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Research paper

A dynamic topical hydrofluoroalkane foam to induce nanoparticle modification and drug release *in situ*

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ABSTRACT

Topical nanoparticles are usually applied using semi-solid formulations, but the delivery process is often inefficient due to the poor drug release from the particles. The aim of this study was to investigate the capability of a dynamic foam to break open nanoparticles upon application to the skin and enhance drug delivery efficiency. Vitamin E acetate (VEAc) was selected as a model drug and loaded into lipid nanoparticles (50–60 nm) prepared by phase inversion. The highest drug loading was 18.9 ± 1.2 mg/ml and the corresponding encapsulation efficiency was 81.5 ± 4.1%. Dynamic foams were generated by emulsifying VEAc-loaded nanoparticle suspensions with hydrofluoroalkane using pluronic L62D. An *in vitro* permeation study demonstrated that VEAc did not release from the nanoparticles when administered as an aqueous suspension, but attained a flux of 18.0 ± 2.1 ($\mu g\ cm^{-2}\ h^{-1}$) when applied using the foam. Drug release from the foam was shown to be a consequence of nanoparticle modification after dose administration and this led to the foam delivering 0.7 ± 0.3% VEAc into the *stratum corneum* (SC) when applied to human skin.

1. Introduction

The administration of active pharmaceutical ingredients (APIs) topically to achieve local delivery has the benefits of 'first-pass metabolism' avoidance, reduction of adverse side-effects associated with systemic toxicity, direct access to the pathological site and improved patience compliance. However, efficient topical delivery can be extremely difficult to achieve due to the highly efficient barrier function of the *stratum corneum* (SC). A number of strategies have been developed in an attempt to improve the efficiency of drug transport across the SC including: electroporation [1]; iontophoresis and phonophoresis [2]; penetration enhancers [3]; ion pairing agents [4]; prodrugs [5]; eutectics [6]; supersaturation [7]; the manipulation of drug vehicles [8] and use of colloids [9–12], but many of these strategies have had limited success when used in combination with highly hydrophobic therapeutic agents.

Two phase formulations such as emulsions and suspensions enable the inclusion of therapeutically relevant concentrations of highly hydrophobic agents within cosmetically acceptable aqueous topical vehicles, but the drug release from such systems is traditionally very poor. For example, the topical application of hydrophobic levothyroxine cream has previously been reported to deliver only 0.8% of the applied drug into the skin [13]. In an attempt to improve the speed and efficiency of hydrophobic drug release from polar vehicles, a wide variety of colloidal carriers have previously been employed in topical formulations, including: micelles, vesicles, liquid crystals [9], liposomes, niosomes [10], polymeric nanoparticles [11], solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) [12]. Of these colloidal systems, nanoparticles offer the most promise for topical delivery as they are highly versatile, exhibit good physical stability and some nanoparticles such as SLNs can be manufactured on an industrial scale. Nanoparticles can enhance the stability of chemically unstable active agents e.g. those agents that are easily damaged by hydrolysis, oxidation and photochemical decomposition, and they can often be suspended in polar vehicles [14,15].

The benefits of nanoparticles when used to administer APIs topically are constrained to effects in the application vehicle as they are not believed to penetrate the SC. Previous work has used confocal laser scanning microscopy (CLSM) to visualise the distribution of fluorescent polystyrene (PS) nanoparticles when applied to porcine skin and found that PS nanoparticles mainly accumulated in the skin follicular openings in a time-dependent manner [16]. Other independent studies have confirmed this nanoparticle localisation process [17,18]. Although the follicular shunt route is

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one potential pathway for APIs to by-pass the SC, as the appendages constitute <0.1% of the whole skin surface area [19], it is presumed that the majority of encapsulated API does not translocate the uppermost layer of skin via this method. As nanoparticles remain on the apical surface of the skin drug release from the nanoparticles is crucial for its delivery. However, the most commonly used topical vehicles such as creams and gels do not efficiently liberate the API from nanoparticles and this is one of the main reasons for the poor delivery efficiency often reported when colloidal topical formulations are used [20]. In order to improve skin permeation of API when delivered using nanoparticles, it is essential to develop novel vehicles that release the encapsulated agent effectively upon dose application.

One novel method that could facilitate the release of therapeutic agents from nanoparticles when incorporated in topical formulations is the use of dynamic vehicles such as foams [21] and sprays [22]. A dynamic system changes after dose actuation, usually as a result of volatile solvent evaporation, and this can drive a change of state or excipient concentration, which has the potential to break down particles and enhance drug release. Pharmaceutical aerosols are commonly used to generate sprays and foams which usually contain drugs; solvents; co-solvents; surfactants/polymers and propellants, all sealed in a canister, thus held under pressure. The inclusion of API in topical foams has been reported in several previous studies [23,24]. Abram developed a topical foam composition that could form an occlusive layer [25]. Tamarkin et al. created a topical foam formulation containing solid APIs which was suitable for cosmetic and pharmaceutical use [26]. However, a large number of previous pharmaceutical foams utilise flammable hydrocarbon propellants i.e. butane, isobutene, and propane. An alternative to hydrocarbon propellants that are non-explosive and non-flammable is now available in the form of hydrofluoroalkanes (HFAs). Employing HFA in aerosols avoids the inconvenient use of explosive hydrocarbons in the manufacture process, allows *in situ* analysis and reduces ozone layer depletion [27].

