used for the management of the retroviral infection mostly exist as conventional dosage forms. The main short coming of these dosage forms are non specific or non targeting delivery of the drug in the site of action. Drug delivery systems by means of colloidal particulate carriers such as liposomes, niosomes have distinct advantages over conventional dosage forms because the particles can act as drug containing reservoirs, and modification of the particle composition or surface can adjust the drug release rate and/or the affinity for the target site. **Aim**

& Objective: The aim of the present study was to formulate zidovudine proniosomes as stable precurs or for the oral drug delivery system. To get the preferred characteristics of a proniosome formulation of hydrophilic drug zidovudine in the presence of cholesterol, it is important to select theproper surfactant. Another objective of this study is to compare the effect. **Methodology:** Screening of drugs, Evaluate organoleptic property, particle size, solubility and partition co-efficient of selected drug. Drug excipients compatibility studies to confirm absence of any interaction between drug and excipients. Determination of zidovudine amount to be used in a proniosome formulation. Preparation of calibration curve of drug. Formulation development of proniosome formulation Physico chemical characterization of prepared proniosome formulations and selection of best formulation in each category based on the evaluation. *Invitro* drug release study of pronoisome formulations. Stability study of best proniosome formulation. **Results & Discussion:** In the present study a challenge was taken to encapsulate hydrophililic anti retroviral drug zidovudine in malto dextrin based novel proniosome formulations. **Conclusion:** Presently proniosomes have been studied by investigators as a preference of oral drug delivery system for antiretroviral drugs to provide a better oral bioavailability considering, targeted delivery, minimize the adverse effects, prolonged release of the niosome encapsulated medicaments through biological membrane and the stability of them. On conclusion, the zidovudine novel proniosome drug delivery system represent a significant improvement in all evaluation parameters. To summarize the above mentioned outcomes, water soluble drug zidovudine was fruitfully incorporated into proniosomes.

Key Words: Zidovudine, Proniosomes, Oral, Drug Delivery.

INTRODUCTION:

For many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms, including tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols and injectables as drug carriers. Even these conventional drug delivery systems are the primary pharmaceutical products commonly seen in the prescription and over- the- counter drug market place, they known to provide a prompt release of drug. Therefore to achieve as well as to maintain the drug concentration within the therapeutically effective range needed for treatment, it is often necessary to take this type of drug delivery system several times a day[1].

Controlled release systems provide drug release in an amount sufficient to maintain the therapeutic drug level over extended period of time with the release profiles of predominantly controlled by the

special technological construction and design of the system itself. The release of active constituent is therefore, ideally independent of exterior factors.

The novel drug delivery systems are the carriers which maintain the drug concentration in therapeutic range for longer period of time and also, in addition, may deliver the content to the site of action if so desired as per requirements[2].

Proniosomes are dry pharmaceutical formulation of water-soluble drug carrier substances that are encrusted with surface active agent and can be measured out as desired and hydrated to form niosomal suspension immediately prior to use on brief agitation in warm aqueous media within minutes. The resultant niosomes are very similar to conventional niosomes and more homogeneous in size.

Proniosome formulations are minute lamellar arrangements of alkyl or di alkyl poly glycerol non-ionic surfactant, cholesterol and hydrophilic carrier molecule. On hydrolysis they generate uni lamellar or multi-lamellar noisome vesicles. In no isome vesicle hydrophilic ends of surfactant exposed towards out side and hydrophobic chains face each other within the bilayer. Therefore the noisome vesicle seizes hydrophilicdrugs within the gap enfolded in the vesicle and the hydrophobic drugs are entrenched within the bilayer[3].

MATERIALS & METHODS:

Materials:

Zidovudine was provided by Drugs India Limited, Hyderabad and Cholesterol, Malto dextrin, Spans, Tween were obtained from Himalaya Scientifics, Nellore, AP. Potassium di hydrogen ortho phosphate from Hi Media Laboratories Pvt. Ltd, Mumbai. Methanol and n-Butyl Alcohol from Sd fine–Chem Limited, Mumbai. HydrochloricAcid from Merck Specialties Private Limited, Mumbai.

Methodology:

Preparation of phosphate buffer pH 7.4:

Dissolve 6.8 gm of potassium di hydrogen orthophosphate and 1.56 gm of sodium hydroxide in 900 ml of water, adjust the pH 7.4 with sodium hydroxide solution and dilute the water to produce1000 ml [4].

PreparationofphosphatebufferpH6.8:

Dissolve 28.8 gm of disodium hydrogen phosphate and 11.45 gm of potassium dihydrogen phosphate insufficient water to produce1000 ml.

Preparation of 0.1NHCl:

Measure 8.5 ml of hydrochloric acid in 1 litre standard volumetric flask and make up the volume using demineralized water.

Calibration of standard curve:

Preparation of stock solution:

Accurately weighed Zidovudine which is equivalent to100mg of Zidovudine was taken in three 100 ml standard volumetric flask with proper label. The content of first, secondand third flask were dissolved in pH7. Phosphate buffer, pH 6.8 phosphate buffer and 0.1N hydrochloric acid respectively. The volume of each flask was made upto100ml using respective buffer solutions to obtain stock solution-1containing1000µg/ml Zidovudine [5].

Identification of analytical wavelength:

The three stock solutions were appropriately diluted with the irrespective buffer solutions and scanned using the UV visible spectro photometer (UV-260,Shimadzu,Japan) in wavelength range in between400 nm to200 nm.

Standard graph:

From the stock solution-1, of three standard flask 10 ml of the contents were pipetted out into three 100ml standard volumetric flask and made up to the marks using respective buffer solutions (stock solution-2).From these three stock solutions -2, aliquots of 2ml, 4ml, 6ml, 8ml and 10ml were pipetted out into a series of 100 ml standard volumetric flasks and the volume was made upto the mark with respective buffer to get drug concentration in the range of 2 to 10 µg/ml. The absorbance of the resulting solutions was then measured at 267nm [6].

Organo leptic properties of Zidovudine:

Organoleptic properties like color, odor and taste of Zidovudine were recorded.

Particle size analysis:

Particle size distribution of the zidovudine was estimated by analytical sieving method. The sieves were stacked on top of one another in ascending degrees of coarseness [7]. The zidovudine powder was placed on the top sieve. The nest of sieves was subjected to a standard period of agitation. The weight of material retained on each sieve was exactly determined.

