Disrupting drug-drug interactions using charged nanoparticle surfaces: a novel means to enhance percutaneous skin penetration from topical gels

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Abstract

Drug self-association can hinder percutaneous penetration. The aim of this study was to investigate if disrupting drug-drug interactions using charged nanoparticle surfaces could promote delivery of a pharmaceutically active agent into the skin. Tetracaine was chosen as a model drug. It was presented to the skin as a hydroxyl methylcellulose (HPMC) gel and Ametop gel™ with and without the addition of –ve charged silica nanoparticles ($Nano_{SiO_2}$). When the nanoparticles were added to the topical formulations it resulted in the fastest drug permeation (109.95 ± 28.63 μg/cm^2/h), highest accumulative mass at 45 min (76.83 ± 18.92 μg) and shortest lag time (2.02 ± 0.79 min) in porcine skin. $Nano_{SiO_2}$ disrupted the tetracaine aggregates in the HPMC gel whilst not interacting with the polymer to increase the formulation macroviscosity. When $Nano_{SiO_2}$were added to the commercial Ametop gel they also enhanced penetration through the skin. Despite the very different composition of the Ametop system compared to the HPMC the nanoparticles appeared to function in a very similar manner, i.e., they broke up the tetracaine aggregates without interacting the formulation. The similar results for both the gel systems suggested that, provided the drug-nanoparticle interactions were optimised to facilitate drug aggregate break up, the addition of solid nanomaterials to semi-solid topical preparations could be a novel penetration enhancement method in a number of different types of skin product.

Key words: Tetracaine, nanoparticles, skin, aggregation, gel, skin, permeation.

Introduction

Topical drug delivery of a therapeutic agent to the skin presents an attractive alternative to other routes of administration by providing a non-invasive strategy that bypasses the first pass metabolism, reduces the adverse side-effects of systemic toxicity and improves patient compliance [1]. However, efficient topical delivery of therapeutic agents can be problematic to achieve due to the relative impermeability of the *stratum corneum* [2]. If a molecule applied topically to the surface of the skin does not have desirable physicochemical properties to allow it to move easily into the tissue, the skin barrier can reduce or slow the pharmacological response to the administered molecule. A good example of a therapeutic agent that is slowed down due to the barrier properties of the skin is the topical anaesthetic tetracaine.

Tetracaine is commercially available as Ametop, a 4 % gel that has an onset of action of 30 – 45 minutes. However, this is too slow for optimal clinical action and this has driven formulators to design of a number of strategies to try and improve the clinical use of this agent [3-5]. However, it has proven difficult to enhance the permeation of this molecule into the skin without the non-reversible modification to the cutaneous tissue. Recent studies have shown that one reason for this may be that tetracaine is an amphiphilic drug that aggregates in aqueous vehicles [6, 7]. When the drug aggregates it exhibits different physiochemical properties in solution compared to the non-aggregated form of the molecule [8-11] and at the high tetracaine drug concentrations, i.e., those typically found in topical preparations, this physical aggregation can hinder penetration into the skin after topical application [12, 13]. Although it has been shown that formulating tetracaine at

lower concentrations reduces the aggregation and improves drug transport, using a low drug concentration *in vivo* would reduce its clinical effect [12]. A more clinically viable penetration enhancement strategy would be to use a formulation excipient that could break up tetracaine aggregation even at high drug concentrations.

Employing nanomaterials in topical products can enhance the percutaneous penetration of therapeutic agents [14-16]. Nanomaterials can act as drug carriers, but in addition if solid nanomaterials (> 10 nm in size) are added to topical formulations, they can also break up drug aggregates without penetrating into the skin through their powerful surface interactions. Using nanomaterials to modify drug-drug interactions could be an attractive drug delivery approach to improve the action of tetracaine because they may control the detrimental effects of this molecules aggregation on the surface of the skin and if the added nanomaterials are > 50 nm they are unlikely to enter the body and hence raise additional toxicity concerns [17-19].

There have been a number of different mechanisms of action proposed for the penetration enhancement properties of nanoparticles include barrier disruption [20, 21], drug supersaturation [22, 23], skin occlusion [24, 25] and the provision of a drug reservoir to prevent drug depletion [26, 27]. However, there is only one reported study that focuses on the ability of nanomaterials to enhance skin penetration through the modification of drug aggregation [28]. The drug-nanoparticle surface interactions are probably more important when attempting to deliver amphiphilic drugs topically to the skin than the other potential penetration enhancing effects aforementioned because the drug nanoparticle surface

interactions may alter the drug-drug interaction equilibrium [29-32]. According to Ueda *et al.* (2011) [28], the physical interactions between formulation excipients and drug aggregates can be a powerful means to enhanced drug penetration through barriers. In addition, it has been shown that drug-nanomaterial interactions can be manipulated through varying the properties of the nanomaterials [33]. However, to date a topical formulation has not been designed with the purpose of enhancing percutaneous penetration simply by the addition of nanomaterials to the preparation in a manner that encourages the drug to interact physically with the particle surfaces.

