

## **Skin Permeation of Zidovudine from Crosslinked Chitosan Film Containing Terpene Enhancers for Transdermal Use**

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**Abstract:** *The purpose of this study was to develop a crosslinked chitosan film with Sodium tripolyphosphate (TPP). of transdermal controlled delivery system for zidovudine as a model drug to overcome the many challenges associated with antiretroviral drug therapy. Films included 5 % w/w zidovudine of the dry polymer weight were prepared by the solution casting method using 2% chitosan polymer in 1% v/v acetic acid solution. Physical characteristics such as thickness, tensile strength, folding endurance, weight uniformity, moisture content, were performed., The ex vivo studies were carried out on male Wistar rat dorsal skin using Franz diffusion cell. DSC analysis showed that there was no significant interaction between the drug and polymers. Terpenes such as menthol, cineole, Oleic acid and Tween 80 were also employed as a chemical enhancer to improve the skin penetration of Zidovudine. In vitro skin permeation study showed that oleic acid was the most promising enhancer among the enhancers examined in the present study.*

**Keywords:** *Zidovudine, chitosan, penetration enhancers, in-vitro evaluation.*

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### **I. Introduction**

Acquired immunodeficiency syndrome (AIDS), caused by Human immunodeficiency virus (HIV), is an immunosuppressive disease that results in life-threatening opportunistic infections and malignancies [1]. HIV infection is one of the major threats to human health due to the lack of relevant vaccine and drugs to cure AIDS.

Highly active anti-retroviral therapy (HAART) strategy involves the use of combination anti-retroviral agents for synergistic therapeutic outcomes. With the adoption of HAART, the average survival of HIV/AIDS patients has increased from less than 1 year to over 10 years [1,2]. The joint United Nations programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO) reported on global AIDS epidemic showed 38.0 million adults and 2.3 million children were living with human immunodeficiency virus (HIV) at the end of 2005. The annual number of AIDS deaths can be expected to increase for many years to come, unless more effective and patient compliant anti-retroviral medications are available at affordable prices [3]. 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 [4,5]. Transdermal drug delivery systems are devices containing drug of defined surface area that delivers a pre-determined amount of drug to the surface of intact skin at a pre-predefined rate. This system overcomes the disadvantages associated with oral products like first pass hepatic metabolism, reduced bioavailability, dose dumping and dosing inflexibility [6]. 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  $\beta$  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 [7,8]. Chitosan acts as a penetration enhancer by opening the tight epithelial junctions [9]. 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 [10,11]. 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 [12]. 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 [13,14].

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 [15]. AZT shows dose-related toxic effects, especially on bone marrow, which imposes dose reduction or discontinuance of treatment [16]. 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 [17]. 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

[18,19]. 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.

Additionally, a combination strategy using both menthol and oleic acid at lower concentrations (2.5% w/v in vehicle) was able to attain similar ex vivo penetration enhancement to that of individual enhancers at 5% w/v concentration. These penetration enhancers are known to alter the lipid bilayer fluidity and swelling of polar pathways of the skin [20,21]. Terpenes, naturally occurring volatile oils, appear to be promising candidates for clinically acceptable enhancers [22]. 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%) [23]. Moreover, a variety of terpenes have been shown to increase percutaneous absorption of both hydrophilic and lipophilic drugs [24,25]. In the present study, a transdermal delivery system (TDS) for AZT was prepared using a menthol, cineole, tween 80 and oleic acid, as penetration enhancers, incorporated in chitosan membrane. After in vitro evaluation of the formulation, ex vivo as well as in vivo permeation of AZT from it was TDS were determined in addition to testing the skin irritation potential of studied across rat skin. The objective of the present work was to develop and characterize the zidovudine monolithic transdermal therapeutic systems for in vitro permeation and physico-chemical properties.

