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Improvement of Skin Permeation of Zidovudine with Penetration Enhancers: *In vivo – In vitro* Correlations

Nripendra Singh and C.D. Upasani

Shriman Sureshdada Jain College of Pharmacy, Neminagar,
Chandwad, Nashik - 423101, Maharashtra, India

Abstract: The aim of this study was to develop a cross linked transdermal films of zidovudine with high molecular weight chitosan as matrix polymer in which propylene glycol and Glycerin was incorporated as plasticizer. Firstly the physicochemical properties of transdermal films were characterized. The impact of different type of enhancers, namely cineole, menthol, tween 80 and oleic acid on *in vitro* skin permeation of zidovudine from transdermal films were examined. The results showed that the chitosan films consisting of glycerin as plasticizer with penetration enhancers (5% w/w) and zidovudine (5% w/w) have good mechanical properties.. DSC analysis showed that there was no significant interaction between the drug and polymers. X-ray diffraction study indicating that drug in the matrix film is not present completely in the crystalline state, but is in the amorphous solid dispersion state. *Ex-vivo* drug permeation through rat skin studies, Formulations F28 (Oleic acid) and F25 (Cineole) showed a high flux and short lag time as compared to F18 (Zidovudine film without enhancer) and other formulations. Based on the higher regression values (r^2) the best fit model was first order for formulations F18 and F28, where as higuchi's matrix release for F25, F26 and F27 formulations. The release approximates non fickian diffusion and case II transport. A good correlation with R^2 (0.9988) was obtained indicating that the *in vitro* flux and *in vivo* AUC fit the straight line equation Y = 2.3028x. So formulations (F28 and F25) have been successful in achieving the desired plasma concenration for therapeutic effectiveness.

Key words: Chitosan · Zidovudine · Penetration enhancers · *In vitro – in vivo* correlation

INTRODUCTION

The Human Immuno Deficiency Syndrome (HIV) and Acquired Immuno Deficiency Syndrome (AIDS) statistics (World Health Organization and United Nations Educational Scientific Cultural Organization) has sketched more than 34.2 million populations surviving with HIV worldwide in 2011 approximately, 2.5 million people were newly infected with HIV and around 1.7 million people expire due to AIDS [1]. Human Immune deficiency virus (HIV) is a lent virus that causes Acquired Immunodeficiency Syndrome (AIDS). It is a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections [2]. From its discovery in 1981-2006, AIDS has killed more than 25 million people worldwide [3]. HIV infects about 0.6% of the world's population [4]. According to current estimates, HIV is set to infect 90million people in Africa resulting in a minimum estimate of 18 million orphans [5]. AIDS is characterized by gradual destruction of cell-mediated (T-cell) immunity; it also affects humoral immunity and autoimmunity because of the central role of the $CD4^+$ T lymphocyte in immune reactions [6]. HIV infects primarily vital cells in the human immune system such as helper T cell (specifically CD4+ T cells), macrophages and dendritic cells. HIV infection leads to low levels of CD4+ T cells, When CD4+ T cell members decline below a critical level, cell-mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections. Most untreated people infected with HIV eventually develop AIDS. These individuals die mostly from opportunistic infections or malignancies associated with the progressive failure of the immune system [7].

Corresponding Author: Nripendra Singh, Shriman Sureshdada Jain College of Pharmacy, Neminagar, Chandwad, Nashik - 423101, Maharashtra, India.
Tel: +91 2556 252529, Fax: +91-2556 253179.

Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV [8]. Antiretroviral treatment reduces both the mortality rate and the morbidity of HIV infection and increases the life expectancy of infected people even after HIV has progressed to diagnosable AIDS [9]. Although ART is not a cure for HIV/AIDS, it can significantly prolong and improve the lives of HIV-infected people [10]. Antiretrovirals slow down the production of HIV and give the body a chance to build up its CD4+ cell count which in turn helps the body fight against opportunistic infections [11]. The major drawbacks of antiretroviral drugs for the treatment of AIDS are their adverse side effects during long-term therapy, poor patient compliance and huge cost of the therapy [12, 13].

Transdermal therapeutic systems, also defined as "patches", deliver therapeutically effective amounts of drugs to the systemic circulation via skin. Transdermal systems have numerous superiorities over oral dosage forms such as overcoming first-pass metabolism, improved patient compliance and reduced gastrointestinal side effects [14, 15]. Polymers are the most important components of these systems in terms of release and permeation characteristics of drugs as well as mechanical properties of the formulations. Besides, plasticizers and permeation enhancers have considerable effect on the permeability, wearing properties formulation of transdermal patches [16, 17, 18]. Several studies have been performed in which natural polymers used as matrix agent to optimize transdermal systems [19, 20].

Chitosan, a natural, biodegradable, biocompatible, bioadhesive polymer, is gaining attention in the pharmaceutical field for a wide range of drug delivery. Chitosan is a copolymer of glucosamine and N-acetyl glucosamine linked by β 1–4 glucosidic bonds obtained by N-deacetylation of chitin. The molecular weight and degree of deacetylation can be modified during its preparation to obtain tailor-made properties. Also, chitosan has free amine as well as hydroxyl groups, which can be modified to obtain different chitosan derivatives [21, 22]. Chitosan acts as a penetration enhancer by opening the tight epithelial junctions [23]. Chitosan being cationic in nature offers great advantages for ionic interactions. Chitosan is polycationic in acidic media (pKa 6.5) and can interact with negatively charged species such as TPP [24, 25]. This characteristic can be employed to prepare cross-linked chitosan membrane. The interaction of chitosan with TPP leads to formation of biocompatible cross-linked chitosan membrane, which can be efficiently employed in drug delivery [26]. The cross-linking density, crystallinity and hydrophilicity of cross-linked chitosan can allow modulation of drug release and extend its range of potential applications in drug delivery [27, 28].

Zidovudine (AZT) is one of the most important anti-HIV drugs and a number of clinical benefits have been reported for patients with AIDS or AIDS related complex receiving AZT, including increased survival and decreased opportunistic infections [29]. AZT shows dose-related toxic effects, especially on bone marrow, which imposes dose reduction or discontinuance of treatment [30]. After peroral administration, it is rapidly absorbed from the gastrointestinal tract with a peak plasma concentration of 1.2 mg/ml at 0.8 h followed by rapid elimination with a half-life of 1 h [31]. As a result, conventional peroral dosage regimen necessitates the frequent administration of large doses (200 mg every 4 h) leading to a high incidence of toxicity and patient non-compliance. Transdermal delivery of AZT provides sustained plasma concentrations for a prolonged time consequently improving patient compliance apart from reducing the frequency and severity of side effects [32, 33]. However, due to its hydrophilicity, the passive permeation rate of AZT is very poor and below the rate sufficient to achieve a therapeutic effect. With the use of suitable vehicles and penetration enhancers, permeation of AZT across the skin may be significantly enhanced. It was found that terpenes and fatty acids at 5% w/v in vehicle were effective in achieving permeation rates that were sufficient to show a therapeutic effect. Terpenes, naturally occuring volatile oils, appear to be promising candidates for clinically acceptable enhancers [34]. Terpenes are generally considered as less toxic and have less irritant effects compared to surfactants and other skin penetration enhancers and some terpenes have been characterized as Generally Recognized As Safe (GRAS) by the US FDA (1, FDA 2006). They have high percutaneous enhancement ability, reversible effect on the lipids of stratum corneum and minimal percutaneous irritancy at low concentrations (1-5%) [35]. Moreover, a variety of terpenes have been shown to increase percutaneous absorption of both hydrophilic and lipophilic drugs [36, 37].

The objective of the present work was to develop and characterize the zidovudine monolithic transdermal therapeutic systems for physico-chemical properties and *in vitro* as well as in vivo transdermal enhancement potential of the penetration enhancers such as cineole, menthol, tween 80 and oleic acid.

MATERIALS AND METHODS

Materials: Zidovudine was gift sample from Matrix Laboratories Limited, Hyderabad, India. Chitosan was purchased from Marine Chemicals, Cochin, India. SodiumTPP, menthol, Tween 80 and oleic acid were purchased from Loba Chemie Pvt. Ltd. Mumbai, India. Cineole was purchased from Sigma-Aldrich, USA. Glacial acetic acid and absolute ethanol was purchased from Merck Chemicals, India. All other reagents were analytical grade and used as such.

