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RESEARCH PAPER

Triggered *In Situ* Drug Supersaturation and Hydrophilic Matrix Self-Assembly

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ABSTRACT

Purpose To understand *in situ* drug thermodynamic activity when embedded in a supramolecular structured hydrophilic matrix that simultaneously self-assembled during drug supersaturation.

Methods A propylene glycol (PG)/water, hydroxypropyl methyl cellulose matrix containing ethanol was used to support diclofenac supersaturation. Phase behaviour, thermodynamics and drug transport were assessed through the determination of evaporation kinetics, supersaturation kinetics and transmembrane penetration.

Results Initial ethanol evaporation from the drug loaded matrix (2.9 ± 0.4 mg.min^{-1}.cm^{-2}) was comparable to that of the pure solvent (*ca.* 3 mg.min^{-1}.cm^{-2}). When 25% w/w of the total ethanol from the applied phase was lost (ethanol/water/PG molar ratio of 7:5:1.2), an inflection point in the evaporation profile and a sudden decrease in drug solubility demonstrated that a defined supramolecular structure was formed. The 55-fold decrease in drug solubility observed over the subsequent 8 h drove *in situ* supersaturation, the rate of which was a function of the drug load in the matrix ($y=0.0078x$, $R^2 < 0.99$).

Conclusion The self-assembling supramolecular matrix prevented drug re-crystallisation for >24 h, but did not hinder mobility and this allowed the thermodynamic activity of the drug to be directly translated into highly efficient transmembrane penetration.

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KEY WORDS diclofenac · hydrophilic matrix · non-ideal mixing · solvent-solvent interaction · supersaturation · topical drug delivery · transmembrane transport

INTRODUCTION

The vast majority of pharmaceutically active agents must penetrate a barrier in order to elicit their therapeutic effects and this process most commonly occurs via a passive mechanism. The rate of barrier transport, when driven passively, has been shown to be proportional to the level of drug saturation at the apical surface of that barrier, that is, the concentration of drug divided by its saturated solubility in the media in which it is dissolved (1–3). As a consequence, manipulating the degree of drug saturation in an administration vehicle can be used to control drug delivery if the membrane penetration of the agent is the rate limiting step in the process (1,2,4). Forcing the loading of a drug beyond its saturated solubility in an administration vehicle, by rapidly dissolving a high energy solid, inducing changes in solution temperature or the mixing of a solvent-antisolvent system, generates drug supersaturation and this can enhance passive transmembrane transport at the most common entry points for drugs into the body i.e. via the gastrointestinal tract, skin, lung, nose or nail (2,5).

Using preformed drug supersaturated solutions to administer therapeutic agents in a clinical setting can be problematic as they have a tendency to re-crystallise upon storage (typically within 24 h from formation of the supersaturation). Preparing drug administration systems using volatile solvents that induce drug supersaturation *in situ*, that is, only after dose actuation, is one means to solve the issue of premature re-crystallisation (6–10). When delivering a drug using an *in situ* supersaturating system a fine balance exists between maintaining drug supersaturation physical stability, in order to prevent loss of thermodynamic activity post

delivery, and drug mobility in the application solvent, because volatile evaporation often controls both saturation kinetics and application matrix viscosity. Previous work has demonstrated a degree of drug supersaturation (DS) of up to 26 can be supported in matrixes generated by volatile sprays, but the restrictions imposed on the drug mobility when drug supersaturation occur rapidly can limit the duration of which this enhanced thermodynamic activity is translated into efficient drug transport (11). Reducing the speed of the drug supersaturation can reduce the effects of vehicle viscosity and prolong the functional benefit of drug supersaturation but this typically also reduces the maximum DS obtained by the applied formulation (12–14).