## II. Experimental

### 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.

### 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. 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.

**Table 1** Preparation of blank Chitosan films and drug loaded Chitosan films.

Formulation	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% w/w)
F1		2%	0	Glycerin	
F2		2%	5	Glycerin	
F3		2%	10	Glycerin	
F4		2%	20	Glycerin	
F5	5%	2%	0	Glycerin	
<b>F6</b>	<b>5%</b>	<b>2%</b>	<b>5</b>	<b>Glycerin</b>	
F7	5%	2%	10	Glycerin	
F8	5%	2%	20	Glycerin	
F9	5%	2%	<b>5</b>	<b>Glycerin</b>	<b>Cineole</b>
F10	5%	2%	<b>5</b>	<b>Glycerin</b>	<b>Menthol</b>
F11	5%	2%	<b>5</b>	<b>Glycerin</b>	<b>Tween 80</b>
F12	5%	2%	<b>5</b>	<b>Glycerin</b>	<b>Oleic acid</b>

Evaluation of Blank film

### Thickness uniformity test

Film thickness was measured using Mitutoyo corporation CD 6 BS digital vernier caliper, Japan with the smallest possible unit measured count of 0.0001. Results obtained are shown in Table 2.

### 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 clamp 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:

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

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 2.

### Mechanical property measurement

The procedure for thickness uniformity has been already discussed above and results obtained are shown in Table 3.

Moisture Content

Moisture content in the films was measured by using VEEGO Karl-Fisher apparatus.

$$\% \text{ Moisture Content} = \frac{\text{Factor} \times \text{B.R.}}{\text{Weight of sample}} \times 100$$

where,

B.R.: Burette Reading

Factor = Weight of water taken ( $\mu\text{g}$ )/B.R.

Results obtained are shown in Table 4.

### Drug content

The drug loaded chitosan films 1cm<sup>2</sup> 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 sonicated for 3 hrs and filtered through 0.45 $\mu$  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 5.

### **Weight Variation Test**

Disc of 1 cm<sup>2</sup> 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 6.

### **Content Uniformity Test**

To ensure uniform distribution of drug in film, a content uniformity test was performed. 10 disc of 1 cm<sup>2</sup> 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 6.

### **Folding endurance**

The folding endurance was measured manually for the prepared patches. A strip of patch (2 x 2 cm<sup>2</sup>) 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 7.

### **Differential Scanning Colorimetry (DSC)**

Thermograms of pure AZT, chitosan and chitosan film were obtained using Mettler -Toledo DSC 821<sup>e</sup> (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).

### **Scanning Electron Microscopy (SEM)**

Samples for SEM studies were mounted on the specimen slubs using fevicol adhesive. Small samples were mounted directly using scotch double adhesive tape. Samples were coated with gold to the thickness of 100 Å using Hitachi vacuum evaporator (model HUS5GB). Coated samples were analyzed in Hitachi Scanning Electron Microscope model S-450 operated at 1.2kv and photographed.

### **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<sup>2</sup> 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 receptor fluid in each cell. 1 cm<sup>2</sup> 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.

## **III. Results and discussion**

### **Thickness uniformity test**

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 2. Thickness of the films was increased due to the cross-linking of the Sodium TPP with chitosan polymer.

The batches from F5 to F12 were containing drug shows decrease in the thickness because of the interaction of the drug with chitosan polymer. Formation of a compact structure during freeze drying and less porous as observed from SEM studies later.

**Table 2.** Film thickness of blank cross-linked films and drug loaded films.

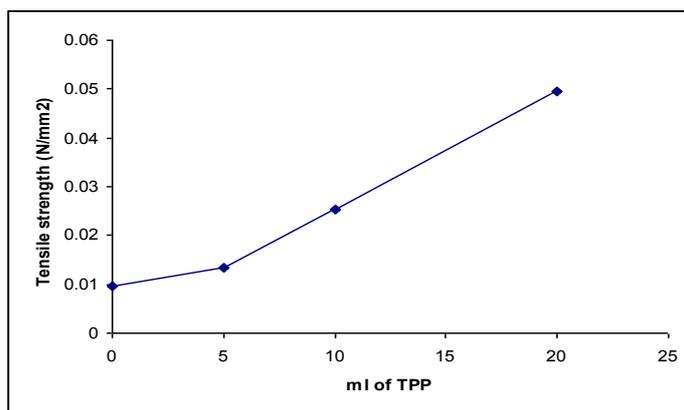
Film code	Film thickness (mm)	± SD
F1	1.201	0.00594
F2	1.4243	0.04656
F3	1.632	0.01195
F4	1.930	0.04987
F5	1.023	0.0483
F6	1.079	0.04817
F7	1.098	0.04572
F8	1.150	0.04306
F9	1.071	0.132
F10	1.073	0.00583
F11	1.081	0.05041
F12	1.077	0.07859