Animals: The animal skin was prepared using a protocol reported earlier [26]. Male Wistar rats (250±10 g) were sacrificed by ether inhalation. The dorsal skin of animal was shaved and subcutaneous tissue was removed surgically and dermis side was wiped with isopropyl alcohol to remove adhered tissue. The skin was washed properly with phosphate buffer and used immediately.

Methodology

Preformulation Studies of Zidovudine

Identification: AZT was scanned in U.V range from 200-400 nm in saline phosphate buffer of pH 7.4 using UV-VIS Double Beam Spectrophotometer, Jasco V500, Japan. Result was given in Table 2.

Melting Point: Melting point of the drug was determined by taking a small amount of the drug in a capillary tube closed at one end. It was placed in Thiel's melting point apparatus and the melting point was noted. Result was given in Table 2.

Partition Coefficient: A known concentration of AZT (50 mg) in 10 ml of saline phosphate buffer of pH 7.4 was shaken for 1h with an equal volume of n-octanol in a separating funnel and allows standing for 4 h. The aqueous phase and organic phase were collected separately. After suitable dilution, the concentration of AZT in aqueous phase was analyzed spectro photo metrically at 266.5 nm against blank. The concentration of the drug in n-octanol was calculated from the difference between the initial and final concentrations in the aqueous phase. Partition coefficient was calculated by taking the ratio of the concentration of drug in oily and in aqueous phase. Result was given in Table 2.

Solubility: The saturation solubility of AZT was determined in phosphate buffer of pH 7.4., water, ethanol, PG and the binary combinations of ethanol in water (group-A), PG in water (group-B) and ethanol in PG (group-C). Excess drug was added to known volumes of the solvent systems, vortexed for 2 min., followed by sonication for 10 min. to dissolve the drug and then equilibrated at 32±0.5°C for more than 48 h. The contents were then centrifuged at 10,000 rev. / min for 15 min and the aliquots of supernatant saturated solvent systems were diluted appropriately and analyzed spectrophotometrically at 266.5 nm. Result was given in Table 3.

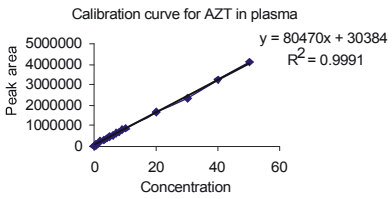

Fig. 1: Calibration curve of AZT

HPLC Method For Estimation Of Zidovudine

Procedure: 10mg of the drug was dissolved in 10ml of Acetonitrile. This 10ml solution (1000µg/ml) was used as a stock for the preparation of further standard solutions of 0.1 to 50µg/ml. From this standard solution take 100µl and spiked (0.1 to 50µl) separately in 200µl plasma and 350µl acetonitrile and vortexed for 2 min. followed by centrifugation for 15 min. at 10,000 rpm. Subsequently, the supernatant was separated into microcentrifuge tubes and evaporated by hot air oven at 40°C for 8h. Dried residues were then reconstituted with 120µl of mobile phase and a 100µl volume was injected onto the column. Calibration curve for zidovudine was obtained at 266nm [38].

Preparation of Chitosan Blank Film: Initially 2% w/w of chitosan solution was prepared in 1% (v/v) acetic acid solution, glycerin was used as a plastisizer at 20% w/w of polymer. This solution of chitosan containing plastisizer was further cross-linked with 0ml, 5ml, 10ml and 20ml of 0.1% w/v sodium TPP given in Table 1. This solution was then stirred gently for 2 hrs. The films were cast onto glass moulds by freeze-drying method. Chitosan film was prepared using 2% w/w chitosan in 1% v/v acetic acid solution. To this solution sodium TPP and Glycerin was added to improve the physical properties of the film and prepared films were characterized for moisture content, tensile strength and thickness uniformity.

Preparation of Drug Loaded Chitosan Film: 2% chitosan solution was prepared in 1% acetic acid solution, glycerin was used as a plastisizer at 20% w/w of polymer.

Table 1: Preparation of blank Chitosan films and drug loaded Chitosan films

Formulation code	Zidovudine (%w/w of polymer)	Polymer (%w/w) in 1% v/v Acetic acid solution	Sod. TPP (in ml)	Plasticiser (20% w/w of polymer)	Permeation enhancer (5% v/v)
F1		2%	0		
F2		2%	5		
F3		2%	10		
F4		2%	20		
F5		2%	0	Propylene Glycol	
F6		2%	5	Propylene Glycol	
F7		2%	10	Propylene Glycol	
F8		2%	20	Propylene Glycol	
F9		2%	0	Glycerin	
F10		2%	5	Glycerin	
F11		2%	10	Glycerin	
F12		2%	20	Glycerin	
F13	2.5%	2%	0	Glycerin	
F14	2.5%	2%	5	Glycerin	
F15	2.5%	2%	10	Glycerin	
F16	2.5%	2%	20	Glycerin	
F17	5.0%	2%	0	Glycerin	
F18	5.0%	2%	5	Glycerin	
F19	5.0%	2%	10	Glycerin	
F20	5.0%	2%	20	Glycerin	
F21	10%	2%	0	Glycerin	
F22	10%	2%	5	Glycerin	
F23	10%	2%	10	Glycerin	
F24	10%	2%	20	Glycerin	
F25	5%	2%	5	Glycerin	Cineole
F26	5%	2%	5	Glycerin	Menthol
F27	5%	2%	5	Glycerin	Tween 80
F28	5%	2%	5	Glycerin	Oleic acid

Table 2: Preformulation data of Zidovudine

Studies	Identification (UV)	Melting point (°C)	Partition coefficient (P)
Result*	266.5	124.33	0.877
Reported	266-267	120-126	

Average of three determinations.

Table 3: Solubility of AZT in various % of solvent system

Solvent System	Saturation Solubility ± SD (mg/ml)
Water	25.17 ± 0.40
Ethanol	77.32 ± 3.84
Propylene glycol	97.90 ± 1.45
Phosphate buffer of pH 7.4	27.77 ± 0.45
Group -A vehicles (% ethanol in water v/v)	
33.3	72.67 ± 1.52
50	153.87 ± 3.82
66.6	261.23 ± 2.05
Group - B vehicles (% pg in water v/v)	
33.3	41.07 ± 2.10
50	70.77 ± 2.12
66.6	135.90 ± 3.82
Group –C vehicles (% pg in ethanol v/v)	
33.3	121.33 ± 2.52
50	135.23 ± 3.36
66.6	170.43 ± 1.10

Table 4: Film thickness of blank cross-linked films and drug loaded films

Film code	Film thickness (mm)	± SD
F1	1.201	0.00594
F2	1.4243	0.04656
F3	1.632	0.01195
F4	1.930	0.04987
F5	0.745	0.132
F6	0.867	0.00583
F7	0.996	0.05041
F8	1.107	0.07859
F9	1.694	0.0483
F10	1.779	0.04817
F11	1.818	0.04572
F12	1.868	0.04306
F13	1.099	0.0383
F14	1.411	0.02817
F15	1.512	0.03572
F16	1.722	0.01306
F17	1.023	0.0483
F18	1.079	0.04817
F19	1.098	0.04572
F20	1.150	0.04306
F21	0.945	0.0183
F22	0.867	0.03817
F23	0.796	0.01572
F24	1.101	0.01306
F25	1.071	0.132
F26	1.073	0.00583
F27	1.081	0.05041
F28	1.077	0.07859

Zidovudine (5 % w/w of polymer) was dissolved in 66.6% v/v hydroalcoholic solution containing 5% w/w of penetration enhancers and mixed in the above polymer solution. It was then cross-linked with 0ml, 5ml, 10ml and 20ml of 0.1% w/v sodium TPP. This solution was then stirred gently for 2 hrs. The films were cast onto glass moulds by freeze-drying method. At the end of the drying period a clean blade was inserted and run all along the edges of film. The film was then lifted off the mould. The dried films were wrapped in butter paper and aluminum foil and stored under vacuum. The optimized film F6 were chosen to prepare drug-loaded films with different penetration enhancers depending on there tensile strength. The prepared drug loaded films were also characterized for thickness uniformity, tensile strength, moisture content etc [39].

