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Research Article

Formulation and Optimization of Zidovudine Niosomes

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Abstract. Zidovudine (AZT) is commonly used to treat patients with AIDS, but it is limited by toxicity and high dosing needs. Alternative formulations have been proposed to overcome these drawbacks. The objective of this study was to evaluate process-related variables like hydration and sonication time, rotation speed of evaporation flask, and the effects of charge-inducing agent and centrifugation on zidovudine entrapment and release from niosomes. Formulation of zidovudine niosomes was optimized by altering the proportions of Tween, Span and cholesterol. The effect of process–related variables like hydration time, sonication time, charge-inducing agent, centrifugation and rotational speed of evaporation flask on zidovudine entrapment and release from niosomes was evaluated. The effect of changes in osmotic shock and viscosity were also evaluated. Non-sonicated niosomes were in the size range of 2-3.5 μm and sonicated niosomes formulated with Tween 80 and dicetylphosphate (DCP) had a mean diameter of 801 nm. Zidovudine niosomes formulated with Tween 80 entrapped high amounts of drug and the addition of DCP enhanced drug release for a longer time (88.72% over 12 h). The mechanism of release from Tween 80 formulation was the Fickian type and obeyed first-order release kinetics. Niosomes can be formulated by proper adjustment of process parameters to enhance zidovudine entrapment and sustainability of release. These improvements in zidovudine formulation may be useful in developing a more effective AIDS therapy.

KEY WORDS: AZT; entrapment efficacy; niosomes; process-related variables; zidovudine.

INTRODUCTION

Nonionic surfactant vesicles (niosomes) formed from self-assembly of hydrated synthetic nonionic surfactant monomers are capable of entrapping a variety of drugs and have been evaluated as an alternative to liposomes (1). Nonionic surfactants form unilamellar and multilamellar vesicles that have similar physical properties to liposomes and are a relatively inexpensive drug delivery system. In niosomes, soluble drug molecules are present in the aqueous compartments between the bilayer whereas insoluble ones are entrapped within the bilayer matrix. The use of niosomes for drug delivery can alter the biodistribution to provide a greater degree of targeting of the drug to selected tissues, sustained release and altered pharmacokinetics (2–4).

Zidovudine (AZT), the first anti-HIV compound approved for clinical use is still widely used alone or in combination with other antiviral agents for treatment of AIDS and AIDS-related complex. The main limitations on the therapeutic effectiveness of AZT are its dose-dependent hematological toxicity, high first-pass metabolism, poor bioavailability and very short biological half-life (5). After oral administration, it is rapidly absorbed from the gastrointestinal tract with a peak plasma concentration of 1.2 μg/ml at 0.8 h. It is also rapidly metabolized to the inactive glucuronide with a mean elimination half-life ($t_{1/2}$) of 1 h. This necessitates frequent administration of large doses (200 mg every 4 h) since it is crucial to maintain the systemic drug concentration within the therapeutic level throughout the treatment course. In order to overcome these disadvantages, some researchers have proposed niosomes as a carrier to deliver AZT. Literature review on niosomal AZT formulations (6) reported that niosomes containing entrapped and free AZT prolonged drug half-life in rabbit serum. AZT aspasomes have also been evaluated for enhancing skin permeation and stability (7).

HIV primarily infects helper T cells, macrophages, and dendritic cells, which are vital to the human immune system. Macrophages are stationed at strategic points like liver, spleen, lungs, and connective tissues where microbial invasions are likely to occur (8) and can become a reservoir for HIV with viral replication occurring in the macrophages after infection (9). Vesicles or particles from 150 nm to 2 μm exhibit maximal phagocytosis by macrophages (10), while vesicles in the size range of 70-150 nm prolong plasma drug concentrations.

The purpose of this study was to evaluate the process parameters that critically affect the formulation of niosomes with respect to entrapment and release in targeting AZT to macrophages. There is little information in the literature on optimizing the different processing variables that are impor-

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tant in the formulation of AZT niosomes for development of an improved drug delivery system. In this investigation, we optimized hydration time, charge-inducing agent, centrifugation, and rotational speed of the evaporation flask. Measurements of *in vitro* drug release were done to assess the effectiveness of the drug delivery system.

MATERIALS AND METHODS

Chemicals

AZT was a gift from Aurobindo Ltd (India). Cholesterol (CHOL), *polyoxyethylene sorbitan monolaurate* (Tween 20), *polyoxyethylene sorbitan monopalmitate* (Tween 40), *polyoxyethylene sorbitan monostearate* (Tween 60), *polyoxyethylene sorbitan monooleate* (Tween 80), *sorbitan monolaurate* (Span 20), *sorbitan monopalmitate* (Span 40), *sorbitan monostearate* (Span 60), *sorbitan monooleate* (Span 80), and dicetyl phosphate (DCP) were from Sigma (USA). All materials used in the study were of analytical grade.

Methods

Formulation of Niosomes

Multilamellar niosomes were prepared by the thin-film hydration method. Accurately weighed quantities of drug, surfactant (Tween or Span), and CHOL were dissolved in chloroform in a round-bottom flask. Different molar ratios of DCP were added to each formulation as a negative charge-inducing agent. The chloroform was evaporated at 60°C under reduced pressure using a rotary flash evaporator (Superfit, India). After chloroform evaporation, the flask was kept under vacuum overnight in a nitrogen atmosphere to remove residual solvent. The thin films were hydrated (11) with 6 ml of phosphate buffered saline (PBS), pH 7.4, and the flask was kept rotating at 60°C at various revolutions per minute (rpms). Formulations were sonicated three times at 50 Hz in a bath-sonicator (Ralsonics model RP 120, Mumbai, India) for 15 min with 5-min interval between successive times. Vesicle suspensions were also sonicated for 5 and 2 min.

