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FORMULATION AND EVALUATION OF A TRANSFERSOMAL VESICULAR CARRIER SYSTEM FOR ENHANCED TOPICAL DELIVERY OF NIPRD-AF1

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ABSTRACT

The potentials of transfersomal formulations for transdermal delivery of NIPRD-AF1 was investigated. NIPRD-AF1 is a phytomedicine derived from the leaves of an indigenous plant, for use in the treatment of fungal infections. A transfersomal vesicular carrier system of NIPRD-AF1 was formulated and evaluated for topical application. Transfersomes of AF1 were prepared by solvent evaporation method using a phospholipid (Phospholipon 90 H) and varying concentrations of a mixture of surfactants (sodium lauryl sulphate, sorbitan monolaurate and Tween 80), and other performance enhancing excipients. The ointment was prepared by the fusion method using British Pharmacopoeia (BP) standard. The transfersomal carrier system was characterized using vesicle morphology, pH, entrapment efficiency (EE) and stability. The *in vitro* drug release of the transfersomes were studied using rat skin and Franz diffusion cell and compared to that of the ointment formulation of AF1. Transfersome formulation with the highest concentration of phospholipon and surfactants had optimal characteristics with an entrapment efficiency of 76.4 ± 0.04%. Optical microscopy showed presence of spherical vesicles in the transfersomal formulation with mean size (diameter) range of 57.5 ± 2.4 – 79.5 ± 3.10 µm. The pH values

ranged between 6.40 and 6.48. The stability study showed that the transfersome formulations were most stable between 4-8^{o}C. All the transfersomal formulations showed potentials for systemic delivery and better permeation profiles than the ointment formulation of AF1.

Key words: NIPRD-AF1, transfersome, ointment, permeation, transdermal drug delivery.

Introduction

Delivery of drugs by application of suitable dosage form to the skin is appreciably a more attractive option to other routes. This is because apart from the convenience of application and safety, it offers other potential advantages such as: avoidance of first-pass metabolism, prolonged and predictable duration of action, minimization of side effects, avoidance of fluctuations in therapeutic drug levels arising from incompatibility with the gastrointestinal contents and variability of absorption [1-3]. Moreover, topical application of drugs is non-invasive and can be self-administered, which encourages patient compliance. Many approaches have been used to enhance delivery of drugs through the skin and other biological membranes [4, 5]. One of such approaches is the use of permeation enhancers in the formulation of vesicular carrier systems.

Transfersomal formulations are one of the vesicular carrier systems developed through liposome technology for enhanced delivery of bioactive materials. They are ultradeformable vesicular carriers formulated with phospholipids and surfactants for effective delivery of drugs into or through the skin [6]. Transfersomes are more elastic than the standard liposomes and thus can overcome skin penetration difficulty by squeezing through the intracellular lipid spaces of the stratum corneum. This capability is due to the high level of vesicle deformabilty arising from the incorporation of surface-active agents. The resulting flexibility of transfersome membrane minimizes the risk of complete vesicle rupture in the skin and allows the vesicles to follow the natural water gradient across the epidermis, when applied under non-occlusive condition [6, 7, 8].

NIPRD-AF1 is the ethyl acetate extract obtained from the leaves of an indigenous plant which has shown significant antifungal activity against common superficial infections. The extract has been shown to have the potential to cure superficial mycosis caused by common dermatophytes [9]. The studies showed that the extract prevented the growth of *Epidermophyton flocossum* ATCC 10227, *Micosporum canis* ATCC

11622, *Trichophyton rubum* ATCC 28941 and *Trichophyton mentagrophytes* ATCC 4808, and suppressed growth of *Aspergillus niger* and *Aspergillus fumigatus*, all of which are dermatophytes commonly implicated in skin fungal infections. It has been used in ethnomedicinal treatment of fungal infections in Nigeria, usually by application of pastes of ground leaves to the affected area of skin. However, only the ointment dosage form has been formulated by NIPRD. This project is intended to formulate a transfersomal vesicular carrier system for topical application of NIPRD-AF1 and compare with the ointment formulation. It is hoped that this dosage design will ensure not only enhanced delivery of AF1 to the affected skin area but also its systemic delivery for treatment of systemic fungal infections.

Materials

The following materials were used in formulating the transfersomes: Phospholipon® 90 H (Phospholipid GmbH, Nattermannallee, Köln), sodium lauryl sulphate (Hopkin & Williams, England), sorbitan monolaurate, polyethylene glycol (Mol. Wt. 200) (Aldrich), Tween 80, choloroform ethyl acetate (Riedel-de Haën, Italy), ethanol (Sigma), methanol, white soft paraffin, liquid paraffin (BDH, England), distilled water, phosphate buffer solution (PBS) (pH 6.5), NIPRD-AF1 extract and emulsifying wax BP. All other reagents were of analytical grade and used without further purification.

