doi.org/10.36721/PJPS.2021.34.1.SUP.313-319.1

Oral bioavailability studies of niosomal formulations of Cyclosporine A in albino rabbits

Mujeeb Ur Rehman*[1], Akhtar Rasul*[1], Muhammad Imran Khan[2], Maria Rasool[3], Ghulam Abbas[1], Feroza Masood[4], Imran Nazir[5], Muhammad Iqbal[6], Nayyer Islam[1], Misbah Hameed[1] and Pervaiz Akhtar Shah[7]

[1]Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad, Pakistan
[2]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore Campus, Lahore, Pakistan
[3]College of Allied Health Professionals, Faculty of Medical & Allied Sciences, Government College University, Faisalabad, Pakistan
[4]Department of Chemistry, Government College University, Faisalabad, Pakistan
[5] Department of Accident & Emergency, Bahawal Victoria Hospital, Bahawalpur, Pakistan
[6]Department of Pharmacy, Gomal University, Dera Ismail Khan, Pakistan
[7]University College of Pharmacy, University of the Punjab, Lahore, Pakistan

Abstract: Cyclosporine A (CsA) is an immunosuppressant agent. Two niosomal formulations of CsA, F_{TS} and F_{SB} were formulated. Both formulations were studied in terms of size, polydispersity index (PDI), morphology and entrapment efficacy etc. Niosomal formulations F_{TS} and F_{SB} and plain aqueous dispersion were given to three assemblies of Albino rabbits (n=8 per group). CsA levels in plasma were determined at appropriate time intervals and pharmacokinetic parameters were evaluated. The percentage entrapment efficiencies of F_{TS} and F_{SB} were found to be 77.29 and 89.31% for respectively. Transmission electron microscopy results indicated spherical nature of niosomes. *In vivo* studies demonstrated that the value of Cmax for the F_{SB} formulation was 1968.419 ng/ml and it was 1498.951 ng/ml and 1073.87 ng/ml for F_{TS} and aqueous dispersion of CsA (control) respectively. It was found that both niosomal formulation F_{TS} & F_{SB} presented significantly high ($p<0.05$) Cmax, AUC0-t, MRT 0-inf and half-life (t1/2) as associated to plain drug dispersion. However niosomal formulation F_{SB} exhibited better *in-vivo* performance as compared to F_{TS}. It was established that CsA can be successfully entrapped in niosomes. So niosomes are promising vehicle for CsA oral delivery.

Keywords: *In vivo* study, niosomes, bioavailability, cyclosporine A, formulation

INTRODUCTION

Oral drug delivery is appropriate route of administration of drug, particularly for chronic diseases, with ease of administration (Poonia *et al.*, 2016). Good oral bioavailability is very important, but many therapeutically significant drugs suffer from reduced oral bioavailability. This can be due to several aspects such as less solubility, partial permeability and hepatic metabolism etc (Kumar *et al*. 2020). In recent years, there has been augmented importance in studying colloidal drug delivery system such as micelles, niosomes, solid lipid nanoparticles, polymeric nanoparticle and self-emulsifying drug delivery systems. Among these systems, niosomes have acquired growing scientific attention as potential drug carriers for solubility and bioavailability enhancement of less water soluble drugs (Arzani *et al.*, 2015). Designing of novel drug delivery systems have gained pronounced interests in recent years (Abhinav *et al.*, 2016). Niosomes are capable of increasing the solubility and hence bioavailability of lipophilic drugs. The use of colloidal drug carriers looks to offer encouraging results in the field of bioavailability improvement of less water soluble drugs. Niosomes are one of the typical colloidal drug delivery systems used for this purpose (Al-mahallawi *et al.*, 2019).

Niosomes can also be employed to control drug diffusion properties. Niosomes have shown advantages as drug delivery systems being cheap and chemically stable alternatives to liposomes. Due to presence of nonionic surfactants in their composition, they may also be used as vehicles for poorly bioavailable drugs. The niosomes are supposed to enhance bioavailability of lipophilic drugs (Jadon PS *et al.*, 2009).

Cyclosporine A (CsA) is used in the prophylaxis and graft rejection in all types of solid organ transplants (Tedesco and Haragsim, 2012). CsA poses some limitations such as poor water solubility, narrow absorption window and p-glycoprotein efflux (Wang *et al.*, 2014). Therefore CsA is a candidate drug to be improved with respect to bioavailability using niosomal approach. Henceforth, the present systematic investigation encompasses the development of two types of niosomes using Tween 60, Span 60, Span 20 and Brij 35. Furthermore, niosomes were studied for entrapment efficiency, vesicle size, PDI, zeta potential and dissolution studies. Vesicles were also

**Corresponding author:* e-mail: mujeebmeopk@yahoo.com; akhtar.rasul@gcuf.edu.pk

subjected to *in vivo* tests using Albino rabbits for assessment of bioavailability and pharmacokinetic parameters.