It is also important, if attempting to include nanomaterials in a topical semi-solid product, to consider the nanoparticle-vehicle interactions [34]. The vehicle can influence drug percutaneous penetration by controlling the drugs thermodynamic activity [35], modifying the membrane structure [36, 37] and influencing the diffusion coefficient of the drug through the vehicle viscosity [38, 39]. Although a solvent matrix must interact with a drug to solubilise it, strong interactions with the solvent should generally be avoided because they can have a detrimental effect on skin permeation through changes in drug diffusion [40]. This is especially true if trying to increase the penetration of a drug from a vehicle using nanoparticles because strong drug-vehicle interactions may influence the nanoparticle-drug interactions.

The aim of this study was investigate if percutaneous drug delivery could be enhanced from topical gels by disrupting drug-drug interactions using charged nanoparticle surfaces. Tetracaine was selected as a model drug as it has previously been shown to

aggregate [6, 7], it has a slow onset of action clinically [41] and nanoparticles with a negative surface charge have been shown to interact with the drug and modify its behaviour [33]. The experiments were conducted in pH 8 because Ametop, the commercially marketed gel, was formulated at pH 8 and the aggregation properties of tetracaine had been previously studied in this pH [12, 33]. Hydroxypropyl methycellulose (HPMC) was chosen as the excipient to produce the novel nanoparticle containing topical product due to its inert nature and ability to form a gel with a large pore size which usually does not hinder diffusion [42]. A spray formulation was used to apply the HPMC system because it was thought to be important to provide the option to apply the nanoparticles and gel from different storage reservoirs in order to prevent thickening of the gel upon storage with the nanoparticles if strong interactions between the components did exist. The sprays were optimised in terms of evaporation kinetics, spray characterization, spray recovery and viscosity to ensure accurate dosing. The tetracaine permeation from the different formulations was assessed using porcine epidermis as a skin model using methods established in previous work [12,13]. Comparisons of the HPMC system with the commercially available Ametop were conducted to assess the potential for the developed formulation to provide superior drug deposition into the skin and to determine if the nanoparticle addition could influence the permeation from different types of formulation. Two drug application protocols were used to dose the drug to the skin: infinite dose studies were performed to understand the effects of vehicle composition on tetracaine delivery; finite dose studies were performed to assess to clinical relevance of any differences observed when nanoparticle were added to the formulations. Silica nanoparticles ($Nano_{SiO_2}$) were used to represent the nanoparticle

surfaces with which the model drug tetracaine could interact. $Nano_{SiO_2}$ were co-administered to the skin with the drug and no drug was encapsulated into the particles or adsorbed onto their surface prior to administration. The semi-solid dosage form's macroviscosity were measured using traditional 'cone and plate' rheometry to have a better understanding of the interactions taking place in the system, i.e., between the drug, nanoparticle and formulation.

Materials and Methods

Materials

HPMC powder (grade 65SH viscosity 400 cP and 50 cP, brand name Metolose) was provided by Shin-Etsu Chemical Ltd, Japan. Tetracaine free base (≥ 98%), hydrochloric acid, acetic acid and sodium acetate were purchased from Sigma Aldrich, UK. Commercially available Ametop gels were supplied by AAH Pharmaceuticals, UK. Silica nanoparticles ($Nano_{SiO_2}$), with a diameter of 200 nm (Psi-0.2), were obtained from Kisker Biotech GmbH and Co., Germany. Ultrapure water (18.2 MΩ) was used throughout this study unless stated otherwise. Phosphate-buffered saline tablets were supplied by Oxoid Limited, UK. Acetonitrile, methanol and water (high-performance liquid chromatography (HPLC grade) were obtained from Fisher Scientific International, UK.

Spray formulation preparation

The HPMC solutions were prepared by stirring HPMC powder slowly into pH 8 water at 70 °C and allowing the system to hydrate for 24 h at 5 °C. The formulations were then transferred into 50 mL plastic spray bottles (Boots, UK). Three HPMC formulations were produced with polymer concentrations of 1 % and 2 % of Grade 65 (viscosity 400 cP, 65SH400) and 3 % of Grade 65 (viscosity 50 cP, 65SH50) because concentrations above these levels were unable to spray through the nozzle of the dosing system used to apply them to the skin in the permeation studies.

Evaporation kinetics

Thirty actuations from each spray formulation (i.e. 1 % 65SH400, 2 % 65SH400, 3 % 65SH50) were applied to a tared weighing boat on an analytical balance and monitored for weight loss after application. Weight of the formulation (g) was plotted against time (min). The rate of solvent evaporation was calculated using a line of best fit. The study lasted for 48 h to ensure the applied formulations were completely dry and no further weight loss occurred. Triplicate experiments were performed.

