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The effects of particle properties on nanoparticle drug retention and release in dynamic minoxidil foams

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ABSTRACT

Nanocarriers may act as useful tools to deliver therapeutic agents to the skin. However, balancing the drug–particle interactions; to ensure adequate drug loading, with the drug–vehicle interactions; to allow efficient drug release, presents a significant challenge using traditional semi-solid vehicles. The aim of this study was to determine how the physicochemical properties of nanoparticles influenced minoxidil release pre and post dose application when formulated as a simple aqueous suspension compared to dynamic hydrofluoroalkane (HFA) foams. Minoxidil loaded lipid nanoparticles (LN, 1.4 mg/ml, 50 nm) and polymeric nanoparticles with a lipid core (PN, 0.6 mg/ml, 260 nm) were produced and suspended in water to produce the aqueous suspensions. These aqueous suspensions were emulsified with HFA using pluronic surfactant to generate the foams. Approximately 60% of the minoxidil loaded into the PN and 80% of the minoxidil loaded into the LN was released into the external aqueous phase 24 h after production. Drug permeation was superior from the PN, i.e. it was the particle that retained the most drugs, irrespective of the formulation method. Premature drug release, i.e. during storage, resulted in the performance of the topical formulation being dictated by the thermodynamic activity of the solubilised drug not the particle properties.

1. Introduction

Loading dermatologically active therapeutic agents in nanoparticles may offer a number of benefits compared to directly incorporating these agents into traditional multiphase semi-solid vehicles including: enhancement of product aesthetics (Souto and Muller, 2008); protection of chemically unstable agents against degradation (Dingler et al., 1999; Jenning and Gohla, 2001; Wissing et al., 2004); sustained/controlled release (Jenning et al., 2000; Puglia et al., 2008) and follicular targeting (Lademann et al., 2006, 2007). In addition, encapsulating water insoluble therapeutic agents in nanoparticles allows loading of these agents in aqueous topical vehicles without the use of irritant organic solvents or surfactants. However, despite their advantages, the use of nanocarriers to administer active agents to the skin in a clinical setting remains disappointingly low (Pardeike et al., 2009).

The purpose of applying most therapeutic agents to human skin is to elicit a local response. In the majority of cases, the active ingredient must permeate directly across the *stratum corneum* (SC), the outermost layer of skin, to access disease targets in the underlying tissue (Barry, 2002). The SC presents a formidable barrier to most low molecular weight (MW) compounds and prevents intact nanoparticle ingress (Alvarez-Roman et al., 2004; Luengo et al., 2006; Stracke et al., 2006; Cross et al., 2007). Therefore, a drug that is loaded into a topically applied nanoparticle must be released by the particle whilst on the surface of the skin if it is to penetrate the SC. This requirement to achieve adequate drug release upon application to the skin is counterbalanced by the need of the particles to retain the drug prior to application. When nanoparticles are formulated and stored in the solid-state prior to application, premature drug release is not usually a significant problem, but designing an effective topical nanoparticle suspension that balances the requirement for suitable drug–particle interactions, to ensure good drug loading, with adequate drug–vehicle interactions, to allow efficient release, is extremely difficult (Mehnert and Mader, 2001; Muller et al., 2002). This paradox is one of the major reasons why nanocarriers are failing to realise their full potential in facilitating drug delivery into the skin.

The balance of the drug release by nanoparticles during storage and upon application to the skin is obviously dependant on the physicochemical properties of the drug and the particle in which it is carried and has a significant effect on product performance. However, as many previous studies have neglected to characterise drug release in the formulation this relationship is hard

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to discern when reviewing the literature (Schafer-Korting et al., 2007). For example, Cohen-Sela et al. (2009) reported that when alendronate, a hydrophilic agent, was loaded into poly(lactic-co-glycolic acid) (PLGA) nanoparticles and suspended in water it was almost completely liberated from the particle carrier within 6 h. The rapid *in vitro* release could be a consequence of the 'free' drug released from the nanoparticles during storage, but this was not assessed. A similar explanation could be applied to the data produced by Shim et al. (2004) who studied the *in vitro* permeation of a hydrophilic agent, minoxdil from poly(ε-caprolactone)-block-poly(ethyleneglycol) nanoparticles, but similarly failed to assess drug release of the particles upon storage. If the drug is not retained in the particle during formulation storage this negates the advantages of using the nanoparticle carrier and it can lead to variable drug release profiles over time, hence there is a requirement to investigate drug release from nanoparticle carriers in more detail.

Dynamic foams represent a new paradigm in topical drug delivery. These systems have the potential to solve the nanoparticle paradox as they trigger the release of agents from nanocarriers only after application to the skin (Tamarkin et al., 2005; Zhao et al., 2009). Proof of concept, i.e. dynamic triggering of drug release, has been demonstrated for these elegant systems, but the versatility and robustness of such an approach is unknown. In a similar manner to traditional nanoparticle containing formulations the influence of the carrier properties on the drug release profiles of therapeutic agents is unknown. To allow judicious use of these novel formulations a fundamental understanding of drug–particle interactions during formulation production, storage and delivery process must be attained.

The advantages of delivering hydrophobic therapeutic agents to the skin using nanocarriers formulated in appropriate vehicles such as foams has been established (Zhao et al., 2009), but the utility of this approach to modulate the delivery of hydrophilic agents is still unclear due to the lack of a systematic investigation in this area. As such the aim of this work was to investigate how the properties of a nanocarrier that was employed to aid the delivery of topical hydrophilic agents influenced the delivery performance of the formulation and ultimately assess if such a nanoparticulate approach can be used to administer topical hydrophilic compounds. Minoxidil was selected as a model hydrophilic therapeutic agent as it has previously been shown to be a problematic to deliver using an aqueous nanocarrier suspension. The hydrophilic drug was loaded into two nanoparticles with different physicochemical properties: lipid nanoparticles (LN); lipid HLB = 2 and polymeric nanoparticles (PN) with a lipid core; lipid HLB = 6. These carriers were formulated as a simple aqueous suspension and a dynamic aqueous foam. The drug release from the two nanocarriers in the two formulations was monitored during storage and the functional effects of the particle and formulation upon drug delivery across a silicone membrane was determined.

