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Investigating the ability of nanoparticle-loaded hydroxypropyl methylcellulose and xanthan gum gels to enhance drug penetration into the skin

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Graphical Abstract

Abstract

Nanoparticle-loaded topical formulations can disrupt drug aggregation through controlled drug-nanoparticle interactions to enhance topical drug delivery. However, the complex relationship between the drug, nanoparticle and formulation vehicle requires further understanding. The aim of this study was to use nanoparticle-loaded hydroxypropyl methylcellulose (HPMC) and xanthan gum gels to probe how the drug, nanoparticle and formulation vehicle interactions influenced the delivery of an aggregated drug into the skin. Tetracaine was chosen as a model drug. It was loaded into HPMC and xanthan gum gels, and was presented to porcine skin using infinite and finite dosing protocols. Gel infinite doses showed no important differences in tetracaine skin permeation rate, but HPMC gel finite doses delivered the drug more efficiently ($46.99 \pm 7.96 \mu\text{g}/\text{cm}^2/\text{h}$) compared to the xanthan gum ($1.16 \pm 0.14 \mu\text{g}/\text{cm}^2/\text{h}$). Finite doses of the nanoparticle-loaded HPMC gel generated a 10-fold increase in drug flux ($109.95 \pm 28.63 \mu\text{g}/\text{cm}^2/\text{h}$) compared to the equivalent xanthan gum system ($14.19 \pm 2.27 \mu\text{g}/\text{cm}^2/\text{h}$). Rheology measurements suggested that the differences in the gels ability to administer the drug into the skin were not a consequence of gel-nanoparticle interactions rather they were a consequence of the dehydration induced diffusional restriction imparted on the drug by xanthan gum compared to the viscosity independent interactions of HPMC with the drug.

Key words: Tetracaine, nanoparticles, skin, aggregation, gel, skin, permeation, HPMC, xanthan gum.

Introduction

When a drug aggregates it exhibits different physiochemical properties in solution compared to the non-aggregated form of the molecule [1-4]. Therefore, at high drug concentrations, i.e., those above the critical aggregation concentration, it can be the properties of aggregates that determine a drug's behaviour rather than the individual molecules from which the aggregates are generated. The process of physical aggregation can hinder drug delivery into the skin after topical application, but it is not traditionally investigated during topical formulation development [5, 6]. Physical drug aggregation that occurs on the surface of the skin, is reversible at lower drug concentrations [5]. However, using low drug concentrations is not an effective solution to combat the problems associated with drug aggregation in topical formulations, as reducing the drug concentration applied to the skin can also reduce the clinical response to the preparation [5].

Nanomaterials can break up drug aggregation even at high drug concentrations through surface interactions that make the drug more available for permeation [7-10]. When nanoparticles act to break up aggregation, rather than acting as a drug carrier, they act on the skin surface, within the topical formulation, as a chemical penetration enhancer [11]. If the nanomaterials are 50 nm or larger they are unlikely to enter the body and therefore this approach raises very few additional toxicity concerns [12-14]. However, presenting a drug-nanoparticle combination to the surface of the skin presents a challenge because semi-solid formulation excipients can interact with both the drug and the nanoparticles [15]. If the drug-nanoparticle equilibrium is disrupted, this may have

deleterious effects on the nanoparticle penetration enhancement effects when administered to the skin [11]. Therefore, more information is required with regards to the drug-nanoparticle-delivery vehicle interactions in topical formulations that attempt to administer and aggregated drug into the skin.

A topical gel is a one-phase system and therefore represents the simplest vehicle in which a drug can be applied to the skin. However, the gelling agent and the solvent that are combined to generate a gel do interact with the drug in order to solubilise it. Through these interactions the topical vehicle controls the drug's thermodynamic activity [16], it influences the drug's diffusion coefficient towards its site of absorption [17, 18] and it interacts with the skin barrier upon which it is deposited during the delivery process [19, 20]. Therefore, a complex relationship between the gel formulation factors and delivery usually exists. This complexity is increased when attempting to administer both an aggregated drug and a nanoparticle to the skin because the drug concentration will influence its aggregation state and the drug's thermodynamic activity, the gelling agent concentration will influence the vehicle viscosity and aesthetic characteristics of the preparation and the gelling agent's chemical and structure features will dictate the vehicle's interactions with the drug, the nanoparticles and the skin. Previous work on nanoparticle behaviour in topical formulations have concentrated on nanoparticle-vehicle interactions as in these published studies the drug is typically incorporated within the nanocarrier and not adsorbed to its surface like in the system where nanoparticles are used to disrupt molecular aggregation [15, 21, 22]. Therefore, in order to generate an effective nanoparticle-loaded gel that facilitates de-aggregation of a drug to enhance its

