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Original Article

Investigation of Polymeric Micellar Nanoparticles of Amlodipine Besylatefor Transdermal Delivery

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ABSTRACT

Objectives: The objective of this study was to formulate and investigate amlodipine besylate loaded polymeric micelles and their effect on enhancing transdermal permeation of the poorly water-soluble drug via transdermal films. **Methods:** Pluronic F127 and Pluronic F68 polymers were chosen to prepare micelles in different drug - polymer ratios. Prepared formulations were characterised for surface morphology, micelle size, polydispersity index, zeta potential, loading efficiency, percentage drug weight in micelles and stability. Optimized amlodipine micelle dispersions were incorporated into hydroxypropyl methylcellulose K100 and polyvinyl pyrrolidone K30 transdermal films prepared by the solvent casting method. These films were evaluated for thickness, percentage weight variation, folding endurance, moisture absorbance, percentage drug content, *ex vivo* permeation and *in vivo* skin irritation. **Results:** Selected micelle formulations had the desired vesicle size, homogeneity and stability. Scanning electron microscopy show spherical vesicles. Micelle incorporated transdermal films were smooth, flexible, translucent and mechanically strong. The *ex vivo* skin permeation studies reveal that the flux of amlodipine besylate from the micelle incorporated transdermal films was significantly higher than that containing the free drug, confirming the drug solubilizing effects of micelles. Reconstitution of micelles from optimized films revealed that the original characteristics of the micelles were intact. Studies in Wistar rats showed that the prepared transdermal films were free of irritation potential when evaluated by the Draize dermal irritation scoring system. **Conclusion:** Beside their solubilizing effect, Pluronic micelles could be useful as novel drug carriers for enhancing transdermal permeation of Amlodipine besylate and thus its bioavailability.

Key words: Hydroxypropyl methylcellulose, Micelle, Nanoparticle, Permeation, Pluronic, Skin irritation.

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INTRODUCTION

For the past few decades, there has been a renewed focus in the area of drug delivery using polymeric delivery systems as carriers for small and large molecules. Novel approaches to drug delivery and formulation using polymers for difficult-to-formulate reagents are nowadays not a new concept and it is revolutionising the future of medicine. Polymeric drug delivery systems are known to dramatically alter the pharmacokinetic and pharmacodynamics properties of drugs. Currently, the most popular subjects in polymeric drug delivery systems are related to polymeric micelles which are generated as a result of self-assemblage of amphiphilic block polymers. Micelles have been extensively studied for the solubilisation and targeted delivery of poorly water-soluble drugs.[1] These vesicles can be used as carriers for drugs with different physicochemical properties.

An important class of polymers known to form micellar nanoparticles are the Pluronic block copolymers or 'poloxamers'. They are made of hydrophilic poly (ethylene oxide) (PEO) and hydrophobic poly (propylene oxide) (PPO) blocks arranged in a tri-block structure i.e., PEO-PPO-PEO. These copolymers behave like surfactants due to their amphiphilic character and are therefore capable of interacting with hydrophobic surfaces and biological membranes. When used in concentrations above critical micelle concentration (CMC) in aqueous solutions, these copolymers self-assemble into micelles ranging in diameters of 10 nm to 100 nm.[2] In the micelle, the copolymer chains are oriented such that the hydrophobic PPO blocks form the core and the hydrated hydrophilic PEO chains form the shell towards the aqueous exterior. Various hydrophobic drugs can be incorporated into the core of these micelles. Thus such micelles can be used as drug carriers for a number of applications.[3]

Transdermal drug delivery provides controlled release of a drug after its application on to the skin and its absorption into the systemic circulation produces pharmacological effects in a region away from the site of application. These systems are capable of maintaining therapeutic levels of the drug in blood as well as the target tissue for prolonged periods.[4]

Amlodipine besylate is an antihypertensive by virtue of its dihydropyridine calcium antagonist action which inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. This drug is a suitable candidate for transdermal delivery, as it undergoes extensive first-pass metabolism, possess low molecular weight and effective in low plasma concentration. Although, several studies have reported the investigation of transdermal patches of amlodipine besylate, it is well known that the poor aqueous solubility of this drug can be a challenge to its systemic absorption.[5] Micellar nanoparticles have great potential in the transdermal delivery of drugs which otherwise show significant problems in cutaneous permeation.[1] Hence, we hypothesize that if the drug is incorporated into polymeric micelles that keep it solubilized and administered via transdermal films or patches, the bioavailability of the drug should be vastly improved. In addition all the advantages of a typical transdermal drug delivery system will be achieved such as reduced toxicity, controlled drug delivery

and improved patient compliance because of reduced frequency of administration.