MATERIALS AND METHODS

Materials

CsA was attained from .Xi'an Lyphar Biotech Co., Ltd China. .Polyoxyl (23) lauryl ether (Brij 35) was acquired from Avonchem Ltd, Macclesfield Cheshire SK116PJ, U K. Polyethylene glycol sorbitan monostearate (Tween 60), Sorbitan monostearate (Span 60), Polyethylene glycol sorbitan monooleate (Tween 80), Sorbitone monooleate (Span 80) and Sorbitan monododecanoate (span 20) were acquired from Daejung Chemicals & Metals Co., Ltd. Cho and 1-Hexadecylppyridinium chloride monohydrate and were attained from Alfa Aesar GmbH & Co.KG. In the research lab of GC University distilled water was prepared.

Methods

Preparation of niosomal formulations

The niosomes of CsA were formulated by thin film hydration method (TFH) (Yeo *et al.*, 2019). In table 1 composition of formulations is specified. Briefly, accurately weighed quantities of surfactant and cholesterol (Cho) in different molar ratios (6:4 and 1:1) were dissolved in 15ml of chloroform/methanol mixture (2:1, v/v) in a 250-ml round-bottomed flask. It was followed by addition of 2.5% of 1-Hexadecylppyridinium chloride (charge inducer). CsA 25mg was added into each formulation. This mixture was placed in rotary evaporator keeping temperature at sixty degree centigrade till a thin film was designed on the wall of rotary flask. Then 20ml of phosphate-buffered saline (PBS) was added. Then it was retained in water bath at sixty degree centigrade for 60 minutes with gentle stirring. The niosomal system was allowed to mature overnight at 4°C.Then formulations were kept in refrigerator at 4°C (De *et al.*, 2018, Ghadi *et al.*, 2019).

Size of vesicle and zeta potential

The size of optimized vesicles, PDI and zeta potential were assessed by zetasizer (Malvern zetasizer version 7.11, UK) at 25°C. The optimized formulation (100µl) was diluted with ten ml of distilled water for analysis. All readings were taken in triplicate and then its mean value was determined (Ruckmani and Sankar, 2010).

Entrapment efficiency studies

The entrapment efficacy of niosomes of CsA was evaluated by validated HPLC method (Romero *et al.*, 2016). Ultracentrifugation method was utilized to separate the niosomes of CsA at 12000×g. Niosomes were centrifuged for 0.5 hourat 4°C. Methanol was employed to disrupt the niosomes. Mobile phase comprised of water - acetonitrile in (3:7, v/v). It was filtered and delivered at a flow rate of 1.2ml/min. Column used was nucleosillC18 (25cm × 3.2mm, 5µm particle size). Sample was injected using 20µl fixed loop. Wavelength of 210 nm was used to assess effluents (Kamboj *et al.*, 2014).

Transmission electron microcopy

The morphology of niosomal formulation (F_{SB}) was observed by transmission electron microscopy (TEM). Niosomal dispersion was plunged onto carbon-coated 200-mesh copper grids and held parallel to permit the infiltration. For staining one drop of 2% uranyl acetate was incorporated on the sample. The sample was then viewed by utilizing TEM (Jeol JEM-1010, Jeol ltd, Japan) (Sezgin-Bayindir *et al.*, 2015).

Oral Bioavailability studies

The formulations (F_{TS} and F_{SB}) were evaluated for *in vivo* behavior of niosomes in twenty four albino rabbits, wash out period was set as two weeks.

The rabbits were administered three carriers of CsA (F_{TS}, F_{SB} and CsA aqueous dispersion) respectively by oral route. The dose of CsA given was 10mg/kg (Wang *et al.*, 2014).

Single dose (parallel study) was employed for *in vivo* evaluation of optimized formulations. The protocols of *in vivo* study were approved by Institutional Review Board, GC University Faisalabad Ref. No. GCUF/ERC/1992 dated 13-09-18. Twenty four healthy albino rabbits (average weight of 1.5Kg) were selected. Each carriers of CsA was administered to group having 8 rabbits (n= 8).