Spray characterisation

The spray formulation placed at a distance of 5 cm vertically above a piece of filter paper and two shots was actuated from the spray canister holding the formulation onto the filter

paper. The spray was allowed to dry and the film residue shape was outlined using a marker. The shortest diameter and the longest diameter of film residue shape were measured. The measurements were used to calculate the area covered by the product based on a perfect circle (Eqs, (1) and (2)) and the aspect ratio (AR, Eq. (3))). Triplicate measurements were performed for each formulation.

$$D_{mean} = \frac{D_{min} + D_{max}}{2} \quad (1)$$

$$Area = \pi \left(\frac{D_{mean}}{2}\right)^2 \quad (2)$$

$$AR = \frac{D_{min}}{D_{max}} \quad (3)$$

Spray recovery

Ten actuations from the spray were applied to a tared weighing boat on an analytical balance and individually measured. The recovery was calculated as a percentage of the sum of the mass of the formulation in the container (C_{final}) and on the tared weighing boat (s) at the end of 10 actuations to the initial mass of formulation in the container ($C_{initial}$) for each sample, n=3 (Eq. 4). In addition, the amount of formulation recovered in the nozzle ($n_{recover}$) was measured by the subtraction of the initial mass of nozzle ($n_{initial}$) from the final mass of the nozzle (n_{final}) (Eq. 5).

$$Recovery\ (\%) = \frac{C_{final+s}}{C_{initial}} \times 100\ \% \quad (4)$$

$$n_{recovery} = n_{final} - n_{initial} \quad (5)$$

Viscosity of formulation

The rheological measurements were performed using CSL a cone and plate rheometer (Carri-med, USA) with plate diameter of 4.0 cm and cone angle of 1.5° at a 100 mm fixed gap. The test was performed over a 1-10 Hz frequency range at constant stress amplitude of 0.798 Pa. All the measurements were carried out at 20°C. Twenty data points were recorded for each rheogram and triplicates were performed for each formulation.

Tetracaine transport studies

Fresh white adult porcine ears were obtained from a local abattoir (Evans and Sons, UK). Damaged ears were discarded. After cleaning with deionized water and wiping the residue with clean wipes, visible hairs were trimmed carefully. The preparation of epidermal porcine skin was carried out by heat separation [43]. Porcine skin was immersed and gently stirred in deionised water at 60°C for 1 min. After removal from the deionized water, the skin as put on a corkboard with the dermal side down and the epidermis was carefully separated from the dermis with tweezers. The separated epidermis was washed with deionized water and floated on filter paper (Whatman no. 1, UK) to act as a support before it is dried with clean wipes. The samples were wrapped in aluminium foil and stored at - 20°C for a maximum of up to 1 month [44]. The samples were thawed before use.

The transport studies were carried out using upright individually calibrated Franz diffusion cells with an average of 2.1 ± 0.1 cm^2 surface areas and 9.2 ± 0.5 mL receptor compartment volume. The porcine skin was cut, mounted and sealed with parafilm between two chambers of the glass diffusion cell with a 13 mm magnetic flea in the receiver chamber. The cell was inverted and filled with previously filtered and sonicated receiver fluid. Phosphate buffered saline (pH 7.4) was employed as a receiver fluid for the porcine epidermis transport studies to mimic the skin environment. The transport studies were performed on a submergible magnetic stirrer plate in a pre-heated water bath set at 37°C to provide a membrane surface temperature of 32°C. After cell equilibration for 1 h, the cells were checked for leaks by inversion and visual inspection for back diffusion. The tetracaine test systems were prepared and adjusted to 8.0 using hydrochloric acid and equilibrated at 32°C unless stated otherwise. Solutions were stirred for at least 24 h and the pH rechecked prior to analysis to ensure they were at equilibrium. The vehicle containing the nanoparticles was corrected to the necessary pH using hydrochloric acid prior to addition to the tetracaine solutions. The infinite dosing studies used 1 mL of tetracaine formulations, which were applied uniformly to the surface of the test membrane and the donor compartment was covered with a parafilm to minimise donor phase evaporation. In the infinite studies, a saturated tetracaine solution of pH 8 was compared to a HPMC formulation (developed in the study) and the commercial preparation Ametop. None of the systems contained nanoparticles as the objective was to understand the drug-vehicle interactions. The finite dosing tested the addition of nanoparticles on the formulations ability to deliver tetracaine in to the skin. The nanoparticles were added at a concentration of 50 mg/mL to the tetracaine

formulations immediately prior to application to the skin to avoid any potential problems induced by chemical or physical instability. The finite dosing transport study used 10 μL, of Ametop or 3% HPMC formulation. The exact weight of the donor solution applied corresponded to 4.87 and 4.85 mg/cm^2 respectively. To these two 151 mM drug-loaded gels (drug loading was matched across the two formulations) an equal amount (10 μL) of with silica nanoparticles ($Nano_{SiO_2}$) or water (control) were added to the formulation at the 0 h time point after correcting the suspension medium pH to 8. At predetermined time intervals, 1 mL aliquots were removed from the Franz cell receiver phases and replaced with fresh receiver fluid to keep the liquid volume in the receiver compartment constant. The collected receiver fluid samples were analysed by HPLC. A total of 5 replicates of each experiment were performed.