By preparation of polymeric micellar nanoparticles of amlodipine besylate incorporated in a transdermal delivery system, a "depot effect" can be credited to the composition of polymeric micellar nanoparticle and stratified skin deposition upon topical application, which maintains a concentration gradient across the skin. This technique will be able to improve the transdermal permeation of poorly water-soluble drug such as amlodipine besylate delivery.[6] Therefore, in this study we propose to formulate polymeric micellar nanoparticles of amlodipine besylate incorporated in transdermal films.

MATERIALS AND METHODS

Materials

Amlodipine besylate was received as a kind gift sample from USV, Baddi. Pluronic F127, Pluronic F68, hydroxypropyl methylcellulose K100 (HPMC K100) and polyvinyl pyrrolidone K30 (PVP K30) were obtained from Yarrow Chemicals, Mumbai and all other chemicals were produced from local sources and were of analytical grades.

Methods

Preparation of polymeric micellar nanoparticles

By direct dissolution dialysis method

An attempt was made to prepare micelles from two Poloxamer (Pluronic F127 and Pluronic F68) of different concentrations as shown in Table 1. Solutions of a constant amount of drug (0.5 mg/ml) in dimethylsulfoxide (DMSO) were added to different amounts (5mg/ml, 10mg/ml and 15 mg/ml) of the polymeric solution of Pluronic F127 and Pluronic F68 in DMSO separately. Samples were mixed by vortexing for 15-20 min followed by incubation in a shaker water bath overnight. To produce drug-loaded micelles, this solution was placed in a dialysis bag and dialysed against distilled water for three days to remove the organic solvent and the free drug. During this process, the DMSO is exchanged with water inducing micelle assembly.[7]

Preparation of polymeric micellar nanoparticles

By thin film hydration method

The two poloxamers were used in the same concentration as described above. Amlodipine besylate (0.5 mg/ml) solution in chloroform was added to 5mg/ml, 10mg/ml and 15 mg/ml solution of polymers in chloroform to obtain concentration ratios as given in Table 1. The rotary evaporator was used to obtain a thin film of drug and the copolymers and a vacuum was applied to remove the organic solvent. Further drying of the film was done under an atmosphere of nitrogen for 30 min. The film was hydrated by the addition of 5 ml of distilled water and vortexing the contents to form the micelle dispersion. The un-entrapped drug was separated by the centrifugation of the dispersion at 13000 rpm for 20 min and finally filtered through a 0.22 µm membrane filter.[8]

Table 1: Composition of micellar dispersions.

Method of preparation	Formulation code	Polymer	Drug : Polymer ratio	Solvent
Dialysis	F1	Pluronic F127	0.5 : 5	DMSO (10 ml)
	F2		0.5 : 10	
	F3		0.5 : 15	
Thin Film Hydration	F4		0.5 : 5	Chloroform (10 ml)
	F5		0.5 : 10	
	F6		0.5 : 15	
Dialysis	F7	Pluronic F68	0.5 : 5	DMSO (10 ml)
	F8		0.5 : 10	
	F9		0.5 : 15	
Thin Film Hydration	F10		0.5 : 5	Chloroform (10 ml)
	F11		0.5 : 10	
	F12		0.5 : 15	

Characterisation of Prepared Polymeric Micelles

Scanning Electron Microscopy (SEM)

The surface morphology of polymeric micelles was determined using SEM (Zeiss Sigma 300). Before analysis, samples were freeze dried and a small amount was placed on a metal stub and subjected to gold sputtering. These coated samples were then scanned and photomicrographs were taken at an acceleration voltage of 15 Kv.