Before the administration of dose the rabbits were not permissible to eat for 12 hours. They are provided with water *ad libitum*. Light controlled room was used for rabbits. Its temperature was conserved at (22±2°C) & humidity at (50-60%) (Campos *et al.*, 2016). Carriers of CsA were administered by oral route in dose of (10 mg/Kg) to respectively group of rabbits by employing flexible catheter at 8:00 am.

After dose administration, samples of blood (2ml) were collected from jugular vein of rabbits at specific intervals of time. EDTA tubes were used for collection of samples (Wang K *et al.*, 2014).

Analysis of plasma levels of CsA

The HPLC technique of Agilent 1200 series was engaged to analyze CsA in rabbit plasma. The HPLC was equipped with auto sampler, heated column and quaternary pump. The column used was Nucleosil C18 (25cm ×z3.2mm, 5 µm particle size). At wavelength of 210nm the effluents were studied.

For twenty four hours the Plasma level time profiles of three carriers of CsA were evaluated. To evaluate

pharmacokinetic parameters software Pk solver was used. To prepare samples for HPLC analysis protein precipitation method was used. In 1.5ml polypropylene micro centrifuge tube acetonitrile (protein precipitating agent) 500µL was incorporated into rabbits whole blood. The tube was vortex mixed for 3 minutes and then centrifuged for ten minutes at 11,000 × g. The supernatant was relocated into another clean tube (Tuzimski and Petruczynik, 2020). Then it was filtered from 0.45 µm filter. After filtration it was used for plasma concentration analysis by HPLC technique as explained above.

STATISTICAL ANALYSIS

To assess results of mean values of size of niosomes, entrapment efficacy and for evaluation of pharmacokinetic parameters for *in vivo* studies one way ANOVA method was employed. The significance level was set to $p< 0.05$. ANOVA was conducted by Graph Pad Prism 6 software at 95% confidence interval.

RESULTS

Preparation of niosomes

The niosomal formulations of CsA F_{TS} and F_{SB} were effectively prepared with the appropriate ratios of surfactants and Cho by TFH method. Results of zeta potential, PDI, size and percentage entrapment efficiency are given in table 2.

Transmission electron microcopy

Morphology of niosomal formulation F_{SB} was evaluated by transmission electron microscope (TEM). Fig. 1 shows the TEM image it demonstrated the formation of spherical vesicles.

Bioavailability study

To evaluate and compare three carriers of CsA (F_{TS}, F_{SB} and CsA aqueous dispersion) were used. Single dose was administered to albino rabbits and CsA concentration was evaluated.

In fig. 2 the blood concentration verses time plots of F_{TS}, F_{SB} and aqueous drug dispersion is demonstrated. In table 3 the values of C_{max}, T_{max}, AUC 0-t and $MRT_{0\text{-}inf\_obs}$ etc are presented.

DISCUSSION

Niosomes of CsA were formulated by TFH method. The average size of niosomes of the formulation F_{TS} & F_{SB} was 1049.3 and 562.5 nm respectively. Greater vesicle size was achieved with surfactants having higher HLB value and vice versa. Approximate same vesicle size was obtained, prepared from Span 20 and Span 60. The incorporation of 1-hexadecyl pyridinium chloride as charge inducing agent in the bilayers may improve the stability of niosomes (Khazaeli *et al.*, 2007). In niosomal formulations as quantity of Cho upsurges, the chain order in niosome bilayer also rises which result in stabilization of niosomal vesicles (Shilakari Asthana *et al.*, 2016). The small vesicles are produced with high Cho concentration. As in the present work the size of niosomes in F_{SB} is small as compared to F_{TS} (Kazi *et al.*, 2010). F_{SB} has value of PDI value 0.321 which indicates a better distribution of size and sufficient uniformity of the formulation (Moawad *et al.*, 2017). The zeta potential of the niosomal formulation F_{SB} was 35.2 (mV). This zeta potential maintains electrostatic stabilization of niosomes. This was due to a positive charge inducing agent hexadecyl pyridinium chloride, which was incorporated in niosomes to increase the stability and to circumvent accumulation of niosomal vesicles (Ag Seleci *et al.*, 2016).