The manner in which the components of an *in situ* supersaturated administration system interact in the solution-state is critical to the drug's conformational freedom in the residual phase. For example, adding poly(ethylene glycol) 400 (PEG 400) (9) and poly(vinyl pyrrolidone) (PVP) (10) into an application vehicle prevents the initiation of drug nucleation during supersaturation, but because these components achieve this physical stabilisation effect via direct hydrogen bond formation with the drug, the resultant supramolecular matrix embeds the drug within it (15). In this scenario the inverse relationship between the vehicle viscosity and drug mobility, that is well-known in polymer solutions, can be applied (Stokes-Eistein equation), suprastructure formation results in drug self-diffusion coefficient and thus transport retardation (16–18). In contrast to dilute polymer solutions the suprastructure of co-solvent systems do not always embed the drug within its matrix (19–21). For example, the propylene glycol (PG): water suprastructure does not embed unionised diclofenac (22). This previous work demonstrated that at PG concentrations of ≥50%v/v the supportive hydrophilic matrix is formed but the suprastructuring in this matrix is such that drug diffusion is not retarded (21,22). The generation of such an environment using high volatile sprays may be ideal to support rapid drug supersaturation in the absence of effects on drug mobility.

The aim of this study was to understand how the residual phase supramolecular structure and drug supersaturation kinetics influenced drug transport using hydrophilic PG/water/hydroxypropylcellulose (HPMC) matrixes. Diclofenac was selected as the model drug as it had previously been shown to display distinct interactions with the supramolecular structures of the PG/water system (21,22). The DS was controlled by varying the diclofenac load in the matrix and hydrofluroalkane (HFA) and ethanol were employed as the volatile constituents. Sprays were formed such that the drug would become supersaturated *in situ* in a residual phase consisting of PG, water and HPMC. In such a system, both the drug saturation and the matrix suprastructure would change post administration and the experimental design attempted to assess both these phenomena through the measurement of 3 supersaturation reference points, these were defined as: a degree of saturation (DS) calculated theoretically (DS_{cal}), a DS calculated in the solution-state using the solution evaporation kinetics (DS_{sol}), and a DS calculated at the membrane interface using the transport studies (DS_{mem}). A series of preformed PG/water/HPMC drug solutions at various levels of drug saturation acted as controls for the study. As the primary focus of this work was to investigate the principles governing the supersaturation kinetics and solvent superstructure using *in situ* supersaturating sprays, a silicone membrane was employed as the barrier for the transport studies and it was not the intention of this work to mimic application of the spray to any specific biological barrier.

MATERIALS AND METHODS

Materials

Diclofenac, as its diethylamine salt (melting point of 154°C, BP grade, 99.9%) was donated by Unique Chemicals, India. The drug was used in its unionised form throughout this study and will therefore be referred to as diclofenac. Propylene glycol (PG) (≥ 99.5%) was supplied from Sigma Aldrich, UK. Hydroxypropyl methyl cellulose (HPMC) grade 65SH (Metolose, viscosity 50 cP) was provided by Shin-Etsu Chemical Ltd, Japan. The propellant hydrofluoroalkane (HFA 134a) was supplied by Solvay (Hanover, Germany). Acetonitrile and methanol (high performance liquid chromatography (HPLC) grade) were obtained from Fisher Scientific International, UK. Ethanol (99.7–100% *v/v*) was provided by BDH Laboratory Supplies, UK. Formic acid (analytical grade, ≥ 96%) was obtained from Sigma Aldrich, UK and phosphate buffered saline (PBS, pH 7.2, 0.172 M) tablets were supplied by Oxoid Ltd, UK. Sheets of silicone membrane (Folioxane®), with a thickness of 120 μm, were purchased from Novatech Ltd, France. De-ionised water (electrical conductivity 0.5–1 μS) was used throughout this study.