Solubility Studies of pure drug:

An excess amount of Zidovudine was added to each of distilled water,chloroform, ethanol, n butanol, methanol, 0.1 N hydrochloric acid and phosphate buffer(pH 6.8 and 7.4). The mixtures were then kept in a well closed air tight containers at ambient temperature for 24 hours in vortex mixer to get equilibrium. The equilibrated samples were centrifuged at 3000rpm for 5minutes. Aliquot portions of the supernatants were taken and properly diluted with phosphate buffer(pH 7.4) for quantification of Zidovudine spectrophotometrically at

267nm. Approximate solubility of drug was indicated from the following limits [8].

Partition co-efficient of pure drug:

Accurately weighed quantity of Zidovudine equivalent to100mg Zidovudine was dissolved in pH 7.4 phosphate buffer and is shaken with the other partitioning organic solvent (n-butanol) for 30 minutes, allowed to stand for 5 minutes, and then removed the lower and upper parts separately. Then the partitioning coefficient was obtained using the formula as follows [9].

Ko/w=Concentrationofdruginorganicphase/Concentrationofdruginaqueous phase

IR spectrum of pure Zidovudine:

The drug Zidovudine was confirmed by comparing the IR spectrum of the drug with published IR spectrum of Zidovudine in Indian Pharmacopoeia.

Drug–excipients compatability studies by FT-IR spectroscopy:

The Drug – excipients compatibility studies were performed in order to confirm absence of any interaction between drug and excipients. Disappearance of an absorption peak or reduction of the peak intensity combined with the appearance of new peaks give clear evidence for interactions between drug and excipient.

Compatibility studies were performed by preparing blend of different excipient with drug.These blends were placed in air tight screw cap amber colored vials at accelerated condition like 40°C/75% RH for one week and carry out FT-IR analysis with saturated potassium bromide using pellet making method. Zidovudine was also stored alone as a reference and each drug - excipient blend is further evaluated for changes like caking, liquefaction, discoloration and odor (or)gas formation.

Formulation:

Concentration and process optimization:

The concentrations of nonionic surfactant and cholesterol were optimized to develop the stable dosage form with different non ionic surfactants (spans and tweens) at 150, 200, 250 and 300 μM concentrations with an equal ratio of cholesterol.Although vesicles were formed in all concentrations, the encapsulation efficiency was found to be very less except at 300Mm ratio. Thus 300:300 μM ratio of surfactant: cholesterol was preferred for present formulation. The process- related variables like speed of rotation of flask, hydration medium and hydration time were optimized by trial and error method.

Zidovudine proniosome preparation:

Proniosome formulations were prepared by the slurry method. The slurry method is comparatively simple and Is mostly useful for the carriers which are not dissolving in organic solvents. In brief, accurately weighed amounts of lipid mixture(500 µM) comprising of surfactant and cholesterol, with 5 µM DCP were dissolved in4mlchloroform. The drug was dissolved in 6ml methanol and there sultant solutions were transferred to a 250ml round bottom flask having maltodextrin carrier.Additional chloroform: methanol solution was added to form slurry in the case of inferior surfactant loading. The flask was attached to a rotary flash evaporator to evaporate solvent at 100-150rpm, a temperature of 60°C, and a reduced pressure of 600mmHg until the mass in the flask had become a dry, free flowing product. After ensuring the complete removal of solvent, the resultant materials were further dried overnight in a desiccator under vacuum at room temperature. This dry preparation is referred to as 'proniosomes' and was used for preparations and for further study on powder properties. These proniosome granules were stored in a tightly closed container at refrigerator temperature until further evaluated [10].

The composition of different batches of Zidovudine proniosomal formulations are represented in Table 1 & 2.

Table 1: Composition of Zidovudine proniosomal formulations without charge inducer

S. No	Formulation code	Surfactant used	300µM surfactant	300µM Cholesterol	Maltodextrin
01	ZP1	Span20	83.30µl	98 mg	500 mg
02	ZP2	Span40	101.64 mg	98 mg	500 mg
03	ZP3	Span60	106.66 mg	98 mg	500 mg
04	ZP4	Span80	109µl	98 mg	500 mg
05	ZP5	Tween20	273.90µl	98 mg	500 mg
06	ZP6	Tween40	390.22µl	98 mg	500 mg
07	ZP7	Tween60	312.5µl	98 mg	500 mg
08	ZP8	Tween80	314.14µl	98 mg	500 mg

#Drug content used 25 mg per batch, ZP-drug loaded proniosome formulations.

Table 2: Composition of Zidovudine proniosomal formulation with charge inducer

S.No	Formulation code	Surfactant used	300 µM Surfactant	300 µM Cholesterol	DCP	Maltodextrin

1	ZPc 1	Span20	83.30µl	98 mg	3mg	400 mg
2	ZPc2	Span40	101.64 mg	98 mg	3 mg	400 mg
3	ZPc3	Span60	106.66 mg	98 mg	3 mg	400 mg
4	ZPc4	Span80	109µl	98 mg	3 mg	400 mg
5	ZPc5	Tween 20	273.90µl	98 mg	3 mg	400 mg
6	ZPc6	Tween 40	390.22µl	98 mg	3 mg	400 mg
7	ZPc7	Tween 60	312.5µl	98 mg	3 mg	400 mg
8	ZPc8	Tween 80	314.14µl	98 mg	3 mg	400 mg

#Drug content used 30mg per batch, ZPc-Charge inducer added drug loaded proniosome formulation

Characterization of Zidovudine proniosome formulations

Preparation of proniosomes

The proniosomal powder was transformed to noisome vesicles by hydrating with phosphate buffer (pH7.4) at 80° C by means of vortexing using vortex mixer for 2min [11].Then iosomal dispersion was placed overa glass slide and the vesicle formation was observed under optical microscope.There resultant niosomal dispersion was subsequently subjected to evaluation of zeta potential, vesicle size, Size distribution, Encapsulation Efficacy and morphology as per the procedure.

Drug content

Zidovudine content in proniosomes was obtained by an UV spectrophotometric method. Niosomes obtained from proniosomal formulation containing 10 mg Zidovudine was taken into a standard volumetric flask. The vesicles were destructed with 50ml propane-1-ol by shaking and 1ml of the mixture subsequently diluted with phosphate buffer pH 7.4. The absorbance was measured spectro photometrically against blank at 267 nm. The average Zidovudine content of three determinations was reported in table.

Dissolution study

Dissolution is the procedure of extracting the active pharmaceutical ingredient out of the solid pharmaceutical dosage form matrix in to solution with in the GIT [12]. Dissolution study

is an ***in vitro*** method that describes how an active pharmaceutical ingredient is take out of a solid dosage form. It is an acceptable tool that predicts and offers rough assessment of the *in vivo* routine of the formulation.