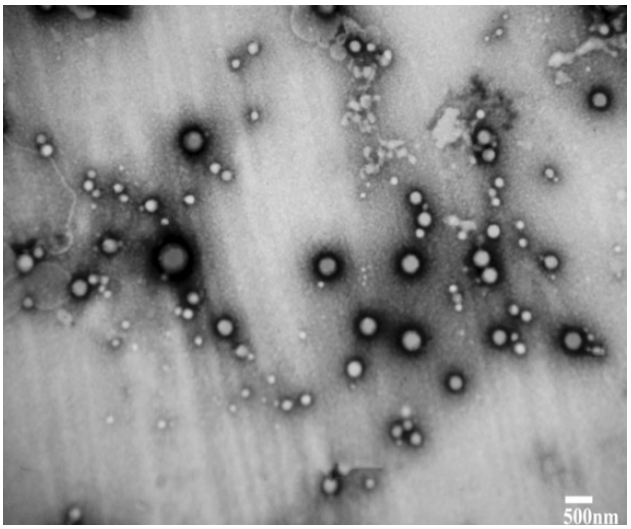

Fig. 1: TEM image of niosomal formulation F_{SB}

TEM images showed that niosomes are present in integral state and the picture predicts the reliability of spherical niosomal vesicles. The size of vesicles depicted by images of TEM for the optimized formulation F_{SB} were relevant to differential light scattering results. Structure of niosomes was also revealed by images of TEM. The external core of niosomal vesicles which includes the hydrophobic drug with in the surfactant/lipid bilayer is darker in contrast to the internal core (Marianecci *et al.*, 2016). The spherical vesicles established in the investigation were found in line with niosomal preparations of flurbiprofen comprising of brij 35 nonionic surfactant which were also spherical (Kumar 2012).

Niosomal formulation F_{TS} and F_{SB} demonstrated more concentration of CsA than aqueous drug dispersion. It was found that F_{SB} demonstrated comparatively higher blood concentrations as associated to the F_{TS} formulation. Span 60 and tween 60 were two nonionic surfactants used in niosomal formulation F_{TS} and span 20 and brij 35 were utilized in F_{SB}. The higher lipid concentration, uniform

vesicle size and mixed nonionic surfactants results in augmented bioavailability (Rasul *et al*, 2020). The peak plasma concentration (C_{max}) is the highest concentration that active pharmaceutical agent accomplishes in the blood circulation after dose administration.

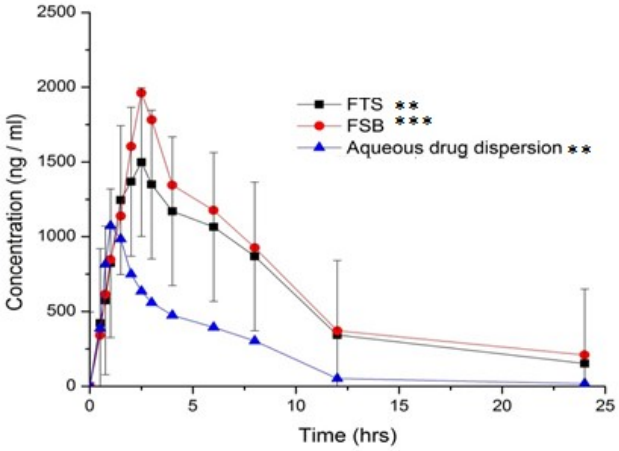

Fig. 2: Comparison of blood concentration of CsA verses time plots of niosomal formulations F_{TS}, F_{SB} and aqueous drug dispersion (**p<0.01 and ***p<0.001).

The mean Cmax of F_{SB} was 1968.419±107.91ng/ml & it was 1498.951±137.57 ng/ml and 1073.87±69.56 ng/ml for F_{TS} and aqueous drug dispersion respectively. One way ANOVA method was used for statistical analysis. Results of ANOVA exhibited a significant difference in the Cmax values (P<0.05) between niosomal formulations F_{TS} and F_{SB} and Cmax of the CsA aqueous dispersion. So the results demonstrated the order of increasing Cmax as F_{SB}>F_{TS}> control aqueous drug dispersion. The superior value of Cmax of niosomes as compare to aqueous drug dispersion shows the improved CsA bioavailability. Furthermore the surface charge of the niosomes F_{TS} and F_{SB} was 32.1 and 35.2 mV respectively, this play a important role in stability (Arzani *et al.*, 2015).