Cumulative amounts of drug (ng) penetrating the unit surface of the membrane area (cm^2) were corrected for sample removal and plotted against time (h). The steady-state flux (J) was calculated from the slope of the linear portion of the curve ($R^2 \geq 0.98$), using at least 3 points with values above the assay limit of detection (LOQ). The permeability coefficient of tetracaine was calculated using equation 6 [45]:

$$J = \frac{k_p}{C_v} \qquad (6)$$

where J represents the flux, k_p is the permeability coefficient of the permeant across the membrane and C_v is the concentration of the drug in the vehicle. The flux enhancement ratio (ER) of the different formulations was determined using the following equation:

$$ER = \frac{J_2}{J_1} \quad (7)$$

where J_1 and J_2 are the steady-state transmembrane transport rate of tetracaine from the tetracaine solutions and tetracaine-nanoparticle gel mixtures respectively. The accumulative mass of tetracaine transported through the skin at 45 min was recorded as this was the usual onset of action time for this agent. The permeation lag time was estimated from the X-axis intercept from the linear regression of the model applied to the permeation data in order to determine the flux.

Tetracaine quantification

The quantification of tetracaine was performed using a reverse-phase HPLC system consisting of a pump with autosampler (Hewett-Packard series 1050, Agilent Technologies UK Ltd., UK) connected to a fluorescence detector (Shimadzu detector RF-551, Shimadzu corp., Japan). The system was controlled via a computer with Chromeleon software (Dionex Corp., USA), which was also used to record and interpret the analytical data. The HPLC mobile phase comprised acetonitrile-methanol-acetate buffer (0.1 M) (25:25:50 (v/v), pH 5.1) set at a flow rate of 1.0 $mL.min^{-1}$. Tetracaine was separated using a Luna 3 μm C18(2) (150 X 4.6 mm) stationary phase (Phenomenex, UK) at room temperature with a 100 μL injection volume and the fluorescence detection at an excitation wavelength of 310 nm and an emission wavelength of 372 nm. The retention time for tetracaine was 4.2 min. The calibration curves were constructed on the basis of

the peak area measurements using standard solutions of known tetracaine concentrations dissolved in an identical fluid as the receiver phase for the transport studies, 10^{-4} HCl (pH 4 water). The assay was shown to be "fit for purpose" in terms of sensitivity (LOD – 4.08 ng/mL, LOQ – 74 ng/mL, n=25), precision (6% CV), and linearity ($R^2 \geq 0.99$).

Statistical Analysis

All values were expressed as their mean ± standard deviation (SD). The statistical analysis of data was performed using the statistical package for social sciences, SPSS version 21, (IBM Corp., USA) with a significance level of 0.05. The normality (Sapiro-Wilk) and homogeneity of variances (Levene's test) of the data were assessed prior to statistical analysis. Transport data were analysed statistically using one-way analysis of variance (ANOVA) tests for normally distributed data and a non-parametric Kruskal-Wallis tests for non-Gaussian distributed data. Post hoc comparisons of the means of individual groups were performed when appropriate using Dunnet's test for normal distributed data and Games Howell test for non-Gaussian distributed data. For all pair-wise comparison of means, Student's independent t-test or Mann-Whitney test was applied. Data were presented using OriginPro software (OriginPro version 8.6, OriginLab Corporation, US).

Results

Formulation optimization and characterization

A high spray volume, a high percentage of spray recovery, a low amount of residual in the nozzle, a large spray deposit area and a moderately high viscosity were all thought to be desirable product characteristics for the spray systems. The rate of evaporation did not discriminate between the three formulations (Table 1, $p > 0.05$, 0.2426 ± 0.0350 g/h), but the 2% 65SH400 was thought not to be ideal because it had the lowest spray actuation mass (66.7 ± 43.7 mg), lowest percentage spray recovery ($99.72 \pm 0.09\%$), highest residual nozzle mass after spray actuation (66.0 ± 15.3 mg), lowest mean spray deposit diameter (2.4 ± 0.3 cm), lowest mean spray deposit area (4.5 ± 1.1 cm^2) and highest viscosity (Fig. 1).