Evaluation of Blank Film

Thickness Uniformity Test: Film thickness was measured using Mitutoyo corporation CD 6 BS digital verneer caliper, Japan with the smallest possible unit measured count of 0.0001. Results obtained are shown in Table 4.

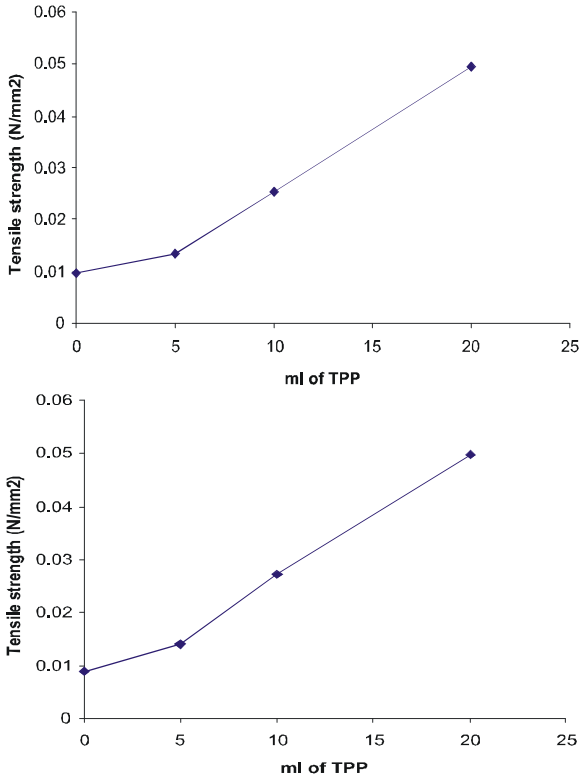

Fig. 2: Effect of TPP on tensile strength of blank chitosan film and Cross-linked films with Plastisizer and Drug

Mechanical Property Measurement: The mechanical properties of chitosan films were evaluated using an Ultra Test (Mecmesin, Japan). Film strip in dimension of 10 mm by 50 mm and free from air bubbles or physical imperfections was held between two clamps positioned at a distance 30 mm. During measurement top clam at a rate of 0.5 mm/s pulled the film to a distance of 50 mm before returning to the starting point. The force was measured when the films breaks. Measurements were run four times for each film. The tensile strength was calculated as:

$$\text{Tensile Strength } (N/mm^2) = \frac{\text{Breaking Force (N)}}{\text{Cross-section area of the sample } (mm^2)}$$

Results obtained are shown in Table 3.

Evaluation of Drug Loaded Film

Thickness Uniformity Test: The procedure for thickness uniformity has been already discussed above and results obtained are shown in Table 4.

Mechanical Property Measurement: The procedure for thickness uniformity has been already discussed above and results obtained are shown in Table 5.

Table 5: Tensile strength of blank Cross-linked films and cross-linked films containing Plastisizer with Drug.

Film code	Tensile strength (N/mm^2)	± SD
F1	0.051	0.003162
F2	0.044	0.001162
F3	0.072	0.004848
F4	0.096	0.001899
F5	0.0931	0.001286
F6	0.0320	0.002051
F7	0.0265	0.00222
F8	0.0180	0.003808
F9	0.00958	0.000912
F10	0.0134	0.000574
F11	0.0253	0.001304
F12	0.0496	0.007127
F13	0.00908	0.000512
F14	0.0114	0.000474
F15	0.0213	0.000304
F16	0.0416	0.002127
F17	0.00883	0.001162
F18	0.0140	0.002162
F19	0.0271	0.005848
F20	0.0499	0.007899
F21	0.00784	0.000212
F22	0.0111	0.000374
F23	0.0221	0.000204
F24	0.0324	0.006127
F25	0.0141	0.001286
F26	0.0143	0.002051
F27	0.0137	0.00222
F28	0.0139	0.003808

Table 6: Folding endurance data of patches

Film code	Trial 1	Trial 1	Trial 1	Mean ± SD
F17	150	165	158	157.66 ± 7.505
F18	113	129	125	122.33 ± 8,326
F19	106	109	103	106 ± 3.0
F20	104	92	97	98 ± 6.027
F25	76	76	67	72.66 ± 4.932
F26	86	89	93	89.33 ± 3.511
F27	99	91	95	95 ± 4.0
F28	82	76	88	82 ± 6

Folding Endurance: The folding endurance was measured manually for the prepared patches. A strip of patch (2 x 2 cm^2) was cut and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of folding endurance given in Table 6.

Drug Content: The drug loaded chitosan films 1cm^2 area were cut from various regions and dissolved in 5 ml of the casting solvent and the volume was adjusted to 50 ml with phosphate buffer pH 7.4. It was sonicate for 3 hrs and filtered through 0.45µ membrane filter. The solution was then, suitably diluted and content per film was estimated spectrophotometrically at 266 nm using standard curve. Results obtained are shown in Table 7.

Weight Variation Test: Disc of 1 cm^2 were cut from the film and weights of each determined using Mettler Toledo digital balance (Switzerland) with sensitivity upto 1mg. Results obtained are shown in Table 8.

Content Uniformity Test: To ensure uniform distribution of drug in film, a content uniformity test was performed. 10 disc of 1cm^2 area were cut from drug-loaded film and each dipped in sufficient quantity of casting solvent and the volume was adjusted to 50 ml with Phosphate buffer pH 7.4. It was sonicate for 3 hrs and filtered through 0.45µ membrane filter. The solution was then, suitably diluted and content per film was estimated spectrophotometrically at 266 nm using standard curve. Results obtained are shown in Table 8.

Differential Scanning Colorimetry (DSC): Thermograms of pure AZT, chitosan and chitosan film were obtained using Mettler -Toledo DSC 821^e (Mettler –Toledo, Switzerland) equipped with intracooler. Indium/Zinc standards were used to calibrate DSC temperature and enthalpy scale. Weighed sample of AZT, chitosan and chitosan film were hermetically sealed in aluminum pans and heated at a constant rate of 10°C/min, over a temperature of 25-250°C. Inert atmosphere was maintained by purging nitrogen gas (flow rate, 50 ml/min) [39].

***In-vitro* Permeation Study:** To see the pattern of AZT release from the different formulations, diffusion studies were carried out using cellulose membrane and rat skin. All formulations were subjected to *in vitro* diffusion through cellulose membrane by using Franz diffusion cell with diffusional surface area of 3.14 cm^2 and a receptor volume of 18 ml. Cellulose membrane was used as barrier between donor and receptor compartment. The membrane was mounted on a Franz diffusion cell. The receptor compartment was filled with phosphate buffer pH 7.4. It was jacketed to maintain the temperature 37 ± 0.5°C and was kept under constant stirring. Prior to application, the membrane was allowed to equilibrate at this condition for 30 min. *Ex-vivo* permeation studies across rat skin were carried out using a permeation set up described above except that instead of cellulose membrane, rat skin was used. The skin pieces were mounted over diffusion cells with the dermal side in contact with the receptor phase, equilibrated for 2 h. Magnetic stirrer at 300 rpm stirred the

Table 7: Drug content in the films

Film code	Area of the film (cm^2)	Loaded drug content (mg)	% Drug content ± SD
F17	49.066	50	99.19 ± 1.75
F18	49.066	50	99.62 ± 0.75
F19	49.066	50	99.94 ± 0.37
F20	49.066	50	99.65 ± 2.84
F25	49.066	50	99.12 ± 0.95
F26	49.066	50	99.92 ± 0.71
F27	49.066	50	99.89 ± 2.24
F28	49.066	50	99.66± 0.71

Table 8: Weight variation and content uniformity test for drug loaded film

Film code	Weight (mg)	± SD	Drug content (mg)	± SD
F17	1.54	0.11	1.005	0.01
F18	1.52	0.13	0.989	0.01
F19	1.26	0.08	1.142	0.01
F20	1.34	0.21	1.001	0.02
F25	1.62	0.31	1.022	0.01
F26	1.96	0.24	1.044	0.02
F27	1.74	0.21	1.033	0.01
F28	1.82	0.22	1.078	0.02

receptor fluid in each cell. 1 cm^2 area of drug-loaded film was clamped between donor and receptor compartments against rat skin. Samples were withdrawn at definite time intervals from the receptor compartment followed by replacement with fresh volume of phosphate buffer solution and concentration of AZT in receptor samples was analyzed by UV spectrophotometric method. For each skin specimen, AZT permeated per unit area was calculated and plotted against time.