The aim of this study was to investigate the release of a lipophilic drug from nanoparticles when administered using a dynamic HFA foam. The foam was designed to take the form of a nanoparticle-in-water-in-HFA emulsion that would collapse upon dose application as a result of HFA evaporation. It was anticipated that the resultant high surfactant concentration in the foam residue after application would break down the nanoparticles and facilitate the release of the encapsulated agent. In an attempt to achieve this *d*-alpha tocopherol acetate, commonly known as vitamin E acetate (VEAc) was selected as a model API and a lipid nanoparticle (LN) was prepared via phase inversion, based on previous work [28], was used to encapsulate VEAc. The drug release and permeation of VEAc when formulated as an aqueous nanosuspension, a foam and a VEAc-saturated oil was tested using silicone membrane and human skin *in vitro*. The poor aqueous solubility of VEAc prevented the use of water as a control vehicle in the permeation studies. As a consequence, despite it not being a good representation of a commercial VEAc product (due to poor consumer acceptability), a saturated oil was used to represent a topical formulation with rapid release.

2. Materials and methods

2.1. Materials

Pluronic L62D, VEAc and heptafluoropropane (HFA 227) were obtained from BASF (New Jersey, USA), Adina Cosmetic Ingredients (Kent, UK), and DuPont de Nemours Int'l SA (Geneva, Switzerland), respectively. Medium chain triglyceride (Labrafac® WL 1349), hydrogenated soybean lecithin (Lipoid® S75-3) and macrogol 15 hydroxystearate (Solutol® HS 15) were kindly supplied by Gattefossé S.A. (Saint-Priest, France), Lipoïd GmbH (Ludwigshafen, Germany) and BASF (Ludwigshafen, Germany), respectively. Sodium chloride and *dl*-alpha tocopherol were purchased from Sigma–Aldrich Ltd. (Gillingham, UK). The medium chain triglyceride (Miglyol 810N) was sourced from S Black Limited (Hertford, UK). Dow Corning 200/350 cS fluid (silicone oil) was purchased from VWR (Leicestershire, UK). HPLC (High Performance Liquid Chromatography) grade ethanol was from BDH (Poole, UK). HPLC grade water and methanol were from Fisher (Leicestershire, UK).

2.2. Vitamin E acetate assay

A HP 1090 liquid chromatography system coupled to a HP 1050 UV detector was used for quantitative analysis of VEAc. The method was based on previously published work [29]. Separation was achieved at 30 °C using a Gemini C18 column (5 μm, 250 mm × 4.6 mm) protected by a Gemini C18 4 mm × 3.0 mm security guard cartridge (Phenomenex, Cheshire, UK). The mobile phase was a mixture of methanol and ethanol (1:1, v/v) and the flow rate was maintained at 1.0 ml/min. The injection volume was 50 μl and the detection wavelength was set at 284 nm.

2.3. Vitamin E acetate encapsulation

The preparation of VEAc-loaded LN involved three main steps [28]. Step 1: VEAc at three levels (1%, 2% and 4%, w/w), Labrafac® WL 1349 (17%, w/w), Solutol® HS 15 (17%, w/w), Lipoid® S75-3 (1.75%, w/w), and a 3% (w/v) aqueous solution of sodium chloride (to 100%, w/w) were mixed to form a homogenous oil-in-water emulsion at ambient temperature (23 ± 2 °C). Step 2: the temperature of the mixture was raised to 85 °C at a rate of 4 °C/min, and then cooled down back to 60 °C. This procedure was repeated three times to induce phase inversion. Step 3: the mixture maintained at 85 °C was diluted with an equal volume of cold water to generate LNs. Slow magnetic stirring was applied to the nanosuspension for 5 min to homogenise the final suspension. The suspension was centrifuged at a speed of 110,000 g at 20 °C for 60 min using a Beckman L8-80 ultracentrifuge (Beckman Coulter, Buckinghamshire, UK). After centrifugation three layers were obtained. The purified nanoparticle aqueous suspension was collected from the middle layer of the centrifuged material and stored at ambient temperature prior to use. The top layer (which solidified, hence presumably contained excess lipids/surfactants) and the sediment (which presumably contained precipitated drug/excipients) were separated, stored and analysed if a full mass balance was required.

2.4. Drug loading, encapsulation efficiency and mass balance

A centrifugal filtration technique was employed to separate the nanoparticles from the aqueous nanosuspension. A Millipore centricon® YM-100 centrifugal concentrator with the MWCF (molecular weight cut-off) of 100 kDa (Fisher Scientific, Leicestershire, UK) was used at 2700 g for 90 min (MSC centaur 2 centrifuge, DJB Labcare Ltd., Buckinghamshire, UK). The nanoparticles retained on the filter were recovered by inverting the sample reservoir and centrifuging at 1000 g for 5 min. The nanoparticles and other layers from the purification step were diluted using HPLC grade ethanol to facilitate VEAc content assay by HPLC. A mass balance of the drug was calculated from the process to get the total drug recovery after encapsulation and purification. The drug loading, encapsulation efficiency (EE) and drug recovery were calculated (Eqs. (1)–(3)):

$$\text{Drug loading} = \frac{W_1}{V} \quad (1)$$

$$\text{Encapsulation efficiency} = \frac{W_1}{W_0} \times 100\% \quad (2)$$

$$\text{Drug recovery} = \frac{W_1 + W_2 + W_3 + W_4}{W_0} \times 100\% \quad (3)$$

where V is the final volume of purified nanosuspension; W_0 is the mass of initial VEAc input; W_1 and W_2 are the mass of the VEAc within the purified nanoparticles and aqueous suspending media; W_3 and W_4 are the VEAc amounts in the top layer and sediment layer after the ultracentrifuge purification.