Test Solution Preparation

Preformed Drug Supersaturated Calibration Solutions

PG/water (50:50 v:v) solutions (SS) of a predefined DS were produced by mixing equal volumes of a PG (the final pH of the PG solutions was adjusted to 3 ± 0.1 with phosphoric acid), and PBS (pH 3) solution containing 3%w/v HPMC (physical stability maintained for at least 36 h, data not shown), in a glass vial. All the solutions were prepared on v/v basis using a calibrated positive displacement pipette (Gilson Microman 1 channel Positive Displacement Pipette, Gilson Inc., USA). The absence of crystal formation in the calibration solutions was confirmed visually using an inverted microscope (Wilovert S, Hund Wetzlar, Germany) at magnification of 200× by

monitoring a 1 ml aliquot of the solutions in a multiwell plate (Greiner® multiwell plates, Sigma Ltd., UK) for 36 h.

In Situ Supersaturating Sprays

Aerosol sprays that formed a drug supersaturated solution *in situ* (ISA) were composed of 5% PG, 5% HPMC solution (containing 3% w/v HPMC in PBS), 35% EtOH and 55% HFA (all w/w). The appropriate amount of diclofenac to generate a DS_{cal} of 5, 10, 20, 40 and 60 (ISA_5, ISA_{10}, ISA_{20}, ISA_{40}, ISA_{60}) after the complete removal of ethanol and HFA equated to adding 0.0044, 0.0087, 0.0174, 0.0348, 0.0522% w/w of diclofenac (calculated theoretically using Eq. 2) to each spray, respectively. All the components were weighed directly into a 10 ml Purgard® canister (Adelphi Tubes, Haywards Heath, UK) at the desired w/w ratios. A 13 mm magnetic flea was added to each canister, and the final pH was checked and adjusted to pH 3±0.1 using phosphoric acid prior to the canisters being sealed with a 100 μL metered valve (Bespak Europe Ltd, UK). HFA 134a was pressure-filled into the sealed glass canister using metered dose aerosol filler (Model 2016, Pamasol Willi Mader AG, Pfäffikon, Switzerland). The sprays were vigorously stirred for 1 min and inspected visually for formulation miscibility and drug stability. The absence of crystal growth in the residual phases of the formulations after dose actuation was again confirmed visually for at least 36 h post dose actuation using the optical microscope (Wilovert S, Hund Wetzlar, Germany) at a magnification of 200× as described previously.

Evaporation Kinetics

Sixty actuations from the ISA_5 and ISA_{40} systems were applied to a tared weighting boat that was placed on an analytical balance (type R160 P, Sartorius, Goettingen, Germany). The formulation weight loss post spray application was monitored and recorded, over 30 h at room temperature (21±1°C). The formulation weight at 30 h was assumed to be equivalent to the weight of the residual mixed cosolvent phase in the absence of ethanol (PG:PBS solution containing 3%w/v HPMC) and this was termed WF_f. Assuming HFA evaporated in the first 2 min of the experiment (9,11) and water did not evaporate to any significant degree, due to involvement in the supersturaturing, allows the EtOH weight to be estimated by subtracting the final weight of the formulation from the weight at any time point after applying the spray (WF_t), hence the changing composition (% w/w) of the residual phase over time could be tracked using Eq. 1:

$$\%WEt_t = \frac{WF_t - WF_f}{WF_f} \times 100 \quad (1)$$

% WEt_t was the % weight of EtOH in the residual phase at a given time (t).