Characterization of Sonicated Vesicles by Transmission Electron Microscopy

A drop of the sample was placed onto a carbon-coated grid and allowed to dry to a thin film. Before drying of this film on the grid, it was negatively stained with 1% phosphotungstic acid. For this, a drop of staining solution was pipetted onto the film and the excess drained off with filter paper. The grid was allowed to air dry thoroughly and then examined using a transmission electron microscope (12) with an accelerating voltage of 80 kV.

Determination of Vesicle Diameter

The size, shape, and lamellar nature of vesicles in non-sonicated formulations were observed by optical microscopy (13) using a calibrated eyepiece micrometer, and photographs were taken at ×400 magnification with a digital camera (Olympus, 8.1 megapixel, Japan).

The *z*-average diameter of sonicated vesicles was determined by dynamic light scattering (14) using a Zetasizer, Nano ZS 90 (Malvern instruments). For the measurement, 100 µl of the formulation was diluted with an appropriate volume of PBS, pH 7.4 and the vesicle diameter, polydispersity index, and zeta potential were determined.

Determination of Drug Entrapment in Vesicles

Zidovudine niosomal formulations were centrifuged at 15,700×*g* for 90 min at 4°C using a refrigerated centrifuge (Eppendorf, 5415 R, Germany) to separate niosomes from non-entrapped drug. Concentration of the free drug in the supernatant was determined by measuring absorbance at 267 nm with a UV spectrophotometer (Shimadzu, UV 1650 PC, Kyoto, Japan). The percentage of drug entrapment in niosomes was calculated (15). This process was repeated thrice to ensure that free drug was completely removed.

$$\%\text{drug entrapment} = [\text{total drug} - \text{drug in supernatant} / \text{total drug}] \times 100$$

Percent drug entrapment was confirmed by lysing the niosomes with n-propanol after centrifugation and measuring absorbance at 267 nm.

Osmotic Shock

The effect of osmotic shock on niosomal formulations with and without DCP was investigated by monitoring the change in vesicle diameter after incubation of niosome suspensions in media of different tonicity (16): 1 M NaI (hypertonic), 0.9% NaCl (normal) and 0.5% NaCl (hypotonic). Suspensions were incubated in these media for 3 h and the change in vesicle size was measured by optical microscopy with a calibrated eyepiece micrometer.

Determination of Viscosity

Viscosity of the formulations was determined (17) using an Ostwald viscometer (Yash Enterprises, Pune, India) at room temperature.

In Vitro Release Studies

In vitro release was studied using a dialysis bag (Himedia dialysis membrane, 12,000-14,000 molecular weight cut-off) as a 'donor compartment'. Niosomes containing entrapped zidovudine obtained after centrifugation of 2 ml of the formulation were resuspended in 1 ml of PBS, pH 7.4, and used for the release study. The dialysis membrane was soaked in warm water for 10 min, one end was sealed with a clip, the niosome preparation or free zidovudine solution was pipetted into the bag and the bag was sealed with another closure clip to prevent leakage. The dialysis bag was placed in 100 ml of PBS, pH 7.4, at 37±2°C. The medium, which acted as the receptor compartment, was stirred at 100 rpm. Samples of medium (5 ml) were withdrawn hourly and replaced with fresh buffer and zidovudine absorbance at 267 nm was

measured using PBS as blank (18). Results were the mean values of three runs. The mechanism of AZT release from niosomal formulations was determined using the following mathematical models: zero-order kinetics, first -order kinetics, Higuchi kinetics, and the Korsmeyer-Peppas and Hixson-Crowel models.

Data Analysis and Statistics

Data are expressed as mean±SD. Statistical analysis was performed by Students' *t* test using GraphPad Prism (version 3.0). Significance was defined at p values <0.05.

RESULTS AND DISCUSSION

Preformulation Studies

Preformulation studies were carried out with nonionic surfactant Tween 80 at 30, 50, 60, 70, 90, and 120 μM with a fixed concentration of AZT and 20 μM cholesterol. Stable vesicles did not form with 30, 50, or 60 μM Tween 80; but a crystal-like structure was seen. At 70 and 120 μM Tween, spherical vesicles were seen along with aggregates (Fig. 1a). In 90 μM Tween, spherical vesicles without crystals were obtained. The process-related variables of sonication time, hydration medium, hydration time, speed of rotation of flask