Determination of Micelle size, Polydispersity Index (PDI) and Zeta Potential

The mean micelle size, PDI and zeta potential were measured by dynamic light scattering method using Malvern zeta sizer (Malvern Instrument, UK). The analysis was performed at a scattering angle of 90° at 25°C. The average of three determinations for these parameters were recorded.

Determination of percentage drug content, percentage drug incorporated and the percentage drug weight in micelles

Drug content in micelles was determined using the UV spectrophotometer, after dissolution of a sample of the micelle dispersion in dimethyl sulphoxide (DMSO) and dilution suitably with phosphate buffer of pH 7.4. After estimating drug content, the percentage drug incorporated or entrapment efficiency and percentage drug weight in micelles was calculated using the equations below:

$$\text{Drug incorprated (\%)} = \frac{a}{b} \times 10 \quad (1)$$

$$\text{Drug weight in micelles} = \frac{a}{b+c} \times 10 \quad (2)$$

Where 'a' is the amount of drug-loaded in micelles, 'b' the amount of drug used in micelle preparation and 'c' is the amount of polymer used in preparation.[9]

Optimization of prepared micelles

Results obtained from the evaluation of micelle size, percentage drug incorporated, zeta potential and PDI was taken into consideration while optimising the micelles. The optimised micelles are then incorporated into the transdermal films.

Preparation of transdermal films loaded with amlodipine besylate micelles

Transdermal patches containing amlodipine besylate micelles were prepared by solvent casting method. The polymers, HPMC K100M and PVPK30 were dissolved in 10 ml of water. Micellar dispersion equivalent to 32 mg of the drug was incorporated into the above solution. Propylene glycol was used as plasticizer. The resulting uniform solution, free of bubbles was cast onto a circular glass mould of an area of 44.15 cm^2 and dried in a constant temperature oven at 40°C for 24 h. The dried films were removed from the petri dish and were cut into square dimensions of 2.65 x 2.65 cm, such that each cut film had a drug load of 5 mg. They were wrapped in aluminium foil and stored in a desiccator for further studies.[10] Films (Control) were also prepared from similar compositions but containing the free drug instead of the drug incorporated polymeric micelles. The compositions are shown in Table 2.

Characterisation of transdermal films

Percentage Moisture absorption

The prepared films were weighed and were set aside in desiccators containing a saturated solution of potassium chloride at room temperature for 24 h in order to maintain 84% RH.[11] After 24 h, the films were weighed again and the percentage moisture uptake was determined from the below-mentioned formula:

$$\text{Percentage Moisture Absorption} = \frac{\text{Final weight-Initial weight}}{\text{Initial weight}} \times 100 \quad (3)$$

Thickness, weight variation and folding endurance

The thickness of the films was measured at five different places using micrometre screw gauge and the mean value was calculated.[12]

Weight variation between the formulated films can lead to a difference in drug content and hence in the *in vitro* release behaviour. The weights of the three films were evaluated.[12]

The folding endurance of the film was determined by repeatedly folding it 300 times manually at the same place, which was considered satisfactory to reveal good films properties, till it broke. The average of three determinations was considered.[10]

Tensile strength and Percentage Elongation at break

The mechanical property was evaluated using Linus Bursting/Tensile Strength Apparatus. The Pressure gauge was selected depending on the sample to be tested by turning the gauge selector switch. A film strip of 2.65 cm x 2.65 cm dimension and free from air bubbles or physical imperfection was placed on the diaphragm plate and the wheel on the top of the diaphragm plate was rotated till it fits securely on the sample and does not rotate any further. The 'Push' button was pressed until the sample bursts. The pressure gauge directly gives reading in kg/cm^2. The test was repeated thrice for each formulation. Tensile Strength is the maximum stress applied to a point at which the specimen bursts.[13]

The percentage elongation can be obtained by the following equation.

$$\% \text{ Elongation} = \left[\left(\frac{L_1 - L_2}{L_2}\right)\right] \times 100 \quad (4)$$

Where L_1 is the final length of each strip and L_2 is the initial length of each strip.