**Mechanical property of the film**

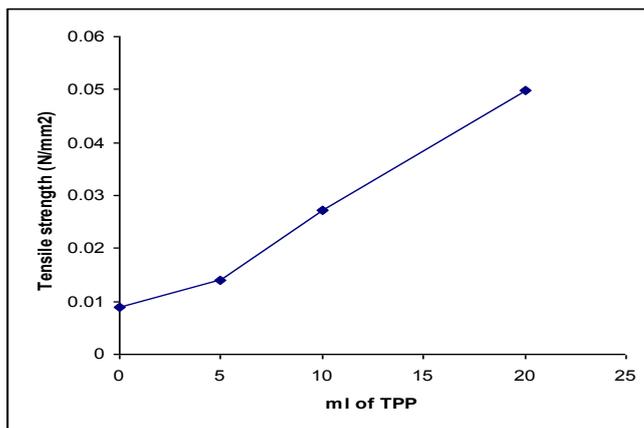
The tensile strength testing provides an indication of the strength at the film. It is suggested that films suitable for transdermal drug delivery should preferably be strong but flexible. The mechanical properties are given in the Table 3. Increasing the concentration of cross-linking agent from 0 ml 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.

**Table 3** Tensile strength of blank Cross-linked films and cross-linked films containing Plastisizer with Drug.

Film code	Tensile strength (N/mm <sup>2</sup> )	± SD
F1	0.00958	0.000912
F2	0.0134	0.000574
F3	0.0253	0.001304
F4	0.0496	0.007127
F5	0.00883	0.003162
F6	0.0140	0.003162
F7	0.0271	0.004848
F8	0.0499	0.004899
F9	0.0141	0.001286
F10	0.0143	0.002051
F11	0.0137	0.00222
F12	0.0139	0.003808



**Fig. 1** Effect of TPP on tensile strength of blank chitosan film.



**Fig. 2** Effect of TPP on tensile strength of Cross-linked films with Plastisizer and Drug.

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.

**Moisture content**

The moisture present in the matrix films helps in maintaining suppleness thus preventing drying and brittleness. Low moisture uptake protects the films from the microbial contamination as well as bulkiness of the transdermal patch. Moisture content % range was found in the range of 3.21 to 3.89 given in Table 4 that was acceptable and within limit.

**Table 4** Moisture content in drug loaded film

Film Code	Moisture content (%) ± SD
F5	3.76 ± 0.06
F6	3.87 ± 0.01
F7	3.21 ± 0.02
F8	3.42 ± 0.03
F9	3.86 ± 0.20
F10	3.85 ± 0.21
F11	3.88 ± 0.33
F12	3.89 ± 0.18

**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 5.

**Table 5** Drug content in the film

Film code	Area of the film (cm <sup>2</sup> )	Loaded drug content (mg)	% Drug content ± SD
F5	49.066	50	99.19 ± 1.75
F6	49.066	50	99.62 ± 0.75
F7	49.066	50	99.94 ± 0.37
F8	49.066	50	99.65 ± 2.84
F9	49.066	50	99.12 ± 0.95
F10	49.066	50	99.92 ± 0.71
F11	49.066	50	99.89 ± 2.24
F12	49.066	50	99.66 ± 0.71

**Content and weight uniformity test**

In the formation of film there was very less drug loss during the process, which is shows in Table 6. It shows that the films have uniformity in its weight and drug content.