Methods

Preparation of transfersomal formulation of AF1

The transfersomal formulation was prepared by adoption of the rotary solvent evaporation method described by Shivanand *et al*. [1]. The phospholipid was added to a mixture of the surfactants and dissolved in a chloroform-methanol mixture (3:1) by shaking in a round-bottomed flask. Four (4) different formulations were prepared based on varying Phospholipon and surfactant concentrations.

Formlations A, B, C and D had phospholipid and surfactant concentrations of 2, 4, 8, 10 and 5, 10, 15, 20%, respectively (Table 1). The organic solvents were then evaporated using rotary evaporator (Byb Bibby Sterilin L.t.d. Stone, Staffordshire,UK) (formulation A) and by manual rotary movement of the flask in water bath (formulations B, C and D) at 55^{o}C. A thin whitish film formed round the flask, which was then allowed to stand overnight (12 h) for complete evaporation of traces of the solvent. The film was hydrated with ethanolic solution of AF1 in PBS (pH 6.5) and shaken vigorously on a Vortex mixer in a suitable container. While still mixing, polyethylene glycol was added. The volume was then made up to 100 ml with PBS and mixed well. On preliminary physical evaluation with respect to homogeneity and appearance of creaming, formulation D had the best appearance and was then optimized. The formulations were sonicated using bath sonicator (Decon®, FS 100b, England) in three cycles of 20 min each, with 2 min between each cycle, at 31^{o}C. The preparations were kept on the laboratory bench to cool to room temperature after which each batch was divided into 20 ml portions, some of which were stored in the refrigerator for further studies.

Preparation of ointment

Table 2 shows the formula for preparation of the ointment. The ointment formulation of AF1 was prepared by the fusion method [10] using an evaporating basin on a water bath. After melting the ointment base the AF1 was incorporated gradually and mixed with a spatula until it was homogeneous.

Characterization of the vesicular formulation

Vesicle morphology

The vesicle shape was visualized using an optical microscope (Leica CME) attached with a digital camera [11]. The microscopic slide was prepared by putting 2 drops of the transfersomal suspension on the slide, spreading with a cover slip and kept to dry overnight. A drop of glycerine was placed on the tail end of the smear, positioned on the microscope stage and clear view obtained at x40 magnification. Photomicrographs of vesicles in the preparations were obtained and their sizes measured using a ruler.

Entrapment Efficiency (EE)

The entrapment efficiency (EE) was determined by ultracentrifugation method [4]. 2 ml of each transfersomal suspension was first centrifuged (CENTRI NAPCO 2019R, Termo Electron Corporation, France) at 14,000 rpm for 2 h at 4^{o}C. 0.5 ml each of the supernatant and sediment was then diluted to 10 ml with PBS (pH 6.5), after lysing the sediment (vesicles) with 0.1% Triton X-100 solution. 0.5 ml of the diluted sample was further diluted to 10 ml and the diluted samples were then analyzed spectrophotometrically using a UV spectrophotometer (UV-160A, Shimadzu, Japan) and EE calculated from the expression [4]:

EE (%) = [(T – C)/T] x 100 …… eqn 1

where C = amount of the drug in supernatant, T = total amount of the drug in both supernatant and sediment, (T – C) = amount of the drug entrapped in the vesicles. The test was performed thrice and the mean value calculated.

pH

A 1.0 ml quantity of each transfersomal formulation was diluted to 20 ml with distilled water and the pH was determined using a pH meter (Accumet Research, model AR10, Fisher Scientific).

Temperature stability of formulations

The formulations were stored for 29 days at different storage conditions and monitored for physical changes. The storage conditions used were room temperature (laboratory bench) (27^{o}C), refrigerator (4-8^{o}C) and hot air oven at (40^{o}C).