Drug Content

To determine the drug content, a film of dimensions, 2.65 x 2.65 cm was shredded and dissolved in 10 ml methanol and the flask was shaken continuously for 24h in a mechanical shaker. After ultra-sonication for 15 min, the solution was filtered and diluted suitably with phosphate buffer pH 7.4. The concentration was estimated by an UV Spectrophotometer at a wavelength of 238 nm to determine the drug content.[10]

Reconstitution and characterization of pluronic micelles from transdermal films

Reconstitution of the amlodipine loaded micelles from the films was done to understand whether micellar properties are affected during the processes of the solvent casting and drying of the latter. The amlodipine besylate loaded film of each of the formulations, FD-1, FD-2 and FD-3 were reconstituted by placing the film of 2.65 x 2.65 cm dimensions in a conical flask containing 20 ml of distilled water which is then shaken and stirred for several hours until a colloidal system is obtained. After reconstitution, the micelle solutions were characterised for percentage drug incorporated, micelle size and morphological properties and compared with the corresponding data of the micelle formulation prior to incorporation into the films.[14] This determination was repeated thrice for each formulation.

In vitro drug release from transdermal films

The *in vitro* drug release studies were performed by using a Modified Keshery-Chein diffusion cell. A dialysis membrane of pore size, 0.22-0.45 micron and molecular weight cut-off (MWCO) of 14000-18000 (Himedia) was fixed to the bottom of the donor cell. The donor compartment was in contact with the ambient conditions. To mimic *in vivo* skin condition as far as possible and keeping in mind the larger cutaneous absorptive area, sink conditions were maintained by placing 900 ml of phosphate buffer of pH 7.4 in the receptor compartment and the contents were stirred at 50 rpm at 37 ± 0.5°C. The prepared transdermal films FD1-FD3 (2.65 x 2.65 cm^2) were placed on the cellophane membrane in the donor compartment. At predetermined time intervals, aliquots of the release medium were withdrawn and replaced immediately with the same volume of phosphate buffer pH7.4. The samples were analysed for drug content at 238 nm using an UV Spectrophotometer after suitable dilutions.[15]

Ex vivo permeation study through rat abdominal skin

An *ex vivo* permeation study was conducted after obtaining the ethical clearance for the handling of experimental animals from the Institutional Animal Ethics Committee with the reference number, NGSM/IAEC/2016-17/07. For this study, excised rat skin was used as the model membrane with the Modified Keshery-Chein diffusion cell. The entire thickness of the abdominal skin of male rat weighing 200 g to 250 g was used. Hair from the abdominal skin was removed by shaving. After excision, the dermal side of the skin was rinsed with Kreb's buffer to remove any adhering tissues or blood vessels and equilibrated for an hour in phosphate buffer pH 7.4 before the start of the experiment.

The receptor compartment contained 900 ml of the phosphate buffer and its temperature was maintained at 37±0.5°C by a thermostatically controlled water bath. The rat skin was clamped between the donor and receptor compartments, with the epidermal side up. At regular intervals, samples of 5 ml were removed from the receptor compartment and replaced with equal volumes of fresh buffer.[16,17] The samples were analysed using an UV Spectrophotometer. The study was continued for 12 h.

The amount of drug permeated through the membrane in 12 h was determined and plotted against time. Using regression analysis the slope of the linear portion of the plot was calculated and divided by the mucosal surface area to give the permeation flux. The steady state permeability coefficient was calculated by dividing the permeation flux by the donor phase drug concentration.[18]

Table 2: Composition of casting solution for optimised ratio with drug and optimised micellar dispersion.

Formulation	FD-1	FD-2	FD-3
Weight of drug (mg)	32	-	-
F5 dispersion equivalent to 32 mg of drug (ml)	-	2	-
F6 dispersion equivalent to 32 mg of drug (ml)	-	-	2
HPMC K100M(mg)	150	150	150
PVPK-30 (mg)	50	50	50
Propylene glycol (ml)	1	1	1
Water(ml) qs	10	10	10