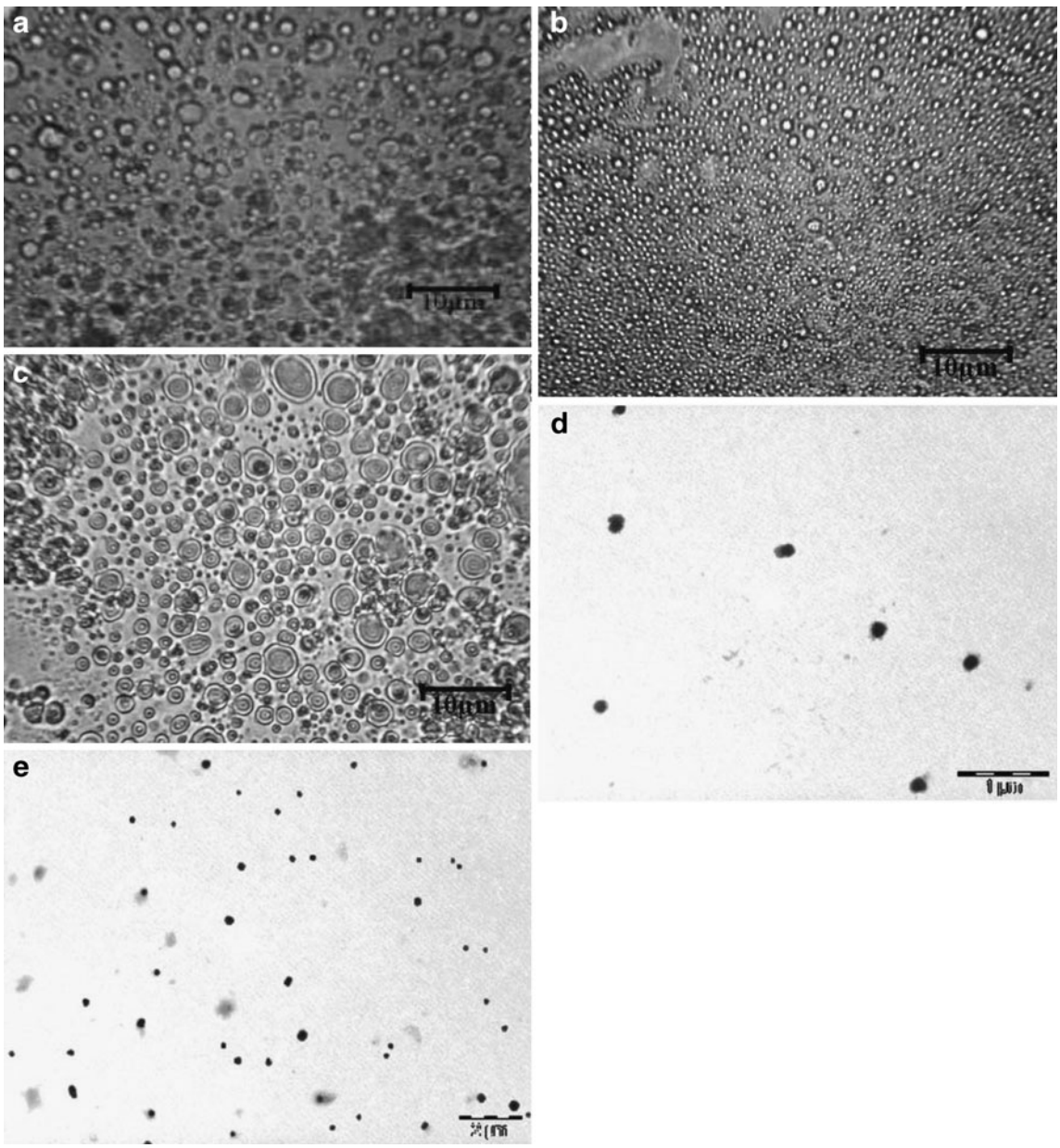

Fig. 1. Photomicrographs of zidovudine-loaded niosomes; **a** Tween 80 formulation at 1:6 Cholesterol/Tween molar ratio showing vesicles with aggregates. **b** Tween 80 formulation at 1:4.5 Cholesterol/Tween molar ratio showing unilamellar vesicles after 2-min sonication. **c** Tween 80 formulation at 1:4.5 Cholesterol/Tween molar ratio showing multilamellar vesicles without sonication. **d** TEM image of sonicated Tween 80 formulation at 1:4.5 Cholesterol/Tween molar ratio with DCP. **e** TEM image of sonicated Tween 80 formulation at 1:4.5 Cholesterol/Tween molar ratio without DCP

evaporator, and charge-inducing agents were investigated in vesicle formation with different concentration of Tween 80 and 20 μM cholesterol with a fixed amount (75 mg) of zidovudine (Table I).

Optimization of Process-related Variables

Effect of Sonication Time

Spherical niosome vesicles were not observed after 5 or 15 min sonication suggesting that ≥5 min exposure to ultrasound may damage the vesicles. Two minutes of sonication produced vesicles with a uniform unilamellar structure (Fig. 1b).

Hydration Medium and Time

Tween 80 formulations hydrated with 6 ml of phosphate buffered saline, pH 7.4, formed stable vesicles. Increasing the volume of hydration medium increased drug leakage (data not shown). Increasing the hydration time of the film from 20 to 45 min resulted in a higher percentage of drug entrapment (Table I).

Rotational Speed of Evaporator Flask

The thickness and uniformity of the film depended upon the rotational speed of the flask. A speed of 100 rpm yielded a uniformly thin lipid film resulting in spherical vesicles on hydration. Lower and higher rpm produced thick films that formed aggregates of vesicles on hydration (data not shown).

Influence of Charge-inducing Agent

Niosomes were formulated with various amounts of the charge-inducing agent, DCP. The inclusion of a charge-inducing agent in the lipid layer prevents the aggregation and fusion of vesicles, and maintains their integrity and uniformity (12). The optimal concentration of DCP in niosomes was identified based on entrapment efficiency. It was found that 5 μM DCP produced spherical vesicles with increased drug entrapment and without aggregation (Table I).

Effect of Ultracentrifugation on Separation of Entrapped Drug

Ultracentrifugation at 15,700×*g* for 90 min at 4°C sediments the vesicles effectively (Table I).

From the above studies, formulation with a Tween 80/cholesterol ratio of 1:4.5 μmol, 10 ml of chloroform as solvent, 45 min hydration time with phosphate buffered saline at pH 7.4, 100 rpm rotation speed of the evaporator flask, 5 μM charge-inducing agent, and 2 min of sonication gave the maximum drug entrapment of 80.3%. Hence, these parameters were used to prepare other formulations with different non-ionic surfactants for further studies.