The dissolution studies were carried out according to the US Pharmacopeia (USP) type I apparatus (basket method). The Zidovudine proniosome formulations corresponding to 10 mg Zidovudine were filled into hard gelatin capsule. The dissolution medium was 900ml 0.1NHCl/ phosphate buffer solution (pH6.8) in six dissolution jars to maintain sink conditions. The capsules were placed in basket and immersed in dissolution medium. The stirring speed was 50rpm, and the temperature was maintained at 37°C±0.5°C. The samples (3 ml) were withdrawn at fixed time intervals using a syringe and passed through 0.2 μm membrane filter [13].

Withdrawn samples from dissolution jars were replaced by fresh medium. The Zidovudine content was evaluated by UV spectrophotometer at 267 nm. The cumulative percentage of Zidovudine release from formulation was plotted as a function of time.

Release Kinetics:

Release kinetics is an essential part for the dosage form development. Mathematical approach is important scientific method to evaluate and optimize the error in terms of deviation in the drug release profiles of formulated dosage form during the formulation development phase. In formulation and development, mathematical model approach used to diminish the number of trials in ultimate optimization. In order to realize the kinetic of drug release, the release data got from different formulations were subjected with various kinetic equation models like zero order, first order, Higuchi's model and Hixson model. Zidovudine release from proniosome formulations were integrated into Korsmeyer & Peppa's equation and the exponent was computed from slope of the straight line [14].

Osmotic shock studies

The consequence of osmotic shock on optimized pronoisome formulations was evaluated by incubating of niosomal suspensions obtained from proniosomes in media of diverse tonicities. The formulation was divided into three parts incubated with hypotonic (0.5%NaCl), isotonic (0.9%NaCl), and hypertonic solutions (1mol/Lsodium iodide solution) for 3 hours. Then the changes in the vesicle size in the formulations were observed and specified in table.

Scanning electron microscopy (SEM)

The exterior characteristics of the proniosome powder and maltodextrin was examined by scanning electron microscope (JSM 6390LA, Jeol,Tokyo, Japan). Each sample was smeare don a small piece of adhesive carbon tape which was fixed on abrass stub and subjected to gold coating using sputtering unit for 10 sec at 10mA of current. The gold coated samples were placed in chamber of SEM and images were recorded [15].

Fourier transform infrared (FT-IR) spectroscopy

FTIR spectra of pure Zidovudine, surfactants, cholesterol, maltodextrin, blank proniosome formulation(CBPF7) and optimized proniosome formulation(ZPC 7) were obtained using FT-IR spectrophotometer (FTIR-6300, Jasco, Japan) bythe usual KBr pellet method to scrutinize the interactions between drug and excipients in formulation. The scanning range was 4000-400 cm^{-1} at ambient temperature. The smoothing of the IR spectra and the baseline correlation procedures were applied [16].

Differential scanning calorimetry

The physical nature of Zidovudine in optimized proniosome formulation was evaluated by performing differential scanning calorimetry analysis of pure Zidovudine, maltodextrin, tween60, cholesterol and formulation (ZPC 7). The DSC thermograms of the samples were gained by a differential scanning calorimeter (DSC6000, Perkin Elmer) [17]. Each sample was held in an aluminum pan and then crimped with an aluminum cover. All the five samples were scanned at10°C/min from 30 to 400°C under a nitrogen purgeat 20 mL/min.

Stability study

Physical stability study was carried out to investigate the degradation of drug from proniosome during storage. The optimized pronoisome formulation with the composition of tween 60 and cholesterol in 250:250 µM ratio with 5µM DCP wasdivided into 3sets of samples. The samples were sealed in glass vials and stored at (2-8°C) in refrigerator, room temperature 25±2°C and 45±2°C for a period of 3 months. Samples were with drawn at definite periods of time and analyzed for vesicle size, % drug remaining and percent drug entrapment [18].

RESULTS & DISCUSSION

Calibration of standard curve:

Preformulation Study:

Table 3: Standard graph data of zidovudine in phosphate buffer pH6.8

Sl.No	Concentration µg/ml	Absorbance at267 nm
1	2	0.128
2	4	0.255
3	6	0.365
4	8	0.475
5	10	0.569
6	12	0.669

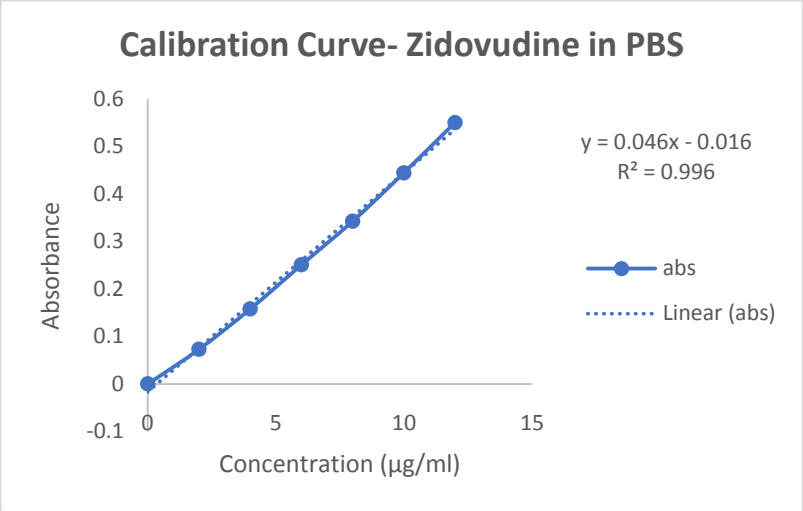


Figure 1: Standard graph of zidovudine in phosphate buffer pH 7.4

Standard graph of zidovudine in 0.1N hyrdrochloric acid:

Table 4: Standard graph data of zidovudine in 0.1N hyrdrochloric acid

Sl.No	Concentration µg/ml	Absorbanceat 267 nm
1	2	0.094
2	4	0.188
3	6	0.275
4	8	0.375
5	10	0.474
6	12	0.573