Skin Irritation Studies in Albino Wistar Rats

As stated earlier prior ethical clearance for the handling of experimental animals for this study was obtained from the Institutional Animal Ethics Committee Albino rats of either sex were housed in cages with free access to laboratory diet and water. The rats were anaesthetized using aesthetic ether and 3 cm^2 area of the skin from either dorsal side was shaved. Rats were used after 24 h of hair depilation. Rats were divided into four groups (n=6). Two groups were treated with the optimized transdermal films containing micellar-drug dispersions. The third group received the films containing the free drug. Control films (placebo) made only from polymers and excipients were used on the fourth group. These films were cut into 2 x 2 cm square strips and each film was applied on the rat's dorsal sides, right side serving as test and left as control (without treatment) and was secured using adhesive tape. The films were applied for 24 h and after removal, the site of application was critically observed at 1h, 24 h, 48 h and 72 h for any sign of erythema or oedema and graded according to the Draize scoring criteria.[20,21]

RESULTS

Amlodipine Besylate was first entrapped in nano-sized pluronic micellesprepared by dialysis and thin film hydration methods using Pluronic F127 and Pluronic F 68. The morphology of nanomicelles of optimised samples was studied by Scanning Electron Microscopy (SEM). The images obtained showed spherical particles as seen in Figure 1.

Drug loading is due to the interaction between the hydrophobic drug and the core forming block of the pluronic polymer.[22] As seen in Table 3, the maximum drug incorporated or entrapment efficiency in micellar dispersions was found to be 91.82 % in F5 and 85.26% in F6, which were both prepared from Pluronic F127 by the thin film hydration method.

The particle size of the prepared polymeric micellar nanoparticles was in the range of 150 nm to 700 nm withF5 and F6 having the smallest micelle size. The PDI of formulation F5 and F6 was 0.347 and 0.497, respectively. The data for micelle size, zeta potential and PDI for all formulations are given in Table 3.

The transdermal films in which F5 and F6 were loaded were evaluated for various physico-mechanical properties and the data obtained are displayed in Table 4. It was observed that there was no significant change in weight variation and thickness among the prepared optimised films.

The reconstituted micellar dispersions of FD-2 and FD-3were evaluated for percentage drug incorporated or entrapment efficiency, particle size and morphological properties. These results of comparison are presented in Table 5.

The *in vitro* drug release from the transdermal films was studied using Modified Keshery–Chein diffusion cell and comparison of drug release profiles is illustrated in Figure 2.

The *ex vivo* skin permeation profiles between films with micelles (FD-2, FD-3) and film with free drug (FD-1) are compared as shown in Figure 3. FD-1 showed least drug permeation of 24.34% or 172.85 μg/cm^2 while FD-2 exhibited the greatest at 74.81% (495.28 μg/cm^2) during the 12 h study. FD-3 achieved a maximum drug release of 69.34% (534.35 μg/cm^2). The permeation flux of FD-2 and FD-3 was found to be 44.52

Table 3: Data for percentage drug incorporated, drug weight in micelles, micelle size, Zeta potential and PDI.

Formulation code	Drug in corporated (%)*	Drug weight in micelle (%)*	Micelle size (nm)*	Zeta potential (mV)	PDI
F1	5.23 ± 2.79	0.249 ± 0.043	690.9 ± 8.26	-5.52	0.628
F2	42.56 ± 5.70	2.026 ± 0.01	356.3 ±17.24	-3.68	0.573
F3	43.86 ±0.19	2.088 ± 0.02	368.1 ± 23.56	-4.56	0.845
F4	75.72 ± 2.78	3.60 ± 2.59	361.8 ± 5.28	-8.50	0.504
F5	91.82 ± 0.01	4.37 ± 0.973	159.2 ± 13.27	-10.3	0.347
F6	85.26 ± 2.12	4.06 ± 1.75	168.4 ± 27.43	-4.56	0.497
F7	25.43 ± 0.01	1.21 ± 0.74	523.7 ± 3.47	-5.29	1.000
F8	55.67 ± 1.14	2.56 ± 0.12	384.1 ± 11.8	-3.66	0.982
F9	49.41 ± 0.49	2.35 ± 0.074	375.2 ± 2.86	-2.84	0.699
F10	54.38 ± 3.49	2.58 ± 0.004	404.5 ± 23.51	-8.98	0.628
F11	65.29 ± 2.01	3.11 ± 0.025	265.8 ± 5.57	-7.95	0.458
F12	78.93 ± 0.857	3.75 ± 0.11	261.8 ± 2.20	-12.9	0.420

*Average of three determinations with standard deviation

Table 4: Data on physico-mechanical properties of transdermal films.