**Table 6** Weight variation and content uniformity test for drug loaded film.

Film code	Weight (mg)	± SD	Drug content (mg)	± SD
F5	1.54	0.11	1.005	0.01
<b>F6</b>	1.52	0.13	0.989	0.01
F7	1.26	0.08	1.142	0.01
F8	1.34	0.21	1.001	0.02
F9	1.62	0.31	1.022	0.01
F10	1.96	0.24	1.044	0.02
F11	1.74	0.21	1.033	0.01
F12	1.82	0.22	1.078	0.02

Folding endurance

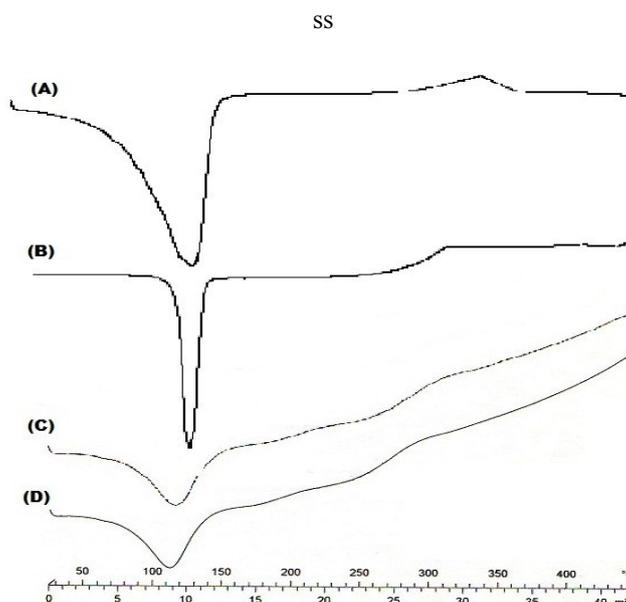
The recorded folding endurance of the patches was shown in Table 7. It depicts all formulations have good film properties. The folding endurance of the patches were containing enhancers decreases the folding endurance of the patches. This test is important to check brittleness, less folding endurance indicates more brittleness.

**Table 7** Folding endurance data of F5 to F12 formulations.

Film code	Trial 1	Trial 1	Trial 1	Mean ± SD
F5	150	165	158	157.66 ± 7.505
<b>F6</b>	113	129	125	122.33 ± 8.326
F7	106	109	103	106 ± 3.0
F8	104	92	97	98 ± 6.027
F9	76	76	67	72.66 ± 4.932
F10	86	89	93	89.33 ± 3.511
F11	99	91	95	95 ± 4.0
F12	82	76	88	82 ± 6