2. Materials and methods

2.1. Materials

Citric acid, docusate sodium, hydrochloric acid, sodium chloride, sodium phosphate dibasic and poly(D,L-lactic acid) (PLA) (MW 75,000–120,000) were purchased from Sigma–Aldrich Ltd. (Gillingham, UK). Poloxamer 188 and Solutol® HS 15 (macrogol 15 hydroxystearate) were kind gifts from BASF (Ludwigshafen, Germany). Pluronic L62D and heptafluoropropane (HFA 227) were obtained from BASF (New Jersey, USA) and DuPont de Nemours Int'l SA (Geneva, Switzerland), respectively. The lipophilic Labrafac® WL 1349 (medium chain triglycerides) and Capryol® 90 (propylene glycol monocaprylate) were provided by Gattefossé S.A. (Saint-Priest, France) and Lipoïd® S75-3 (soybean lecithin at 69% of phosphatidylcholine) was furnished by Lipoïd GmbH. (Ludwigshafen, Germany). High performance liquid chromatography (HPLC) grade water, methanol, glacial acetic acid and analytical grade acetone were purchased from Fisher Scientific (Leicestershire, UK). Minoxidil (Ph Eur) and Rogaine® solution (2%) was sourced from Spodefell (Kingston, UK) and AAH Hospital Services (Coventry, UK), respectively. Silicone (polydimethylsiloxane) membrane (50 μm thickness) was obtained form BioPlexus (Ventura, USA).

2.2. Methods

2.2.1. Minoxidil analysis

Quantitative determination of minoxidil was performed using either a stand-alone UV (ultraviolet) spectrophotometer or a high performance liquid chromatography system coupled to a flow-through UV detector. The liquid chromatography system consisted of a HP 1090 pump and autosampler (Agilent Technologies UK Ltd., Wokingham, UK). The flow-through UV detector was a HP 1050 (Agilent Technologies UK Ltd., Wokingham, UK). Separation was achieved using a Gemini C18 column (5 μm, 250 mm $\times$ 4.6 mm) (Phenomenex, Macclesfield Cheshire, UK), maintained at 50 °C. The injection volume was 10 μl and the detection wavelength was 281 nm. The mobile phase for minoxidil analysis was a mixture of methanol and water (75:25, v/v) containing 1% (v/v) glacial acetic acid and 0.3% (w/v) docusate sodium (final pH adjusted to 2.5 using hydrochloric acid). The mobile phase flow rate through the column was set at 1.0 ml min^{-1}. The HPLC system was used to analyse all the minoxidil samples from this study except for those that contained PLA when the stand-alone UV spectrometer (Cary 100, Varian Ltd., Oxford, UK) at a UV absorbance wavelength of 281 nm was employed. The minoxidil sample matrix demonstrated no detectable absorption at 281 nm (data not shown).

2.2.2. Aqueous solubility determination

A series of minoxidil saturated aqueous solutions at different pHs (pH 3.0–7.0, citric acid-phosphate buffer, 0.5 M) were prepared by adding an excess of drug to 15 ml scintillation glass vials containing 10 ml buffer. The suspensions were agitated for 24 h and hydrochloric acid was used to adjust any pH drift, a consequence of drug solubilisation, when appropriate. The samples were filtered using 0.2 μm cellulose acetate syringe filter (Fisher Scientific, Leicestershire, UK), diluted using mobile phase and analysed by HPLC. All samples were stored under light protected conditions at ambient temperature (23 $\pm$ 2 °C) during the solubility study (n = 3). The percentage drug ionisation at a particular pH was calculated using Eq. (1) and a drug pK_a of 4.6 (Moffat et al., 2004):

$$\text{Ionisation\%} = \frac{100}{1 + 10^{\text{charge}(\text{pH} - \text{p}K_a)}} \quad (1)$$

Where Ionisation% was the percentage of minoxidil ionisation in an aqueous solutions at different pHs, charge was +1 as minoxidil is a base and the pK_a was the dissociation constant. The pH of the aqueous solutions ranged from 3.0 to 7.0 in the solubility study.

2.2.3. Nanoparticle preparation

Minoxidil loaded LN were prepared using a phase inversion method developed by Heurtault et al. (2002). Minoxidil (0.3–1.0%, w/w), Labrafac® WL 1349 (17.0%, w/w) and Lipoid® S75-3 (1.75%, w/w) were weighed directly into a 100 ml Duran® glass bottle and mixed at ambient temperature for 30 min using a magnetic stirrer bar. Solutol® HS 15 (17%, w/w) and a 3% (w/v) aqueous solution of sodium chloride (to 100%, w/w) were mixed in a 100 ml beaker at 50 °C until the Solutol® HS 15 had dissolved. The solution was transferred to a Duran® bottle with continuous stirring to form a homogenous emulsion. This emulsion was

Table 1
The effect of minoxidil input concentration on its recovery, loading and encapsulation efficiency (EE) when manufacturing lipid nanoparticles (LN) and polymeric nanoparticles (PN). All data are expressed as mean ± standard deviation ($n = 3$).