Drug Release Kinetics: The results of *in vitro* permeation studies are subjected for different kinetic models such as zero order, first order, Higuchi and Korsemeyer and Peppas equations. The regression coefficient values were given in Table 9.

Permeation Data Analysis

Flux: The flux (μg $cm^{-2}hr^{-1}$) was calculated from the slope of the plot of the cumulative amount of drug permeated per cm^2 of skin at steady state against the time using linear regression analysis. In steady state situation, the flux J, is defined as the slope of line.

$$J = dQ/dtA$$

Enhancement Ratio (ER): Enhancement ratio was used to evaluate the effect of permeation enhancer on diffusion and permeation of selected drug molecules. It is calculated by:

Enhancement Ratio = Kp with permeation enhacer/ Kp without permeation

Results of flux and enhancement ratio weregiven in Table 11.

***In-vivo* Transdermal Studies:** Male wistar rats were anaesthetized with thiopental sodium (45 mg/kg) and the hair on the dorsal side were removed with an electric hair clipper. A polyacrylate donor cap with a surface area of 3.77cm^2 was affixed on the dorsal side of rat skin by application of a thin film of cyanoacrylate adhesive to its rim. Subsequently, gel formulations was placed within the donor cap and spread evenly using a pipette tip to achieve complete surface coverage. After application of a unit dose, the donor cap was occluded with aluminium foil and then the entire area was tightly wrapped with adhesive tape in order to prevent removal of the donor cap by the animal. Blood samples were collected for 24 h and the plasma concentrations of AZT were determined by a validated HPLC method of analysis (40).

RESULTS AND DISCUSSION

Preformulation Studies of Zidovudine

Identification: The AZT was identified by the light absorption in the U.V. range of 200-400 nm. The absorbance of drug solution was 0.487 at λmax 266.5 nm. The results are shown in Table 2.

Melting Point: The melting point of AZT was found to be 125.33°C, which was in agreement with the USP XXIV and reported value. The results are shown in Table 2.

Partition Coefficient: Drug without sufficient lipophilicity encounters difficulty in crossing lipid bilayer. However when the lipophilicity is too prominent, the drug may form a reservoir with these layers. Hence a balance of hydrophilicity and lipophilicity is desirable in the structure of drug. Octanol/phosphate buffer of pH 7.4 partition coefficient is thought to be a good indicator in predicting transdermal delivery of AZT. The partition coefficient was found to be 0.877. The results are shown in Table 2.

Solubility: The saturation solubility of AZT was determined in phosphate buffer of pH 7.4, water, ethanol, PG and the binary combinations of ethanol in water (group-A), PG in water (group-B) and ethanol in PG (group-C). The results are shown in Table 3.

Evaluation of Blank and Drug Loaded Crosslinked Chitosan Films

Physical Appearance: The patches formed were smooth and translucent in appearance.

Film Thickness: It was observed that increasing the concentration of cross-linking agent then there were increase in the thickness of the film as shown in Table 4. Thickness of the films was increased due to the cross-linking of the Sodium TPP with chitosan polymer.

The batches from F13 to F28 were containing drug shows decrease in the thickness because of the interaction of the drug with chitosan polymer.

Mechanical Property Measurement: The tensile strength testing provides an indication of the strength at the film. It is suggested that films suitable for transdermal drug delivery should preferably be strong but flexible. The mechanical properties are given in the Table 5. Increasing the concentration of cross-linking agent from 0 ml to 20 ml increased the tensile strength of the film. The tensile strength of film was increased due to the formation of complex with the chitosan polymer. More the concentration of the cross-linking agent more will be the formation of ionic cross-linking and hence the tensile strength is also high.

The tensile strength of the films containing glycerin as a plastisizer was flexible because glycerin may have decreased the rigidity of the network, providing a less ordered film structure and increasing the ability of movement of polymer. Thus glycerin acts as a better plastisizer used in the same concentration.

Folding Endurance: The recorded folding endurance of the patches was shown in Table 6. It depicits all formulations have good film properties. The folding endurance of the patches were containing enhancers decreases the folding endurance of the patches. This test is important to check brittleness, less folding endurance indicates more brittleness.

Drug Content in the Film: The % drug content of all formulations were more than 99.12 indicating good loading efficacy and suitability of method adopted for preparing chitosan film. There was no significant difference in drug content was observed within the studied batches given in Table 7.

Content and Weight Uniformity Test: In the formation of film there was very less drug loss during the process, which is shows in Table 8. It shows that the films have uniformity in its weight and drug content.

Differential Scanning Colorimetry: DSC thermogram of zidovudine showed a sharp endotherm peak that corresponds to melting in the range of 125°C. However this endothermic peak shifted to 126°C in case of zidovudine -chitosan films given in Figure 3. Overall, the DSC results suggest the absence of any physical interactions between the polymer matrix and zidovudine [39].

X-Ray Diffraction: X-ray diffraction patterns of pure drug (curve A), placebo film (curve B) and drug-loaded film (curve c) are presented in Figure 4. Placebo film shows an envelope, at 24°. Diffractogram of AZT has several characteristic sharp peaks. The highest crystalline AZT peaks occurred at 2θ of 8.22°, 14.3°, 15.4°, 16.4°, 20.2°, 22°, 24.2° and 26.4°, but series of smaller peaks are observed at 2θ of 9.8°, 19.2°, 30.2°, 32°, 34.6° and 36.4°. In case of drug-loaded film, a peak at 26.7° is observed corresponding to placebo, but no peaks were observed in drug-loaded films corresponding to the crystalline AZT indicating that drug in the matrix film is not present completely in the crystalline state, but is in the amorphous solid dispersion state.

***In-vitro* and *Ex-vivo* Permeation Study:** The *in-vitro* diffusion studies were carried out as described earlier.

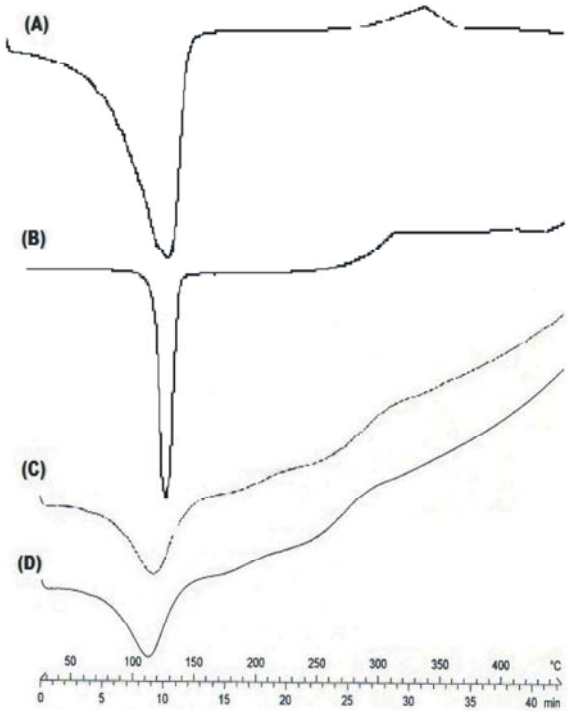

Fig. 3: DSC of A) Zidovudine, B) Zidovudine –chitosan film. C) chitosan flakes and D) Blank chitosan film