Formulation Code	Appearance	Weight variation (mg)*	Thickness (mm)*	Folding Endurance*	*% Drug Content (mg)*	% Moisture absorption*	Tensile strength (kg/cm^2)	% Elongation
FD-1	Non uniform, Flexible	25 ± 2.5	0.22 ± 1.9	250 ± 12	93.83 ± 0.1	5.9744 ± 0.847	0.69 ± 0.019	35.2 ± 0.015
FD-2	Smooth and Flexible and uniform	20 ± 0.04	0.17 ± 0.08	285 ± 10	96.68 ± 0.4	8.2436 ± 0.723	0.84 ± 0.011	31.2 ± 0.14
FD-3	Smooth, Flexible and uniform	20 ± 0.09	0.18 ±0.13	280 ± 9	98.75 ± 0.3	8.9200 ± 0.175	0.83 ± 0.019	32.1 ± 0.09

*Average of three determinations with standard deviation

μg/cm²/h and 41.27 μg/cm²/h, respectively, which was significantly higher compared to 14.40 μg/cm²/h for FD-1.

Skin irritation testing of transdermal film formulations, FD-1, FD-2 and FD-3 was conducted on albino Wistar rats, in accordance with the OECD Guideline number 402.[23] The appearance of redness or erythema at the site of application is a sign of skin irritation. Based on the extent of erythema or edema, a score from 0 (no irritation) to 4 (severe erythema or irritation) was assigned to each ratas per the Draize dermal irritation scoring system.[24] The scoring for the rats in all the four groups is presented in Table 6.

DISCUSSION

The drug loading efficiency of Pluronic micelles can be attributed to its micellar solubilisation of the poorly soluble drug, amlodipine. It was observed that, irrespective of the type of Pluronic used, micellar nanoparticles prepared by the thin film hydration method showed superior drug loading. This observation is an indication of the greater number of micelles produced by this method that led to greater drug solubilisation and entrapment.[25]

The small size of the micelles is important for stability as well as for better skin permeation capability. Low PDI of these micelle formulations showed that they had a narrow size distribution and also had the homogeneity of the micellar dispersion. The negative zeta potential values obtained for all formulations were close to reported values for pluronic micelles and indicate their colloidal stability in aqueous solution.[22,26] From these observations, F5 and F6 were considered optimal and were used for incorporation into the transdermal films

Transdermal Films incorporated with the optimized micelle formulations were prepared using HPMC K100 and PVP K30 in the optimal ratio of 3:1. These polymers were selected since they were compatible with the micellar dispersion. Selection of the polymer composition was based on the results obtained after the physical characterisation (appearance, folding endurance, tensile strength and percentage elongation) of the films prepared from HPMC K100 to PVP K30 ratios of 1:1, 1:3 and 3:1. Control films were also prepared in which drug-loaded polymeric micelles were replaced with free drug.

Films with micelles (FD-2 and FD-3) were comparatively more translucent, uniform, smooth and flexible than the films without micelles, FD-1. This may be because, in case of films with micelles, the drug is incorporated into micellar core; therefore the drug remains solubilised and does not precipitate. However, films in which the drug is directly added are comparatively whitish because of poor solubility of the drug in the aqueous polymer solution that could have caused some degree of precipitation of the drug during drying.

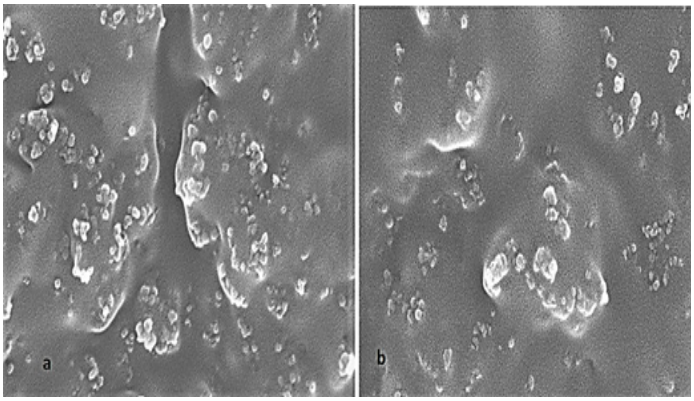

Figure 1: Scanning Electron Microscopy (SEM) images of (a) F5 and (b) F6.