Nanoparticles	Drug input (mg/ml)	Drug loading (mg/ml)	Drug recovery (%)	EE (%)
LN030	3.0	1.4 ± 0.1	96.2 ± 2.0	42.2 ± 2.0
LN060	6.0	1.4 ± 0.1	98.1 ± 2.8	20.6 ± 1.0
LN100	10.0	1.5 ± 0.1	98.6 ± 1.6	12.7 ± 0.5
PN010	1.0	0.3 ± 0.0	99.0 ± 4.0	26.4 ± 3.7
PN020	2.0	0.4 ± 0.0	96.1 ± 3.5	20.6 ± 0.8
PN030	3.0	0.6 ± 0.0	98.6 ± 1.4	20.4 ± 1.0

heated and cooled between 60 and 85 °C at a rate of 4 °C/min for three cycles to induce phase inversion. The mixture was reheated to 85 °C and diluted with cold water (0 °C) to generate the lipid nanoparticles. Slow magnetic stirring was applied to the aqueous nanosuspension post-manufacture for 5 min to facilitate homogenisation. The suspensions were centrifuged (110,000 × *g*, 20 °C for 1 h) using a Beckman L8-80 ultracentrifuge (Beckman Coulter, Buckinghamshire, UK) to allow isolation of the purified nanoparticles from any excess additives (the nanoparticles isolation procedure previously described in detail in Zhao et al., 2009).

PN with a Capryol® 90 lipid core were prepared by solvent displacement (Fessi et al., 1989). A 5 ml sample of drug solution was prepared containing 60 mg PLA, 240 mg Capryol® 90 and 10–36 mg minoxidil in a mixture of acetone and methanol (3:2, v/v). The organic solution was injected into an aqueous solution containing 0.5% (w/v) poloxamer 188 at a speed of 0.5 ml/min using a 5 ml plastic syringe housed in an infusion pump (Precidor, Infors, Basel, Switzerland). The mixture was homogenised at 5000 rpm for 10 min using a Silverson L4RT laboratory mixer (Silverson Machines Ltd., Waterside, UK) and left overnight in the fume hood to allow complete solvent removal.

Excess drug was separated from the LN and PN nanosuspensions using a Millipore centricon® YM-100 centrifugal concentrator with a molecular weight cut-off of 100 kDa (Fisher Scientific, Leicestershire, UK) by centrifugation at 2700 × *g* for 90 min (MSC centaur 2 centrifuge, DJB Labcare Ltd., Buckinghamshire, UK). In this system drug–loaded nanoparticles were retained on the filter and the excess 'free' drug in the solution was removed that passed through the filter. After purification the nanoparticles were collected by inverting the sample reservoir and centrifuging at 1000 × *g* for 5 min and re-suspended in water (LN) or poloxamer 188 (0.5%, w/v) aqueous solution (PN). The pH of the purified nanosuspension was controlled at 6.0 ± 0.0 ($n = 3$) for both LN and PN. A full mass balance recovery of minoxidil was performed on samples from both nanoparticle manufacturing methods. All samples during the preparation and purification of minoxidil loaded LN were diluted with HPLC grade methanol and analysed by HPLC. Minoxidil loaded PN were diluted with HPLC grade acetonitrile and assayed using a stand-alone UV spectrometer, but the remainder (filtrate) of the minoxidil samples from the minoxidil loading process was analysed by HPLC. The total drug recovery, drug concentration and encapsulation efficiency were calculated (Eqs. (2)–(4)):

$$\text{Drug concentration} = \frac{W_1}{V} \quad (2)$$

$$\text{Encapsulation efficiency} = \frac{W_1}{W_0} \times 100\% \quad (3)$$

$$\text{Drug recovery} = \frac{W_1 + W_2 + W_3}{W_0} \times 100\% \quad (4)$$

Where V was the final volume of purified nanosuspension; W_1 and W_2 represent the mass of the minoxidil within the purified nanoparticles and aqueous filtrate; W_0 was the mass of initial minoxidil input; W_3 was for LN only and represented the drug mass in the remaining solution after LN isolation via ultracentrifugation.

2.2.4. Nanoparticle characterisation

The measurements of nanoparticle size and particle size distribution indicated by polydispersity index (PI), were performed using a dynamic light scattering (DLS) instrument equipped with a 30 mW laser at 676 nm (Brookhaven ZetaPlus, Brookhaven Instruments Ltd., Worcestershire, UK). A polydispersity index above 0.2 implied a broad distribution of nanoparticles. Analyses were carried out at a scattering angle of 90° at 25 °C in triplicates. All the samples were diluted with HPLC grade water prior to analysis. The Zeta potential of nanoparticles was also determined using a Brookhaven ZetaPlus with palladium electrodes (Brookhaven instruments Ltd., Worcestershire, UK) at fixed light scattering angle of 90°. The purified nanoparticle sample was diluted in 1 mM sodium chloride solution prior to analysis. The measurement was performed three times on three samples from the same stock solution and data were presented as mean ± standard deviation of the three measurements.

2.2.5. Foam preparation

Nanoparticle-loaded dynamic foams were prepared following the method previously published by Zhao et al. (2009). The LN (minoxidil 1.4 mg/ml) and PN (minoxidil 0.6 mg/ml) were transferred to 10 ml plastic coated glass canisters (Schott UK Ltd., Stafford, UK) directly after purification, the pH of the suspensions checked and adjusted if required and an appropriate amount of pluronic L62D surfactant was added. The canisters were sealed with 100 μl metered spray valves (Valois UK Ltd., Bletchley, UK) and heptafluoropropane propellant was filled into the canister using a pressurised filler (Pamasol Willi Mäder AG, CH-8808 Pfäffikon SZ, Switzerland). The mixture in the canister was left to stir overnight at 1000 rpm using a Variomag® Telesystem HP15 stirrer plate (Florida Scientific Services, Inc., Daytona Beach, USA) to ensure homogenisation. Six foams were prepared: PNFM05, PNFM20, PNFM75, LNFM05, LNFM20 and LNFM75. The first two letters of foam nomenclature represent the type of aqueous nanoparticle suspension included in the foam (at 40%); FM represents foam and the last two digits relate to the surfactant level, i.e. 0.5% for '05', 2.0% for '20', and 7.5% for '75'. The remaining component of the foam was the propellant which constituted the rest of the formulation. For example, the PNFM05 foam comprised of PN 40% (w/w) aqueous suspension, 0.5% (w/w) pluronic L62D surfactant and 59.5% (w/w) propellant. The apparent pH of these foams was examined prior to use by releasing 2 sprays on a pH indicator paper (KAAGAT Ltd., Tipton, UK) and comparing the colour with the manufacture standard.