The 1% 65SH400 and 3% 65SH50 formulations were not significantly different ($p > 0.05$) in terms of the amount of spray actuated (157.7 ± 14.1 mg) and the recovery from the spray container (99.83 ± 0.05 %). However, 3% 65SH50 system was chosen for further investigation rather than 1% 65SH400 because, although it covered a relatively small area (5.9 ± 0.5 cm^2), it showed appropriate viscosity to remain on the skin (Fig. 1) and it deposited 104-fold less residue on the spray nozzle ($p < 0.05$). Furthermore, the 3% 65SH50 gel was the most efficient in terms of ejecting the dose of the three spray formulations.

Infinite transport studies

Infinite doses of tetracaine were used to understand the effect of vehicle composition on tetracaine transport (Table 2, Fig. S2 for permeation profiles). According to manufacturer's data, the commercially available Ametop gel consisted of saturated concentrations of tetracaine with sodium hydroxide, sodium methyl-o-hydroxybenzoate, sodium propyl-p-hydroxybenzoate, monoboasic potassium phosphate, xanthan gum, sodium chloride and purified water. The HPMC formulation consisted of saturated concentrations of tetracaine with 3% HPMC. No nanoparticles were added to these systems. There was no significant difference ($p > 0.05$) in steady-state tetracaine transport rate ((110.23 ± 40.93 μg/cm^2/h) and lag time (9.15 ± 2.05 min) when infinite doses of tetracaine were delivered by Ametop and the HPMC formulation compared to a simple saturated tetracaine solution, i.e. with no formulation additives.

Finite transport studies

Finite studies were performed to study the transport of tetracaine from the semi-solid formulations using a dosing regime that matched clinical conditions (Table 3, Fig S3 and S4 for permeation profiles of Ametop and HPMC formulations respectively). Based on manufacturer's data, the $\mathrm{Nano}_{\mathrm{SiO}_2}$, which were added to the formulations, only consisted of amorphous silica and water. Thus, water acted as a control to mimic the drug dilution effects that were experienced upon addition of the nanoparticles to the formulations. Calculation of the lag time showed that the HPMC spray, with the added $\mathrm{Nano}_{\mathrm{SiO}_2}$, had

the shortest permeation lag time (2.02 ± 0.79 min). In addition, the tetracaine permeation rate (109.95 ± 28.63 μg/cm^2/h) and the amount of tetracaine permeating at the 45 min time point (76.83 ± 18.92 μg) were the highest when the drug was formulated as the HPMC spray containing the $Nano_{SiO_2}$. The addition of water to the HPMC semi-solid formulation did not induce a significant change in tetracaine steady-state flux (30.51 ± 12.16 μg/cm^2/h) and accumulative mass permeating the skin at 45 min (21.99 ± 10.48 μg), but it did induce a 1.7 times reduction in lag time from 7.09 ± 1.80 min to 3.97 ± 1.07 min. (Table 3). Compared with the water control, the $Nano_{SiO_2}$ significantly enhanced ($p<0.05$) percutaneous tetracaine transport 3.6 fold when added to the HPMC formulation (Table 3). In addition, the $Nano_{SiO_2}$ significantly increased the accumulative mass at 45 min (m_{45min}) by ca. 3 times and reduced the lag time (t_{lag}) by ca. 2-fold (Table 3).

In contrast to the HPMC formulation, the addition of water to the Ametop formulations significantly enhanced tetracaine permeation rates by 5-fold, increased accumulative mass by 10-fold and reduced lag time by 1.5 times (Table 3). Compared to the water control, the $Nano_{SiO_2}$ addition to the Ametop formulation increased the skin permeation by 2.7. However, of the two formulations tested the HPMC formulation was superior, it significantly enhanced the tetracaine flux (46.99 ± 7.96 μg/cm^2/h) by 40-fold, increased accumulative mass (31.13 ± 4.04 μg) by 124-fold and reduced lag time (7.09 ± 1.80 min) by 8-fold compared to the Ametop formulation.

Rheology measurements

The rheological characteristics of the semi-solid formulations were examined to try and further understand the observed differences in the tetracaine permeation profiles (Table 3). All the Ametop formulations were significantly ($p < 0.05$) more viscous (higher storage and loss modulus) than the HPMC formulations. The addition of water in both formulations significantly decreased ($p < 0.05$) the viscoelasticity of the formulations. However, there were no significant changes ($p>0.05$) in rheological behaviour when the additions of water and $Nano_{SiO_2}$ to the gels were compared.