Transmembrane Transport

Infinite dose transmembrane transport studies were conducted using individually calibrated upright Franz diffusion cells (MedPharm Ltd, UK) with diffusional area of ~2.1 cm^2 and receptor volume of ~10.8 ml. Silicone membrane (measured thickness of 124.17 μm±6.01 (n=54) was fitted, and sealed between the two diffusion cell chambers. The receptor compartment was filled with a previously sonicated and filtered receiver phase consisting of 20:80 EtOH:PBS (pH 7.4). The cells were placed in a temperature controlled water bath (Grant SSD40, Grant Instruments, Chelmsford, UK) at 29°C to obtain 25°C at the membrane interface (this temperature was selected in attempt to maintain consistency across the evaporation, solubility and transport studies). The membrane interface temperature was measured using a wire probe attached to a thermocouple (Hanna Instruments, UK) which was placed at the membrane surface. Uniform mixing of the receiver phase was achieved with the aid of magnetic followers and magnetic stirring bed (Grant SSD40, Grant Instruments, UK). The cells were equilibrated, inverted and visually checked for leaks prior to the initiation of the transport studies. A 1 ml aliquot of the preformed transport calibration solutions; $SS_{2.5}$, $SS_{3.4}$, SS_5, SS_7, SS_{10} and SS_{15}, or sixty actuations of the *in situ* supersaturating sprays; ISA_5, ISA_{10}, ISA_{20}, ISA_{40} and ISA_{60} were applied to the apical surface of the silicone membrane. A transfer tube was fitted on the actuation valve and a constant 10.5 cm distance between the membrane surface and the spray was maintained to avoid loss of actuated dose outside the donor chamber. Diclofenac transport across silicone membrane was monitored by withdrawing aliquots (1 ml) of the receiver medium, at appropriate intervals, and assaying the drug content by HPLC. The withdrawn volume was immediately replaced by an equal volume of prethermostated receptor solution.

Determination of the Degree of Saturation

Calculated Degree of Saturation

Since the maximum equilibrium solubility of the diclofenac in the 50:50 co-solvent was known, the theoretical degree of diclofenac saturation in the absence of the volatile EtOH and HFA components could be predicted, as per Eq. 2:

$$DS_{cal} = \frac{[Dic]_{PG}}{[Dic]_{eq} \times DF} \quad (2)$$

where $[Dic]_{PG}$ was the initial diclofenac concentration ($mg.ml^{-1}$) in PG, $[Dic]_{eq}$ the saturated equilibrium solubility ($mg.ml^{-1}$) in a 50:50 v:v cosolvent solution (drug saturated data obtained from Benaouda *et al.* (21)), DF was the

dilution factor (in this case, DF was 2) and DS_{cal} was the theoretically calculated DS. The DS_{cal} was calculated assuming no crystal formulation occurred and instantaneous molecular mixing of the PG solution and the antisolvent solution (i.e. 3%w/v HPMC in PBS (pH 3)) was achieved.

Solution-State Degree of Saturation

Since the amount of drug (mg) applied in the spray was known and did not change over time, the drug concentration and thus the *in situ* supersaturation kinetics could be determined from the knowledge of the residual phase composition, gained by the gravimetric measurement, according to equation 3:

$$DS_{sol} = \frac{WD_{App}/WF_t}{C_{ss}} \tag{3}$$

where DS_{sol} was the degree of drug saturation in solution determined from the gravimetric measurement of the formulation, WD_{App} was the weight of the drug applied (g), WF_t the weight of the formulation at time t, and C_{ss} the saturated solubility of drug in the applied phase at time t. The C_{ss} in the applied phase at different time points was experimentally determined by adding excess DDEA to the mixture with the appropriate weight ratio of PG, HPMC solution (pH 3) and EtOH. Each sample was stirred for 48 h in sealed vials at room temperature (21±2°C). The mixtures were then centrifuged at 13000 rpm for 20 min (Biofuge pico Heraeus Instruments, Germany), the drug-saturated supernatant was diluted and drug content assayed by HPLC.

Membrane Interface Degree of Saturation

The Membrane interface DS (DS_{mem}) produced *in situ* using the ISAs was calculated via a calibration curve generated from the membrane transport enhancement ratio. This was constructed by the comparison of the rate of drug mass transfer from the supersaturated solutions (SS) with the compositionally similar saturated solutions through a silicone membrane, according to equation 4:

$$DS_{mem} = \frac{Flux_{\sup}}{Flux_{sat}} \tag{4}$$

Where $Flux_{sup}$ was the steady-state transport rate across silicone obtained membrane from supersaturated solutions and $Flux_{sat}$ the steady-state transport rate from the drug saturated control. The calibration solutions were of equivalent composition to that of the supersaturated formulation, in order to account for drug-vehicle interactions (21).