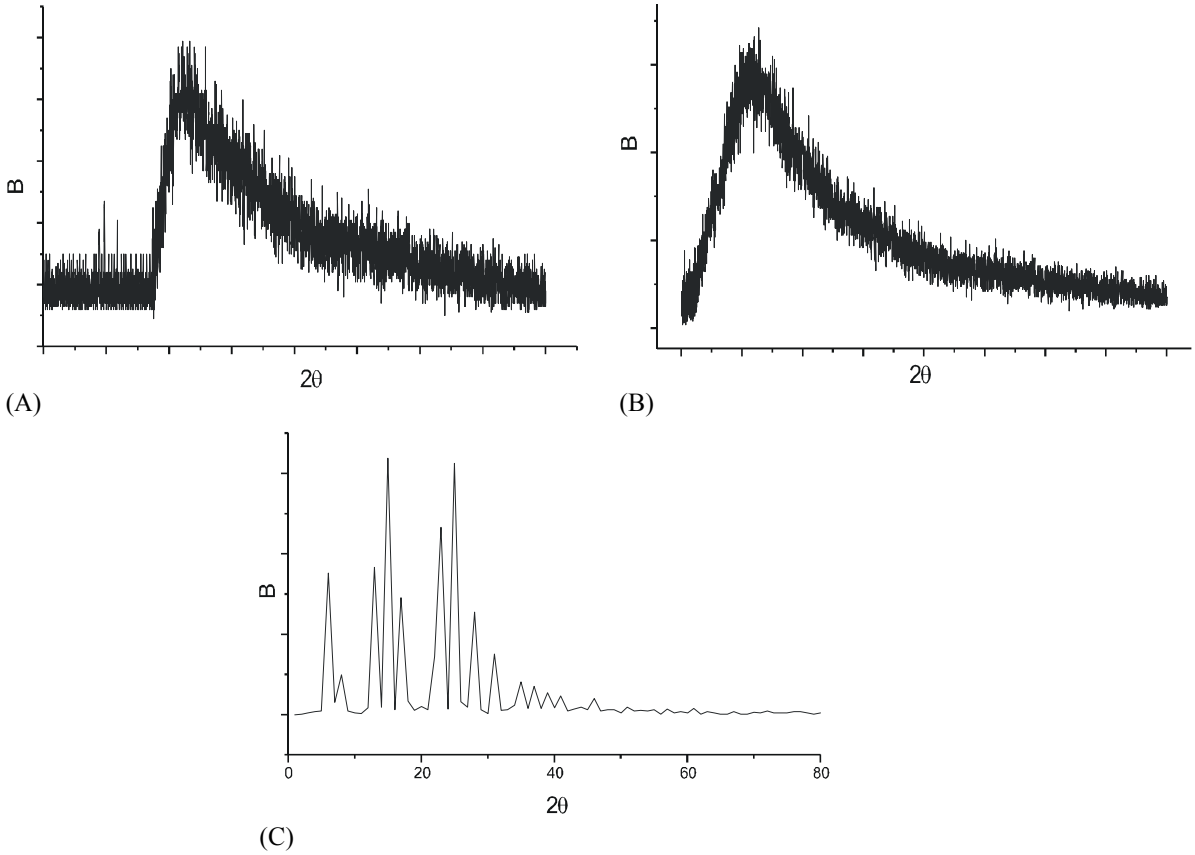

Fig. 4: XRD of (A) Zidovudine, (B) Chitosan blank crosslinked film, (C) Zidovudine loaded crosslinked film

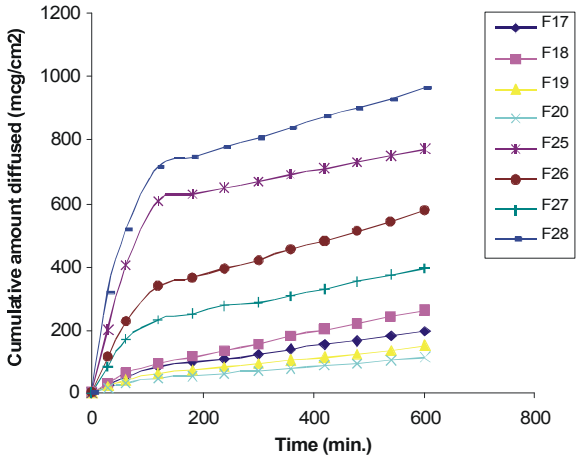

Fig. 5: Drug diffused from different formulations through cellophane membrane

The amount of drug diffused from F17, F18, F19 and F20 are shown in Figure 5. The drug diffused from F18 was more as compared to F17, F19 and F20 because of low concentration (5 ml of TPP) of cross-linking agent. Increase in the concentration of cross-linking agent leads to as increase in the density of the cross-linked network of chitosan. Due to the more complex network formed in the F19 and F20, the drug diffused from these films were low. On the basis of drug diffusion F18 was selected for incorporating enhancers to increasse drug diffusion for further study. All the enhancers formulated at 5% (w/w) in chitosan film increase the penetration of Zidovudine through the skin. All the molecules tested increase Zidovudine flux relative to control F18. All enhancers studied do not increase Zidovudine flux to the same extent. Oleic acid significantly increases the flux of Zidovudine across the skin respective to the control. Oleic acid has been found to increase the epidermal permeability through a mechanism involving the stratum corneum lipid membranes. Oleic acid is incorporated into skin lipid, disrupts molecular packaging and alters the level of hydration, thus allowing drug penetration [41]. It has also been described that at high concentrations oleic acid can exist as a separate phase within the lipid bilayers [42, 43]. Our results confirm that it can work synergistically with ethanol and glycerol, as proposed by other authors [44]. It has shown a moderate enhancing activity on the transdermal flux of Zidovudine, probably due to its moderate capacity for disrupting the stratum corneum lipid packing. It was reported that oxygen containing terpenes, such as menthol, cineole etc form new aqueous pore pathways in the skin thus increasing the permeation of hydrophilic drugs like AZT [45]. According to the lipid protein and partitioning theory, penetration enhancers may act by one or more of the three main mechanisms: disruption of the highly ordered lipid structure, interaction with intracellular protein to promote permeation through the corneocyte and increased partitioning [46]. As terpenes are highly lipophillic, it is less likely that they interact with keratin, which has been proved by calorimetric studies where they did not alter protein endotherm [46, 47]. On the other hand, it was found that terpenes alter neither thermodynamic activity nor partioning of AZT into stratum corneum from vehicle [48]. Hence, the only other possible mechanism was disruption/alteration in the barrier property of stratum corneum. So oxygen containing terpenes, such as menthol, cineole predominantly act as, first by disrupting the existing lateral/transverse hydrogen bond network of the stratum corneum lipids bilayer probably by preferential hydrogen bonding of terpenes with ceramide head groups and secondly by facilitating the excessive hydration of ceramide head groups and thereby increase the breath of existing polar pathways or form new polar pathways. Based on the pharmacokinetics of AZT, a target flux between 0.2 and 0.6 mg/cm^2/h is required across human skin from a 50 cm^2 patch in order to reach therapeutically effective plasma concentrations.

Maximum drug diffusion was observed from F28 containing 5% oleic acid and F25 containing 5% cineole were added as penetration enhancers while minimum drug diffusion was observed from F18 (Control) are illustrated Figure 5. It was observed that only 261 µg/cm^2 of drug diffused from control preparation F18 in 10 h which is not sufficient to achieve and maintain therapeutic concentration of Zidovudine. So diffusion of Zidovudine can be significantly enhanced by penetration enhancer 5% oleic acid and 5% cineole. At 5% w/w enhancer concentration, diffusion of AZT increased from 261 µg/cm^2 to 965 µg/cm^2 in F28 containing 5% oleic acid and 773 µg/cm^2 in F25 containing 5% cineole attained in 10 h which is sufficient to achieve and maintain therapeutic concentration of Zidovudine from chitosan film formulations.

Flux values of Zidovudine from different formulations in decreasing order are as follows F28 > F25 > F26> F27>F18 (Control). Flux of Zidovudine in different from control and other formulations. However among all the enhancers, flux of Zidovudine with of 5% oleic acid and 5% cineole were maximum and not significantly different from each other in their flux enhancement activities, although 5% oleic acid showed higher flux values.