Vesicle size, shape, lamellarity, entrapment efficacy, and *in vitro* release of AZT from niosomes formulated using Tween 20, 40, 60 and Span 20, 40, 60, 80 with and without DCP were investigated.

Vesicle Size and Shape

Photomicrographs revealed that niosomes were spherical and multilamellar (Fig. 1c). Sonicated vesicles remain non-aggregated up to 15 days compared to non-sonicated vesicles (data not shown).

Non-sonicated vesicles were 2-3.5 μ (Table II). Addition of cholesterol to the formulation led to spherical vesicles that were stable on sonication under the study conditions. Cholesterol abolished the phase transition in a manner that may be analogous to that observed for liposomes (17).

Niosomal vesicles from Tween 80 formulations with cholesterol were larger than those with Tween 60. Vesicles of Tween 20 were larger than those of 40, 80, and 60. This suggests that when the hydrophilicity of the surfactant increases, the vesicle size increases. Similar results were observed by Agarwal *et al.* (13).

Table I. Optimization of Process-related Variables in the Formulation of Zidovudine Niosomes with Different Concentrations of Tween 80 and Fixed Amounts of Cholesterol (20 μm) and Zidovudine (75 mg)

		CHOL/Twn 80										
S. no	Batch no.	μM ratio	WT (mg)	H M		HV in ml	H T in min	% E	SLT (ml)	CS rpm	RSF rpm	B S T min
1	Zid 01	1:1.5	7.6:39.3	PBS	pH 7.4	6	20	72.18±0.36	10	5,000	200	2
							45	74.8±0.21				
2	Zid 02	1:2.5	7.6:65.5	PBS	pH 7.4	6	20	75.82±0.64	10	5,000	200	2
							45	77.39±0.26				
3	Zid 03	1:3.0	7.6:78.6	PBS	pH 7.4	6	20	73.16±0.82	10	10,000	150	2
							45	74.12±0.38				
4	Zid 04	1:3.5	7.6:91.7	PBS	pH 7.4	6	20	73.26±0.27	10	10,000	150	2
							45	76.0±0.26				
5	Zid 05	1:4.5	7.6:117.9	PBS	pH 7.4	6	20	78.23±0.18	10	13,000	100	2
							45	80.3±0.84				
6	Zid 06	1:6.0	7.6:157.2	PBS	pH 7.4	6	20	78.64±0.92	10	13,000	100	2
							45	80.32±0.91				

Data are means±SD (n=3)
Twn Tween, *CHOL* cholesterol, *WT* weight, *HM* hydration medium, *HV* hydration volume, *HT* hydration time,% *E* percentage entrapment, *SLT* solvent, *CS* centrifugation speed, *RSF* rotation speed of flask, *BST* bath sonication time

Table II. Average Vesicle Size, Viscosity, and Percent Zidovudine Entrapped in Niosomes

S. no	Formulation cholesterol/ surfactant ratio (1:4.5)	Average vesicle size of unsonicated formulation (μm)	Viscosity (centipoise)	% drug entrapped
1	Tween 20	3.04±0.314	2.096	83.8±1.2
2	Tween 40	2.75±0.125	2.267	81.6±0.9
3	Tween 60	2.48±0.288	2.277	82.4±1.4
4	Tween 80	2.66±0.190	1.955	79.5±0.8
5	Span 20	2.62±0.450	3.248	81.2±1.0
6	Span 40	2.66±0.314	2.133	82.1±1.6
7	Span 60	2.66±0.260	1.986	86.6±1.6
8	Span 80	2.04±0.144	2.936	78.7±0.9
9	Tween 20 with DCP[a]	<2	2.347	82.3±0.9
10	Tween 40 with DCP[a]	<2	2.620	79.7±1.3
11	Tween 60 with DCP[a]	<2	2.583	79.8±1.6
12	Tween 80 with DCP[a]	<2	2.274	79.7±1.2
13	Span 20 with DCP[a]	3.0±0.180	3.487	82.3±1.3
14	Span 40 with DCP[a]	3.52±0.195	3.422	81.1±1.84
15	Span 60 with DCP[a]	2.48±0.250	2.267	85.7±1.7
16	Span 80 with DCP[a]	2.50±0.214	3.240	78.8±1.6

Data are means±SD (n=3)
[a] Concentration of DCP used in the formulation is 5 μm

TEM images of niosomes (Fig. 1d, e) show that multilamellar niosomes prepared by thin film hydration were spherical and that the sonicated Tween 80 vesicles with and without DCP were nanosize with mean diameter of 149.62 and 137.28 nm, respectively. Sonicated vesicles without DCP in Tween 80 formulation had a narrower size distribution [polydispersity index, (PDI)] and were smaller than those with DCP (Table III), while for Span 20 formulation the vesicle size and PDI without DCP is comparable to that with DCP. Vesicle size in niosomes depends upon the properties of the molecules in the bilayers but also the interaction between bilayers. The increased size with DCP may be due to the increased separation between bilayers of niosome vesicles

Measurements of zeta potential show that Tween 80 niosomes possess negative charge (−0.0925 mV) at pH 7.4 indicating that a weak electrostatic repulsive force exists in the niosomal bilayer. Also, the inclusion of DCP in Tween 80 niosomes increased the zeta potential (−7.79 mV) compared to the Tween 80 formulation without DCP. Particles with zeta potential close to zero are less able to be phagocytosed than charged particles (19).

Entrapment Efficiency

AZT entrapment was influenced by the affinity of the drug for the niosome material, the thickness of the niosome bilayers, the drug solubility in water, and the compatibility between the drug and niosome material (10).