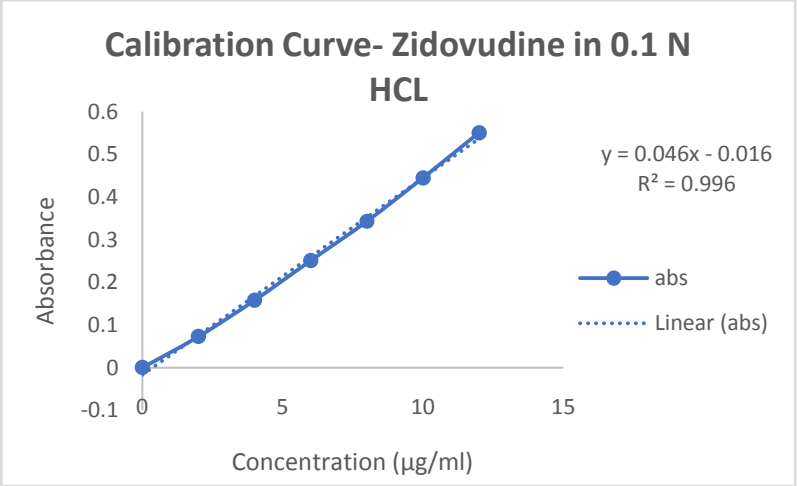


Figure 2: Standard graph of zidovudine in 0.1N hyrdrochloric acid

Organoleptic properties:

The organoleptic properties like color, odor, and taste of the API were evaluated. The color of zidovudine was found to be white to off white powder, no characteristic odor was observed in the study and the taste was found to be bland.

Particlesize:

Pre weighed zidovudine powder was passed through the series of sieve numbers 20, 30, 40, 60, 80, 100 and 120. Noparticles were retained in any sieve. All the drug particles completely passed through the sieves. Thus the particle size of the API was found to be less than 115 microns.

Solubility studies of zidovudine:

The outcome of zidovudine solubility in various solvents are summarized in Table 5.

Table 5: Solubility studies data of zidovudine.

S.No	Solvents ystemml	Solubility	
		Zidovudine mg/ml	Solubilitydescription
1	0.1NHCl	61.34	Soluble
2	PBSpH6.8	68.13	Soluble
3	Water	59.32	Soluble
4	PBSpH7.4	61.32	Soluble
5	Methanol	2.31	Slightly soluble
6	Ethanol	1.32	Very slightly soluble
7	Chloroform	1.11	Very slightly soluble
8	n-butanol	0.68	Very slightly soluble

The results suggest that the drug zidovudine was soluble in aqueous solvents such as 0.1N hydrochloric acid, phosphate buffer pH6.8, water and phosphate buffer pH 7.4, slightly soluble in methanol and very slightly soluble in ethanol, chloroform and n-butanol.

IR spectrum of pure Zidovudine:

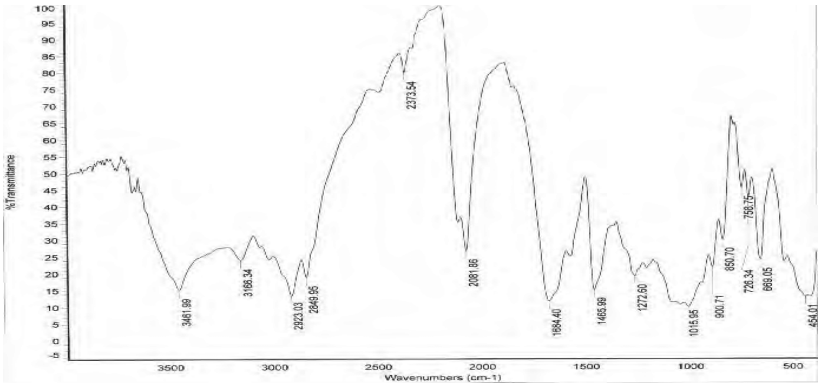


Figure 3: FT-IR Spectra of pure zidovudine

Table 6: FT-IR Spectra observations of pure zidovudine

Range	Observed	Functionalgroup
3200-3400	3481.99	NHstretch
2900-2950	2923.03	OH-Stretching
1400-1450	1465.99	C-NStretching
1340-1380	1272.60	C=CStretching
1100-1150	1015.65	CH_2group
1010-1040	900.71	C-HStretching

The characteristic peaks of IR spectrum which is taken for the obtained drug zidovudine was found to be similar with IR spectrum of zidovudine published in Indian Pharmacopoeia.

Partition Co-efficient Of Pure Drug:

Partition coefficient of zidovudine was determined using organic solvent n-butanol with pH 7.4 phosphate buffer and the result was found to be 0.053. The Result revealed that the pure zidovudine exhibit slighly hydrophilic nature.

Drug–excipients compatibility studies:

Compatibility studies were carried out by preparing physical mixture of different pronoisome excipients with zidovudine and stored at 40°C/75%RH for a week. The presence and possible interactions were evaluated by FT-IR analysis and found that there is

no any interaction between drug and excipients by seeing the characteristic peaks of the drug.