Discussion

Loading tetracaine into a two very different semi solid formulation and applying an infinite dose of the systems to the surface of the skin showed no important differences in terms of the drug permeation. The data demonstrated that neither the xanthan gum or HPMC had a large effect on tetracaine permeation behaviour, i.e. the excipients were considered to be relatively inert, when the drug was presented to the skin as in its aggregated state (the drug concentration in the formulation was above its measured critical aggregation concentration at pH 8 [12]). Similar observations were observed by Kim [46] and Charoo [47]. However, HPMC is not inert in all circumstances. In supersaturated conditions, HPMC has been shown to interact with a drug and act as an anti-nucleating agent [48-50]. To some extent the influence of HPMC on drug permeability is dependent on its viscosity and the nature of the drug with which the

polymer is formulated with. In the infinite dose studies without nanoparticles tetracaine would show strong intermolecular interactions and this may why the interactions with the HPMC were limited [12, 13]. The specificity of the drug-vehicle interactions in semi-solid preparations is one reason why constructing a semi-solid system that facilitates efficient release of a drug from a topically applied formulation is not a trivial task. Not optimising the drug-vehicle interactions can lead to inefficient delivery. For example, an investigation by Shah et al. [51] demonstrated that only 5 – 10 % of the applied hydrocortisone was released from commercial creams. To circumvent this problem the type of formulation must be matched to the issues presented by the drug. For example, Reid et al. [52] showed that EtOH/PEG HFA spray could generate a 6-fold enhancement of BMV delivery through the skin as compared to commercial cream, but not one approach works for all systems. For example, Fiala et al. [53] used a similar approach to Reid et al. [47] when trying to improve the skin penetration of lidocaine, but this was not successful. It should be noted that although HPMC did not show the ability to hider the delivery of the aggregated tetracaine in the current study viscosity modifiers do generally have the potential to hinder drug diffusion from the formulation into the skin. For example, the addition of a 7 % gelling agent to thicken a Transcutol:isopropyl myristate binary mixture resulted in a four-fold decrease in clebopride diffusion [54].

In the finite dosing studies the –ve charged nanomaterials suspended in water were added on the skin just after application of the tetracaine formulations. These systems were compared to equivalent semi-solid formulations applied to the skin with an equal amount of pH-adjusted water in order to account for the drug dilution effects upon dosing. When

the systems with and without nanomaterials were compared across both types of semi-solid vehicles used in this work, the nanomaterials enhanced drug permeation into the skin. It has previously been shown in simple aqueous solvents that $\mathrm{Nano}_{\mathrm{SiO}_2}$ breaks up the tetracaine aggregates due to weak surface interactions between -ve charged $\mathrm{Nano}_{\mathrm{SiO}_2}$ surfaces and the + ve charged tetracaine molecules [33]. Although it was likely that the same mechanism of action was responsible for the changes in drug permeation shown in this study it was thought necessary to further investigate the nature of the drug-vehicle-nanoparticles interactions in the gels using rheology measurements.

It was interesting that the infinite and finite dosing studies did not show the same results. The lack of discrimination between the formulation types in the infinite dosing studies was not replicated in the finite dosing studies where the HPMC formulation showed a far superior penetration rate into the skin. There is often a significant difference between infinite finite dose studies. For example, in a study by Cross et al. [55], thickening agents were shown to retard drug penetration through the skin in infinite dose studies but the opposite effect was observed in finite dose studies. The authors attributed the different results to the increased hydration due to less water evaporation and enhanced drug diffusivity through the *stratum corneum* when using finite doses. It seemed reasonable that the same reasons for the differences in the infinite and finite doses from Cross et al.'s work [55] could be applied to the current study results.

According to the rheological data, there was no difference between the viscoelasticity of the semi-solid formulations when either water or silica particles in water were added to

the semi-solid systems. This was unlike the results obtained by Moddaresi et al. [56], where an increase in viscoelasticity was observed when lipid nanoparticles were added to hyaluronic acid vehicle. This previous work attributed the change in rheological behaviour to the presence of strong interactions between the nanoparticles and the hyaluronic acid. In the current study the rheology data implied that the formulation excipients and the nanoparticles did not display a strong interaction because the addition of water and the addition of the nanoparticles suspended in water had the same effect on the gel rheology. These results suggested that the superior tetracaine permeation upon the addition of nanoparticles was largely due to the other interactions taking place in the system, i.e., tetracaine-nanoparticle and/or tetracaine-formulation interactions. The fact that the enhanced permeation was observed both in the in-house manufactured HPMC formulation and the commercially available Ametop preparation, even though the two preparations exhibited very different rheological behaviour, indicated that the tetracaine-nanoparticle influenced the drug permeation to a greater extent compared to the tetracaine-formulation interactions. If the reverse was true then a much larger difference in the effects of adding nanoparticles to the gels would be expected.

The addition of water to the tetracaine formulations, regardless of whether or not the liquid contained nanoparticles, altered the drug permeation through the skin. This type of viscosity dependant permeation behaviour is usually a consequence of the drug forming hydrogen bonds with the thickening agent in the topical formulation [59] which reduces the drug permeation. The data from the current study showed the opposite effect, i.e. the permeation of the drug increased upon the addition of water. As mentioned previously

this may have been because of the relatively strong drug-drug affinity of the tetracaine molecules (CAC – 7.38 μM [12]) and the weak interactions between the drug on the formulation vehicles (as shown by the infinite dosing studies). Alternatively it is also possible that the semi-solid system to showed viscosity-independent permeation behaviour and the dilution of the system reduced the drug aggregation [57, 58].