In vitro skin permeation study

Permeation of transfersomes through hairless rat skin was determined by using Franz diffusion cell (PS 80067 A, Copley Scientific, UK) [4, 12]. The skin was obtained by scalp removal method from Swiss albino Wister rats. It was carefully mounted onto the diffusion cell with stratum corneum side facing the donor compartment and dermal side bathed in receptor media. A 0.5 ml quantity of each AF1 transfersomal preparation was placed into the donor compartment over the skin while the receptor compartment was filled with 6.0 ml of phosphate buffer solution (PBS, pH 6.5). The temperature of the diffusion cell was maintained at 32 ± 1^{o}C throughout the experiment and the receptor compartment was stirred continuously by means of a magnetic stirrer in the cell. 1.0 ml samples were withdrawn through the sampling port at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 12.0 h and automatically replaced with equal volumes of fresh diffusion medium (PBS) from the supply reservoir, thus maintaining the sink condition throughout the experiment. The samples were analyzed for drug content spectrophotometrically at predetermined AF1 absorption maximum of 257 nm, in conformity with Beer's plot, using a UV spectrophotometer (UV-160A, Shimadzu, Japan). This was repeated using 500 mg of the ointment formulation. The test was performed thrice for each formulation and the mean amount of NIPRD-AF1 permeated per unit area of the rat skin (µg/cm^2) and flux (µg/cm^2/h) were calculated for all the formulations.

Statistical analysis

The results were analyzed by ANOVA and Student's *t*-test using Microsoft Office Excel 2007 and values obtained at ($P < 0.05$) considered significant.

Results and Discussion

On physical examination, formulation D was the best in terms of homogeneity. This is an indication that the relatively high content of phospholipids (10%) and surfactants (20%) compared to the other formulations was the best.

The photomicrographs showed the presence of spherically shaped vesicles dispersed in all the batches prepared. The mean vesicular size (diameter) obtained ranged between 57.5 ± 2.4 and 79.5 ± 3.10 µm for formulations B and D, respectively. The values for other formulations lie within the range. With availability of requisite equipment for formulation and evaluation, further research efforts will aim at producing transfersomes of vesicle sizes within or close to nanoscale. Fig. 1 shows photomicrographs obtained for formulations B and D. The presence of vesicles indicated that the phospholipid used in the transfersome formulation actually formed vesicular carriers. Similar observation had been reported for an ethosomal vesicular carrier formulation [11].

All the formulations were stable between 4-8^{o}C (refrigeration) and at 27^{o}C (room temperature) as there was no phase separation after 24 h of storage. However, samples stored at 40^{o}C (oven) showed signs of instability within 3 h, ranging from phase separation (cracking) to reduction in volume, perhaps due to evaporation of the volatile components. By day 2, samples stored at room temperature and in the refrigerator were still relatively stable although there were signs of particle sedimentation especially with those at room temperature. Samples stored at 40^{o}C continued to deteriorate and it was concluded that the AF1 transfersomal formulation was highly unstable above room temperature. After 14 days, samples stored at 27^{o}C had clearly creamed, with formulations B and C forming clearly two phases, though still redispersible on shaking. The refrigerated samples sedimented but redispersed easily on shaking. After 29 days, the formulations kept at room temperature had creamed and cracked while those in the refrigerator had also creamed to a lesser extent and were easily redispersable on shaking. The results indicated that storage of transfersomal vesicular formulation of NIPRD-AF1 is best under refrigeration. This result is in

line with previous findings that vesicular carriers are best stored under refrigeration [4, 5]. Formulating the suspension into a gel using suitable bases improves the stability and residence time of transfersomes when applied topically. In the gel form, it could be stored beyond the shelf-life at room temperature without losing homogeneity.

The pH of the various transfersomal formulations of AF1 were found to be 6.40 ± 0.05, 6.48 ± 0.01, 6.46 ± 0.03, and 6.48 ± 0.03 for formulation A, B, C and D, respectively. This indicates that the formulations were only slightly acidic therefore may not cause discomfort or irritations on application to the skin which has normal average pH of 5.5 (4.5-6.0) [13].

The EE values obtained were 48.6 ± 1.52, 51.3 ± 2.14, 43.7 ± 1.50 and 76.4 ± 0.04%, for formulations A, B, C and D, respectively. Therefore, in all the formulations appreciable amounts of AF1 particles were successfully entrapped in the vesicles formed by the Phospholipon, with the value for formulation D being significantly higher than the other batches. Formulations B and D prepared using water bath to evaporate the organic solvents recorded higher EE values than those produced with rotary evaporator. Therefore, the water bath method seems to be more efficient than the rotary evaporator method on a very small scale. On the industrial scale, however, the rotary evaporator will be preferred due to solvent recovery and reduced exposure of personnel to solvent hazards.