2.5. Particle size and zeta potential analysis

The nanoparticle size and polydispersity index (PI) were analysed by photon correlation spectroscopy (PCS) (Brookhaven ZetaPlus, Brookhaven Instruments Ltd., Worcestershire, UK). The samples were diluted with HPLC grade water in a ratio of 1:20 (w/w) prior to analysis. All measurements were carried out at a scattering angle of 90° at 25 °C, $n = 5$, three batches of each particle suspension were assessed. A PI above 0.2 indicated the broad distribution of nanoparticles. Zeta potential was assessed using the same Brookhaven ZetaPlus and the purified nanoparticle sample was diluted in 1 mM sodium chloride solution (1:50, v/v) prior to analysis.

2.6. Foam preparation

The VEAc-loaded LN aqueous suspension (LN10, Table 1), after purification (*ca.* 10 mg/ml), was transferred to a 10 ml plastic coated glass canister (Schott UK Ltd., Stafford, UK) and an appropriate amount of pluronic L62D surfactant was added. The canister was sealed with a 100 μl metered spray valve (Valois UK Ltd., Bletchley, UK) and HFA 227 was filled into the canister using pressurised filler (Pamasol Willi Mäder AG, CH-8808 Pfäffikon SZ, Switzerland). This was left to stir overnight at 1000 rpm using a Variomag® Telesystem HP15 stirrer plate (Florida Scientific Services, Inc., Daytona Beach, USA) to homogenise the system. The formulation compositions, all of which contained *ca.* 0.3% (w/w) VEAc, were as follows: FM002 (40.0% LN10 nanoparticle suspension, 2.0% pluronic L62D, 58.0% HFA 227, w/w), FM075 (40.0% LN10 nanoparticle suspension, 7.5% pluronic L62D, 52.5% HFA 227, w/w), and FM150 (40.0% LN10 nanoparticle suspension, 15.0% pluronic L62D, 45.0% HFA 227, w/w).

2.7. Foam drug content uniformity

The drug content uniformity in the foam formulations (FM002, FM075 and FM150) was tested over 4 weeks at ambient temperature. On days 1, 7, 14, and 28, two foam sprays were released from the canister and the weight of the sprays was calculated by the weight loss. The released foam residue was dissolved in ethanol and the drug amount was assayed by HPLC. The VEAc content uniformity in foam formulations was calculated as a percentage of VE recovery (Eq. (4)):

$$\text{VEAc recovery} = \frac{W_a}{W_b} \times 100\% \quad (4)$$

where W_a is the mass of the actual VEAc in two sprays of foam and W_b is the theoretical VEAc amount in two sprays of foam. The VEAc content determination was conducted 30 times for each formulation and the mean recovery was calculated.

2.8. In vitro membrane release

Static vertical individually calibrated Franz-type diffusion cells (MedPharm Ltd., Guildford, UK) with an average diffusional surface area of *ca.* 2 cm^2 and a receiver volume of *ca.* 10 ml were used for the *in vitro* release experiments. Each cell contained a small magnetic stir bar that was used to ensure that the receiver fluid remained homogenous during the experiments. A silicone membrane (120 μm thick, Kapitex Healthcare Ltd., West Yorkshire, UK) was cut to size and mounted in the diffusion cells held thermostatically at 32 °C in a SS40-5 water bath (Philip Harris, Staffs, UK) with a Variomag® stirrer plate. Due to the extremely lipophilic properties of VEAc (clear viscous liquid), Miglyol 810N which has been used in a variety of topical preparations was selected as the receiver fluid to maintain sink conditions since VEAc showed a good solubility (up to 50%, w/w) therein. The chemical stability of VEAc in the receiver fluid was examined by dispersing 0.5, 1.0, and 1.5 mg/g VEAc in the fluid at 32 °C over 48 h and testing the VEAc recovery ($n = 3$). After cell equilibration at 32 °C for 1 h, three types of test formulation were applied to the surface of each membrane: (1) 1.0 ml VEAc saturated in silicone oil (the maximum miscibility of VEAc was *ca.* 5%, w/w); (2) 1 ml VEAc-loaded LN aqueous suspension at three concentrations (LN05, LN10 and LN20, details in Table 1); and (3) 2.5 g of the foam formulations (FM002, FM075 and FM150) containing approximately 1 ml VEAc-loaded LN suspension. At predetermined time intervals, 0.5 ml of receiver fluid was drawn from the Franz cell receiver compartment and diluted with ethanol prior to HPLC assay. The receiver fluid was replenished with an identical volume of fresh Miglyol 810N, held at 32 °C, in order to keep the liquid volume in the receiver compartment constant. All the diffusion experiments were carried out at least five times. Cumulative amounts of drug (μg) penetrating the unit surface area of the silicone membrane (cm^2) were corrected for sample removal and plotted against time (h). Steady-state flux was taken from the line of best fit over at least six time points with a linearity of $r^2 \geqslant 0.98$. The lag time was determined from the intercept of the time axis with the steady-state regression curve.