Conclusions

In this study, an efficient tetracaine topical spray formulation with superior drug permeability and a more rapid onset of action compared to commercial Ametop was generated. This was obtained through the addition of nanoparticles to a semi-solid preparation that was sprayed onto the skin. Evidence was gathered to suggest that the added nanoparticles primarily interacted with the drug and disrupted the tetracaine aggregates, released more free drug to improve drug permeation and lead to a shorter lag time. The HPMC spray formulation was thought to be superior to the commercial preparation due to the lower macroviscosity, which made tetracaine more readily available to permeate through the skin. However, the nanoparticle addition to the commercial formulation was also effective in improving the delivery of tetracaine, which suggested that this novel drug delivery strategy could even be applied to currently marketed products to enhance their performance.

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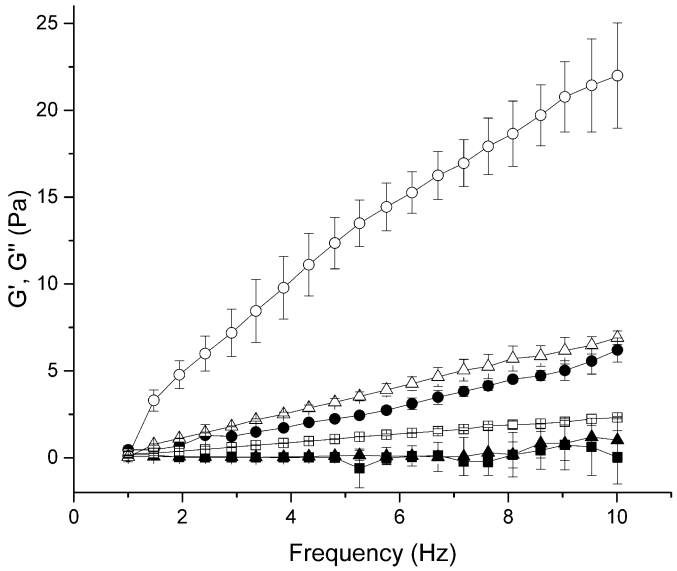

Fig. 1 Storage modulus (G', solid) and loss modulus (G'', open) measured as a function of frequency (Hz) for various 1 % 65SH400 (■), 2 % 65SH400 (●) and 3 % 65SH50 (▲) HPMC formulations. Data points represent mean ± standard deviation, n=3.