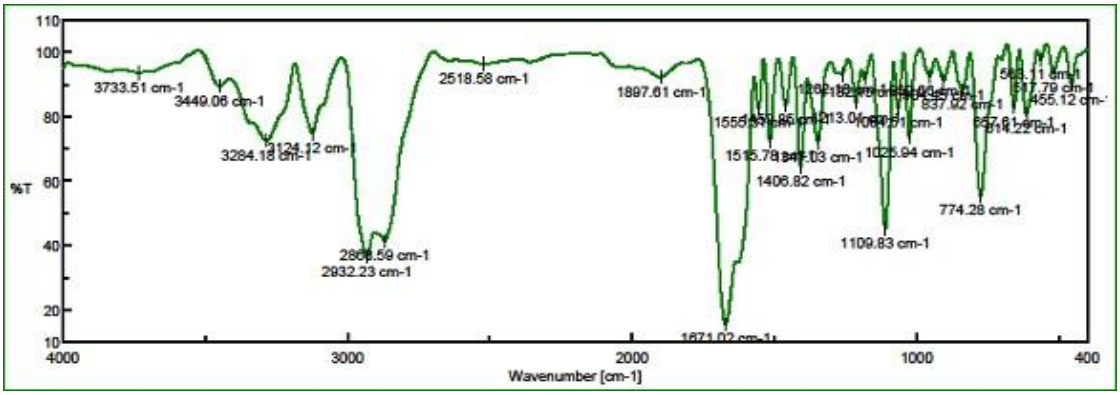


Figure 4: FT-IR Spectra of zidovudine with cholesterol

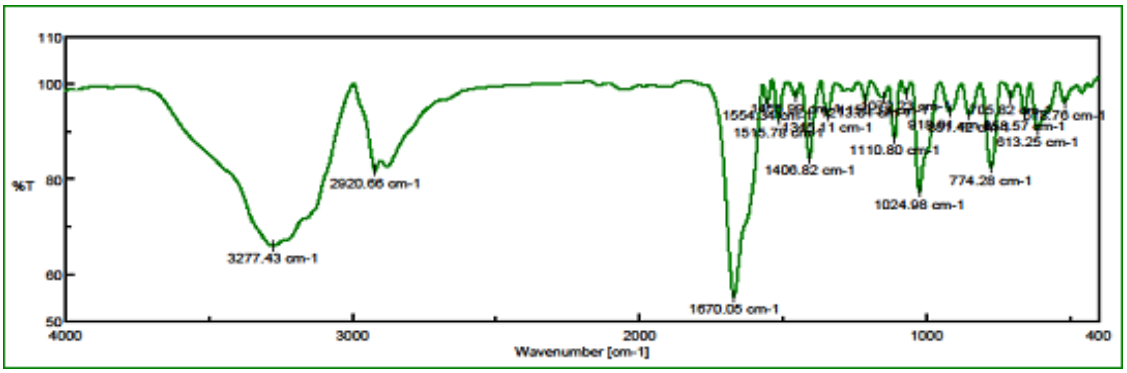


Figure 5: FT-IR Spectra of zidovudine with Maltodextrin

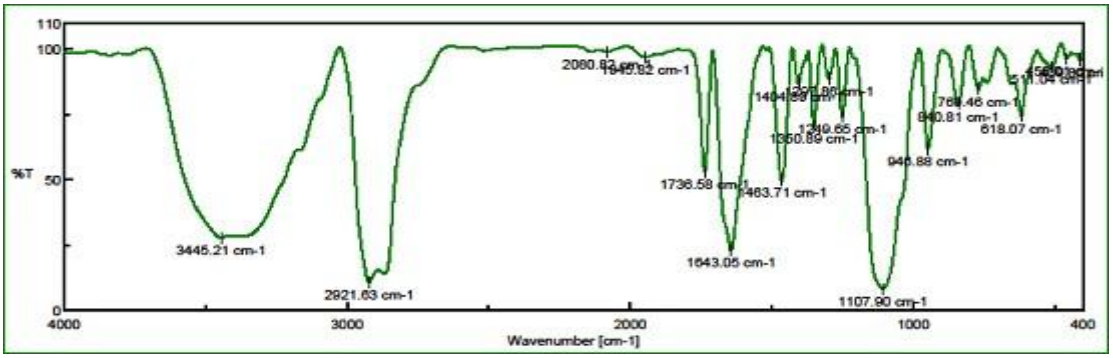


Figure 6: FT-IR Spectra of zidovudine between with Span

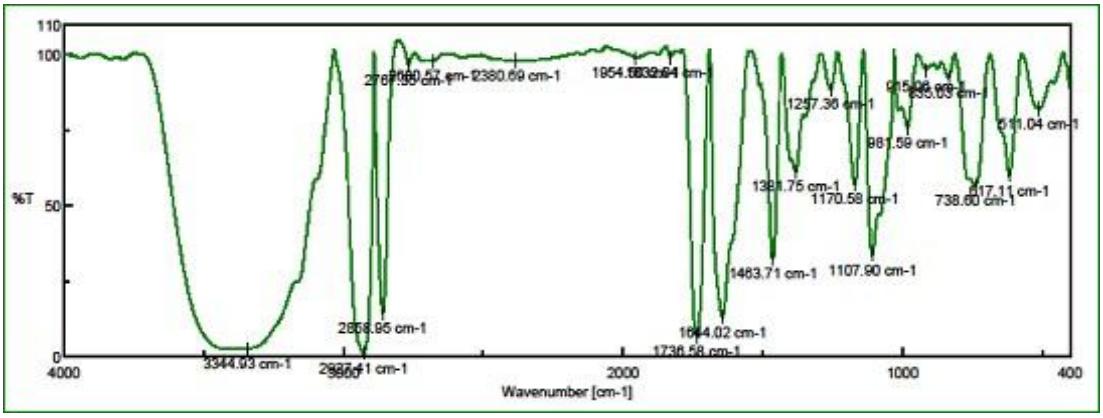


Figure 7: FT-IR Spectrabof zidovudine between cholesterol

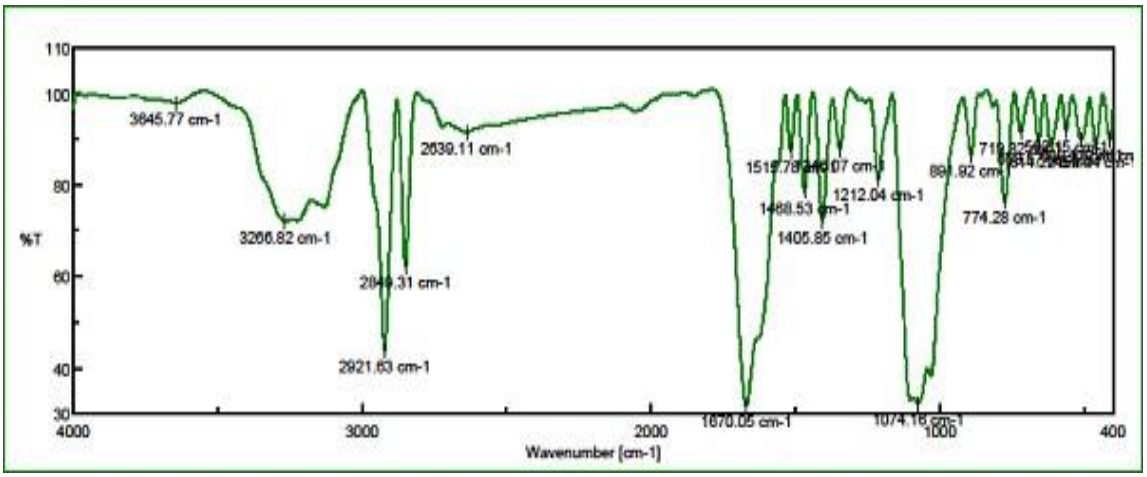
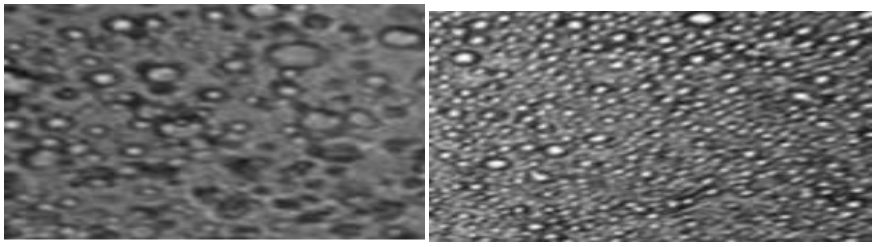


Figure 8: FT-IR Spectra of zidovudine with DC

Characterization of zidovudine proniosome formulations

Optical microscopy:

The morphology of prepared zidovudine proniosome formulations were studied using optical microscopy and the images are illustrated in figure



ZP6 **ZP7**

Figure 9: Optical photo micro graph of various batches of proniosome

Encapsulation Efficiency:

Table 7: Encapsulation efficiency of various pronoisome formulations.