penetration into the skin using nanomaterials, further research is needed to determine the relationships between the drug aggregation, gel properties and nanoparticle interactions such that drug permeation into the skin from this novel preparation can be controlled.

The aim of this study was to use nanoparticle-loaded hydroxyl methylcellulose (HPMC) and xanthan gum gels to probe how the drug, nanoparticle and formulation vehicle interactions influenced the delivery of an aggregated drug into the skin. Tetracaine was selected as a model drug as it has previously been shown to aggregate [23, 24]. It has a slow onset of action clinically [25] and nanoparticles with a negative surface charge have been shown to interact with the positively charged drug and modify its behaviour [11]. 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 [5, 11]. Hydroxypropyl methylcellulose (HPMC) was chosen to produce the semi-solid formulation due to its ability to form a gel with a large pore size, which minimises the potential for the gel structure to impart diffusional restriction on the drug [26]. A spray formulation was used to apply the HPMC system as this allowed the mixing of the nanoparticles and the drug only upon drug application, which avoided any physical instability issues that could reduce the homogeneity of dosing. The sprays were optimised in terms of evaporation kinetics, spray characterization, spray recovery and viscosity to ensure accurate dosing in the skin permeation studies. The HPMC system was compared with xanthan gum as it presented a negatively charged, tight gel and it allowed the direct use of the commercial Ametop formulation in the studies. Two drug application protocols were used to dose the drug to

the skin: infinite dose and finite dose studies because it was anticipated that the formulation viscosity, which may increase during finite dosing, could be influential in the delivery of the drug into the skin. Silica nanoparticles (NanoSiO_2) were used to represent the nanoparticle surfaces with which the model drug tetracaine could interact. NanoSiO_2 were co-administered to the skin 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 ($\geq 98\%$), hydrochloric acid, acetic acid and sodium acetate were purchased from Sigma Aldrich, UK. Commercially available Ametop gels were supplied by AAH Pharmaceuticals, UK. The Ametop gel contained tetracaine, sodium hydroxide, sodium methyl-p-hydroxybenzoate, sodium propyl-p-hydroxybenzoate, monobasic potassium phosphate, xanthan gum, sodium chloride and purified water. Silica nanoparticles (NanoSiO_2), with a diameter of 200 nm (Psi-0.2), were obtained from Kisker Biotech GmbH and Co., Germany. Ultrapure water (18.2 M Ω) 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.

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. The xanthan gum formulation used the commercial Ametop formulation and was used as supplied and did not need further characterisation.

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.

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

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

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

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).

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

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

Formulation rheology

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 (this stress was in the linear viscoelastic region) 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 permeation 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 [27]. 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 [28]. The samples were thawed before use.

The permeation studies were carried out using upright individually calibrated Franz diffusion cells with an average of $2.1 \pm 0.1 \text{ cm}^2$ surface areas and $9.2 \pm 0.5 \text{ mL}$ 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 permeation studies to mimic the skin environment. Sink conditions were maintained throughout the permeation studies (drug concentration in the receiver fluid does not exceed 10% of its saturated solubility). The saturated solubility of tetracaine was $(6.29 \pm 0.07) \text{ mM}$ in pH 8 water and $(7.16 \pm 0.05) \text{ mM}$ in phosphate buffered saline. The permeation studies were performed on a submersible 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 (151 mM to ensure drug loading was matched across 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, an aqueous tetracaine solution of pH 8 was compared to a HPMC formulation (developed in the study) and the xanthan gum gel. None of the systems contained nanoparticles, as the initial 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 permeation study used 10 μ L, of xanthan gum gel or 3% HPMC formulation. The exact weight of the donor solution applied corresponded to 4.87 and 4.85 mg/cm² respectively. To these two 151 mM drug-loaded gels, an equal amount (10 μ L) of silica nanoparticles (NanoSiO₂) 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 [29]:

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

where k_p represents the permeability coefficient of the permeant across the membrane, J is the flux 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 permeation rate of tetracaine from the tetracaine solutions and tetracaine-nanoparticle gel mixtures respectively. The accumulative mass of tetracaine permeated 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 (Hewlett-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 permeation studies (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 $\pm$ 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. Permeation 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 pairwise 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 cm²) 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 ($99.83 \pm 0.05\%$). 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 cm²), 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 permeation studies

Infinite doses of tetracaine were used to understand the effect of vehicle composition on tetracaine permeation (Table 2, Fig. S2 for permeation profiles). According to manufacturer's data, the xanthan gum gel consisted of saturated concentrations of tetracaine with sodium hydroxide, sodium methyl-o-hydroxybenzoate, sodium propyl-p-hydroxybenzoate, monobasic 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 permeation rate (110.23 ± 40.93 µg/cm²/h) and lag time (9.15 ± 2.05 min) when infinite doses of tetracaine were delivered by xanthan gum gel and the HPMC formulation compared to a simple saturated tetracaine solution, i.e. with no formulation additives.

Finite permeation studies

Finite studies were performed to study the permeation of tetracaine from the semi-solid formulations using a dosing regimen that allowed formulation drying on the skin surface, which mimicked clinical application conditions (Table 3, Fig S3 and S4 for permeation profiles of xanthan gum and HPMC formulations respectively). Based on manufacturer's data, the NanoSiO₂, 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 NanoSiO₂, had the shortest permeation lag time (2.02 ± 0.79 min). In addition, the tetracaine permeation rate (109.95 ± 28.63 $\mu\text{g}/\text{cm}^2/\text{h}$) 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 NanoSiO₂. 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 $\mu\text{g}/\text{cm}^2/\text{h}$) 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 NanoSiO₂ significantly enhanced ($p < 0.05$) percutaneous tetracaine permeation by 3.6 fold when added to the HPMC formulation (Table 3). In addition, the NanoSiO₂ significantly increased the accumulative mass at 45 min ($m_{45\text{min}}$) 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 xanthan gum 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 NanoSiO₂ addition to the xanthan gum formulation increased the skin permeation by 2.7. However, of the two gels tested the HPMC formulation was superior, it significantly enhanced the tetracaine flux ($46.99 \pm 7.96 \mu\text{g}/\text{cm}^2/\text{h}$) by 40-fold, increased accumulative mass ($31.13 \pm 4.04 \mu\text{g}$) by 124-fold and reduced lag time ($7.09 \pm 1.80 \text{ min}$) by 8-fold compared to the xanthan gum 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). The xanthan gum formulations behaved as an elastic semi-solid ($G' > G''$) while the HPMC formulations behaved as a viscous liquid ($G'' > G'$). The addition of water in both formulations significantly decreased ($p < 0.05$) G' and G'' values which indicated the macromolecular interactions in both systems were weakened. However, there were no significant changes ($p > 0.05$) in rheological behaviour when the additions of water and NanoSiO₂ to the gels were compared.

Discussion

Loading tetracaine into two semi-solid formulations, one containing HPMC and a second containing xanthan gum showed no important differences in terms of the drug

permeation when an infinite dose was applied to the surface of the skin. The data suggested that when the drug and excipient supply did not deplete on the surface of the skin the xanthan gum and the HPMC did not have a large effect on tetracaine delivery; the two formulations delivered the drug with a similar rate to the saturated solution. Both formulations presented the drug to the skin surface as an aggregate (the drug concentration in the formulation was above its measured critical aggregation concentration at pH 8 [5]) and the experimental data suggests that the drug aggregates moved through the gel at the same rates in the two different gel systems. These results accord with previous studies that have shown that gelling agents can be considered to be relatively inert [30, 31]. However, there have also been instances reported where HPMC has been actively involved in the process of drug delivery from the topical formulations. For example, in supersaturated conditions, HPMC has been shown to interact with a drug and act as an anti-nucleating agent [32-34]. The different effects of HPMC in semi-solid gels appear to be linked to the nature of the drug which the polymer delivers. In the case of tetracaine, it presents as positively charged aggregate which has strong self-association at pH 8 and this may minimise the drug's interactions the macromolecules used to form topical gels [6]. As the gels showed no important differences, the nanomaterials were not added to infinite dosing studies.