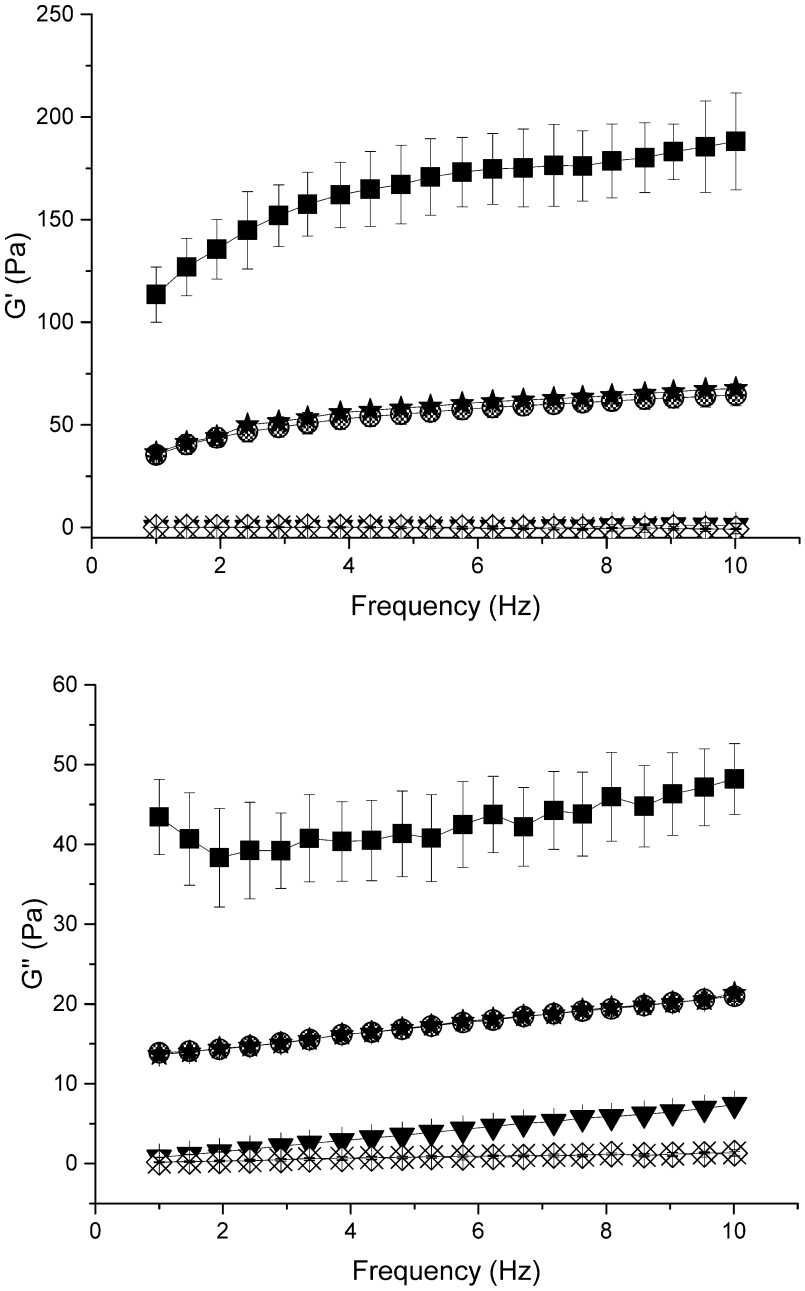

Fig. 2 Storage modulus (G', top) and loss modulus (G'', bottom) measured as a function of frequency (Hz) for Ametop (■), Ametop with the addition of water (■), Ametop with the addition of silica nanoparticles, $Nano_{SiO_2}$ ( ), HPMC formulations (▼), HPMC formulation with the addition of water (◇), formulation with the addition of $Nano_{SiO_2}$ ( ). Data points represent mean ± standard deviation, n=3.

Table 1. Characteristics of various HPMC formulations. Data represent mean ± standard deviation of 3 independent tetracaine samples. * Significant differences were observed based on one-way ANOVA test.

	1 % 65SH400	2% 65SH400	3% 65SH50
Evaporation rate (g/h)	0.24 ± 0.04	0.27 ± 0.03	0.25 ± 0.04
Spray mass (mg)	157.7 ± 14.1	66.7 ± 43.7*	145.5 ± 27.5
Spray recovery (%)	99.83 ± 0.05	99.72 ± 0.09*	99.92 ± 0.02
Nozzle recovery (mg)	31.4 ± 18.2*	66.0 ± 15.3*	0.3 ± 0.1
D_{mean} (cm)	3.5 ± 0.1*	2.4 ± 0.3	2.7 ± 0.1
Area (cm^2)	9.8 ± 0.6*	4.5 ± 1.1	5.9 ± 0.5
Aspect ratio	1.0 ± 0.0	0.9 ± 0.1	0.7 ± 0.1

Table 2. Steady state flux and permeability constants, k_p, accumulative mass at 45 minutes, m_{45min}, and lag time, t_{lag}, of infinite dosages of Ametop and tetracaine HPMC in the presence of different additives in pH 8 across porcine epidermis membrane. Data represent mean ± standard deviation of 3 independent tetracaine samples.

	Flux ($\mu g/cm^2/h$)	k_p (10^{-3} cm/h)	m_{45min} (μg)	t_{lag} (min)
Saturated solution	110.23 ± 40.93	66.20 ± 24.58	64.5 ± 21.11	9.15 ± 2.05
Ametop formulation	105.36 ± 21.97	63.27 ± 13.19	59.80 ± 9.31	10.10 ± 1.50
HPMC formulation	107.28 ± 28.31	64.43 ± 17.00	67.19 ± 18.21	8.79 ± 2.43

Table 3. Steady state flux, flux enhancement ratio, ER, accumulative mass at 45 min, m_{45min}, and lag time, t_{lag}, of finite dosages of Ametop and tetracaine HPMC in the presence of different additives in pH 8 across porcine epidermis membrane. Data represent mean ± standard deviation of 3 independent tetracaine samples. * Significant differences were observed based on one-way ANOVA.

	Flux ($\mu g/cm^2/h$)	ER	m_{45min} (μg)	t_{lag} (min)
Ametop	1.16 ± 0.14	-	0.25 ± 0.06	31.60 ± 3.00
Ametop + water	5.62 ± 2.25	4.71 ± 1.40*	2.53 ± 1.77*	20.20 ± 1.34*
Ametop + $Nano_{SiO_2}$	14.19 ± 2.27	12.86 ± 3.16*	8.12 ± 1.21*	10.69 ± 1.98*
HPMC	46.99 ± 7.96	-	31.13 ± 4.04	7.09 ± 1.80
HPMC + water	30.51 ± 12.16	0.69 ± 0.35	21.99 ± 10.48	3.97 ± 1.07*
HPMC + $Nano_{SiO_2}$	109.95 ± 28.63	2.48 ± 1.08*	76.83 ± 18.92 *	2.02 ± 0.79*