2.2.6. Minoxidil in situ release study

A 10 ml aliquot of freshly prepared (the release test was conducted immediately upon manufacture) aqueous nanosuspension (LN (minoxidil, 1.4 mg/ml) or PN (minoxidil, 0.6 mg/ml)) (Table 1) was employed to mimic the *in situ* minoxidil release in canister

before dose application using the centricon® YM-100 centrifugal concentrators (Section 2.2.3). At predetermined time points (1, 2, 4, 6, 8, 20, 22 and 24 h) a 0.2 ml sample was removed from the suspensions and the nanoparticles were separated from the aqueous suspending vehicle. The drug content in the aqueous vehicle was analysed by HPLC. The minoxidil remaining in the PN and LN particles was assayed by stand-alone UV spectroscopy and HPLC, respectively, to achieve a mass balance. The cumulative amount of minoxidil released into the suspending medium was plotted against time (h). Before and after the release study an Axioskop 50 microscope (Carl Zeiss Ltd., Herts, UK) was used to check the presence of drug crystals in the suspensions. The particle size of the nanoparticles was also assessed after 24 h to monitor the integrity of particles during the release study.

2.2.7. Silicone membrane permeation

Static vertical Franz diffusion cells (MedPharm Ltd., Guildford, UK) were employed to assess the permeation of minoxidil through silicone membrane. The cells, which had an average diffusional surface area of *ca.* 2 cm^2 and a receiver volume of 10 ml, were calibrated individually prior to use. A small magnetic stir bar was placed in each cell, they were mounted with silicone membrane (50 μm thick), they were sealed and the receiver compartment of the cells filled with citric buffer (pH 3.6, 0.5 M). The drug showed adequate solubility in the fluid to maintain sink conditions through the experiments (data not shown). The permeation study was performed at 32 °C in a SS40-5 water bath (Philip Harris, Staffs, UK) on a motorless electronic magnetic stirrer plate (Variomag® Telesystem HP15) (Florida Scientific Services, Inc., Daytona Beach, USA). After cell equilibration at 32 °C for 1 h, the cells were inverted and checked visually for leaks. The test formulations were applied to the surface of the silicone membrane and the donor compartment was covered with parafilm to minimise donor phase evaporation. The test formulations were as follows: (1) 1.0 ml commercial Rogaine® solution (2%); (2) 1 ml saturated minoxidil aqueous solution at pH 6.0 and (3) 2.5 g of the foam formulation (PNFM05, PNFM20, PNFM75, LNFM05, LNFM20 and LNFM75). A 2.5 g aliquot of foam was used as this would dose sufficient formulation to produce 1 ml of nanosuspension. At predetermined time intervals (24, 48, 72, 96 and 120 h) 0.5 ml of receiver fluid was withdrawn from the Franz cell receiver compartment and analysed by HPLC. The receiver fluid was replenished with an identical volume of fresh receiver fluid, held at 32 °C. All the permeation experiments were carried out at least five times and the cumulative amount of drug in the receiver compartment was calculated at each time point.

2.2.8. Statistical analysis

Statistical analysis of data was carried out using SPSS version 16.0 and a statistically significant difference was determined at a minimal level of significance of 0.05. All data were checked in terms of normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene's test) prior to analysis. Repeated measures of ANOVA (analysis of variance) were employed to analyse minoxidil release from nanoparticles; all other data was analysed using a one-way ANOVA or Student's *t*-test. Post hoc comparisons of the means of individual groups were performed when appropriate using Tukey's HSD (Honestly significant difference) test.

3. Results

3.1. Minoxidil loading

Increasing the minoxidil input during LN formation from 3.0 to 10.0 mg/ml had no statistically significant effect ($p > 0.05$) on drug loading (remained at *ca.* 1.4 mg/ml, Table 1), but it did significantly ($p < 0.05$) reduce encapsulation efficiency (EE) from 42.2 ± 2.0% to 12.7 ± 0.5%. Increasing initial minoxidil input from 1.0 to 3.0 (mg/ml) during PN manufacturing increased drug loading significantly ($p < 0.05$) from 0.3 to 0.6 (mg/ml), but this also produced a significant reduction ($p < 0.05$) in EE from *ca.* 26% to 20%. The LN was shown to exhibit a higher loading capacity compared to the PN at the same drug input concentration (Table 1). No drug losses were observed in any of the loading experiments; minoxidil recovery after encapsulation from both process was not significantly ($p > 0.05$) different from 100%.

Table 2
Particle size, polydispersity index (PI) and zeta potential of minoxidil loaded lipid nanoparticles (LN) and polymeric nanoparticles (PN). The details of nanoparticle composition can be found in Table 1; all data are represented as mean ± standard deviation ($n = 3$).