In our investigation, the mixture of a spans and tweens were employed for the formulation of niosomal vesicles. Span 60 / Tween 60 mixtures with mean HLB values 9.8, designed stable niosomes with the addition of Cho and exhibited good entrapment efficiency. The firmness of bilayers of Span 60 / Tween 60 in niosomal formulation F_{TS} can explain the mentioned high encapsulation ability (77.28±0.35) which results in high C_{max}. This finding is consistent with high entrapment efficiency of ciprofloxacin by Span 60 / Tween 60 containing niosomes (Akbari *et al.*, 2015). In niosomal formulation $F_{SB,}$ Span 20 and Brij 35 were used. Span 20 / Brij 35 mixtures with mean HLB values 12.75, has designed stable niosomes along with Cho and demonstrated highest entrapment efficiency (89.31±0.37) and also Cmax. When the HLB of surfactants raises beyond ten, the quantity of Cho required to formulate vesicles also upsurges. So relatively additional quantity of Cho is needed to recompense for a higher head group. Two nonionic surfactants were used in formulation F_{SB}, sorbitan monolaurate (span 20) having (HLB 8.6) and Brij 35 (HLB 16.9). Despite brij 35 having a high HLB value, when it is assorted with span 20, they formed niosomes with maximum CsA entrapment at appropriate Cho concentration. It is found that the affinity of CsA for the system of mixed surfactant was sufficiently greater, which result in improved entrapment efficiency. Furthermore greater entrapment accomplished with the system of mixed surfactants also depend on the chain length of surfactants, a lengthier chain generates high entrapments due to enhanced reliability of the monolayer. This finding is consistent with high entrapment efficiency of diacerein by Span 20 / poloxamer 184 mixed surfactant system niosomes (Khan MI *et al.*, 2016).

The Tmax for the control aqueous dispersion was 1.15625 ± 0.296 hours. Whereas for niosomal formulations F_{TS} and F_{SB} it was 2.125 ± 0.44 and 2.375 ± 0.2314 hours respectively. The higher Tmax of niosomal formulations was owing to the slow dissolution and extended release of the drug from niosomes (Abdallah *et al.*, 2016).

AUC is utilized for evaluation of relative effectiveness of diverse pharmaceutical formulations. In this study, the mean ± SD values of AUC 0-t for niosomal formulations F_{SB} and F_{TS} were 15671.27 ± 935.36 ng.h/mL^{-1} and 13703.09 ± 1135.13 ng.h.mL^{-1} respectively. While it was 5253.501 ± 868.30 ng.h.mL^{-1} for the aqueous dispersion of CsA. The data of ANOVA revealed that values of AUC 0-t of both the niosomal formulations are significantly higher (p<0.05) as associated to the aqueous drug dispersion. This can be result of Span 20 and brij 35 in F_{SB}.

The value of MRT 0-inf of F_{SB} and F_{TS} were calculated as 10.50651 ± 0.5519 and 9.757969 ± 0.257 hours, respectively. While the values of $MRT_{0\text{-}inf}$ for aqueous dispersion of CsA was 5.427286 ± 0.6929. The results of ANOVA demonstrated that the value of MRT 0-inf for niosomal preparations were significantly high as compared to control plain dispersion (p<0.05). Nevertheless, no significant difference was perceived amongst the niosomal preparations F_{TS} and F_{SB}. The values of AUC and MRT evaluated in the current study were found in line with niosomal preparations of clarithromycin which also exhibited higher values of AUC and MRT as associated to plain drug (Shilakari Asthana *et al.*, 2016). Related results have been presented by Sakthivel *et al*., 2013. They explained niosomal vesicles of oxcarbazepine exhibited significantly high values of the AUC and MRT in contrast to the plain oxcarbazepine dispersion (Sakthivel *et al.*, 2013). Additionally the niosomes of losartan potassium also exhibited significantly (p<0.05) high results of $AUC_{0\text{-}inf}$, C_{max}, T_{max}, $T_{1/2}$ and MRT as compared to pure losartan potassium solution (Kamboj *et al.*, 2016).

Table 1: Composition of niosomal formulations

S No.	Code of Formulation	Molar ratio (Surfactant : CHO)	Surfactants (mg)		Cholesterol (mg)
			Tween 60	Span 60	
1	F_{TS}	6:4	196.35	64.59	77.33
			Span 20	Brij 35	
2	F_{SB}	1:1	43.31	149.94	96.66

Table 2: Average size, PDI, zeta potential, and Entrapment efficacy (%) of different niosomal preparations

S No	Parameters	F_{TS}	F_{SB}
		(Tween 60+Span 60: Cho)	(Span 20 + Brij 35 : Cho)
		6:4	1:1
1	Size (nm)	1049. 3± 0.118	562.5 ± 0.236
2	polydispersity index	0.463 ± 0.17	0.321 ±0.043
3	Zeta potential (mV)	32.1 ± 2.5	35.2 ± 2.3
4	% Entrapment efficiency	77.29 ± 0.35	89.31 ± 0.37