Fig. 2 illustrates results obtained from the skin permeation study. All the transfersome and ointment formulations progressively delivered their AF1 content through the rat skin in the order: formulation D > B > A > C > Ointment. This correlated with the results of the flux calculated (Table 4) and EE values obtained for the transfersomes. Formulation D with the highest EE had the highest flux throughout the test period compared to formulations B, A, C and the ointment, respectively. Flux (μg/cm^2/h) gives an indication of the amount of drug or active substance that permeates the skin per unit time. The result showed that flux decreased with time for all the formulations. The transfersome formulations significantly performed better than the ointment formulation, and would therefore be expected to produce better

therapeutic effect. This result follows earlier findings that vesicular carrier formulations offer enhanced transdermal drug delivery into systemic circulation, not only over other topical but also oral dosage forms [5]. It is thought that transfersomes are capable of permeating through the layers of skin by means of a hydrating gradient, under non-occluded condition, from the outer skin surface to the more hydrated viable tissues [14, 15]. The surfactant constituent of transfersomes provides flexibility, to the vesicles and the horny stratum corneum layer, which enhances penetration of the intact skin. Transfersomes have been reported to be up to 10 times more ultradeformable than conventional liposomes and so can squeeze through pores in stratum corneum, which are less than one-tenth of the vesicle diameter [6, 16].

Conclusion

The phytomedicine, NIPRD-AF1 was successfully formulated into transfersomes. Formulation D having 10% phospholipid and 20% surfactants was the best formular developed. Transfersomal vesicular carrier formulation offers enhanced delivery of NIPRD-AF1 across the skin over the ointment formulation and may therefore be further developed for the treatment of not only susceptible superficial but also systemic mycotic infections. The useful applications of transfersomal vesicular carrier systems can be exploited for transdermal delivery of NIPRD-AF1 and possibly for the development of some other phytomedicines.

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Table 1: Formula for preparation of NIPRD-AF1 transfersomes

Ingredient	**Quantity used per batch**			
	A	B	C	D
NIPRD-AF1 extract (g %w/v)	1.0	1.0	1.0	1.0
Phospholipon® 90 H (g %w/v)	2.0	4.0	8.0	10.0
Sodium lauryl sulphate (g %w/v)	5.0	10.0	15.0	20.0
Sorbitan monolaurate (ml %v/v)	5.0	10.0	15.0	20.0
Tween 80 (ml %v/v)	5.0	10.0	15.0	20.0
Chloroform (ml)	7.5	7.5	7.5	7.5
Methanol (ml)	2.5	2.5	2.5	2.5
Ethanol (ml)	10.0	10.0	10.0	10.0
Polyethylene glycol (Mol. Wt. 200) (ml)	7.5	7.5	7.5	7.5
PBS (pH 6.5) (ml)	qs 100.0	qs 100.0	qs 100.0	qs 100.0

Table 2: Formula for preparation of NIPRD-AF1 ointment

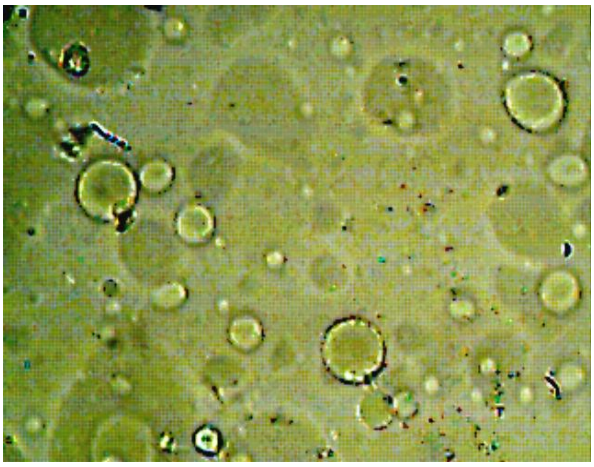
Ingredient	Quantity used (%)
NIPRD-AF1	1.50
Emulsifying wax BP	30.00
White soft paraffin	48.50
Liquid paraffin	20.00
Total weight	100.0 g

Table 3: Amount of NIPRD-AF1 permeated per unit area of rat skin (± S.E.M)