Diclofenac Assay

A HPLC method that had previously been shown to be fit for purpose in terms of accuracy, reproducibility and limit of detection was employed for this study (21). The method used a mobile phase flow rate of 1.2 $ml.min^{-1}$, a UV detector (Jasco Corporation Ltd, UK) at a detection wavelength of 275 nm, a Gemini column (250 x 4.6 mm) and a mobile phase that comprised acetonitrile:methanol:formate buffer (25 mM) (50:20:30%v/v), pH 3.5. Samples of 20 μl were injected by an autosampler (Jasco Corporation Ltd, UK). The retention time for diclofenac was approximately 7.6 min and the calibration curves were constructed on the basis of the peak area measurements, using standard solutions of known diclofenac concentrations dissolved in an identical fluid as the receiver phase for the permeation studies. Cumulative amounts of diclofenac penetrating into the receiver chamber per unit surface area ($\mu g.cm^{-2}$), corrected for sample removal, were plotted against time (h), and the steady state flux (J) was calculated from determination of the slope of the linear portion of the curve ($R^2 \geq 0.97$), using at least five points with values above LOD (0.68 $\mu g.ml^{-1}$ $n=30$).

Statistical Analysis

All values were expressed as their mean±standard deviation. When statistics were employed to compare the data, the data were checked in terms of normality (Sapiro-Wilk) and homogeneity of variances (Levene's test) and if appropriate one way analysis of variance (ANOVA) was employed (using post-hoc, Tukey analysis where appropriate) using the statistical package for social sciences SPSS version 16.0 (SPSS Inc., Chicago, IL. USA).

RESULTS AND DISCUSSION

Volatile Evaporation Triggered Superstructure Formation

The hydrophilic matrixes investigated in this work were composed of HFA, ethanol, water, HPMC and diclofenac. They were formed using a volatile spray which exposed the components to atmospheric conditions only upon dose actuation. The solvents respective enthalpies of vaporisation suggested that exit from the spray canister would lead to a three phase of evaporation, the first phase dominated by HFA loss (19.6 $kJ.mol^{-1}$ (23)) the second by ethanol (41.43 $kJ.mol^{-1}$ (24)) and the third by water (43.99 $kJ.mol^{-1}$ (25)). The gravimetric data recorded for the sprays did display a triphasic profile and the rate and extents of evaporation in each of the three phases suggested that applying theoretical model based upon respective enthalpies of evaporation to interpret the data was appropriate, but that the influence of the matrix