These flux values were above the theoretical Zidovudine flux values (0.2 and 0.6 mg/cm^2/h) [49]. The steady state flux of Zidovudine from film formulation calculated from the linear portions of the cumulative amount permeated against time plots was 31µg/cm^2/h from F28 respectively and the flux values were very high in comparison with passive Zidovudine flux across rat skin in the absence of ethanol and penetration enhancers [50].

According to the lipid protein and partitioning theory, penetration enhancers may act by one or more of the three main mechanism: disruption of the highly ordered lipid structure, interaction with intracellular protein to promote permeation through the corneocyte and increased partitioning [51]. As terpenes are highly lipophilic it is less likely that they interact with keratin and it has been proved by calorimetric studies where they did not alter protein endotherm [52]. On the other hand, it was found that terpenes alter neither thermodynamic activity nor partioning of AZT in to SC from vehicle [53]. Hence, the only other mechanism possible is disruption/alteration in the barrier property of SC. So oxygen containing terpenes, such as menthol, cineole predominantly acting as: 1) disrupt the existing lateral/transverse hydrogen bond network of the SC lipids bilayer probably by preferential hydrogen bonding of terpenes with ceramide head groups 2) Facilitate the excessive hydration of ceramide head groups and thereby increase the breath of existing polar pathways or form new polar pathways.

A target flux between 0.2 and 0.6 mg/cm^2/h is required across human skin from a 50 cm^2 patch in order to reach therapeutically effective plasma concentrations. However, as rat skin is 3-5 fold more permeable than human skin [53]. The minimum target flux would be approximately 1 mg/cm^2/h across rat skin. As the steady state flux of AZT across rat skin from formulation F28 was above 31 µg/cm^2/h, from 1cm^{2}area achieving therapeutically effective plasma concentrations would be possible with these formulations.

The release kinetics was evaluated by making use of zero order, first order Higuchi's diffusion and Korsemeyer -Peppas equation. Calculated regression coefficient values for different formulations are tabulated in Table 9. These values are compared with each other for model and drug equation. Based on the higher regression values (r^2) the best fit model was first order for formulations F18 and F28 formulations, whereas higuchi's matrix release for F25, F26 and F27 formulations. The Figure 7-11 shows release kinetic profile of zidovudine patches for zero order, first order, Higuchi and Peppas respectively.

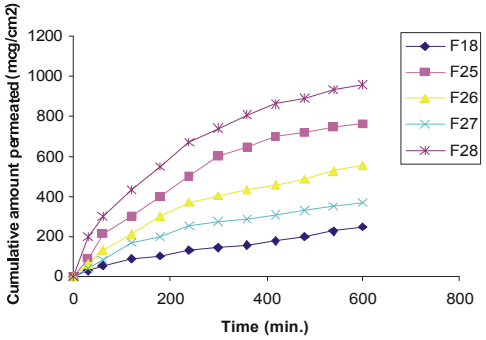

Fig. 6: *Ex vivo* permeation profiles of AZT across rat skin from films

Drug release kinetics graphs

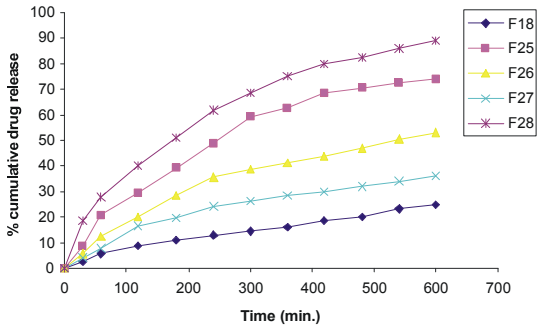

Fig. 7: Cumulative % drug release from transdermal patches

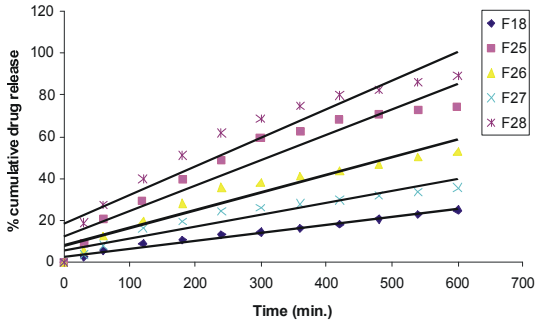

Fig. 8: Zero order release kinetic profile of zidovudine loaded patches

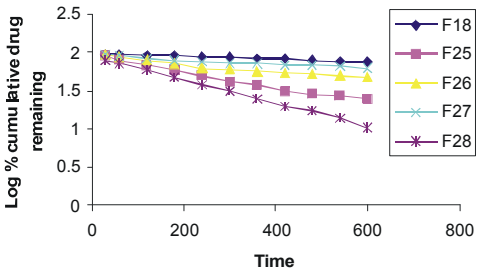

Fig. 9: First order release kinetic profile of zidovudine loaded patches

Table 9: Drug release kinetics of zidovudine from the films

Formulation code	Zero order	First order	Higuchi matrix	Peppas kinetics	'n' values for peppas
F18	0.977	0.990	0.978	0.806	0.878
F25	0.918	0.977	0.983	0.623	1.131
F26	0.930	0.972	0.987	0.683	0.978
F27	0.914	0.945	0.985	0.728	0.935
F28	0.907	0.999	0.977	0.502	1.016

Table 10: Mechanism of drug release

'n'	Mechanism
n<0.5	Fickian diffusion
0.5 < n < 1	Non fickian diffusion
Above 1	Case II transport

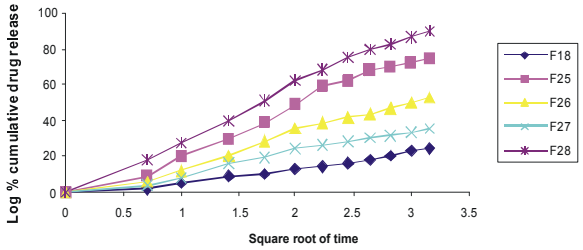

Fig. 10: Higuchi release kinetic profile of zidovudine loaded patches

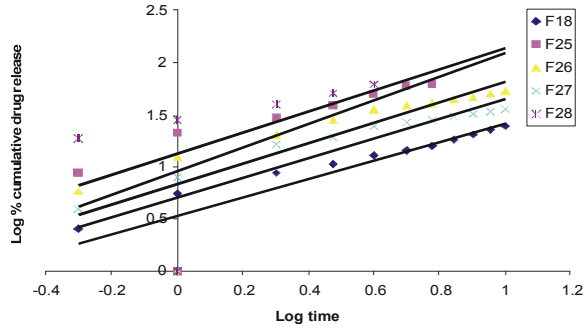

Fig. 11: Peppas release kinetic profile of zidovudine loaded patches

The Peppas model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved. 'n' value could be used to characterize different release mechanism. Peppas-Korsmeyer equation is given as,

$$\% R = K t^n$$

Or

$$\text{Log } \%R = \text{Log } k + \text{Log } t$$

where,

R = Drug release,
K = Constant,
n = Slope,
t = Time

Flux and Enhancement ratio

Table 11: Permeation parameters of AZT through rat skin

Formulations	Steady stateFlux (µg /cm^2/h)	Enhancement ratio
F18	38.2	1
F25	122.4	3.13
F26	84.3	2.10
F27	56.5	1.4
F28	136.2	3.13

The 'n' values obtained graphically from peppas plot were shown in Table 10. The values obtained between 0.878 to 1.131 which indicates that the release approximates non fickian diffusion and case II transport.

***In vivo* Transdermal Studies:** The pharmacokinetic parameters of AZT after transdermal application of optimized formulations to rats for 24 h are given in table 12. It was found that from the control gel (F18) formulation, reached maximum plasma concentration of 2.8 µg/ml within 3 hours and AUC was 71.0±3.23 hr*µg/ml which was not sufficient to achieve therapeutic plasma concentration but in case of enhancers it was found 25.4±1.00 µg/ml for F28 and 21.6µg/ml for F25 within 3 hours and AUC was 311.5±27.89 hr*µg/ml for F28 and 275.5±21.34hr*µg/ml for F25 suggesting that was sufficient to achieve therapeutic plasma concentration. The targeted/predicted plasma concentration value for AZT is reported as 20 to 60 µmol[88]. HIV could be safely inhibited by this concentration. In the present study F28 and F25 formulations gave 25.4and 21.6µmol as the maximum plasma concentration. These values is between reported values for AZT which safely inhibited HIV in Humans. So it can be concluded that these formulations have been successful in achieving the desired plasma concenration for therapeutic effectiveness.