2.9. In vitro skin tape stripping

Human abdominal skin was obtained from plastic surgery with informed patient consent and approval from the Research Ethics Committee of the University of Hertfordshire. The subcutaneous fatty tissue was removed using a scalpel and scissors. The skin was covered with aluminum foil and stored below −20 °C until required. Full thickness skin was thawed at ambient temperature prior to use and punched into appropriate size using a cork borer to fit the Franz diffusion cells (MedPharm Ltd., Guildford, UK). The individually calibrated cells had an average diffusional surface area of *ca.* 0.6 cm^2 and a receiver volume of 2 ml. Miglyol 810N was the receiver fluid maintained at 37 °C using a SS40-5 water bath with a Variomag® stirrer plate in order to achieve the skin surface

Table 1
Vitamin E acetate (VEAc) loading, encapsulation efficiency and recovery from the lipid nanoparticles (LNs) ($n = 3$).

Nanoparticles	VEAc input (mg/ml)	Drug loading (mg/ml)	Encapsulation efficiency (%)	Drug recovery (%)
LN05	5.0	5.1 ± 0.2	90.1 ± 3.7	101.0 ± 2.3
LN10	10.0	9.8 ± 0.1	88.6 ± 2.8	100.3 ± 1.7
LN20	20.0	18.9 ± 1.2	81.5 ± 4.1	100.3 ± 2.4

temperature of 32 °C. Once the skin disks were mounted in the cells with the dermal side bathed with Miglyol 810N fluid, they were equilibrated in the 37 °C water bath for 1 h and the cells were checked for leaks by inversion. At equilibrium, 400 μg/cm^2 of VEAc was applied onto the skin surface from three formulations (n = 6): (1) VEAc-saturated solution in silicone oil; (2) VEAc aqueous nanosuspension (~10 mg/ml); and (3) FM075. The donor compartment was covered with a parafilm followed by aluminum foil to prevent water loss and to protect from light during the experiment. After 24 h, 0.2 ml receiver fluid was withdrawn and diluted with 1 ml ethanol for HPLC assay. The donor and receiver compartments were separated and both soaked in 5 ml ethanol for 6 h. The skin disks were isolated and stretched with pins on a corkboard in order to avoid any furrows on the surface. The excess VEAc on the skin surface was cleaned using cotton buds and two tape strips to remove any adhered formulation. Tape stripping was performed by adhering a strip of Sellotape® (Henkel consumer adhesives, Winsford, UK), with a defined surface area slightly larger than the area to be stripped, onto the skin. The Sellotape® was covered with a 300 g weight and rapidly removed with forceps. The first two strips and cotton buds were put in a glass scintillation vial and 5 ml ethanol was added to extract the VEAc. The subsequent 20 successive tape strips were used to remove SC under the aforementioned conditions and all 20 tape strips were placed in a glass vial and soaked with 5 ml ethanol. After removal of the SC the underlying viable epidermis was peeled away from the dermis carefully with a scalpel and the remaining dermis was cut into pieces, placed in a 5 ml ethanol and cold homogenised using a Silverson L4RT laboratory mixer (Silverson Machines Ltd., Waterside, UK). The samples from the surface of the skin, the SC, the viable epidermis and the dermis were soaked in ethanol separately, sonicated for 60 min (Decon F5300b, Sussex, UK) and then shaken overnight at ambient temperature whilst being protected from the light. The samples were filtered through a 0.2 μm syringe filter with which VEAc showed no binding and an aliquot of each sample was centrifuged for 10 min at 11,000g (Eppendorf miniSpin, VWR, Leicestershire, UK). The drug in the supernatant was analysed by HPLC. The amounts of VEAc on the skin surface, in the Franz cell washing, and those present in the different skin layers were calculated to get the VEAc skin disposition and adding the VEAc found in these compartments gave its recovery. All the extraction methods were proven 'fit for purpose' i.e. good and reproducible drug recovery. The mean recovery from surface washing, tape stripping, and whole skin extraction was 98.5%, 99.8% and 97.9%, respectively, with standard deviations of below 4% ($n \geqslant 4$) for each compartment.

2.10. Effect of pluronic L62D on nanoparticle stability

The effect of pluronic L62D surfactant on the stability of the LN was investigated by incubating placebo LN and the LN10 nanoparticle suspension (details in Table 1) with and without surfactant at 25 and 32 °C. The mass ratio of LN to L62D was set at 40:2, 40:7.5, and 40:15 (w/w), respectively, to match the excipient ratios in the foam formulations after ejection from the canister. The particle size and size distribution of LN in these mixtures were examined over 24 h using the light-scattering method previously described.

2.11. Statistical analysis

Statistical analysis of data was performed using SPSS version 16.0 with a minimal level of significance of 0.05. Non-parametric Kruskal–Wallis and Mann–Whitney tests were used to analyse the permeation data. Repeated measures of ANOVA (analysis of variance) were employed to analyse the effect of surfactant level in foams on dose uniformity over 4 weeks. All other data were analysed using ANOVA and post hoc comparisons of the means of individual groups were performed using Tukey's Honestly significant difference test.

3. Results

3.1. Vitamin E acetate encapsulation

With the increase of VEAc input from 5.0 to 20.0 mg/ml, the drug loading in the LN aqueous suspension increased from 5.1 ± 0.2 to 18.9 ± 1.2 (mg/ml) ($p < 0.05$) (Table 1). As the loading of VE increased the encapsulation efficiency reduced ($p > 0.05$), but still remained high with the lowest value of 81.5 ± 4.1%. For all concentrations of VE tested in the loading experiments the drug recovery was *ca.* 100% with no significant difference between each group ($p > 0.05$).