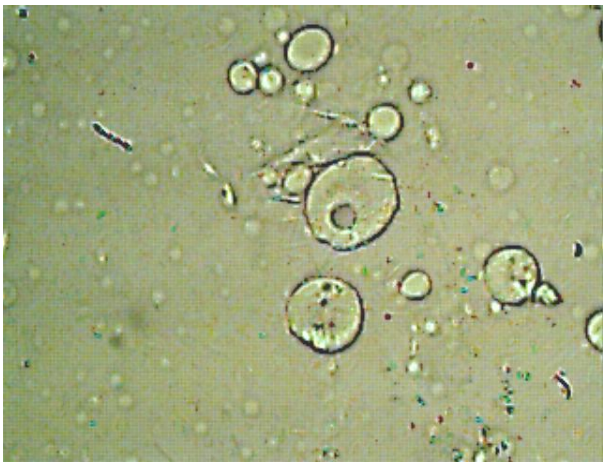
Time (h)	**Amount permeated per unit area for each formulation (µg/cm^2)**				
	A	**B**	**C**	**D**	**Ointment**
0.5	1.65 ± 0.0002	1.27 ± 0.0005	2.41 ± 0.0006	2.66 ± 0.0001	0.51 ± 0.0015
1.0	2.15 ± 0.0016	2.47 ± 0.0001	2.66 ± 0.0001	3.48 ± 0.0005	1.24 ± 0.0002
2.0	2.72 ± 0.0006	3.42 ± 0.0002	2.66 ± 0.0003	3.92 ± 0.0006	1.77 ± 0.0006
3.0	3.16 ± 0.0001	4.56 ± 0.0006	2.72 ± 0.0005	4.43 ± 0.0004	2.28 ± 0.0001
4.0	3.48 ± 0.0001	4.62 ± 0.0001	2.97 ± 0.0001	4.81 ± 0.0002	2.66 ± 0.0006
6.0	4.30 ± 0.0006	4.81 ± 0.0006	3.04 ± 0.0001	6.14 ± 0.0003	3.04±0.0001
12.0	4.50 ± 0.0001	4.85 ± 0.0001	3.52 ± 0.0006	6.55 ± 0.0003	3.20 ± 0.0005

Table 4: Flux of the NIPRD-AF1 transfersome and ointment formulations

Time (h)	Flux for each formulation ((µg/cm^2/h)				
	A	**B**	**C**	**D**	**Ointment**
0.5	3.30	2.54	4.82	5.32	1.02
1.0	2.15	2.47	2.66	3.48	1.24
2.0	1.36	1.71	1.33	1.96	0.89
3.0	1.05	1.52	0.91	1.48	0.76
4.0	0.87	1.16	0.74	1.20	0.67
6.0	0.72	0.80	0.51	1.02	0.51
12.0	0.38	0.40	0.29	0.55	0.27



(a)x 40



(b) x 40

Fig. 1: Photomicrographs of transfersomes of formulations B (a) and D (b) showing presence of vesicular carriers.

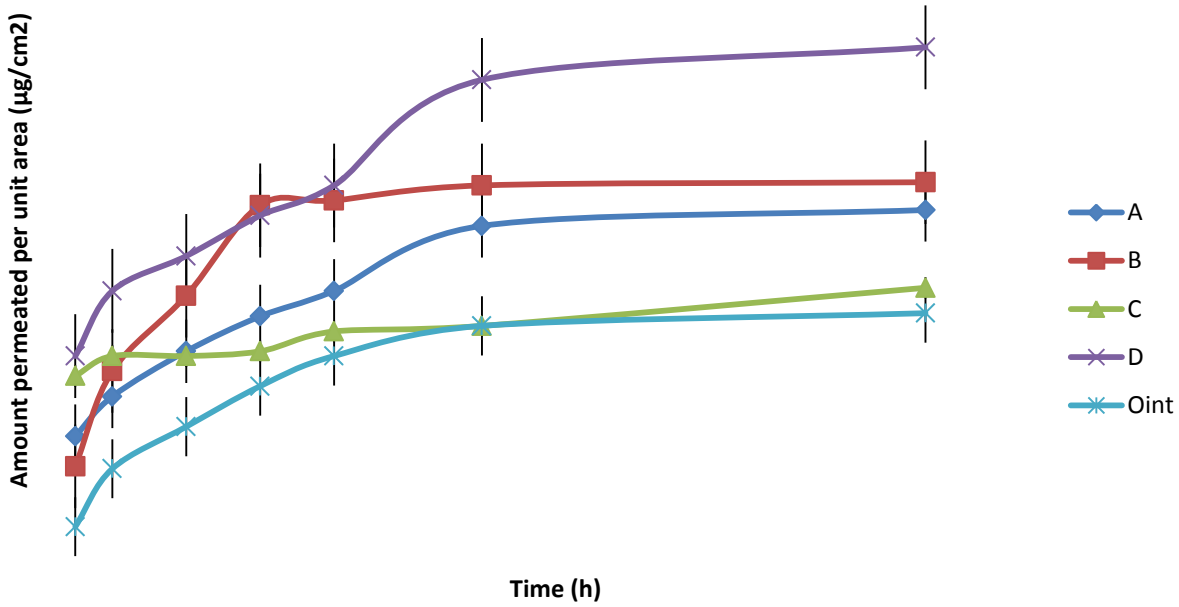


Fig. 2: Plot of amount permeated per unit area of rat skin (µg/cm^2) against time (h) for NIPRD-AF1 transfersomes and ointment formulations.