Nanoparticles	Particle size (nm)	PI	Zeta potential (mV)
LN Placebo	51.9 ± 0.5	0.07 ± 0.02	−0.4 ± 0.3
LN030	49.8 ± 0.6	0.07 ± 0.06	−3.2 ± 1.6
LN060	51.1 ± 0.5	0.03 ± 0.03	−2.5 ± 2.5
LN100	54.3 ± 0.7	0.06 ± 0.02	0.0 ± 0.0
PN placebo	261.6 ± 3.5	0.06 ± 0.01	−18.8 ± 2.7
PN010	256.2 ± 4.7	0.18 ± 0.01	−27.9 ± 0.6
PN020	267.3 ± 6.4	0.13 ± 0.03	−20.4 ± 1.6
PN030	258.8 ± 3.8	0.12 ± 0.03	−23.3 ± 0.4

3.2. Nanoparticle characterisation

The particle size of all the LN was very similar (between 49 and 55 nm, Table 2) regardless of the drug loading level. Only the LN100 particles were significantly ($p < 0.05$) larger than the other LN. The PN were much larger (~260 nm) compared to LN, but again no significant size change ($p > 0.05$) was observed when the drug loading was varied. The polydispersity index (PI) was very low for all the particles produced (PI < 0.2) which indicated a very narrow particle size distribution. The LN was neutral, whilst the PN was found to be negatively charged. No link between increasing drug input and particle surface charge was observed for either the LN or the PN suggesting that the drug was not adsorbing to the particle surface.

3.3. In situ minoxidil release

The aqueous solubility of minoxidil decreased dramatically as the pH of the aqueous vehicle in which it was dissolved increased due to the suppression of drug ionisation (Fig. 1). For example, the aqueous drug solubility was 82.6 ± 1.7 mg/ml at pH 3.0 where the drug ionisation was 97.5%, but this changed to 1.9 ± 0.0 at pH 7.0 where the drug ionisation was 0.4%. Drug ionisation and solubility followed identical trends throughout the series of solubility experiments. The pH of all the freshly prepared nanoparticle suspensions was set to 6.0 to minimise drug release and maximise drug loading, however despite this both LN030 and PN030 (selected for comparison due to their good drug loading) produced a biphasic drug release profile immediately after production (Fig. 2). The most rapid rate of drug release for both LN (0.22 ± 0.01 mg ml^{-1} $h^{-1/2}$) and PN (0.11 ± 0.01 mg ml^{-1} $h^{-1/2}$) was over the first 8 h after production, but both nanoparticles continued to release drug up until 24 h, though at a slower rate of *ca.* 0.08 mg ml^{-1} $h^{-1/2}$ (LN) and 0.02 mg ml^{-1} $h^{-1/2}$ (PN) (Fig. 2). After 24 h, no drug crystals were observed in the solutions (analysed microscopically); there was no significant ($p > 0.05$) size change for both types of particles when compared to their original size prior to the release experiment (LN030: 49.4 ± 0.8 nm and PN030: 257.5 ± 5.1) and ≥95% of minoxidil was recovered.

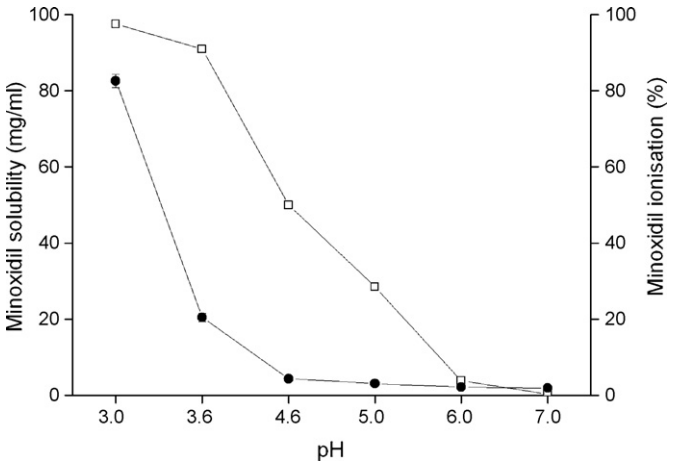

Fig. 1. The aqueous solubility at ambient temperature (23 ± 2 °C) (*filled circle*) and ionisation percentage (*empty square*) of minoxidil at different pHs from 3.0 to 7.0. Points represent mean ± standard deviation, but the standard deviation was too small to be seen ($n = 3$).

3.4. Minoxidil membrane permeation

The same two sets of nanoparticles, PN030 and LN030, again after correcting the suspension medium pH to 6, were incorporated into two dynamic foam formulations and their ability to deliver minoxidil across a silicone membrane compared against the commercial Rogaine® (2%) solution (pH 8.0) and a minoxidil saturated aqueous solution (pH 6.0) (Fig. 3). The data was not presented as traditional membrane permeation profile as the limited capability of minoxidil to cross the silicone membrane restricted the number of drug samples that could be taken from the Franz cell receiver fluid. Despite this the amount of drug that cumulated in the receiver fluid did gradually increase over time and allowed accurate analysis (i.e. the samples were above the limit of detection for the drug assay). At each time point, Rogaine® delivered significantly ($p < 0.05$) more drugs across the membrane than the saturated aqueous solution (pH 6.0), although the total quantity of drug in the receiver fluid was minimal in both cases, i.e. less than 1% of the applied dose. After 120 h Rogaine® delivered twice as much minoxidil ($10.1 \pm 2.2\ \mu g\ cm^{-2}$) compared to that of the drug saturated aqueous solution at pH 6.0 ($4.6 \pm 0.8\ \mu g\ cm^{-2}$).