3.2. Nanoparticle characterisation

The size of LN irrespective of the drug loading was between 50 and 60 nm both before and after the purification method (Table 2). Increasing drug input from 5 to 20 mg/ml increased the size of LN from 52.5 ± 0.5 to 56.9 ± 0.3 nm before purification and from 53.6 ± 0.5 to 57.4 ± 0.4 nm after purification ($p < 0.05$). The polydispersity index (PI) was very low for the placebo and for all the VEAc-loaded LN batches (PI < 0.1) indicating a very narrow particle size distribution. The particle surface was not charged according to the zeta potential analysis. For example, the zeta potential of the placebo LN was −0.4 ± 0.3 mV and the values of drug-loaded LN were 0.7 ± 0.4 mV (LN05), −1.8 ± 0.7 mV (LN10) and −2.5 ± 1.8 mV (LN20), respectively.

3.3. Foam uniformity

The foam uniformity was represented as the VEAc recovery at designated time points. The test was conducted over 4 weeks at ambient temperature. The average recovery of VEAc from all three foam formulations (FM002, FM075 and FM150) was in the range of 95–115% (data not shown). There was no significant difference between FM020 and FM150 in terms of VEAc recovery during the 4 weeks ($p > 0.05$). However, the VEAc recovery from FM075 was significantly higher than that from FM020 and FM150 ($p < 0.05$) (data not shown).

3.4. In vitro membrane permeation

The recovery of VEAc from a Miglyol 810N solution was *ca.* 100% over 48 h, hence the drug was considered to be stable in the receiver fluid. VEAc did not permeate through silicone membrane, i.e. it was not detected in the receiver fluid after 48 h of permeation, when the VEAc-loaded LN was applied as an aqueous suspension. The highest VEAc flux across the membrane was obtained from VEAc-saturated silicone oil solution which displayed a permeation profile that started to plateau after 22 h of permeation (Fig. 1). The change in the permeation of VEAc when applied using silicone oil was a consequence of dose depletion, i.e. >10% of the applied VEAc had penetrated the membrane. Dose depletion caused the VEAc thermodynamic activity in the formulation to drop below 1, which leads to a decline in the driving force. The fluxes from three foam formulations were 13.3 ± 1.0 (FM020), 18.0 ± 2.1 (FM075) and 11.7 ± 0.8 μg cm^{-2} h^{-1} (FM150), which were almost 30 times lower than the flux of VEAc when applied from the saturated silicone oil (533.4.0 ± 61.0 μg cm^{-2} h^{-1}). The flux from FM075 was significantly greater than the fluxes from FM020 and FM150 ($p < 0.05$) and no significant difference in flux was found between FM020 and FM150 ($p > 0.05$). The lag time of

Table 2
Particle size, polydispersity index (PI) and zeta potential of vitamin E acetate (VEAc)-loaded lipid nanoparticles (LNs) suspended in water. All data were represented as mean ± standard deviation (n = 3).

Nanoparticles	Before purification		After purification		Zeta potential (mV)
	Particle size (nm)	PI	Particle size (nm)	PI	
Placebo	55.8 ± 0.9	0.08 ± 0.01	51.8 ± 0.3	0.07 ± 0.02	−0.4 ± 0.3
LN05	52.5 ± 0.5	0.07 ± 0.02	53.6 ± 0.5	0.03 ± 0.01	0.7 ± 0.4
LN10	53.9 ± 0.1	0.09 ± 0.01	54.7 ± 1.0	0.08 ± 0.01	−1.8 ± 0.7
LN20	56.9 ± 0.3	0.09 ± 0.01	57.4 ± 0.4	0.03 ± 0.00	−2.5 ± 1.8

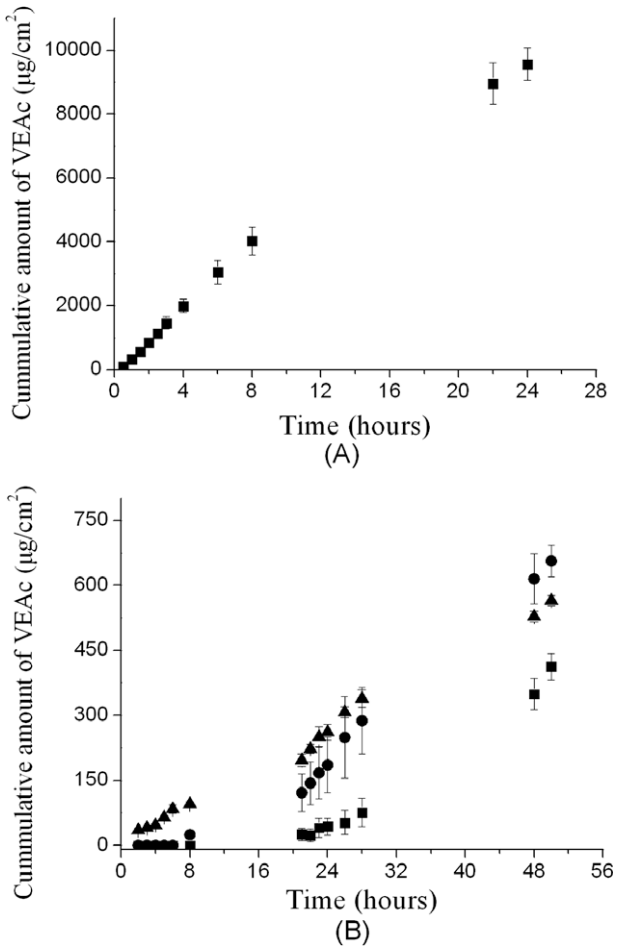

Fig. 1. The permeation profile of vitamin E acetate (VEAc) across silicone membrane from (A): saturated silicone oil and (B) foam formulations: FM020 (2.0% surfactant, *filled square*), FM075 (7.5% surfactant, *filled circle*), and FM150 (15.0% surfactant, *filled triangle*) (w/w) (n = 5).