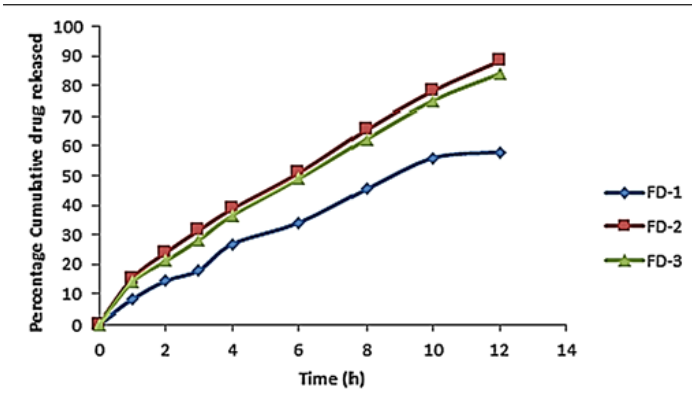

Figure 2: *In vitro* drug release profiles of FD-1, FD-2 and FD-3 through dialysis membrane.

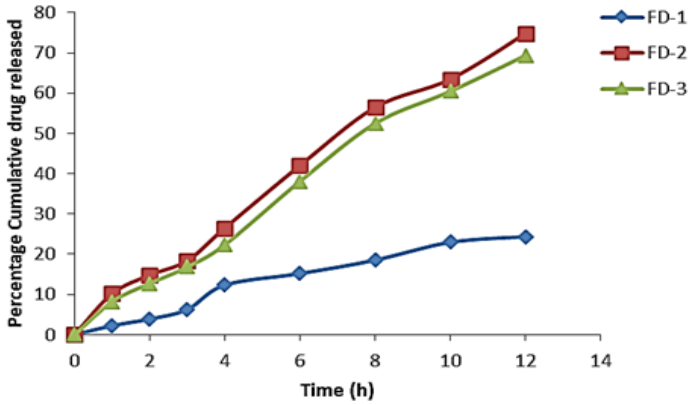

Figure 3: *Ex vivo* drug permeation profiles of FD-1, FD-2 and FD-3 through rat skin.

Table 5: Comparison of micelle parameters between initial and reconstituted micelle dispersions.

Parameters	Initial micelle dispersion (F5)	Reconstituted micelle dispersion from FD-2	Initial micelle dispersion (F6)	Reconstituted micelle dispersion from FD-3
Percentage drug incorporated*	92.35± 0.12	90.24 ± 0.10	86.73± 0.24	84.95 ± 0.14
Micelle size* (nm)	159.2 ± 13.27	163 ±11.26	168.4 ± 27.43	170.2 ± 11.43
PDI	0.347	0.315	0.497	0.492
Zeta potential (mV)	-10.3	-10.7	-4.56	-4.37

*Average of three determinations with standard deviation

Table 6: Data on skin irritation studies of optimised transdermal films.

Placebo group treated with transdermal film devoid of drug								
Reaction	1 h		24 h		48 h		72 h	
	Con.	Trt	Con.	Trt	Con.	Trt	Con.	Trt
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Treatment group treated with Transdermal film, FD-1								
	1 h		24 h		48 h		72 h	
	Con.	Trt	Con.	Trt	Con.	Trt	Con.	Trt
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Treatment group treated with Transdermal film, FD-2								
	1 h		24 h		48 h		72 h	
	Con.	Trt	Con.	Trt	Con.	Trt	Con.	Trt
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Treatment group treated with Transdermal film, FD-3								
	1 h		24 h		48 h		72 h	
	Con.	Trt	Con.	Trt	Con.	Trt	Con.	Trt
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0

Con. – Control; Trt. - Treatment

The low folding endurance of FD-1 which lacked micelles could be due to the precipitation of the drug during the drying of the films. The presence of the drug crystals in the films would have reduced the flexibility of the latter. Those films with micelles were smooth, uniform and had optimum flexibility.