***In vitro-In vivo* Correlation:** The approach of *in vitro-in vivo* correlation was based on establishing a linear relationship between *in vitro* flux and *in vivo* bioavailability. *In vitro-in vivo* correlation was done by carrying out regression analysis. The correlation coefficient value (R^2) was obtained by correlating the *in vitro* flux and *in vivo* AUC values as given in Fig. 13.

Table 12: Pharmacokinetics parameters of AZT after transdermal application of various film formulations to rats for 24 h.(mean ± SD, n = 6)

Parameter	Units	F18 (Control)	F25	F28
Elimination rate constant	1/hr	0.02±0.01	0.16±0.01	0.17±0.01
Half life	hr	1.3±0.87	4.1±0.35	4.3±0.23
Tmax	hr	3.0±0.00	3.0±0.00	3.0±0.00
Cmax	µg/ml	2.8±0.19	21.6±1.14	25.4±1.00
AUC	hr*µg/ml	71.0±3.23	275.5±21.34	311.5±27.89
Vd	ml	17102.7±2097.8	779.0±103.33	670.3±87.73
Clearance (Cl)	ml/hr	355.0±91.09	123.2±8.16	110.2±8.47
MRT(mean residential time)	hr	4.1±0.19	8.4±0.13	8.7±0.36

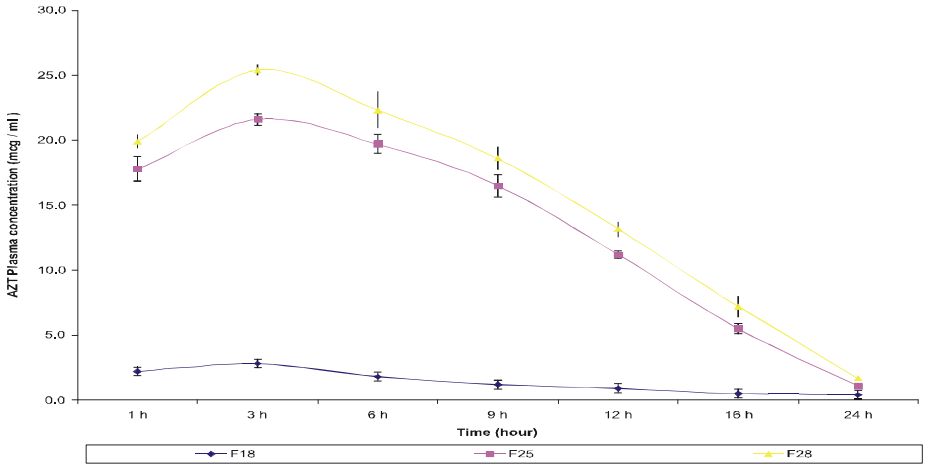

Fig. 12: Plasma concentration-time profile of AZT after transdermal application of various film formulations to rats for 24 h (mean ± SD, n = 6)

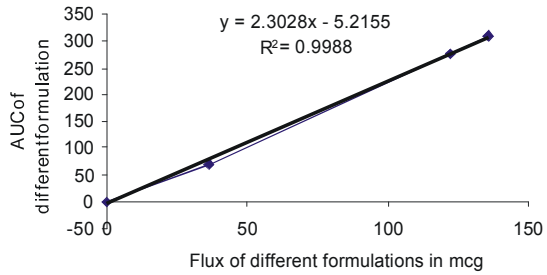

Fig. 13: Linear relationship between *in vitro* flux and *in vivo* bioavailability of different formulations

As observed from the Fig. 13, a good correlation with R^2 (0.9988) was obtained indicating that the *in vitro* flux and *in vivo* AUC fit the straight line equation Y = 2.3028x. This would serve as a measure for further formulation development

CONCLUSION

In conclusion, oleic acid was shown to be exclusively the best enhancer zidovudine chitosan film as F28 when tried in 5% concentration. Also, cineole was shown to be the good enhancer for drug permeation when tried in 5% concentration compared with the control Zidovudine patches. *Ex-vivo* drug permeation through rat skin studies, F28 (oleic acid), F25 (cineole) showed a high flux and short lag time as compared to F18 (control) and other formulation, so achieving therapeutically effective plasma concentrations would be possible with these formulations. A good correlation with R^2 (0.9988) was obtained indicating that the *in vitro* flux and *in vivo* AUC fit the straight line equation Y = 2.3028x. It can be concluded that formulations (F28 and F25) have been successful in achieving the desired plasma concenration for therapeutic effectiveness.

REFERENCES

1. World Health Organization, Media centre, HIV/AIDS Face Sheet, July 2012.
2. Douek, D.C., M. Roederer and R.A. Koup, 2009. "Emerging concepts in the immunopathogenesis of AIDS". Annu. Rev. Med., 60: 471-84.
3. Levin, Aviad, Hayouka, Zvi, Friedler, Assaf, Loyter and Abraham, 2010. "Specific eradication of HIV-1 from infected cultured cells". AIDS Research and Therapy, 7(31): 31.

4. Greener, R., 2002. "AIDS and macroeconomic impact". In: S. Forsyth (ed.). State of The Art:AIDS and Economics. IAEN, pp: 49-55.
5. Morgan, D., C. Mahe, B. Mayanja, J.M. Okong, R. Lubega and J.A. Whitworth, 2002. "HIV-1 infection in rural Africa: is there a difference in median time to AIDS and survival compared with that in industrialized countries?". AIDS, 16(4): 597-632.
6. Alimonti, J.B., T. Blake and K.E. Fowke Mechanisms, 0000. of CD4+ T lymphocyte cell death in human immunodeficiency virus infection and AIDS DOI 10.1099/vir.0.19110-0.
7. Klein Leichman and Abigail, 2010. "On the HIV warpath". Israel 21c Innovation News Service. http://israel21c.org/201010038374/health/on-the-hiv-warpath. Retrieved 11 October 2010.
8. Botes, M.E., 2003. Antiretroviral therapy: pharmacology. In Disease Review, pp: 334-341, Johnnic Publishing.
9. Kitahata, M.M., S.J. Gange and A.G. Abraham, 2009. "Effect of early versus deferred antiretroviral therapy for HIV on survival". N. Engl. J. Med., 360(18): 1815-26.
10. Hicks, L.M., R.E. Cahoon, E.R. Bonner, R.S. Rirard, J. Sheffield and J.M. Jez, 2003. Current management challenges in HIV: Tolerability of Antiretrovirals and metabolic complications. aids patient care and STDs. 17(5).
11. Henry, C.A., 2000. Semitogenesis in Zebrafish. Ph.D Thesis University of Washington.
12. Richman, D., M.M. Fischl, M.H. Grieco, M.S. Gottlieb, P.A. Volberding, O.L. Laskin, J.M. Leedom, J.E. Groopman, D.Mildvan, M.S. Hirsch, G. Jackson, D.T.Durack, D. Phil and S. Nusinoff-Lehrman, 1987. N. Engl. J. Med., 317: 192-197.
13. Lewis, L.D., S. Amin, C.I. Civin and P.S. Lietman, 2004. Hum. Exp. Toxicol., 23: 173-185.
14. Tanner, T. and R. Marks, 2008 Delivering drugs by the transdermal route: Review and comment. Skin Res.Technol., 14: 249-260.
15. Guy, R.H., 2010. Transdermal Drug Delivery. In Drug Delivery, Handbook of Experimental *Pharmacology*; Schäfer-Korting, M., Ed., Springer Verlag: Berlin, Germany, pp: 11-13.
16. Valenta, C. and B.G. Auner, 2004. The use of polymers for dermal and transdermal delivery. Eur. J. Pharm. Biopharm., 58: 279-289.
17. Güngör, S., M.S. Erdal and Y. Özsoy, 2012. Plasticizers in Transdermal Delivery Systems. In Recent Advances in Plasticizers; Luqman, M., Ed.; Intech: Rijeka, Crotia, pp: 91-112.
18. Williams, A.C. and B.W. Barry, 2012. Penetration enhancers. Adv. Drug Deliv. Rev., 64: 128-137.
19. Güngör, S., A. Bektaş, F.I. Alp, B.S. Uydeş-Doğan, O. Özdemir, A. Araman and Y. Özsoy, 2008. Matrix type transdermal patches of verapamil hydrochloride: *In vitro* permeation studies through excised rat skin and pharmacodynamic evaluation in rats. Pharm. Dev. Technol., 13: 283-289.
20. Aktar, B., M.S. Erdal, O. Sairli, S. Güngör and Y. Özsoy, 2011. Transdermal films of metoclopramide hydrochloride with terpenes as penetration enhancers: Design, characterization, *in vitro* evaluation and ATR-FTIR spectroscopic investigation on excised pig skin. Asian Chem. Lett., 16: 67-78.
21. Ilium, L., 1998. Chitosan and its use as a pharmaceutical excipient. Pharm Res., 15: 1326Y1331.
22. Felt, O., P. Buri and R. Gurny, 1998. Chitosan: a unique polysaccharide for drug delivery. Drug Dev Ind Pharm. 24:979Y993.
23. Van Der Lubben, I.M., J.C. Verhoef, G. Borchard and H.E. Junginger 2001. Chitosan and its derivatives in mucosal drug and vaccine delivery. Eur. J. Pharm. Sci., 14: 201Y207.
24. Calvo, P., C. Remunan-Lopez, C.L. Vila-Jato and M.J. Alonso 1997. Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. J. Appl. Polym. Sci., 63: 125Y132.
25. Calvo, P., C. Remunan-Lopez, C.L. Vila-Jato and M.J. Alonso, 1997. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharm Res., 14: 1431Y1436.
26. Akbuga, J. and G. Durmaz, 1994. Preparation and evaluation of cross-linked chitosan microspheres containing furosemide. Int. J. Pharm., 111: 217Y222.
27. Berger, J., M. Reist, J.M. Mayer, O. Felt, N.A. Peppas and R.. Gurny, 2004. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. Eur. J. Pharm. Biopharm., 57: 19Y34.
28. Knaul, J.Z., S.M. Hudson and K.A.M. Creber, 1999. Improved mechanical properties of chitosan fibres. J. Appl. Polym. Sci., 72: 1721Y1731.
29. McLeod, G. and Hammer S. Zidovudine, 1992. Five years later. Ann. Intern. Med., 117: 487-501.
30. Bonina, F., C. Puglia, M.G. Rimoli, *et al*., 2002. Synthesis and *in vitro* chemical and enzymatic stability of glycosyl 3'-azido-3'- deoxythymidine derivatives as potential anti-HIV agents. Eur. J. Pharm. Sci., 16: 167-174.