the foam formulations ranged from 20.6 ± 1.6 h (FM020) to 3.2 ± 1.7 h (FM150) compared to that of the saturated silicone oil solution at 0.3 ± 0.1 h (p < 0.05).

3.5. In vitro tape stripping

VEAc did not pass the SC irrespective of the formulation that was used to apply it (Table 3). Furthermore the aqueous nanosuspension failed to deliver any drug into SC. The silicone oil vehicle delivered 1.7 ± 0.4% of its applied VEAc dose into the SC which was significantly more (p < 0.05) than the foam delivered at 0.7 ± 0.3% (FM075) (w/w). The VEAc recoveries after the 24 h from Franz cell rinsing, skin surface, and different skin layers were 92.7 ± 5.2% (silicone oil), 88.4 ± 5.8% (nanosuspension) and 81.1 ± 9.7% (FM075), respectively (p > 0.05).

Table 3
Distribution of vitamin E acetate (VEAc) after 24 h in human skin. A 400 μg/cm² dose of VEAc was applied using three formulations: VEAc silicone oil (saturated solution), VEAc-loaded lipid nanoparticle (LN) aqueous suspension (*ca.* 10 mg/ml), and a LN containing foam (FM075, compositions in Section 2.6) (n = 6). SC relates to *stratum corneum*.

Item (μg/cm²)	Silicone oil	Nanosuspension	FM075
Applied dose	408.9 ± 13.5	400.5 ± 10.2	404.8 ± 8.2
Donor compartment	124.5 ± 32.7	136.9 ± 15.4	201.6 ± 30.7
Skin surface	248.3 ± 34.3	216.9 ± 14.5	124.9 ± 24.3
SC, tape strippings	6.4 ± 1.3	0	2.3 ± 1.1
Viable epidermis	0	0	0
Dermis	0	0	0
Receptor fluid	0	0	0
Total recovery	379.2 ± 31.0	353.7 ± 19.8	328.8 ± 44.2
SC recovery (%)	1.7 ± 0.4	0	0.7 ± 0.3
Total recovery (%)	92.7 ± 5.2	88.4 ± 5.8	81.1 ± 9.7

3.6. Effect of pluronic L62D on nanoparticle stability

The presence of additional L62D to model foam application induced an increase in the size of LN after 24 h irrespective of the temperature. The more L62D used, the bigger the final size of LN obtained (p < 0.05) (Table 4). At the highest L62D concentration (LN:L62D = 40:15, w/w), the size of the placebo LN increased to 329.6 ± 8.3 nm at 25 °C and 367.4 ± 7.5 nm at 32 °C after 24 h which was more than six times bigger compared to that of the LN control (i.e. LN suspension without surfactant). Similarly the sizes of LN10 after 24 h incubation with the highest surfactant level were 221.6 ± 3.7 nm (25 °C) and 400.6 ± 20.4 nm (32 °C). The size change of both the LN suspensions over 24 h was significantly larger at 32 °C than at 25 °C (p < 0.05) irrespective of the surfactant concentration employed. For example, at the LN, surfactant ratio of 40:7.5 (LN:L62D, w/w), the particle size of placebo LN was 98.0 ± 0.6 nm after incubation at 25 °C, almost twice the control value of 51.9 ± 0.5 nm, whereas at 32 °C the particle size dramatically increased to five times its original value of 264.6 ± 6.9 nm.

4. Discussion

The high drug loading, encapsulation efficiency (EE), and 100% drug recovery demonstrated that the LN produced in the current study was an effective carrier in which to load the lipophilic VEAc. During the encapsulation process, the poor aqueous solubility of VEAc and full miscibility with medium chain triglycerides used to form the LN prevented the drug loss in the water medium and drove the active agent into the particle. The high drug particle affinity resulted in the high loading and encapsulation efficiency. Heurtault et al. employed differential scanning calorimetry (DSC) to investigate the LN excipient interactions and found that the nanoparticle exhibited a core–shell structure [28]. The oily medium chain triglycerides (Labrafac® WL 1349) constituted the particle core that was surrounded by a tensioactive rigid shell made of a mixture of hydrogenated soybean lecithin (Lipoid® S75-3) and macrogol 15 hydroxystearate (Solutol® HS 15). VEAc is highly

Table 4
The effect of pluronic L62D surfactant on the swelling of the lipid nanoparticles (LNs); the particle size and polydispersity index (PI) of placebo LN and vitamin E acetate-containing LN10 nanoparticle (Tables 1 and 2) after 24 h incubation at different surfactant levels and temperatures were presented (w/w, $n = 3$).