Table III. Mean Diameter and Polydispersity Index of Sonicated Zidovudine Niosomes

Formulation cholesterol/ surfactant ratio (1:4.5)	Mean diameter (μm)	Polydispersity index
Tween 80	0.282±0.13	0.49±0.05
Tween 80 with DCP[a]	0.801±0.23	0.76±0.16
Span 20	2.793±0.14	0.43±0.04
Span 20 with DCP[a]	2.713±0.15	0.49±0.18

Data are means±SD (n=3)
[a] Concentration of DCP used in the formulation is 5 μm

Role of Cholesterol Content in Drug Entrapment

Many nonionic surfactants form vesicles when cholesterol is included in the bilayer to the level of 30-50 mole% (6). In this study, AZT niosomal formulations contained 20 μM cholesterol and the vesicles formed without aggregation. High entrapment was found in Span 40 and Span 60 formulation at a cholesterol/surfactant micromolar ratio of 1:4.5 (Table II). Incorporation of cholesterol into niosomes at ratios up to 1:6.0 increased the encapsulation efficiency of zidovudine (Table I). Inclusion of cholesterol increases the viscosity of the formulation indicating more rigidity of the bilayer membrane. Moreover, drug partitioning will occur more easily in highly ordered systems of surfactant and cholesterol. The ability of the lamellar surfactant phase to

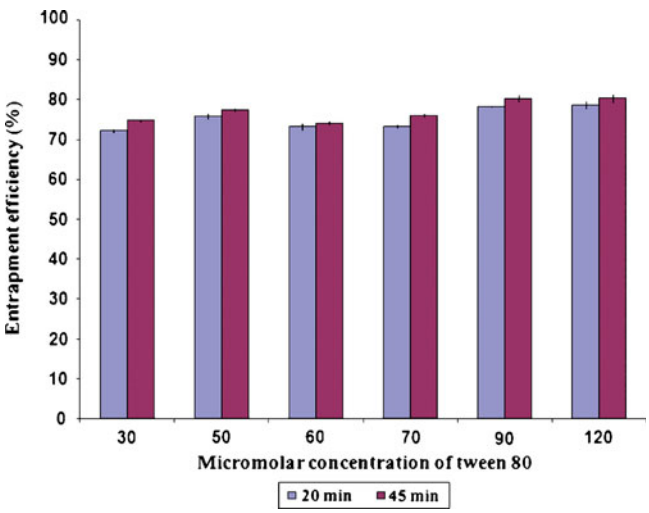

Fig. 2. Effect of different micromolar concentrations of Tween 80 on percentage entrapment

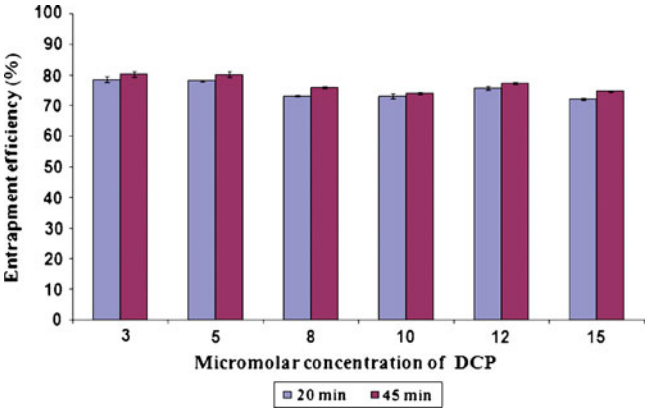

Fig. 3. Effect of different micromolar concentrations of DCP on percentage entrapment

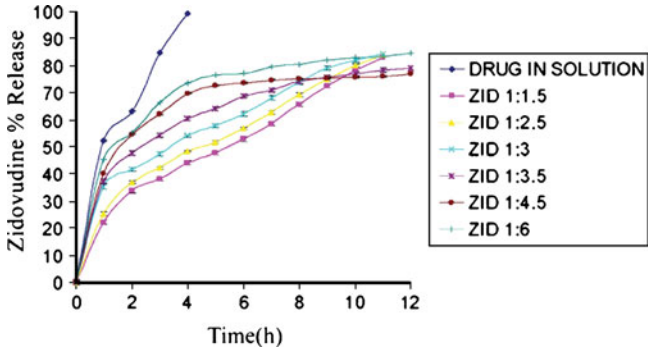

Fig. 4. *In vitro* release of zidovudine from niosome formulations of Tween 20 and cholesterol at various ratios. All values shown as mean ± standard deviation (n=3)

accommodate drug, depends upon the structure of the surfactant phase (20). The percentage of drug entrapment was high in Span 60 and Tween 20 formulations and low in Span 80 and Tween 80 formulations (Table II). Entrapment efficiency increased with decreased cholesterol content in Tween 80 formulations (Table I).

Role of Surfactant in Drug Entrapment

The effect of different concentrations of Tween 80 on percent entrapment is depicted in Fig. 2. An increase in Tween 80 beyond a ratio of cholesterol/surfactant of 1:4.5 results in spherical vesicles along with aggregates. This shows that surfactant beyond a certain concentration with a low amount of cholesterol will not form stable vesicles with good entrapment (Fig. 1a).

Entrapment efficiency for niosomes prepared with Tween 20 was higher than that with Tween 60. Similarly, the entrapment efficiency of Tween 60 was higher than Tween 40 and Tween 80 (Table II). This shows that the longer the alkyl chain of the surfactant, the less drug will be entrapped. Tween 80 has a longer saturated alkyl chain than Tween 60 and lower entrapment efficiency. The length of the alkyl chain influences the hydrophilic-lipophilic balance (HLB) value of the surfactant and the lower the HLB value of the surfactant, the lower will be the entrapment efficiency. A low HLB value was found with Tween 80 (15) and a higher HLB value with Tween 20 (16.7). Our results are dissimilar to the results reported by Ahmed *et al.* (21) which indicate that the lower the HLB of the surfactant, the higher will be the entrapment efficiency.