Thermal Studies of Films

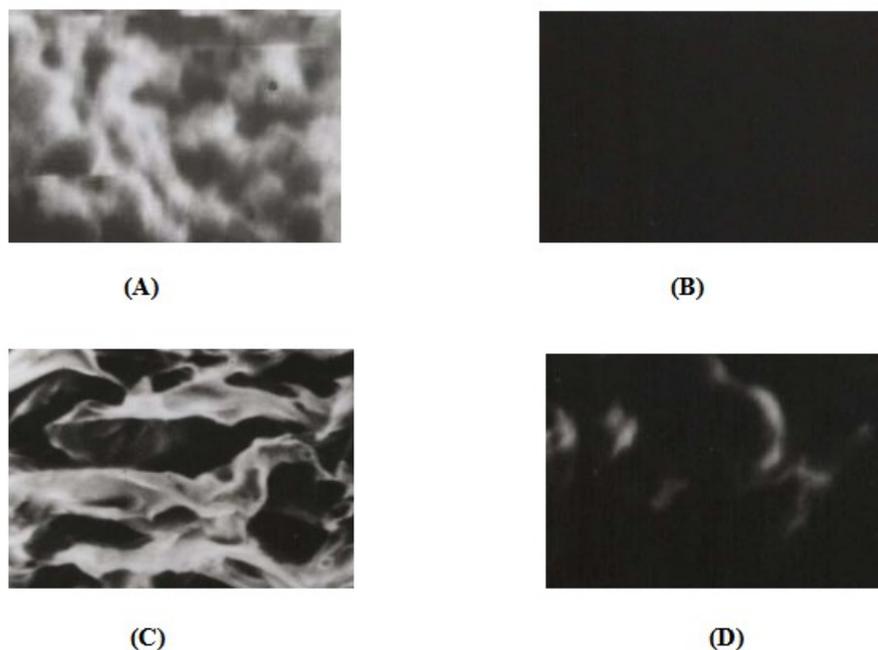
The DSC thermograms of zidovudine, chitosan flakes, chitosan films and zidovudine -chitosan films are shown in Figure 3. DSC studies were performed to understand the behaviour of the zidovudine, cross-linked chitosan & drug loaded chitosan on application of thermal energy. Chitosan flakes showed broad endotherm from 57.16<sup>0</sup>C to 114.68<sup>0</sup>C indicating moisture loss from the sample. The endotherm related to evaporation of water is expected to reflect the molecular changes brought in after cross-linking. DSC thermogram of blank chitosan film showed broad endotherm from 78.43<sup>0</sup>C to 136.59<sup>0</sup>C indicating moisture loss from the sample. DSC thermogram of zidovudine showed a sharp endotherm peak that corresponds to melting in the range of 125<sup>0</sup>C [27]. However this endothermic peak shifted to 126<sup>0</sup>C in case of zidovudine -chitosan films. Overall, the DSC results suggest the absence of any physical interactions between the polymer matrix and zidovudin.



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

### Film morphology studies

SEM microphotographs revealed that commercial sample of Zidovudine has a rough surface, as shown in Figure 4 A. In addition, SEM microphotographs of blank film and Zidovudine films were acquired and compared with that of Zidovudine sample. The morphology of blank film was plain, indicating that the blank film had a smooth surface (Figure 4 B). In contrast to the blank film, features of Zidovudine -chitosan film showed rough surface as compared to blank film (Figure 4 C). This typical surface appearance suggests that Zidovudine is not only dispersed in the film matrix but also projected onto the surface of the film. After diffusion of Zidovudine the morphology of Zidovudine -chitosan film showed smooth surface (Figure 4 D).



**Fig. 4** Scanning Electron Photomicrographs of A) Zidovudine, B) Blank film, C) Zidovudine – Chitosan film, D) Zidovudine – Chitosan film after diffusion. Magnification: A) x 180, B) x 900, C) x 180, D) x 180.

### In-vitro Permeation study

The in-vitro diffusion studies were carried out as described earlier. The amount of drug diffused from F5, F6, F7 and F8 are shown in Figure 5. The drug diffused from F6 was more as compared to F5, F7 and F8 because of low concentration (5 ml of TPP) of cross-linking agent. Increase in the concentration of cross-linking agent leads to an increase in the density of the cross-linked network of chitosan. Due to the more complex network formed in the F7 and F8, the drug diffused from these films were low. On the basis of drug diffusion F6 was selected for incorporating enhancers to increase 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 F6. All enhancers studied do not increase Zidovudine flux to the same extent. Oleic acid significantly increases the flux of Zidovudine across the skin relative 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 [28]. It has also been described that at high concentrations oleic acid can exist as a separate phase within the lipid bilayers [29,30]. Our results confirm that it can work synergistically with ethanol and glycerol, as proposed by other authors [31]. 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 (32). 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 (33). As terpenes are highly lipophilic, it is less likely that they interact with keratin, which has been proved by calorimetric studies where they did not alter protein endotherm (33-34). On the other hand, it was found that terpenes alter neither thermodynamic activity nor partitioning of AZT into stratum corneum from vehicle (35). 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<sup>2</sup>/h is required across human skin from a 50 cm<sup>2</sup> patch in order to reach therapeutically effective plasma concentrations.