Both aqueous nanosuspensions and foams tested in this work delivered significantly less ($p < 0.05$) minoxidil compared to the Rogaine® formulation and drug saturated aqueous solution after 120 h of permeation (Fig. 4). In addition, the aqueous nanosus-

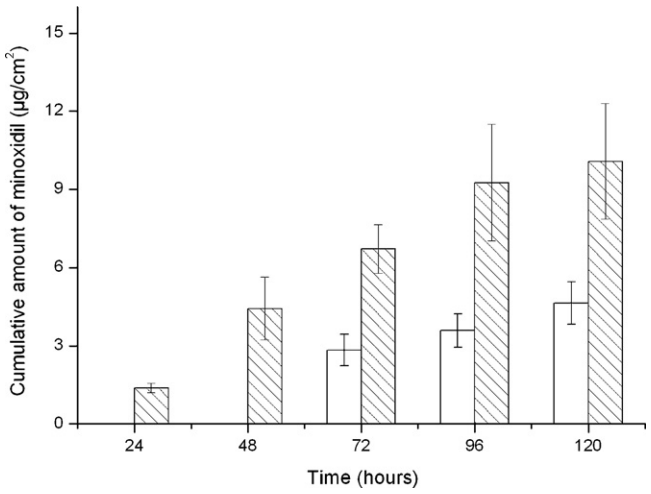

Fig. 3. The permeation behaviour of minoxidil across a silicone membrane at 32 °C when applied using a commercial Rogaine® formulation (2%, w/v) (*diagonal lines*) and a drug saturated aqueous solution at pH 6.0 (*white*), data points represent the mean cumulative minoxidil amount over 120 h ± standard deviation ($n = 5$).

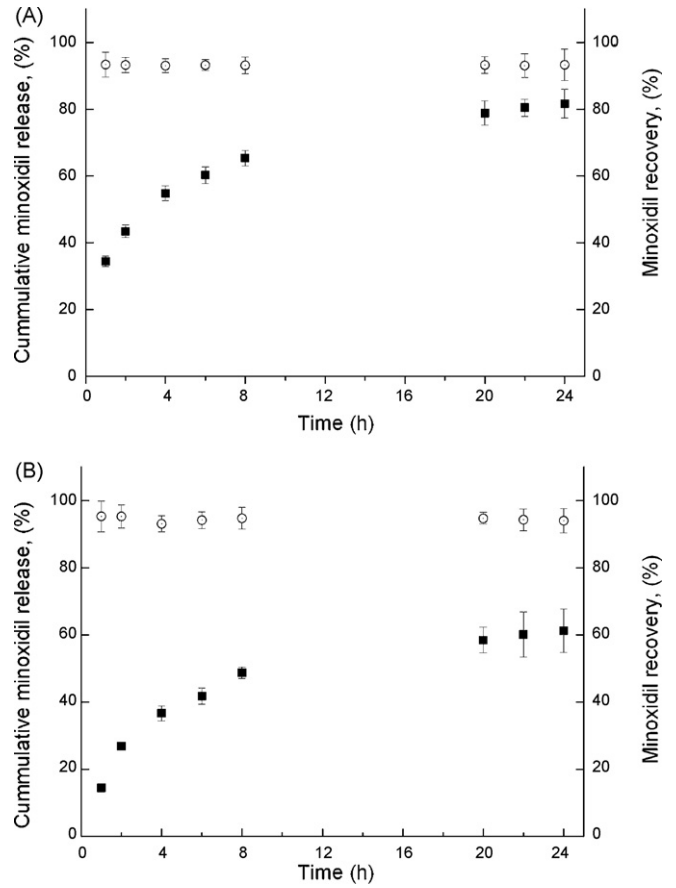

Fig. 2. Minoxidil *in situ* release profile from both lipid nanoparticles (A) and polymer nanoparticles (B) that were suspended in water (pH 6.0). The data presented illustrates the cumulative minoxidil released in the suspending medium over 24 h at 32 °C (*filled square*) and minoxidil recovery from both nanoparticles and suspending medium (*empty circle*). Data points represent mean ± standard deviation ($n = 3$).

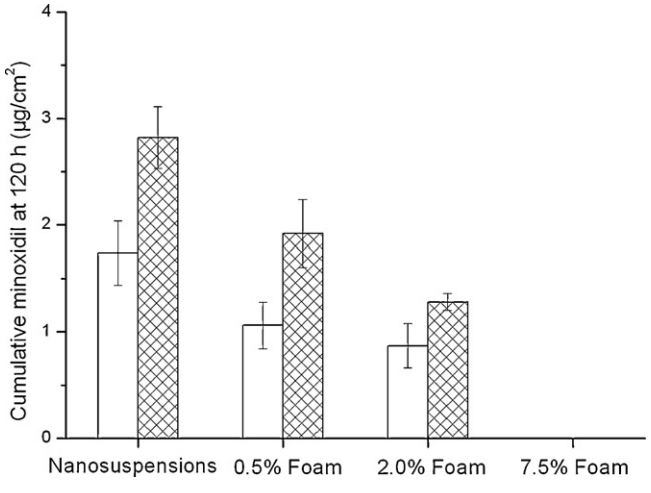

Fig. 4. The cumulative amount of minoxidil delivered across silicone membrane at 32 °C from lipid nanoparticles (LN030, Table 1) (*white*) and polymeric nanoparticles (PN030, Table 1) (*hatched*) formulations presented either as a simple aqueous suspension (nanosuspension) or foams containing different levels of pluronic L62D surfactant. All data represent the mean cumulative minoxidil amount at 120 h ± standard deviation ($n = 5$).

pensions, i.e. the nanocarriers prior to formulation within the foams, delivered significantly more ($p < 0.05$) minoxidil across the silicone membrane during the 120 h study compared to the identical suspensions when formulated as foams. For both types of nanoparticles suspended in the dynamic foams, the cumulative minoxidil amount that permeated across the silicone membrane in 120 h significantly decreased ($p < 0.05$) with increasing pluronic L62D surfactant concentration. In fact when the L62D surfactant level in the foams was set at 7.5% (w/w), the drug concentration over 120 h in receiver fluid was too low to quantify. At each surfactant level, the drug delivered across the membrane was significantly higher ($p < 0.05$) from the PN030 loaded foam compared to LN030 loaded foam. For example, at 0.5% (w/w) pluronic concentration, the amount of minoxidil in the receiver fluid after 120 h was 1.9 ± 0.3 ($\mu g\,cm^{-2}$) for PNFM05 compared to 1.1 ± 0.2 ($\mu g\,cm^{-2}$) for LNFM05.