The entrapment efficiency of Span 60 was higher than Span 20, 40, and 80. The higher entrapment may be due to the solid nature, hydrophobicity, and high-phase transition temperature of the surfactant (22). Entrapment was found to be greater in span 60 formulations (86.6%) than in span 80 formulations (78.7%; Table II), even though both have the same head group. Our results were similar to those reported in the literature for carboxyfluorescein niosomes (22). The

Table IV. Effect of Osmotic Shock on Non-sonicated Zidovudine Niosomal Formulations

S. no	Formulation cholesterol/ surfactant ratio (1: 4.5)	Average vesicle size (μm) after incubation with			
		PBS pH 7.4	Hypotonic 0.5% NaCl[b]	Normal 0.9% NaCl[b]	Hypertonic 1 M NaI[b]
1	Tween 20	3.04±0.314	3.4±0.440	3.2±0.356	Shrunk
2	Tween 40	2.75±0.125	3.0±0.260	2.8±0.285	Shrunk
3	Tween 60	2.48±0.288	2.8±0.324	2.6±0.200	Shrunk
4	Tween 80	2.66±0.190	3.0±0.214	2.8±0.346	Shrunk
5	Span 20	2.62±0.450	3.0±0.350	2.8±0.288	Shrunk
6	Span 40	2.66±0.314	3.0±0.250	2.8±0.365	Shrunk
7	Span 60	2.66±0.260	2.8±0.185	2.6±0.165	Shrunk
8	Span 80	2.04±0.144	2.6±0.268	2.4±0.240	Shrunk
9	Tween 20 with DCP[a]	<2	2.2±0.150	<2	Shrunk
10	Tween 40 with DCP[a]	<2	2.3±0.230	<2	Shrunk
11	Tween 60 with DCP[a]	<2	2.5±0.195	<2	Shrunk
12	Tween 80 with DCP[a]	<2	2.1 ±0.260	<2	Shrunk
13	Span 20 with DCP[a]	3.0±0.180	3.4±0.280	3.0±0.380	Shrunk
14	Span 40 with DCP[a]	3.52±0.195	3.8±0.256	3.5±0.390	Shrunk
15	Span 60 with DCP[a]	2.48±0.250	3.6±0.145	3.4±0.248	Shrunk
16	Span 80 with DCP[a]	2.50±0.214	3.0±0.368	2.5±0.390	Shrunk

Data are means±SD (n=3)
[a] Concentration of DCP used in the formulation is 5 micromolar.
[b] Hypertonic solution, 1 M sodium iodide (NaI); hypotonic solution, 0.5% sodium; chloride; normal saline solution, 0.9% sodium chloride

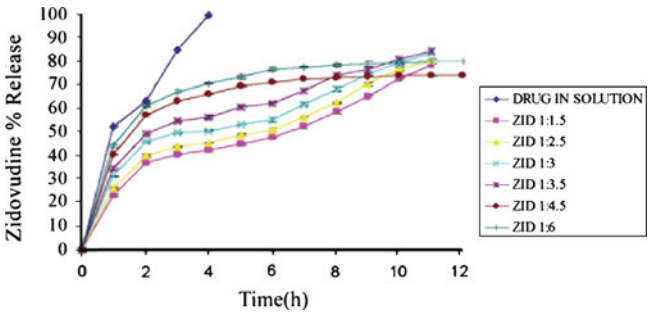

Fig. 5. *In vitro* release of zidovudine from niosome formulations of Tween 40 and cholesterol at various ratios. All values shown as mean± standard deviation (n=3)

introduction of double bonds into the paraffin chains in liposomes causes lower entrapment efficiency in span 80 systems (23). Our results are similar to those with liposomes. Sorbitan monostearate (Span 60 C_{18}) vesicles showed increased entrapment efficiency (86.6%) when compared to Sorbitan monopalmitate (Span 40 C_{16}) niosomes (82.1%; Table II). These results are similar to those reported for non-sonicated sorbitan monoester niosomes loaded with doxorubicin confirming the hypothesis that entrapment efficiency may be correlated with the hydrophobicity of the alkyl chain of the sorbitan esters (24).

Role of Charge-inducing Agents in Drug Entrapment

The effect of charge on niosome bilayers is a matter of debate. Some reports (25,26) have concluded that negatively charged liposomes are more effective in drug entrapment than neutral liposomes. The effect of different micromolar concentrations of DCP on percentage entrapment of Tween 80 formulation is shown in Fig. 3. Formulations with 5 μM DCP show good entrapment at a surfactant/cholesterol ratio of 1: 4.5. Formulations of Tween with DCP decreased drug entrapment except with Tween 80 where a marginal increase in entrapment was observed. In Span 40 and 60 formulations, DCP decreased entrapment whereas in Span 20 and 80 formulations, entrapment was increased (Table II). These results show that inclusion of DCP alters the entrapment; but that it also depends upon the alkyl side-chain of the surfactant. Tween and Span formulations containing oleate