This data is mean along with standard deviation

Table 3: Pharmacokinetic parameters of F_{TS}, F_{SB} and CsA aqueous dispersion

Pharmacokinetic Parameters	Cmax (ng.mL-1)	Tmax (h)	AUC 0-t (ng/ml*h)	MRT 0-inf_obs (h)	Vz/F_obs (mg/kg)/ (ng/ml)	$t_{1/2}$ (h)	Lambda_z (1/h)
Niosomal formulation (F_{TS})	1498.951 ±137.57	2.125 ±0.44	13703.09 ±1135.13	9.757969 ±0.257	0.006074 ±0.00050	6.304108 ±0.1157	0.109983 ±0.00197
Niosomal formulation (F_{SB})	1968.419 ±107.91	2.375 ±0.2314	15671.27 ±935.36	10.50651 ±0.5519	0.005335 ±0.00023	6.515562 ±0.3380	0.106631 ±0.0055
CsA aqueous dispersion	1073.87 ±69.56	1.15625 ±0.296	5253.501 ±868.30	5.427286 ±0.6929	0.009919 ±0.0016	3.683 ±0.70309	0.195661 ±0.0451

Vz/F is apparent volume of distribution in terminal phase after oral administration. The values of Vz/F calculated for selected niosomal formulation F_{SB} and F_{TS} were 0.005335 ± 0.00023 and 0.006074 ± 0.00050 (mg/kg) / (ng/ml), respectively. The value of Vz/F aqueous drug dispersion was 0.009919 ± 0.0016 (mg/kg) / (ng/ml). The results of ANOVA showed that the value of Vz/F for control plain dispersion was significantly high ($p<0.05$) as associated to niosomal formulations F_{SB} and F_{TS}. The pure drug dispersion has high value of Vz/F as compare to formulation F_{TS} and F_{SB}. It showed that niosomal formulations have slower rate of clearance which render the accessibility of the active pharmaceutical ingredient more freely in the blood inspite of being gathered in organs or excreted. The results of Vz/F established in the current study were similar with niosomal preparation entrapping morin hydrate which also demonstrated the small volume of distribution and slow rate of clearance, which facilitates the accessibility of the active pharmaceutical ingredient more swiftly in the blood rather than being gathered in the tissues (Waddad AY *et al.*, 2013). This may be an additional conceivable cause for reasonably low values of Vz/F acquired with niosomal vesicles in the current evaluation, which result in increased bioavailability.

The $t_{1/2}$ is the time interval in which the quantity of active pharmaceutical ingredient in the body is lessened by one half. The half-life ($t_{1/2}$) determined in the current study was 3.683 ± 0.703 hours, 6.304 ± 0.1157 hours and 6.515 ± 0.3380 hours for control plain dispersion, F_{TS} and F_{SB} respectively. The results of ANOVA demonstrated a significant difference amongst the $t_{1/2}$ of niosomal formulations & aqueous drug dispersion whereas minor difference was seen among the niosomal preparations (F_{SB} and F_{TS}). The enhanced half-life of niosomes can be described by the influence of nonionic surfactants. Similarly in diltiazem niosomes span 60 was used as nonionic surfactant. These niosomes have increased half-life as compared to control free drug, which also result in enhanced bioavailability. This might be owing to deliberate rate of absorption of drug and also slow release which prolongs the half-life ($t_{1/2}$). Similarly the niosomes of acyclovir also exhibited significantly higher values of $T_{1/2}$ as associated to free solution of drug (Ammar H *et al.*, 2017).

Lambda_z is individual estimate of the terminal elimination rate constant or terminal disposition rate constant. In this study, the mean ± SD values of Lambda_z for niosomal formulations F_{SB} and F_{TS} were

0.106631 ± 0.0055 (1/h) and 0.109983 ± 0.00197 (1/h) respectively. Whereas it was 0.195661 ± 0.0451 (1/h) for the aqueous drug dispersion. The ANOVA results of Lambda_z established a significant difference amongst the niosomal preparations and plain aqueous dispersion of CsA. While an insignificant difference was found amongst the niosomal preparations F_{SB} and F_{TS}. Lesser values of terminal elimination rate constant Lambda_z acquired by niosomal preparations as equated to plain drug dispersion showed less elimination of CsA and thus CsA be present in the body for extended period of time to exhibit it's therapeutic effects. Nanocarriers like liposomes or niosomes, have fewer probabilities for their uptake into the liver which may be a rational justification for low value of Lambda_z achieved in the present study (Kamboj *et al.*, 2014).

The above mentioned results are according to the estimated conduct of niosomal formulations. So the niosomes could be considered as nano drug depots, having capability to sustain release of active pharmaceutical agents with improved bioavailability.