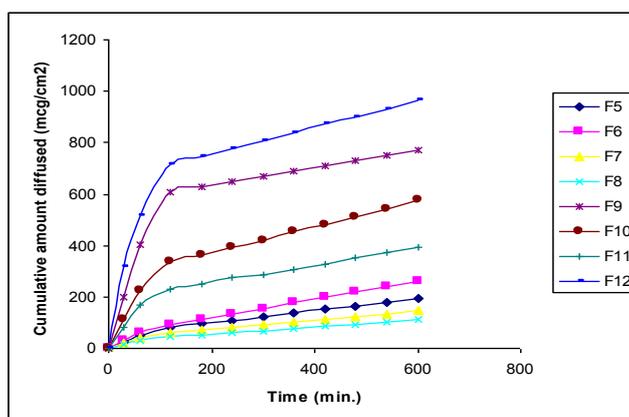
Maximum drug diffusion was observed from F12 containing 5% oleic acid and F9 containing 5% cineole were added as penetration enhancers while minimum drug diffusion was observed from F6 (Control) are illustrated figure 5. It was observed that only 261 µg/cm<sup>2</sup> of drug diffused from control preparation F6 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<sup>2</sup> to 965 µg/cm<sup>2</sup> in F12 containing 5% oleic acid and 773 µg/cm<sup>2</sup> in F9 containing 5% cineole attained in 10 h which is sufficient to achieve and maintain therapeutic concentration of Zidovudine from chitosan film formulations.

In case of ex-vivo diffusion through rat skin, permeation profiles of Zidovudine across rat skin with and without 5% w/w enhancers in chitosan gel formulations are illustrated in figure 6. Flux values of Zidovudine from different formulations in decreasing order are as follows F12 > F9 > F10 > F11 > F6 (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<sup>2</sup>/h) (36). The steady state flux of Zidovudine from film formulation calculated from the linear portions of the cumulative amount permeated against time plots was 31 µg/cm<sup>2</sup>/h from F12 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 (37).

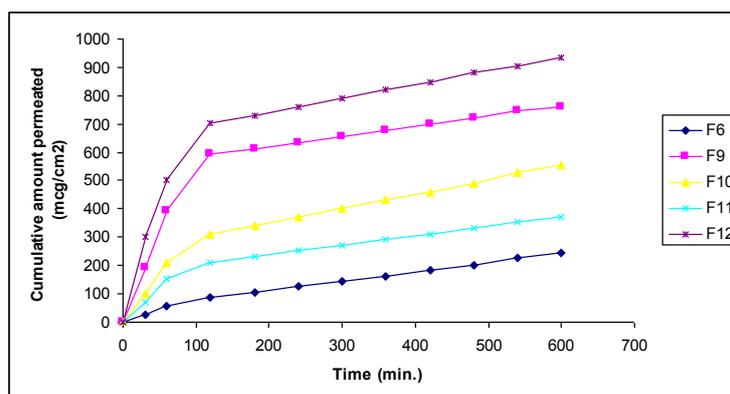
Flux of Zidovudine in terpenes was significantly different from control and other formulations. However, among the enhancers, flux of Zidovudine with cineole was maximum after F12 formulation.

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 [38]. 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 [39]. On the other hand, it was found that terpenes alter neither thermodynamic activity nor partitioning of AZT in to SC from vehicle [40]. 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<sup>2</sup>/h is required across human skin from a 50 cm<sup>2</sup> patch in order to reach therapeutically effective plasma concentrations. However, as rat skin is 3-5 fold more permeable than human skin [41]. The minimum target flux would be approximately 1 mg/cm<sup>2</sup>/h across rat skin. As the steady state flux of AZT across rat skin from our formulations were above 31 µg/cm<sup>2</sup>/h, from 1cm<sup>2</sup>area achieving therapeutically effective plasma concentrations would be possible with these formulations.



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



**Fig. 6.** Ex vivo permeation profiles of AZT across rat skin from gel formulations

#### IV. Conclusion

In conclusion, oleic acid was shown to be exclusively the best enhancer zidovudine chitosan film as F12 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, F12 (oleic acid), F9 (cineole) showed a high flux and short lag time as compared to F6 (control) and other formulation, so achieving therapeutically effective plasma concentrations would be possible with these formulations.

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