Tensile strength and percentage elongation at break are two parameters that reflect the strength and elasticity of the films which can affect the handling properties and usage of the transdermal films.[27] Tensile strength of micelle containing films was found to be substantially higher than that of pure drug-loaded films. Obviously, the presence of precipitated drug in FD-1 makes the film less flexible and more prone to brittleness and breakage. The percentage elongation was found to be in the range of 32.1 ± 0.09 to 31.2 ± 0.014. It was observed that the films which showed maximum tensile strength showed least percentage elongation, indicating that these two parameters are inversely related.

On reconstitution of the films, FD-2 and FD-3, the solutions appeared as clear as the initial micellar solution. This is because the drug is present in highly solubilized form within the micelles present these films. Whereas reconstitution of films with free drug (FD-1) formed a turbid dispersion with precipitation of drug. Thereby indicating the solubilizing power of Pluronic F127 micelles on the drug in the former films (FD-2 andFD-3). Reconstituted micellar dispersions did not show significant variation in percentage drug incorporated, micelle size, PDI or zeta potential from the initial values. When the initial micelle and reconstituted micelles were observed under high power microscope the micelle shape was intact. These results conclude that micelles particle size and shape have been retained during the film formation method. Therefore it can be concluded that the micelles retain their properties even after film formation method and the film formation method does not affect the integrity of the micelles.

A comparison of the *in vitro* drug release profiles between transdermal films containing free drug (FD-1) and that with micelles (FD-2, FD-3) containing the same ratio of HMPCK100M and PVPK-30, show a noticeable difference in drug permeation. The maximum percentage cumulative drug release of FD-2 was 88.32 ± 0.04 and that of FD-3 was 84.04 ± 0.05, which was significantly higher than FD-1 which showed percentage cumulative drug release of 58.02 ± 0.07. It was evident from the drug release study that the incorporation of micelles in the transdermal films improved the drug permeation due to the nanosize of drug-loaded micelles.

The results for the *ex vivo* skin permeation flux confirm that the amount of drug permeated through the skin from FD-2 and FD-3 was substantially higher than from FD-1. This is attributed to the fact that the drug is in the solubilised form in micelles in the former and therefore results in better permeability. The increase in permeability can also be explained by the nanosize of the prepared micelles that enables the particles to permeate the skin and aided by the occlusion effect produced by the transdermal film. The *ex vivo* percentage drug permeated from all formulations was found to be significantly lower than that from *in vitro* permeation. This difference can be attributed to the greater thickness and barrier effect of the skin which can slow down the release of the drug. These results indicate that there is a very good possibility that the micelles carrying the drug could very well permeate through the human skin.[28]

At any time during observation of the Wistar rats in the skin irritation studies, the erythema and edema score in each rat was '0' in the placebo and treatment groups, as there were no signs of skin irritation on the shaven rat skin from the time the films were removed. Applied preparations producing scores of 2 or less are considered to be free of skin irritation potential. Hence, it can be concluded that the prepared transdermal films were free of skin irritants.[29,30]

CONCLUSION

Many approaches have been made to enhance the absorption efficiency and bioavailability of highly hydrophobic drugs. This study throws light in the area of the use of pluronic micellar nanoparticles as a means of solubilizing poorly soluble drugs such as amlodipine besylate while representing a novel approach for facilitating transdermal permeation by administration via transdermal films or patches. This strategy also provides the drug with some level of protection against degradation, thus improving drug absorption. Thus it is possible that such formulations have the potential of combining the benefits of nanoparticles and transdermal delivery of problematic actives, thereby achieving improved therapeutic effectiveness by increasing their bioavailability. Further studies in human subjects will help to confirm the improvement in drug permeation from such systems.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

PEO: Poly ethylene oxide; **PPO:** Poly propylene oxide; **CMC:** Critical micelle concentration; **HPMC K100:** Hydroxypropyl methylcellulose K100; **PVP K30:** Polyvinyl pyrrolidone K30; **SEM:** Scanning electron microscopy; **PDI:** Poly dispersity Index; **DMSO:** Dimethyl sulphoxide; **MWCO:** Molecular weight cut-off; **OECD:** Organisation for Economic Co-operation and Development.

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