In the finite dosing studies the HPMC formulation showed a far superior penetration rate into the skin compared to the xanthan gum formulation, which did not align with the infinite dosing data. Differences between infinite and finite dosing protocols have been previously assigned to the increased sensitivity of the drug to its

environment when it begins to deplete in the formulation [35]. For example, in a study by Cross et al. [36], 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.

As the gels showed a difference in the finite studies, the silica nanomaterials, which displayed a negative surface charge, were added to these systems to understand their effects. The nanomaterials were applied to the skin just after application of the drug-loaded gels and these systems were compared to equivalent semi-solid formulations added to the skin with an aliquot of pH-adjusted water in order to account for the dilution effects that arose as a consequence of the dosing protocol. Both the xanthan gum and the HPMC gels showed enhanced drug permeation into the skin when mixed with the nanomaterials. Based on these results and the fact that the silica particles are known to be able to adsorb tetracaine [11], it was assumed that the drug permeation enhancement induced by the addition of the NanoSiO_2 was attributed to the breaking up of the tetracaine aggregates due to weak surface interactions between the negatively charged NanoSiO_2 surfaces and the positively charged tetracaine molecules. The fact that the HPMC + NanoSiO_2 showed 100-fold increase in drug flux compared to the xanthan gum formulation that is employed commercially suggested that this could be a clinically important means to enhance tetracaine permeation into the skin.

The addition of the silica particles did not change the rheological properties of the formulations and therefore, it seemed reasonable to suggest that the differences observed between the finite and infinite dosing studies should be assigned to the different influence

of the drug-vehicle agent interactions in the two types of experiments, rather than the nanoparticle-vehicle interactions because nanoparticle-vehicle interactions typically increase macromolecular entanglement and hence are translated into changes in the formulation rheological profile. For example, Moddarsi et al. [15] showed an increase in topical formulation viscoelasticity when lipid nanoparticles were added to hyaluronic acid vehicle. However, it made sense, that neither HPMC or xanthan gum would interact with the nanomaterials used in this work given that the HPMC gel was neutral and the xanthan gum was negatively charged and so they would presumably have limited interactions with the negatively charge nanomaterials [37].

The xanthan gum gel appeared to be perturbed more than the HPMC formulation by the addition of an aliquot of water in the rheology studies and this provides some clues as to what effects the gels were having in the finite permeation studies. Xanthan gum is a branched, high molecular weight polysaccharide with acidic characteristics that forms a gel due xanthan chain dehydration [38]. Hydration of the xanthan gel by the addition of water would weaken the intermolecular links and reduce the diffusional restriction imparted upon the tetracaine molecules. In the finite studies, it is likely that solvent evaporation would cause the reverse effects, i.e., an increase in gel dehydration, which would strengthen the intermolecular interactions and impart a greater diffusional restriction on the drug. HPMC in contrast is a very weak gel [39] that forms as a consequence of the amphiphilic characteristics of macromolecule chains providing connections between hydrophobic and hydrophilic sites of the polymer. Although adding water to HPMC would reduce its viscosity, like other neutral polymers, it is likely that

this gel would allow drug permeation to proceed independently of the gel viscosity because the mesh size of the gel is large and probably would not change much upon dilution [40, 41]. As a consequence, in the finite dosing study, the loss of water upon drying on the skin from the HPMC gel and the subsequent increase in viscosity would probably have a very limited effect on the drug permeation behaviour. The differences in the structures of the gels and their capability to interact with the drug to restrict its diffusion is also probably enhanced by the fact the HPMC gel is neutral, the xanthan gum gel is negatively charged at alkaline pHs and the drug is positively charged. Therefore, it is likely that tetracaine would display electrostatic interactions with xanthan gum gel and this would hinder the drug diffusion.