Compositions	Particle size (nm) at 24 h		PI at 24 h	
	25 °C	32 °C	25 °C	32 °C
Placebo LN suspension	51.9 ± 0.5	56.4 ± 1.0	0.03 ± 0.03	0.04 ± 0.02
LN:L62D (40:2)	56.0 ± 1.2	79.2 ± 2.6	0.05 ± 0.03	0.03 ± 0.02
LN:L62D (40:7.5)	98.0 ± 0.6	264.6 ± 6.9	0.09 ± 0.03	0.06 ± 0.04
LN:L62D (40:15)	329.6 ± 8.3	367.4 ± 7.5	0.11 ± 0.05	0.18 ± 0.04
LN10 suspension	53.6 ± 0.5	56.1 ± 1.1	0.03 ± 0.02	0.04 ± 0.03
LN10:L62D (40:2)	57.7 ± 0.8	63.1 ± 1.9	0.05 ± 0.02	0.04 ± 0.02
LN10:L62D (40:7.5)	80.4 ± 0.3	131.9 ± 3.1	0.19 ± 0.03	0.10 ± 0.06
LN10:L62D (40:15)	221.6 ± 3.7	400.6 ± 20.4	0.14 ± 0.05	0.20 ± 0.07

lipophilic with a calculated log P_{ow} (*n*-octanol/water) of 12.2. Therefore VEAc would be expected to exhibit a greater affinity to the LN lipid core (Labrafac® WL 1349) compared to the shell.

The VEAc-loaded LN was stable in aqueous suspension and did not aggregate during the centrifuging purification process, which removed excess lipids, surfactants, and small amounts of VEAc therein. The stabilisation mechanism was thought to be a consequence of steric repulsion generated by the surfactants at the LN shell layer surface. The zeta potential analysis of the nanosuspensions showed a net neutral charge and this inferred that there was a lack of electrostatic repulsion between these particles. The work done by Buszello et al. and Jumaa and Müller supported the hypothesis that Solutol® HS 15 stabilised the suspension via steric and not electrostatic repulsion [30,31]. The fact that the particle size of LN did not change after centrifugation purification was thought to be a consequence of the special structure of the LN employed (lipid core surrounded by solid surfactant shell). It is this solid shell that maintains the sufficient rigidity of LN to prevent aggregation and destruction during purification [28].

The excellent dose uniformity and recovery for the foam formulations during the 4-week stability study inferred that the drug was uniformly distributed in the HFA emulsion and it was chemically stable irrespective of the amount of pluronic L62D stabiliser incorporated into the formulations. The uniform drug content was assumed to be a consequence of the nanoparticle and the emulsion physical stability. The pressurised emulsions used in this work were LN-in-water-in-HFA 227 systems which can be considered analogous to an oil-in-water-in-oil multiple emulsion. In a similar manner to the nanoparticle suspension, the pressurised emulsions were thought to be stabilised via a steric mechanism facilitated by the presence of the pluronic L62D, a triblock copolymer surfactant that has been previously reported in HFA solvents [32]. Since the pressurised emulsions are physically stable, the aggregation, flocculation and coalescence of the internal phase was avoided and the same mass of internal phase (LN-in-water) was delivered upon each actuation of the metered valve, which guaranted good drug content uniformity.

Although a number of model membranes are available for skin penetration studies, the most commonly used, silicone, was employed in current work to assess the permeation behaviour of VEAc from the drug-loaded LN aqueous suspension, saturated silicone oil, and three different foam vehicles. The silicone membrane was selected as it is a non-porous matrix which would allow the study of the drug release and permeation from particulate delivery systems without the confounding effects of particle transport.

VEAc did not penetrate the silicone membrane when applied as an aqueous nanosuspension. The lack of permeation was presumably due to inefficient drug release from the LN. To enable silicone membrane permeation using the Franz cell model VEAc must first be released by the nanoparticle into the water phase as the LN cannot penetrate the membrane (binding studies showed that an insignificant amount of VEAc bound to the membrane, data not shown). The failure of VEAc to release from the LN suggested that the carrier remained unchanged when applied to the silicone membrane surface using the water vehicle. In contrast to the aqueous nanosuspension, VEAc did release from the LN and penetrate the silicone membrane when applied using any of the three foam vehicles. Upon dose actuation and subsequent HFA evaporation, the foam collapsed leaving a mixture of the LN aqueous suspension and L62D surfactant residue on the membrane surface. The high L62D concentration, caused by the HFA evaporation, was thought to modify the LN and aid VEAc release. Subsequent *in vitro* modelling experiments confirmed that the LN did change in size as a result of an increase in pluronic L62D surfactant concentration (from 2% to 15%) (Table 4). Both placebo and drug-loaded LN increased in size over 24 h at 25 and 32 °C. The cause of the NP modification was postulated to be the interaction between the L62D surfactant and lipoid® S75-3 (mainly composed of phospholipid). A number of previous reports have proposed that the interaction between pluronic surfactants and phospholipids is driven by a change in the hydration state of the lipid head group and the alteration of lipid packing caused by pluronic surfactants [33,34].