CONCLUSION

The niosomal formulation of immunosuppressant drug CsA has successfully been developed. Niosomal formulation F_{SB} exhibited the maximum entrapment of CsA (89.31%). *In vivo* studies showed that values of AUC 0-t, Cmax, Tmax, half-life and MRT 0-inf of optimized niosomal formulation F_{TS} & F_{SB} are significantly high as paralleled to plain drug dispersion. So the bioavailability of CsA is improved with sustain release effect of niosomes. However, F_{SB} formulation displayed greater AUC 0-t, Cmax and mean residence time as compared to F_{TS}. So niosomal formulation F_{SB} can be a better alternative dosage form of CsA delivery. All pharmacokinetic parameters showed favorable results. The method of preparation of niosomes is simple, cost effective and scalable as compared to other methods. Consequently, it was demonstrated that niosomal formulation F_{SB} can be utilized successfully for extended delivery of CsA.

ACKNOWLEDGEMENTS

The authors are thankful to the Teacher Incharge, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad and Dr. Muhammad Sher Incharge Hi-ech Lab, University of Sargodha, Pakistan for facilitating in study.

REFERENCES

Abdallah MH, Sabry SA and Hasan AA (2016). Enhancing transdermal delivery of glimepiride via entrapment in proniosomal gel. *J. Young Pharm*., **8**(4): 335-340.

Abhinav M, Neha J, Anne G and Bharti V (2016). Role of novel drug delivery systems in bioavailability enhancement: At a glance. *Int. J. Drug Deliv. Tech*., **6**(1): 7-26.

Ag Seleci D, Seleci M, Walter JG, Stahl F and Scheper T (2016). Niosomes as nanoparticular drug carriers: Fundamentals and recent applications. *J. Nanomater*., 2016(1) 1-13.

Akbari V, Abedi D, Pardakhty A and Sadeghi-Aliabadi H (2015). Release studies on ciprofloxacin loaded non-ionic surfactant vesicles. *AJMB*., **7**(2): 69.

Al-mahallawi AM, Fares AR and Abd-Elsalam WH (2019). Enhanced permeation of methotrexate via loading into ultra-permeable niosomal vesicles: Fabrication, statistical optimization, ex vivo studies, and in vivo skin deposition and tolerability. *AAPS Pharm. Sci. Tech*., **20**(5): 171.

Ammar H, Haider M, Ibrahim M and El Hoffy N (2017). In vitro and in vivo investigation for optimization of niosomal ability for sustainment and bioavailability enhancement of diltiazem after nasal administration. *Drug Deliv*., **24**(1): 414-421.

Arzani G, Haeri A, Daeihamed M, Bakhtiari-Kaboutaraki H and Dadashzadeh S (2015). Niosomal carriers enhance oral bioavailability of carvedilol: Effects of bile salt-enriched vesicles and carrier surface charge. *Int. J. Nanomedicine*., **10**: 4797.

Campos S, Félix L, Venâncio C, de Lurdes Pinto M, Peixoto F, de Pinho PG and Antunes L (2016). *In vivo* study of hepatic oxidative stress and mitochondrial function in rabbits with severe hypotension after propofol prolonged infusion. *SpringerPlus*., **5**(1): 1349.

De A, Venkatesh N, Senthil M, Sanapalli BKR, Shanmugham R and Karri VVSR (2018). Smart niosomes of temozolomide for enhancement of brain targeting. *Nanobiomedicine*., **5**: 1849543518805355.

Ghadi ZS, Dinarvand R, Asemi N, Amiri FT and Ebrahimnejad P (2019). Preparation, characterization and in vivo evaluation of novel hyaluronan containing niosomes tailored by box-behnken design to co-encapsulate curcumin and quercetin. *Eur. J. Pharm. Sci.,* **130**: 234-246.

Kamboj S, Bala S and Saini V (2016). Formulation and evaluation of niosomes containing an antihypertensive drug. World Academy of Science, Engineering and Technology. *Int. J. Med. Health, Biomed, Bioeng Pharm. Eng*., **10**(4): 178-182.

Kamboj S, Saini V and Bala S (2014). Formulation and characterization of drug loaded nonionic surfactant vesicles (niosomes) for oral bioavailability enhancement. *The Sci. World J*., 2014(1): 1-8.

Kazi KM, Mandal AS, Biswas N, Guha A, Chatterjee S, Behera M and Kuotsu K (2010). Niosome: A future of targeted drug delivery systems. *J. Adv. Pharm. Technol.,* **1**(4): 374.