Formulation code	%DrugEn capsulated	Formulation code	%DrugEn capsulated
ZP1	42.72 ±2.727	ZPC1	45.38 ±1.924
ZP2	61.02 ±2.555	ZPC2	63.62 ±1.541
ZP3	68.73 ±1.740	ZPC3	70.72 ±2.479
ZP4	33.49 ±0.978	ZPC4	35.13 ±1.404
ZP5	64.80 ±2.807	ZPC5	68.38 ±2.395
ZP6	72.27 ±2.491	ZPC6	77.32 ±2.113
ZP7	80.24 ±1.886	ZPC7	85.02 ±1.560
ZP8	49.47 ±0.919	ZPC8	57.59 ±2.025

Drugcontent:

Table 8: Drug content of zidovudine proniosomal formulations

Formulationcode	Drugcontent	Formulationcode	Drugcontent
ZP1	100.17 ±0.766	ZPC1	99.03 ±0.245
ZP2	99.58 ±0.474	ZPC2	99.94 ±0.652
ZP3	99.32 ±1.082	ZPC3	99.19 ±0.854
ZP4	99.95 ±0.578	ZPC4	100.13 ±1.234
ZP5	99.16 ±1.524	ZPC5	99.04 ±0.573
ZP6	99.93 ±0.541	ZPC6	99.01 ±0.949

ZP7	99.54 ±1.125	ZPC7	99.67 ±0.963
ZP8	100.04 ±0.769	ZPC8	99.03 ±0.245

***Invitro* drug release study of proniosome formulations:**

Table 9: *In vitro* zidovudine release data of proniosome formulations of zidovudine without DCP in 0.1N HCl

TIME Hours	ZP1	ZP2	ZP3	ZP4	ZP5	ZP6	ZP7	ZP8
0.15	15.38	11.19	8.20	16.44	10.46	7.75	5.71	13.04
0.45	18.08	13.51	11.32	22.52	12.77	9.70	6.49	15.24
1	26.58	15.42	12.50	30.24	14.63	11.89	10.57	21.52
2	36.37	24.49	17.54	43.49	22.46	15.46	13.63	26.68
3	50.18	37.58	30.82	53.61	34.64	27.52	22.64	45.73
4	61.75	44.59	37.52	72.40	41.68	33.52	25.58	56.68
5	70.63	58.90	51.25	81.08	56.62	45.67	41.66	64.45
6	77.82	64.45	57.52	87.58	62.52	50.69	45.60	72.43
9	84.67	74.59	63.41	91.46	69.68	59.52	54.42	79.29
10	89.55	81.40	72.57	95.68	78.35	65.57	60.51	85.37
12	94.44	86.55	75.40	97.13	81.50	71.47	68.29	92.42
16	97.79	90.30	81.57	99.68	85.51	77.42	72.58	96.01
18	99.69	93.40	86.64	-	90.53	83.68	79.59	98.56
20	-	98.56	92.44	-	95.39	89.31	85.62	100.31
22	-	98.88	96.40	-	97.40	95.54	93.51	-
24	-	99.69	98.53	-	99.59	97.56	96.56	-

Table 10: *Invitro* zidovudine release data of proniosome formulations of zidovudine without DCP in PBS 6.8

Time Hours	ZP1	ZP2	ZP3	ZP4	ZP5	ZP6	ZP7	ZP8
0.15	12.71	8.28	6.37	14.13	7.17	5.17	3.33	11.29
0.45	16.20	10.25	7.78	20.40	8.67	6.82	4.31	13.38
1	23.56	16.36	9.66	28.30	12.62	8.45	7.52	18.35
2	32.45	22.52	15.05	39.22	19.22	12.51	10.38	26.97
3	47.68	34.37	27.79	51.48	31.52	24.48	19.19	42.46
4	59.62	40.19	33.47	69.38	38.78	29.14	22.33	53.43
5	67.52	55.58	48.07	78.40	53.64	42.52	37.67	61.36
6	74.35	62.54	54.35	85.65	59.40	47.51	43.30	69.29
9	81.52	71.42	61.42	89.41	66.89	56.37	51.32	76.36
10	87.41	78.42	69.44	93.25	74.30	63.49	57.37	82.29
12	91.12	83.43	72.43	95.06	79.38	68.44	64.58	89.37
16	95.31	88.37	78.42	97.45	82.49	74.50	69.39	92.29
18	97.08	91.44	83.33	99.14	87.60	80.37	77.46	96.81

20	99.30	95.38	89.31	-	93.52	86.55	83.39	98.32

Table 11: *Invitro* zidovudine release data of proniosome formulations of zidovudine with DCP in0.1 N HCl

Time Hours	ZPC1	ZPC2	ZPC3	ZPC4	ZPC5	ZPC6	ZPC7	ZPC8
0.15	12.03	9.18	6.45	14.68	7.72	5.45	3.66	11.52
0.45	17.28	11.08	8.47	20.45	9.55	7.44	5.53	13.78
1	24.56	14.79	10.46	29.66	12.51	9.44	7.77	19.37
2	33.47	22.59	16.61	38.5	19.56	13.45	11.41	25.51
3	48.53	34.67	27.78	50.49	31.44	24.50	16.63	42.54
4	59.64	40.49	34.42	69.56	37.57	27.63	20.39	49.50
5	67.51	56.65	48.56	77.61	50.50	43.21	38.43	62.36
6	75.66	60.56	54.44	84.72	57.47	48.37	43.46	70.49
10	87.49	77.61	70.44	92.52	75.41	63.42	57.59	82.49
12	91.53	83.55	73.74	96.39	78.61	69.52	65.59	89.21
16	96.44	88.53	78.38	98.24	82.50	75.04	70.43	93.54
18	98.11	91.52	84.67	99.51	88.33	80.23	77.44	96.58
20	99.77	94.55	90.31	-	94.36	86.48	83.49	98.64

22	-	96.50	93.49	-	97.78	91.12	90.61	99.56
24	-	99.04	97.57	-	98.39	95.36	94.46	

Proniosome formulations with DCP in 0.1N HCl

Table 12: *Invitro* zidovudine release data of proniosome formulations of zidovudine with DCP in pH 6.8 phosphate buffer