4. Discussion

Loading therapeutic agents into nanoparticles and then including these nanocarriers within topically acceptable vehicles for drug delivery to the skin is a complex and costly process compared to the traditional one-step emulsification procedures that are employed to incorporate active agents into a cream. Therefore, nanotechnology must afford a significant advantage to justify its commercial use in this application. Controlling the delivery of hydrophilic agents such as minoxidil to the scalp and minimising the number treatment interventions required to elicit an effect is one way that nanocarriers could potentially improve clinical outcomes. However, in order to realise their true benefits particulate carriers must maintain their integrity and drug load during formulation storage and allow controlled drug release upon dose delivery. Employing water soluble drugs in nanocarrier systems significantly increases the chances of dose dumping prior to administration. If the drug is released from the nanoparticle into the delivery vehicle prior to dose application this may negate the positive influence of improved drug chemical stability and sustained/controlled release that a particulate carrier can provide.

Minoxidil, which is an ionisable (pK_a of 4.6), hydrophilic compound ($\log P_{(octanol/water)}$ 1.2) was successfully loaded into both the LN and the PN produced in this study. The LN contained a liquid medium chain triglycerides core (HLB = 2) and the PN contained a liquid propylene glycol monocaprylate core (HLB = 6) (Moffat et al., 2004). The superior minoxidil loading capacity of the LN was surprising and suggested that the drug had a higher affinity for more hydrophobic lipid used in the LN. However, this conclusion assumes that the production method and different excipient ratios used in the LN and PN particles did not affect drug loading, which is probably not the case. The most striking difference between the two sets of nanoparticles was that the LN contained *ca.* 10% lipid where as the PN only contained *ca.* 2% (w/v) lipid and this was probably the reason for the superior LN loading of minoxidil. Although the lipid content of the PN could be increased, more than 2% (w/v) lipid was found to produce particles with a size beyond the nanometer range (data not shown). Attempting to equilibrate LN and PN loading by increasing the lipid content of the PN was therefore deemed inappropriate. The encapsulation efficiency (EE) for both nanoparticles was low (<50%) and this was presumed to be a result of the hydrophilic nature of minoxidil and its appreciable solubility in the aqueous nanoparticle dispersing medium. Despite not all of the drug loading into the particles, the good drug recovery and reproducibility was indicative of a sound encapsulation process during which the drug did not chemically degrade.

Compared to the LN (*ca.* 50 nm, PI < 0.1), a bigger particle with wider size distribution was obtained for the PN (*ca.* 260 nm, PI < 0.2). The discrepancy in the particle size of LN and PN was attributed to the different manufacturing methods for the two particles. LN was generated by phase inversion, whereas PN was produced by solvent displacement. The size and size distribution of the LN was determined by the quick inversion of a water-in-oil emulsion to an oil-in-water emulsion, whist the size and size distribution of PN was governed by the mechanical forces of the homogeniser during the rapid diffusion of organic phase through the aqueous phase. Both types of nanoparticles also differed in the surface charge properties according to the zeta potential analysis. LN surface was neutral, whereas the PN surface was negatively charged. This difference in surface charge can be explained by the difference in the nanoparticle shells. The negative zeta potential of PN made from PLA has been described in previous studies to be a consequence of the terminal carboxylic groups in the polymer whereas LN used a neutral surfactant (Musumeci et al., 2006). These two different particle surfaces, probably dominated by the surfactants used in the production methods; both resulted in excellent physical stability in the final aqueous suspensions.

The drug–loaded PN and LN nanosuspensions were readily emulsified in the HFA 227 propellant with the aid of pluronic L62D surfactant in a similar manner to previous work (Zhao et al., 2009). HFA 227 is non-flammable, non-explosive propellant that is approved for human use. It is an ideal propellant system for the production of topical foams as it exists as a liquid when sealed within pressurised, air-tight containers at ambient temperature, but readily vaporises upon exposure to atmospheric pressure to form a self-assembling foam (Sciarra and Cutie, 1996). A nanoparticle-in-water-in-heptafluoropropane emulsion, considered to be analogous to an oil-in-water-in-oil multiple emulsion, has previously been shown to generate fast breaking foams which are well suited to this application as theoretically foam collapse should initiate drug liberation from the nanoparticles (Zhao et al., 2008).