The highest flux through the silicone membrane was obtained when VEAc was applied from saturated silicone oil solution. This was expected as the oil, which is not a suitable vehicle for topical VEAc delivery, made the VEAc instantly accessible to the membrane for permeation. According to Higuchi's equation [35] all vehicles containing drug at the same thermodynamic activity should result in the same penetration rate, provided that the systems behaved ideally. VEAc when presented as a saturated solution was assumed to have a thermodynamic activity of 1 during the steady state of permeation. When formulated as a foam the VEAc could also theoretically exhibit a thermodynamic activity of 1 if the VEAc was released from the nanoparticles rapidly enough to maintain saturation of the external water phase. However, as the VEAc, when delivered from the foam exhibited a much slower permeation rate through silicone membrane than the saturated silicone oil, it is presumed that the thermodynamic activity of 1 was not achieved in the water and that drug release from LN was the rate-limiting barrier. The amount of surfactant employed in the foam vehicle determined the degree of LN modification and thus the drug release rate from LN. However in addition to the effects of surfactant on the LN it probably also solubilised the VEAc in the water vehicle. The solubilisation capability of both the pluronic copolymer and Solutol® HS 15 (a component of the LN) has been shown previously [36,37]. Optimal release from these nanosuspension containing foams is dependant upon attaining a sufficient concentration of surfactant to break down the LN, but limit solubilisation of the VEAc. As the aim of the study was specifically to investigate if 'breaking open' nanoparticles by dynamic foams could enhance drug release, then an aqueous nanosuspension was selected as the control system wherein the nanoparticles remained intact. The use of a nanoparticle-containing semi-solid vehicle as control was considered inappropriate since the LN may

collapse in these vehicles and lose the integrity before dose application but this cannot be assessed using simple analytical methods.

In vitro human skin tape stripping has been shown to be a versatile tool to study drug distribution in human skin both *in vitro* and *in vivo* [38]. For a lipophilic molecule, such as VEAc, with a log *P* of more than 3, the intercellular route has been proposed as the dominant pathway to traverse the SC [39]. Therefore when applying a topical drug-loaded nanoparticle formulation drug release from the particles is a pre-requisite to successful penetration into human skin. In the current study, the lack of VEAc release from intact nanoparticles, when applied as an aqueous suspension (shown by the silicone membrane studies) prevented drug penetration into skin. However, when the foam was employed as the application vehicle, the increase in pluronic surfactant concentration after application modified the LN to enable VEAc release and penetration into skin. Although the drug-saturated silicone oil delivered more VEAc into SC compared to foam, this vehicle was used to represent a system without a barrier to drug release and such an oily vehicle would rarely be used in the clinical environment. For both foam and saturated oil formulations, VEAc only penetrated into the SC and did not partition from SC into viable epidermis (Table 3). This can be explained by the extremely lipophilic nature of VEAc. Liron and Cohen suggested that the solubility parameter of the SC was *ca.* 10 $(\text{cal cm}^{-3})^{1/2}$ and this is very close to the value of VEAc (9.1 $(\text{cal cm}^{-3})^{1/2}$), thus VEAc was assumed to show an appreciable solubility in SC and hence the propensity to remain in the barrier was high [40,41].

Vitamin E exists naturally in human skin predominantly as *dl*-alpha tocopherol which is only found in natural sources e.g. vegetables, cereals and nuts. The levels of *dl*-alpha tocopherol in human skin have been reported to be 14.2 ± 1.7 (SC), 13.3 ± 1.6 (epidermis), and 7.0 ± 0.5 (dermis) (μg/g tissue) [42]. In the current study, a highly selective HPLC method capable of resolving the natural and acetate forms of *d*-alpha tocopherol was used to quantifying the active agents (data not shown). Analysis of the tapes from the stripping process did not show any evidence of *dl*-alpha tocopherol presence, whilst *d*-alpha tocopheryl acetate was detected only in the SC.

As lipophilic topical active agents are commonly formulated in oily vehicles, which are normally greasy and as such aesthetically and cosmetically unpleasant, the successful delivery of VEAc using an aqueous foam is a significant development for increased patient compliance. In addition, the skin irritation problems relating to the less desirable alcohols could be avoided using aqueous foam formulations [43]. Despite the skin tolerability of pluronic L62D not being investigated in current study, it is usually accepted that pluronic surfactants are non-toxic and non-irritant since many of them such as L62, F68 and F127 have previously been formulated in various topical, oral and parenteral products at a level up to 15.5% [44]. The incorporation of nanoparticles in foams also exhibits the potential of improving the chemical stability of the active agents thus foams could prove to be a useful addition to the spectrum of available topical formulations. However, the performance of dynamic foams was lower compared to that of simple oily vehicles. More work is required to improve the speed of release from this dynamic system and this could be achieved by employing other types of nanoparticles and/or surfactants. In addition, further investigations are needed to determine the capability of dynamic foams to enhance the delivery of less lipophilic molecules.

5. Conclusions

The model drug VEAc was efficiently encapsulated in the LN prepared using phase inversion method with a *ca.* 100% drug recovery. VEAc was chemically stable and uniformly distributed within the vehicle owing to the steric stabilisation mechanism of the foaming emulsion over a 4-week period. The application of the nanoparticles in water led to poor drug release from nanoparticles and consequently VEAc did not permeate across silicone membrane and penetrate into human skin using this formulation. In contrast, when the foam was employed to administer the particles, the evaporation of propellant resulted in the concentration of the pluronic L62D surfactant in the foam residue, modification of LN and enhanced release of VEAc into the water medium. The foam allowed the VEAc to permeate across the silicone membrane and penetrate into SC. The dynamic foam delivery system developed herein could provide a promising strategy for the topical delivery of hydrophobic active therapeutic agents to skin in an aqueous vehicle.

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