superstructure must be built into this model (Fig. 1, the drug load did not significantly alter the evaporation profile, $p>0.05$). The rapid loss of over one quarter of the formulation mass 2 min after the final spray was applied (ca. 27% of the applied dose at a rate=24.34±3.67 mg.min^{-1}, n=5) suggested HFA had been completely removed in this initial phase. Such a rapid mass loss has previously been attributed to HFA and the cross correlation of the mass, the rate and the time of applying the sprays both in this work and that in previous work suggested this highly volatile component had completely evaporated within the first 2 min after the final spray application (9,11). Between 2 and 120 min, a linear steady-state phase was noted in the solvent evaporation profile with a rate of 2.92±0.42 mg.min^{-1}.cm^{-2} (R^2=0.99, n=5). This rate was comparable to that of the pure ethanol solvent (*ca.* 3 mg.min^{-1}.cm^{-2} (11)) and thus it was highly likely that this phase of evaporation was consequence of 'free' ethanol loss, that is, ethanol not bound by the hydrophilic matrix suprastruture formed by the residual phase components. At 120 min, there was an inflection point in all the recorded evaporation profiles. Assuming the PG and water evaporative loss was negligible at this point in the profile and HFA had been removed completely the mass of the residual phase suggested a molar ratio composition of 7:5:1.2 (ethanol/water/PG; corresponding to a 0.64 volume fraction of ethanol). The ratio where the inflection in the evaporation kinetics was observed correlated with a marked decline in the drug saturated solubility (Fig. 1) and was consistent with that obtained previously for water- ethanol mixtures (26). A 0.7 ethanol volume fraction has also previously been shown to be important in ethanol water suprastructuring (27,28). Above a ethanol volume fraction of ca. 0.7 (27,28), it is suggested that the highly structured water clusters with hydrated ethanol is broken and ethanol-ethanol hydrogen bonding becomes predominant. Below ethanol co-solvent ratios of 0.7 solvent superstructuring of ethanol-water is more likely to occur (27,28). Such phenomenan resulting from the non-ideal mixing when simple alcohols are mixed with water is well documented (29–32). However, the hydrophilic matrix in the current work is complicated by the presence of PG which at 50:50 (v:v) has been also shown to sustain water structuring (21,33). The experimental observations, that is, the simultaneous change in drug solubility and the evaporation kinetics suggest the 7:5:1.2 (ethanol/water/PG) ratio was the point at which the relatively strong ethanol-ethanol hydrogen bonds in the residual phase of the sprays were disrupted by the assembly of a new supramolecular structure which integrated the evaporative solvent, retarding its loss from the formulation and providing a highly receptive environment for the diclofenac. Through analysis of the evaporation profiles alone it was impossible to determine if the drug was integrated into the new superstructure and direct spectroscopic analysis proved difficult, but subsequent alignment of this data with transport profiles allowed the functional effects of supramolecular formation on diffusion to be established.

In the third phase of the evaporation profile the mass of the hydrophilic matrix declined in a non-linear fashion from 120 min until 600 min post dose application, after which no statistical change in the mass was observed ($p>0.05$). Transforming the data (between 120 and 600 min) on a log-log plot provided an evaporation rate of 0.66 mg.min^{-1}.cm^{-2} (R^2=0.95, n=5) in this region. Water evaporation rate calculated using the equation proposed by Smith *et al.*, (34) yielded a rate of 0.275 mg.min^{-1} and hence if the diclofenac was simply dissolved in water only 8 h would be required for the amount of water applied in the formulation (0.06 g.cm^{-2}) to be lost. However, comparing total mass of the hydrophilic matrix after 600 min with the theoretical mass applied suggested that not only had the water not been lost up to 600 min time point but also 3% of the ethanol also remained. This supported the notion that a supramolecular matrix had been formed. The