Conclusions

Through a combination of skin permeation studies and rheological assessment of nanoparticle-loaded HPMC and xanthan gum gels, this study shed more light on the complex relationship between the nanoparticle-vehicle and drug-vehicle interactions. The experimental data indicated that the interactions of the silica nanoparticles with the gel used to administer the drug to the skin were less important than the drug-vehicle interactions. Xanthan gum appeared to restrict the diffusion of the tetracaine due the dehydration of the formulation when applied to the surface of the skin which increased the gels intermolecular interactions and allowed the -ve charged gel interact with the +ve charged drug. In contrast, the HPMC gel formed a matrix that did not have a great impact on the drug permeation into the skin due to the weak gel that was formed and the inability for this system to display electrostatic interactions with the drug. Although it appeared

that the nanoparticles used in this work showed robust penetration enhancing characteristics when combined with tetracaine, i.e., they functioned to enhance the penetration of the drug into the skin from both gels, a weak, uncharged gel seemed to be the optimal system to deliver this drug into the skin.

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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 $\pm$ standard deviation, $n=3$.

Fig. 2 Storage modulus (G' , top) and loss modulus (G'' , bottom) measured as a function of frequency (Hz) for the xanthan gum gel (■), xanthan gum gel + water (●), xanthan gum gel + silica nanoparticles, NanoSiO_2 (□), HPMC gel (▼), HPMC gel + water (◇), HPMC gel + NanoSiO_2 (◻). Data points represent mean $\pm$ standard deviation, $n=3$. The lines of the xanthan gum gel with and without the addition of water overlay each other. The same applies for the HPMC gel.

Table 1. Characteristics of various HPMC gels. Data represent mean $\pm$ 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 $\pm$ 0.04	0.27 $\pm$ 0.03	0.25 $\pm$ 0.04
Spray mass (mg)	157.7 $\pm$ 14.1	66.7 $\pm$ 43.7*	145.5 $\pm$ 27.5
Spray recovery (%)	99.83 $\pm$ 0.05	99.72 $\pm$ 0.09*	99.92 $\pm$ 0.02
Nozzle recovery (mg)	31.4 $\pm$ 18.2*	66.0 $\pm$ 15.3*	0.3 $\pm$ 0.1
D _{mean} (cm)	3.5 $\pm$ 0.1*	2.4 $\pm$ 0.3	2.7 $\pm$ 0.1
Area (cm ²)	9.8 $\pm$ 0.6*	4.5 $\pm$ 1.1	5.9 $\pm$ 0.5
Aspect ratio	1.0 $\pm$ 0.0	0.9 $\pm$ 0.1	0.7 $\pm$ 0.1

Table 2. Steady state flux and permeability constants, k_p , accumulative mass at 45 minutes, $m_{45\text{min}}$, and lag time, t_{lag} , of infinite dosages of tetracaine formulated in a xanthan gum gel and HPMC gel at pH 8 across porcine epidermis membrane. Data represent mean $\pm$ standard deviation of 3 independent tetracaine samples.

	Flux ($\mu\text{g}/\text{cm}^2/\text{h}$)	k_p ($10^{-3} \text{ cm}/\text{h}$)	$m_{45\text{min}}$ (μg)	t_{lag} (min)
Saturated solution	110.23 ± 40.93	66.20 ± 24.58	64.5 ± 21.11	9.15 ± 2.05
Xanthan gum gel	105.36 ± 21.97	63.27 ± 13.19	59.80 ± 9.31	10.10 ± 1.50
HPMC gel	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, $m_{45\text{min}}$, and lag time, t_{lag} , of finite dosages of tetracaine loaded in xanthan gum and HPMC gels at pH 8 across porcine epidermis membrane. Data represent mean $\pm$ standard deviation of 3 independent tetracaine samples. * Significant differences, one-way ANOVA.

	Flux ($\mu\text{g}/\text{cm}^2/\text{h}$)	ER	$m_{45\text{min}}$ (μg)	t_{lag} (min)
Xanthan gum	1.16 ± 0.14	-	0.25 ± 0.06	31.60 ± 3.00
Xanthan gum + water	5.62 ± 2.25	$4.71 \pm 1.40^*$	$2.53 \pm 1.77^*$	$20.20 \pm 1.34^*$
Xanthan gum + NanoSiO ₂	14.19 ± 2.27	$12.86 \pm 3.16^*$	$8.12 \pm 1.21^*$	$10.69 \pm 1.98^*$
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	$3.97 \pm 1.07^*$
HPMC + NanoSiO ₂	109.95 ± 28.63	$2.48 \pm 1.08^*$	$76.83 \pm 18.92^*$	$2.02 \pm 0.79^*$