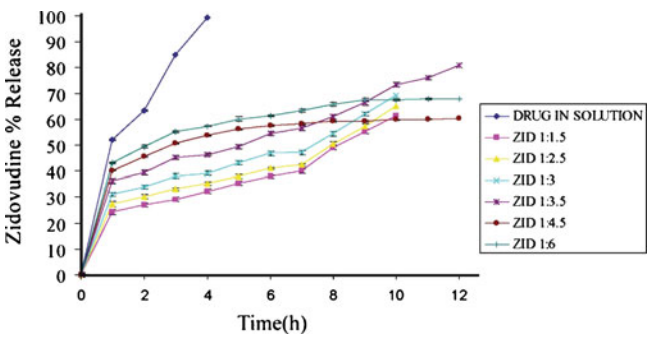

Fig. 6. *In vitro* release of zidovudine from niosome formulations of Tween 60 and cholesterol at various ratios. All values shown as mean± standard deviation (n=3)

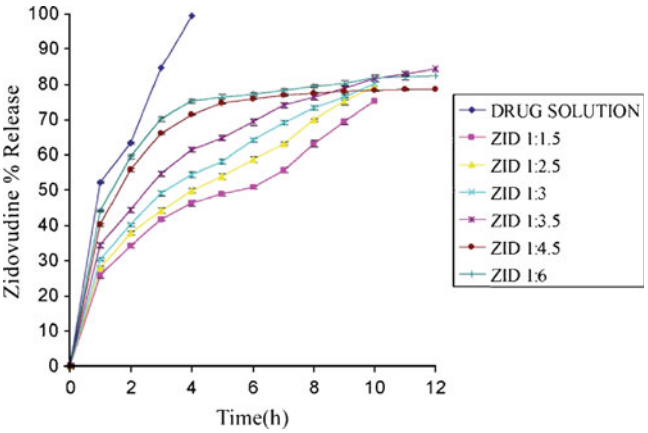

Fig. 7. *In vitro* release of zidovudine from niosome formulations of Tween 80 and cholesterol at various ratios. All values shown as mean± standard deviation (n=3)

as the fatty acid increased drug entrapment in the presence of the negative charge-inducing agent DCP.

Osmotic Shock

Formulations were treated with hypotonic (0.5% NaCl), hypertonic (1 M NaI), or normal saline (0.9% NaCl) solutions. Vesicle size was not increased significantly in formulations with cholesterol. This shows that cholesterol increased the rigidity of the vesicles. Increase in vesicle size was observed in formulations with cholesterol and DCP. Inclusion of DCP reduces the rigidity due to electrostatic repulsion thereby increasing vesicle size. In hypertonic solution, all the formulations shrank uniformly. Formulations incubated with saline showed a slight increase in vesicle size when compared to other media (Table IV). This demonstrates that zidovudine niosomes could be diluted with normal saline for parenteral use.

Viscosity

Inclusion of DCP in Tween and Span formulations increases the viscosity (Table II). Span 20 formulations with

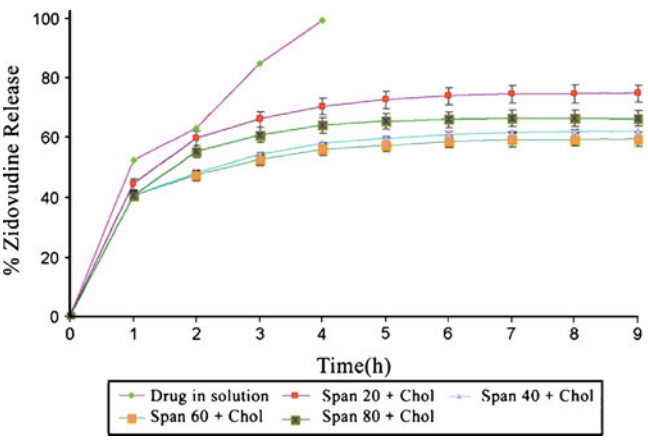

Fig. 8. *In vitro* release of zidovudine from niosome formulations of Span with cholesterol. All values shown as mean±standard deviation (n=3)

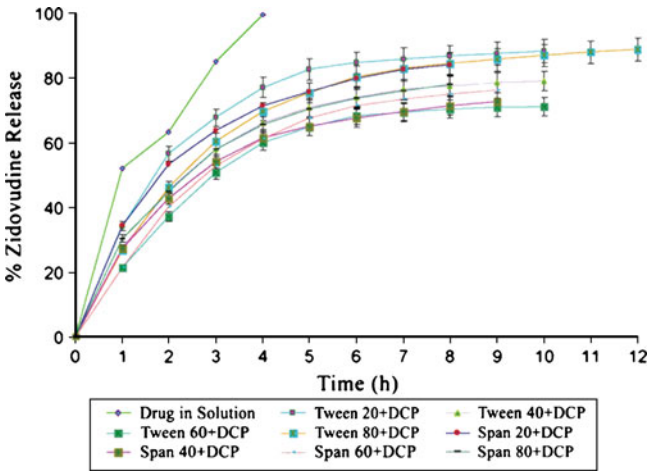

Fig. 9. *In vitro* release of zidovudine from niosome formulations of Tween 20, 40, 60, and 80, and Span 20, 40, 60, and 80 with DCP. All values shown as mean±standard deviation (n=3)

cholesterol were highly viscous, while Tween 80 formulations were less viscous. Polyhedral niosomes were reported to have high viscosity at room temperature when compared to spherical niosomes because of their rigid shape and temperature-dependant shape transformation (18).