Khan MI, Madni A and Peltonen L (2016). Development and *in vitro* characterization of sorbitan monolaurate

and poloxamer 184 based niosomes for oral delivery of diacerein. *Eur. J. Pharm. Sci*., **95**: 88-95.

Khazaeli P, Pardakhty A and Shoorabi H (2007). Caffeine-loaded niosomes: Characterization and in vitro release studies. *Drug Deliv*., **14**(7): 447-452.

Kumar S (2012). Proniosomal gel of flurbiprofen: Formulation and evaluation. *J. Drug Deliv. Ther.,* **2**(1): 1-5.

Kumar R, Dalvi SV and Siril PF (2020). Nanoparticle-based drugs and formulations: Current status and emerging applications. *ACS Appl. Nano Mater.,* **3**(6): 4944-4961.

Marianecci C, Di Marzio L, Del Favero E, Cantu L, Brocca P, Rondelli V, Rinaldi F, Dini L, Serra A and Decuzzi P (2016). Niosomes as drug nanovectors: Multiscale ph-dependent structural response. *Langmuir*., **32**(5): 1241-1249.

Moawad FA, Ali AA and Salem HF (2017). Nanotransfersomes-loaded thermosensitive in situ gel as a rectal delivery system of tizanidine HCl: Preparation, *in vitro* and *in vivo* performance. *Drug Deliv.*, **24**(1): 252-260.

Poonia N, Kharb R, Lather V and Pandita D (2016). Nanostructured lipid carriers: Versatile oral delivery vehicle. *Future Sci. OA*., **2**(3): FSO135.

Romero GB, Arntjen A, Keck CM and Muller RH (2016). Amorphous cyclosporin a nanoparticles for enhanced dermal bioavailability. *Int. J. Pharm.,* **498**(1-2): 217-224.

Rasul A, Khan MI, Rehman MU, Abbas G, Aslam N, Ahmad S, Abbas K, Shah PA, Iqbal M and Al Subari AMA (2020). *In vitro* characterization and release studies of combined nonionic surfactant-based vesicles for the prolonged delivery of an immunosuppressant model drug. *Int. J. Nanomed*., **15**: 7937.

Ruckmani K and Sankar V (2010). Formulation and optimization of zidovudine niosomes. *Aaps Pharmscitech*., **11**(3): 1119-1127.

Sakthivel M, Kannan K, Manavalan R and Senthamarai R (2013). Formulation and *in vivo* evaluation of niosomes containing oxcarabazepine. *J. Pharm. Sci*. **5**(1): 8.

Sezgin-Bayindir Z, Antep MN and Yuksel N (2015). Development and characterization of mixed niosomes for oral delivery using candesartan cilexetil as a model poorly water-soluble drug. *AAPS. Pharm Sci. Tech*., **16**(1): 108-117.

Shilakari Asthana G, Sharma PK and Asthana A (2016). In vitro and in vivo evaluation of niosomal formulation for controlled delivery of clarithromycin. *Scientifica*., 2016(2016): 1-10.

Tedesco D and Haragsim L (2012). Cyclosporine: A review. *J. Transplant*., **2012**(1): 1-7.

Tuzimski T and Petruczynik A (2020). Review of chromatographic methods coupled with modern detection techniques applied in the therapeutic drugs monitoring (TDM). *Molecules*, **25**(17): 4026.

Waddad AY, Abbad S, Yu F, Munyendo WL, Wang J, Lv H and Zhou J (2013). Formulation, characterization and pharmacokinetics of morin hydrate niosomes prepared from various non-ionic surfactants. *Int. J. Pharm.,* **456**(2): 446-458.

Wang K, Qi J, Weng T, Tian Z, Lu Y, Hu K, Yin Z and Wu W (2014). Enhancement of oral bioavailability of cyclosporine A: Comparison of various nanoscale drug-delivery systems. *Int. J. Nanomedicine*, **9**: 4991.

Wang L, Peng M, Zhu Y, Tong S-s, Cao X, Xu XM and Yu JN (2014). Preparation of pluronic/bile salt/ phospholipid mixed micelles as drug solubility enhancer and study the effect of the ppo block size on the solubility of pyrene. *Iranian J. Pharm Res.* **13**(4): 1157.

Yeo LK, Chaw CS and Elkordy AA (2019). The effects of hydration parameters and co-surfactants on methylene blue-loaded niosomes prepared by the thin film hydration method. *Pharmaceuticals*, **12**(2): 46.