Although a number of methods have been previously employed to monitor drug release from nanoparticles, the centrifugal technique was considered to be the most suitable method to monitor release kinetics of nano-encapsulated agents in this work as it could separate free and loaded drug in a matter of minutes (Heng et al., 2008). Despite attempting to minimise the solubility of the drug in the aqueous phase of the formulation by setting the pH to 6, both the LN and the PN demonstrated a biphasic drug release immediately after manufacture i.e. prior to delivery by the foam. Both release profiles are thought to represent a diffusion-based process governed by the diffusivity of the drug and partition coefficient of the drug between the nanoparticles and the aqueous environment. After the first stage of relatively fast drug release (before 8 h), the amount of minoxidil that accumulated in the aqueous dispersion medium probably influenced the concentration gradient between the particle and the medium which lead to a second phase of slow release (over 24 h). Minoxidil is a small molecule with a molecular weight of 209 and thus a relatively high rate of diffusion would be expected out of the nanoparticles. The short drug diffusion distance arising from the small particle size (<300 nm) could also have played a role in the fast drug release (zur Muhlen et al., 1998). The most likely explanation for the differences in the release profiles for the two particles (i.e. the LN releasing more drug, more rapidly compared to the PN) was the different partition processes that occurs in the suspensions. The liquid core (medium chain triglycerides) of the LN was much more lipophilic and demonstrated a much lower minoxidil solubility (less than 0.1% (w/w) at ambient temperature) compared to the PN. Although the LN loading was higher, the drug affinity was low and this would naturally lead to a more rapid partition out of the LN compared to the PN. No drug crystals were observed microscopically in both nanosuspensions throughout the whole study, which excluded the confounding effects of drug recrystallisation on the release. In addition, no particle col-

lapse was witnessed after the release study (shown by particle size monitored over 24 h) which rules out particle erosion-driven release.

The drug release observed in this study from the simple nanosuspensions suggests that the premature release of a hydrophilic drug in an aqueous formulation vehicle i.e. release prior to application of a dose, is difficult to prevent irrespective of the nanoparticle properties. One strategy that could minimise the dumping of the active agent into the aqueous vehicle prior to dose application is to reduce the effective partition coefficient of the active agent between the hydrophilic vehicle and the particles. For example, the selection of more hydrophilic particles (Hillaireau et al., 2006), the regulation of pH to decrease the aqueous solubility of the agents (Govender et al., 1999) and the addition of the third ingredient in the particle to enhance the affinity of the active agent to the particles (Ishihara et al., 2009). All these approaches are capable of reducing the effect of premature drug release but, to our knowledge, no solvents suitable for topical drug delivery are available in which minoxidil shows much lower solubility compared to water and nanoparticle integrity can be maintained, and thus the premature drug release is hard to control using these means.

The effect of the premature minoxidil release from nanoparticles on drug permeation across a barrier was assessed using a polydimethylsiloxane membrane. Silicone membranes are an accepted alternative to skin when attempting to model percutaneous drug absorption through the SC (Cross et al., 2001). The poor membrane permeation of minoxidil from all the formulations tested in this work, despite ionisation suppression, was assumed to be due to the drug's hydrogen bond donor acidity and hydrophilic nature that have been previously reported to adversely affect the permeation across silicone membrane (Geinoz et al., 2002). For example, minoxidil has three hydrogen bond donor sites and polydimethylsiloxane is a hydrogen bond acceptor (Cronin et al., 1998); hence the formation of hydrogen bonding between the drug and silicone membrane is highly plausible. The log P of polydimethylsiloxane has been reported to be *ca.* 10 (Henry et al., 2001) which is significantly more hydrophobic compared to minoxidil (log P = 1.2). When minoxidil was applied using Rogaine®, it was assumed that the presence of ethanol and propylene glycol in the formulation could modify the membrane and reduce its barrier properties, an effect that has been demonstrated in previous work (Cross et al., 2001). As a consequence more minoxidil permeated through the membrane over 120 h from Rogaine® than from other aqueous vehicles such as the nanosuspensions and foams. However, the skin permeation profile of minoxidil from these vehicles still needs further investigation due to the presence of particle-skin interaction and potential particle deposition via follicular route.

For the nanocarriers used in this study to have any chance of controlling drug release from the topical formulations drug ejection from the nanoparticle must be the rate controlling step in the drug delivery process. However, a combination of the premature drug release from the nanocarriers and the lack of minoxidil silicone membrane permeability meant this was not the case. Reviewing the release and permeation data for the nanoparticle systems employed in this work provides evidence that all the carrier systems presented a mixture of 'free' and loaded drug to the surface of the silicone membrane. According to the Higuchi's interpretation of Fick's law (Higuchi, 1960), drug permeation across a membrane is determined by its thermodynamic activity (α) i.e. the amount of drug presented to the membrane in relation to its saturated solubility. Therefore the permeation rate for both the nanosuspensions and the foams tested in this work would simply be dependent upon free drug concentration in the presented vehicle as this would dictate α. The fact that compared to the saturated solution (α = 1) less minoxidil permeated across the silicone membrane after 120 h from the nanosuspensions and foams seemed to support this hypothesis. However, the vehicles were not exactly equivalent for the nanosuspension and foams. The foams also contained pluronic surfactant which probably increased minoxidil solubility and reduced α further. This provides an explanation of why the drug permeation rate was significantly lower for the foams compared to the nanosuspension despite using the same particles. All these results are indicative of α driving the drug permeation which means that the nanocarriers had little positive impact upon minoxidil delivery.

5. Conclusions

Both lipid and polymeric nanoparticles proved to be ineffective at preventing minoxidil release during storage in an aqueous formulation vehicle. The functional consequence of drug release prior to dosing was that the formulations behaved in a similar manner to simple drug solutions. These effects were only discerned by a systematic investigation of the delivery process which involved correlating drug release before dosing with the performance of the formulations after application to a topical membrane. The timing and rate of drug release from a particulate is critical and drug release during storage renders this approach ineffective when attempting to control topical drug administration. Not only does this work highlight the need to always characterise drug release from a topical formulation containing particulates prior to application, but it also demonstrates that effective drug loading into nanoparticles does not always translate into effective delivery. If drug retention is achieved, i.e. not released into the aqueous solvent after dose application to the skin as a result of passive diffusion, then a mechanism of triggering release upon application is required. A dynamic foam has the potential to trigger drug release but the particle design is critical as if the drug and particle properties are not well matched premature dose dumping becomes a significant problem.

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