31. Klecker, R.W., J.M. Collins, R. Yarchoan, *et al*., 1987. Plasma and cerebrospinal fluid pharmacokinetics of 3'-azido-3'-deoxythymidine: A novel pyrimidine analog with potential application for the treatment of patients with AIDS and related diseases. Clin Pharmacol. Ther., 41: 407-412.
32. Kim, D. and Y.W. Chien, 1996. Transdermal delivery of dideoxynu- cleoside-type anti-HIV drugs. 2. The effect of vehicle and enhancer on skin permeation. J. Pharm. Sci., 85: 214-219.
33. Panchagnula, R. and J.R.. Patel, 1997. Transdermal delivery of azi- dothymidine (AZT) through rat skin *Ex-vivo*. Pharm. Sci., 3: 83-87.
34. Williams, A.C. and B.W. Barry, 1991. Terpenes and lipid-protein partitioning theory of skin penetration enhancement. Pharm. Res., 8: 17-24.
35. Okabe, H., Y. Obata, K. Takayama and T. Nagai, 1990. Percutaneous absorption enhancing effect and skin irritation of monocyclic monoterpenes. Drug. Des. Deliv., 6: 229-238.
36. Gao, S. and J. Singh, 1998. *In vitro* percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. J. Control. Release, 51: 193-199.
37. Zhao, K. and J. Singh, 1999. *In vitro* percutaneous absorption enhancement of propranolol hydrochloride through porcine epidermis by terpenes/ ethanol. J. Control. Release, 62: 359-366.
38. Pokharkar, V., S.Dhar and N. Singh, 2011. Effect of penetration enhancers on gel formulation of Zidovudine: In vivo and ex vivo studies. PDA Journal of Pharmaceutical Science and Technology, 64: 337-348.
39. Singh, Nripendra and C.D. Upasani, 2013. Skin Permeation of Zidovudine from Crosslinked Chitosan Film Containing Terpene Enhancers for Transdermal Use. IOSR Journal of Pharmacy and Biological Sciences, 7(2): 31-35.
40. Narishetty, S.T.K. and R. Panchagnula, 2004. Transdermal delivery system for Zidovudine: *in vitro*, ex vivo and in vivo evaluation. Biopharmaceutics and Drug Disposition, 25: 9-20.
41. Singh, N. and C.D. Upasani, 2013. Effects of penetration enhancers on *in vitro* permeability of zidovudine gels. Indo american. J. Pharm. Research, 3(4): 3256-3265.
42. Ongpipattanakul, B., R.R. Burnette, R.O. Potts and M.L. Francoeur, 1991. Evidence that oleic acid exists in a separate phase within stratum corneum lipids, Pharm. Res., 7: 350-354.
43. Tanojo, H., A. Bosvan Geest, J.A. Bouwstra, H.E. Junginger and H.E. Bodde 1997. *In vitro* human skin barrier perturbation by oleic acid: thermal analysis and freeze fracture electron microscopy studies, Thermochim. Acta, 293: 77-85.
44. Aboofazeli, R., H. Zia and T.E. Needham, 2002. Transdermal delivery of nicardipine: an approach to *in vitro* permeation enhancement, Drug Delivery, 9: 239-247.
45. Zaslavsky, B.Y., N.N. Ossipov, V.S. Krivitch, L.P. Baholdina and S.V. Rogozhin, 1978. Action of surface-active-substances on biological membranes. II. Hemolytic activity of non-ionic surfactants, Biochim. Biophys. Acta, 507: 1-7.
46. Yamane, M.A., A.C. Williams and B.W. Barry, 1995. Terpene penetration enhancers in propylene glycol/water co-solvent systems: effectiveness and mechanism of action. J. Pharm. Pharmacol., 47: 978-989.
47. El-Kattan, A.F., C.S., Asbil and B.B. Michniak, 2000. The effect of terpene enhancer lipophilicity on the percutaneous permeation of hydrocortisone formulated in HPMC gel systems. Int. J. Pharm., 198: 179-189.
48. Chiu, D.T. and P.H. Duesberg, 1995. The toxicity of Azidothymidine (AZT) on Human and animal cells in culture at concentrations used for antiviral therapy. Genetica, 95: 103-109.
49. Suwanpidokkul, N., P. Thongnopnua and K. Umprayn, 2004. Transdermal delivery of Zidovudine: the effects of vehicles, enhancers and polymer membranes on permeation across cadaver pig skin. AAPS Pharm. Sci. Tech., 5: 1-8.
50. Barry, B.W. and S.L. Bennett, 1987. Effect of penetration enhancers on the permeation of mannitol, hydrocortisone and progesterone through human skin, J. Pharm. Pharmacol., 39: 535-546.
51. Yamane, M.A., A.C. Williams and B.W. Barry, 1995. Terpene penetration enhancers in propylene glycol/water co-solvent systems: effectiveness and mechanism of action. J. Pharm. Pharmacol., 47: 978-989.
52. Narishetty, S.T.K. and R. Panchagnula, 2004. Transdermal delivery of Zidovudine: Effect of terpenes and their mechanism of action. J. Controlled Release., 95: 367-379.
53. Narishetty, S.T.K. and R. Panchagnula, 2004. Transdermal delivery system for Zidovudine: *in vitro*, ex vivo and in vivo evaluation. Biopharmaceutics and Drug Disposition, 25: 9-20.