Time Hours	ZPC1	ZPC2	ZPC3	ZPC4	ZPC5	ZPC6	ZPC7	ZPC8
0.15	10.37	7.39	5.16	12.38	6.59	4.21	2.43	9.35
0.45	14.45	9.24	6.35	17.37	7.13	5.22	3.32	11.21
1	21.55	13.37	8.37	26.48	10.37	7.39	5.14	16.42
2	30.44	19.47	13.27	35.51	16.39	10.35	8.52	23.56
3	45.30	31.52	24.57	47.47	27.39	21.28	13.45	39.60
4	55.72	37.49	31.22	66.37	33.39	23.36	16.14	45.31
5	64.42	52.48	44.56	74.55	47.31	40.22	34.32	58.30
6	72.52	57.56	51.39	81.55	54.35	47.44	40.41	67.22
9	79.53	68.25	58.80	86.40	62.57	53.38	48.29	73.29
10	84.46	74.31	66.32	90.53	71.42	60.41	54.29	79.30

12	88.46	80.42	69.26	93.49	75.31	65.16	61.21	86.25
16	93.44	85.23	74.29	96.18	79.49	72.29	67.30	90.42
18	95.18	88.37	81.42	98.02	84.55	77.10	74.34	93.36
20	98.49	93.51	87.03	99.52	91.13	82.38	79.35	94.95
22	99.21	95.41	91.34	-	94.36	89.24	87.56	97.52
24	-	98.55	95.48	-	97.16	93.19	92.12	99.13

Table 13:Release kinetics data of selected proniosome formulations

F.Code	Zeroorder		Firstorder		Higuchi'model		HixsonCrowel		Korsmeyer&Peppasequation	
	0.1N HCl	pH 6.8PBS	0.1N HCl	pH 6.8PBS	0.1N HCl	pH 6.8PBS	0.1N HCl	pH 6.8PBS	0.1NHCl	pH6.8PBS
ZP5	0.871	0.882	0.906	0.946	0.973	0.975	0.980	0.980	0.956n=0.515	0.965n=0.590
ZP6	0.932	0.937	0.935	0.959	0.986	0.982	0.984	0.986	0.958n=0.571	0.960n=0.655
ZP7	0.950	0.955	0.928	0.949	0.981	0.977	0.979	0.984	0.965n=0.634	0.971n=0.738

ZPC5	0.896	0.907	0.949	0.963	0.979	0.979	0.984	0.959	0.966n=0.576	0.959n=0.621
ZPC6	0.933	0.938	0.962	0.967	0.981	0.976	0.986	0.984	0.963n=0.636	0.956n=0.701
ZPC7	0.953	0.960	0.952	0.958	0.973	0.965	0.984	0.984	0.959n=0.733	0.962n=0.812

Vesicle size, size distribution and zeta potential determination of proniosomes:

Mean zeta potential, vesicle size, and PDI data of different zidovudine proniosomal formulations prepared using different surfactants were determined by zeta sizer and the data are given in**Table**

Table 14:Vesicle size, size distribution and zeta potential of proniosomes

S.No	Formulation code	Zeta potential mV	Vesicle Size nm	PDI
1	ZP 1	-26.5±0.862	147.8±9.592	0.398±0.043
2	ZP 2	-28.2±1.079	141.6±10.159	0.379±0.016
3	ZP 3	-31.6±1.007	135.5±10.977	0.371±0.036
4	ZP 4	-28.9±1.500	129.1±15.808	0.379±0.059
5	ZP 5	-2.81±0.105	169.9±14.479	0.352±0.023
6	ZP 6	-2.91±0.792	176.2±9.735	0.393±0.042
7	ZP 7	-2.83±0.387	172.7±10.797	0.331±0.046
8	ZP 8	-3.26±0.569	165.2±11.927	0.363±0.069

9	ZPC1	-42.1±0.519	149.2±8.864	0.359±0.036
10	ZPC2	-43.2±1.365	144.5±7.436	0.342±0.015
11	ZPC3	-44.3±1.735	138.5±6.548	0.390±0.035
12	ZPC4	-43.4±0.954	131.8±8.450	0.371±0.056
13	ZPC5	-33.0±1.172	172.2±6.396	0.397±0.029
14	ZPC6	-35.5±1.137	178.8±6.872	0.398±0.066
15	ZPC7	-34.4±1.159	175.0±5.122	0.372 ±0.046
16	ZPC8	-34.5±1.114	168.8±8.435	0.375±0.051

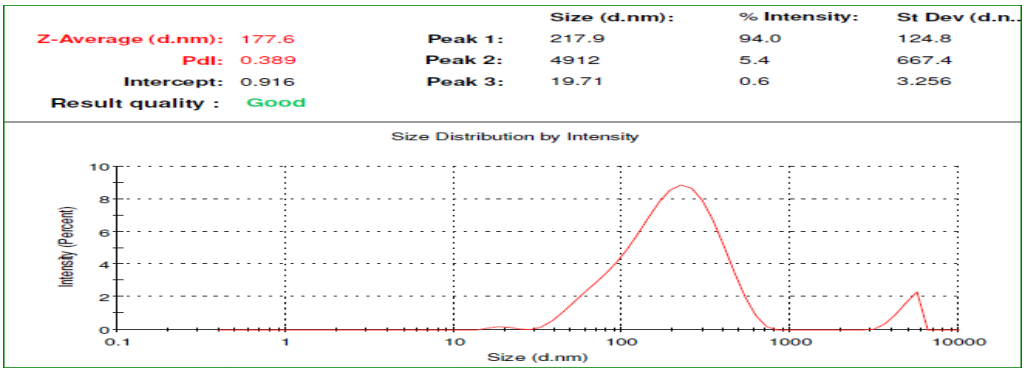


Figure 10: Vesicle size, size distribution report of optimized proniosome ZPC-7

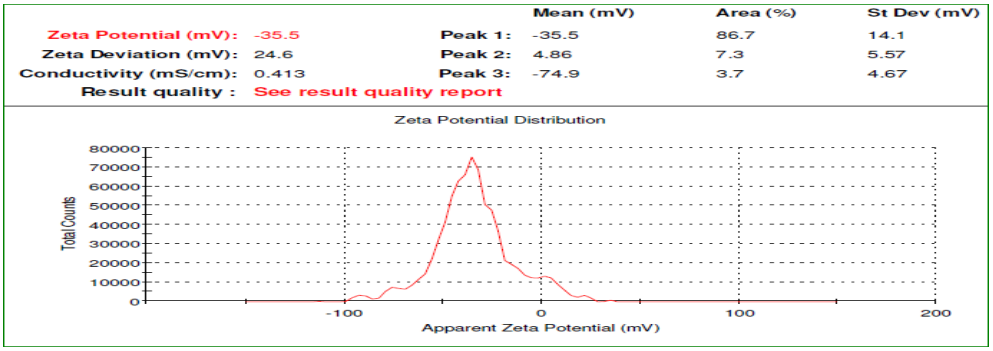


Figure 11: Zeta potential reports of optimized proniosome ZPC 7

Differential scanning calorimetry reports of optimized proniosome formulation:

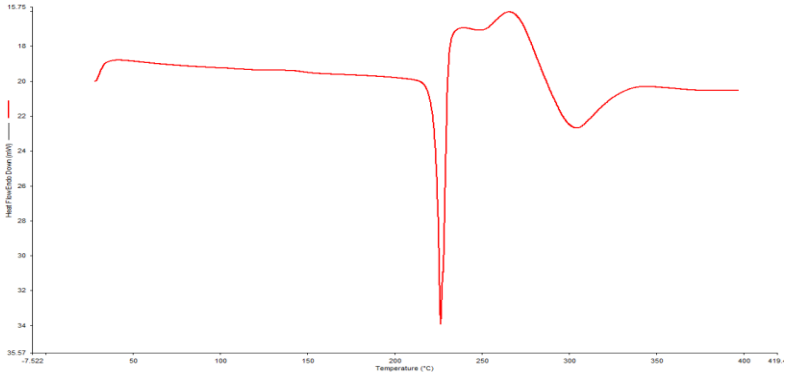


Figure 12:DSC spectrum of pure drug zidovudine

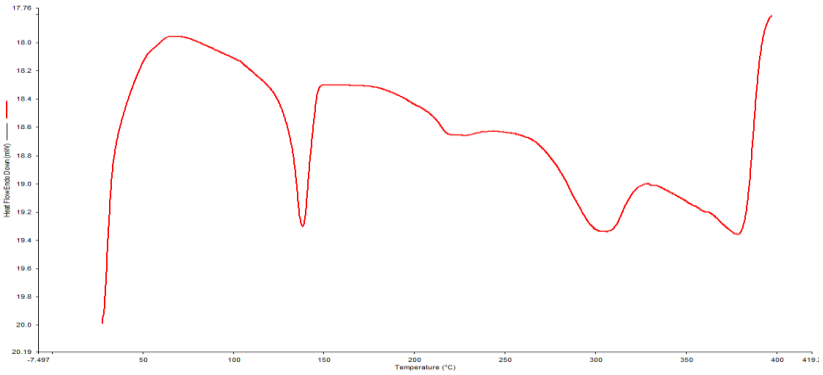


Figure 13: DSC spectrum of optimized proniosome formulation ZPC7

Osmotic shock studies

Table 15: Effect of osmotic shock on zidovudine proniosome formulations

Formulation	AverageVesiclesize			
	PBSpH7.4	Hypertonic 1mol/LNaI	Isotonic0.9% NaCl	Hypotonic 0.5%NaCl
ZP7	171.4 ±6.047	Shrunk	176.2 ±10.627	197.4 ±15.615
ZPC7	175.0 ±5.122	Shrunk	182.7 ±8.059	203.0 ±10.104

Scanning electron microscopy SEM:

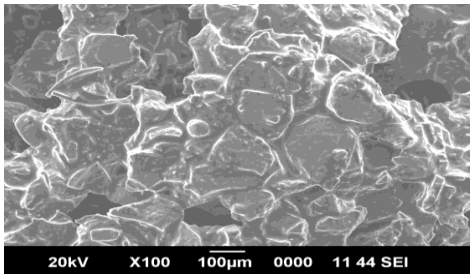


Figure 14: SEM image of optimized proniosome formulation ZPC7

Stability study:

Table 16:Stability study data of optimized proniosome formulation

Temperature	Refrigerator Temperature2-8°C			Room Temperature25±2°C			Elevated Temperature 45°±2°C		
Samplingperiod	1 month	2 months	3 months	1 month	2 months	3 months	1 month	2 months	3 months
PercentageDrugretained	99.23 ±0.89	98.71 ±0.80	98.55 ±1.17	98.56 ±0.81	98.12 ±0.72	97.69 ±0.98	98.66 ±0.92	95.13 ±1.35	90.98 ±1.45

Percentaged rugEncapsulated	84.69 ±1.14	84.07 ±1.45	83.16 ±1.24	83.39 ±0.81	81.89 ±1.18	80.08 ±1.40	80.52 ±1.57	73.42 ±1.63	68.02 ±2.03
VesicleSize	178.63 ±7.16	182.5 ±8.18	188.97 ±8.91	182.97 ±8.91	189.67 ±7.83	196.83 ±11.95	189.67 ±12.24	202.63 ±17.38	224.4 ±18.04

From the stability study results of vesicular size, encapsulation efficiency and drug content of the optimised proniosome formulations it was concluded that the proniosome formulation was quite stable at refrigeration temperature and room temperature as well. Therefore, the zidovudine proniosomal formulations can be stored at either refrigeration or room temperature. Results for vesicular size, encapsulation efficiency and drug content are shown in tables. The outcome suggest that proniosomes offered a more stable structure that could minimize the problems found about conventionally prepared niosomes like degradation by hydrolysis or sedimentation, oxidation, fusion and aggregation during storage.

CONCLUSION

The proniosome formulations were evaluated, scanning electron microscopy, optical microscopic examination, vesicle size, size distribution, zeta-potential,percentage encapsulation efficiency, drug content and *invitro* drug release.The optimized formulation was evaluated for differential scanning calorimetry, fourier transform infrared (FT-IR) spectroscopy, osmotic shock studies, stability studies. The drug release kinetic mechanism was determined for 6 proniosome formulations.

Fourier transform infrared(FTIR) spectroscopy, differential scanning calorimetry revealed that the stability of zidovudine formulation in proniosome formulations. The results of osmotic shock on optimized proniosome formulations of zidovudine proved the stability of vesicle size in isotonic solution.

The stability study data illustrated that the proniosome formulation was quite stable at refrigeration temperature and room temperature as well.

On conclusion, the zidovudine novel proniosome drug delivery system represent a significant improvement in all evaluation parameters. To summarize the above mentioned outcomes, water soluble drug zidovudine was fruitfully incorporated into proniosomes.

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Conflict Of Interest:

The authors declare no conflict of interest, financial or otherwise.

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