In vitro Release

Niosomal zidovudine formulations with Tween 20, 40, 60, and 80 show significant reduction in *in vitro* drug release ($p<$ 0.001) in 4 h compared with drug in solution (Figs. 4, 5, 6, 7). The initial drug release in 3 h from all the formulation ratios was 38-66% for Tween 20 (Fig. 4), 40-67% for Tween 40 (Fig. 5), 29-55% for Tween 60 (Fig. 6), and 41-70% for Tween 80 (Fig. 7). Fast drug release in the initial hours may be due to the release of adsorbed drug from the lipophilic region of niosomes, which will help to achieve the optimal loading dose. When the concentration of cholesterol was high in Tween 80 (cholesterol/surfactant micromolar ratio 1:1.5) the drug release was 41.73% in 3 h, and in formulations with a low concentration of cholesterol (cholesterol/surfactant micromolar ratio 1:6), the percentage drug release was 70.16% (Fig. 7). Increasing cholesterol markedly reduces the efflux of the drug. Inclusion of cholesterol fills the pores in vesicular bilayers and abolishes the gel-liquid phase transition of liposomal and niosomal systems resulting in niosomes that are less leaky (27). This confirms that cholesterol in the formulation acts as a membrane stabilizing agent that helps to sustain drug release. Release of zidovudine from Tween 20, 40, 60, and 80 niosomes (cholesterol/surfactant micromolar ratio 1:4.5) was slow compared to other ratios (Figs. 4, 5, 6, 7). Initial rapid release up to 3 h followed by extended release up to 12 h was observed in Tween 80 formulations. The release from Tween 80 formulations (cholesterol/surfactant micromolar ratio 1:4.5) was 78% (Fig. 7) and from Tween 60 formulations, 82% in 12 h (Fig. 6).

The fatty acid chain length of polyoxyethylene sorbitan-type surfactants influences drug release. Based on the release rate, the different Tweens can be ranked as Tween 80 ($C_{9=9}$)> Tween 20 (C_{12})>Tween 40 (C_{16})>Tween 60 (C_{18}). The greater the chain length, the slower the release rate. When comparing the release of Tween 80 with $C_{9=9}$ and Tween 60 with C_{18}, the unsaturation in Tween 80 was responsible for the higher rate of release. This is in accordance with the concept that the unsaturation in the chain increases chain fluidity and permeability (28).

Drug release from Span 80, 60, 40, and 20 (cholesterol/surfactant micromolar ratio 1:4.5) was found to be 66.8%, 59.9%, 62.5%, and 75.1% in 9 h. The entire amount of loaded drug was not released from the niosomes. This may be due to entrapment of the drug in the lipophilic region (Fig. 8).

Inclusion of DCP in Tween and Span formulations increased the percent release of drug and extended the time of release compared with formulations without DCP (Fig. 9). Similar results were reported for Span 40 and Span 60 by Manconi *et al.* (29).

Tween 80 formulation (surfactant/cholesterol 1:4.5) released 78.56% of the drug at 11 h while the Span 20 formulation (surfactant/cholesterol 1:4.5) released 75% of the drug in 9 h. This means that the viscosity of the formulation (Table II) has no correlation with the molecular weight of surfactant or release of drug.

Differences in the *in vitro* release profiles may be due to vesicle size, lamellarity, and membrane fluidity as a function of chain length of surfactant and cholesterol content (30).

Release Kinetics

Table V shows that Tween 20 with cholesterol follows zero-order kinetics and the other formulations obey first-

Table V. Determination of Order of Release of Zidovudine from Niosomal Formulations

Formulation cholesterol/ surfactant ratio (1:4.5)	Higuchi	Korsemeyer-Peppas		Zero order		First order		Hixson-Crowell		Release mechanism
	r^2	r^2	n	r^2	K_0 (% mg/h)	r^2	K_1 (h^{-1})	r^2	Slope (n)	
Tween 20	0.99839	0.99753	0.34975	0.99997	7.57	0.99783	0.08848	0.99891	0.22626	FD
Tween 40	0.99271	0.99596	0.20464	0.98559	4.34	0.99022	0.04961	0.98875	0.12794	FD
Tween 60	0.99490	0.99729	0.23899	0.98875	4.06	0.99175	0.03507	0.99079	0.09935	FD
Tween 80	0.99114	0.99343	0.36324	0.98342	7.87	0.99227	0.09545	0.98963	0.24109	FD
Tween 20+DCP[a]	0.99999	0.99999	0.44849	0.99865	10.31	0.99919	0.13970	0.99995	0.33929	FD
Tween 40+DCP[a]	0.99795	0.99791	0.54197	0.99361	10.29	0.99893	0.10302	0.99763	0.27727	FD
Tween 60+DCP[a]	0.99848	0.99759	0.69367	0.99458	11.40	0.99919	0.09812	0.99809	0.27779	NFD
Tween 80+DCP[a]	0.99709	0.99673	0.59676	0.99214	11.71	0.99898	0.12422	0.99738	0.32769	NFD

[a] Concentration of DCP used in the formulation is 5 μm
FD Fickian diffusion, *NFD* non-Fickian diffusion

order kinetics. Calculation of Higuchi's correlation coefficient confirms that drug release was proportional to the square root of time indicating that zidovudine release from niosomes was diffusion controlled. The *n* value from the Korsmeyer-Peppas model for zidovudine niosomal formulation was between 0.23 and 0.54 which confirms the Fickian type diffusion, whereas Tween 60 and 80 formulations with DCP follow an anomalous diffusion mechanism with erosion ($n>0.54$) (31). Release profiles fitted into a Hixson-Crowell model further confirmed that drug release from niosomes followed anomalous diffusion

CONCLUSION

Changes in the micromolar ratios of nonionic surfactants with a constant ratio of cholesterol are associated with changes in the entrapment and release of zidovudine from Tween 80 niosomes. Niosomes formulated with Tween 80 entrapped large amounts of zidovudine, and the addition of DCP sustained the drug release for a longer time. We are currently examining the stability, tissue distribution, and pharmacokinetics of zidovudine niosomes.

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