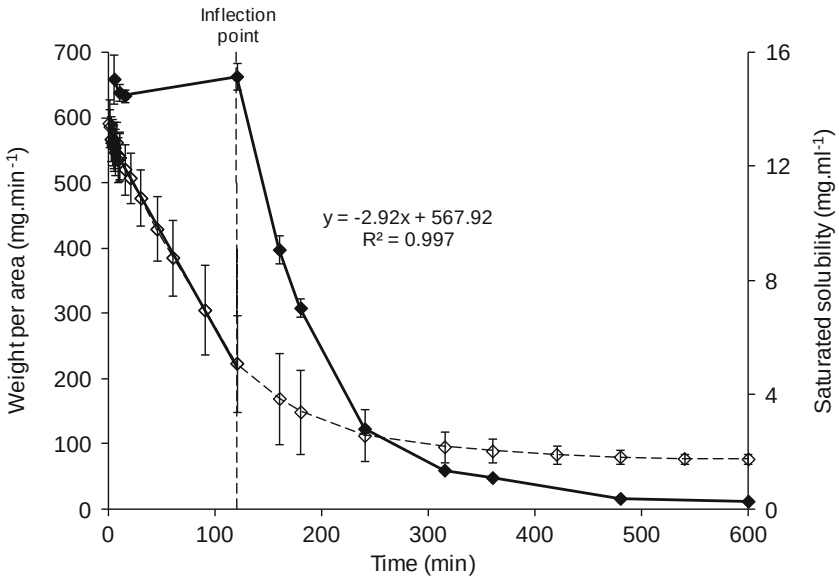

Fig. 1 Representative mean weight loss due to solvent evaporation from supramolecular structured matrix applied using the *in situ* supersaturating aerosols (◊) (n=5 ± SD) and diclofenac saturated solubility in the applied matrix over 600 min post-dose actuation (♦) (n=3 ± SD). The point at 120 min represents the critical point for the matrix supramolecular structure self-assembly.

PG/water were the least volatile components and most likely to form hydrogen bonds when the composition was moving towards a 50:50 mixture as reported by previous work (21). Such a suprastructure has the capacity to trap both the water and a small proportion of the ethanol in the matrix.

Solution-State Drug Supersaturation

Tracking drug supersaturation kinetics in the residual phase after dose actuation using the gravimetric method demonstrated that the DS_{sol} immediately upon application (0 to 2 min), did not change significantly ($p>0.05$). HFA evaporation was proposed to dominate this time period post application and as HFA did not alter the drug solubility no change in supersaturation was anticipated (Fig. 1). Likewise, despite the marked decrease in the amount of ethanol present in the applied phase between 2 and 120 min i.e. from 88 to 63% w/w, the DS_{sol} values remained constant ($p>0.05$) because a loss in the 'free' ethanol i.e. the ethanol that was not associated with the supramolecular matrix structure, did not have a big influence upon the drug solubility (drug-saturate concentration remained constant, $p>0.05$). At 120 min (i.e. 7:5:1.2 molar ratio of ethanol/water/PG), a sudden drop in diclofenac saturated solubility in the residual phase was observed and in the subsequent 8 h the drug saturated solubility declined 55-fold (i.e. when compared to 600 min post dose application). The kinetics of drug saturation fitted a non-linear regression model for all the *in situ* supersaturating systems (ISS) when measured between 120 and 600 min post spray application ($R^2<0.99$, Fig. 2) and the rate was directly proportional to the initial drug load of the sprays ($y=0.00787x$, $R^2<0.99$) (Fig. 2, inset). This direct correlation and the fact that the drug loading in the residual phase of the *in situ* supersaturating sprays did not significantly affect the evaporation profile (there was no statistical difference between the ISA_5 and ISA_{40} formulation weight loss at any time point ($p>0.05$)) demonstrated that the diclofenac was not embedded in the hydrophilic superstructure matrix.

The solubility of diclofenac in the applied phase and hence the DS_{sol} was very sensitive to evaporative solvent loss below the critical ratio of 7:5:1.2 (ethanol/water/PG) presumably because according to the respective molecules solubility parameters ($\delta_{dilcofenac}=11.49$ $(cal/cm^3)^{1/2}$ and $\delta_{ethanol}=12.7$ $(cal/cm^3)^{1/2}$) ethanol is a good solvent for the drug and its loss left the drug stranded in the less favourable water/PG mix. Accordingly, when the suprastructure had formed the *in situ* drug supersaturation kinetics closely followed the second phase of ethanol evaporation and this again provides a strong indication that it was ethanol evaporation that was dominant in the two phases of the triphasic mass loss and not water. The delayed evaporation kinetics of the ethanol bound in the suprastructured hydrophilic matrix led to retardation in the supersaturation kinetics compared to the normal rate expected if a simple ethanol solution was employed and there was a disparity between the DS_{cal} and the final DS_{sol}. The maximum DS_{sol} values calculated using the evaporation data at 600 min were 1.6, 3.2, 6.4, 12.7 and 19. These were 3-fold lower ($p<0.05$) than the DS_{cal} values of 5, 10, 20, 40 and 60, respectively. However, taking

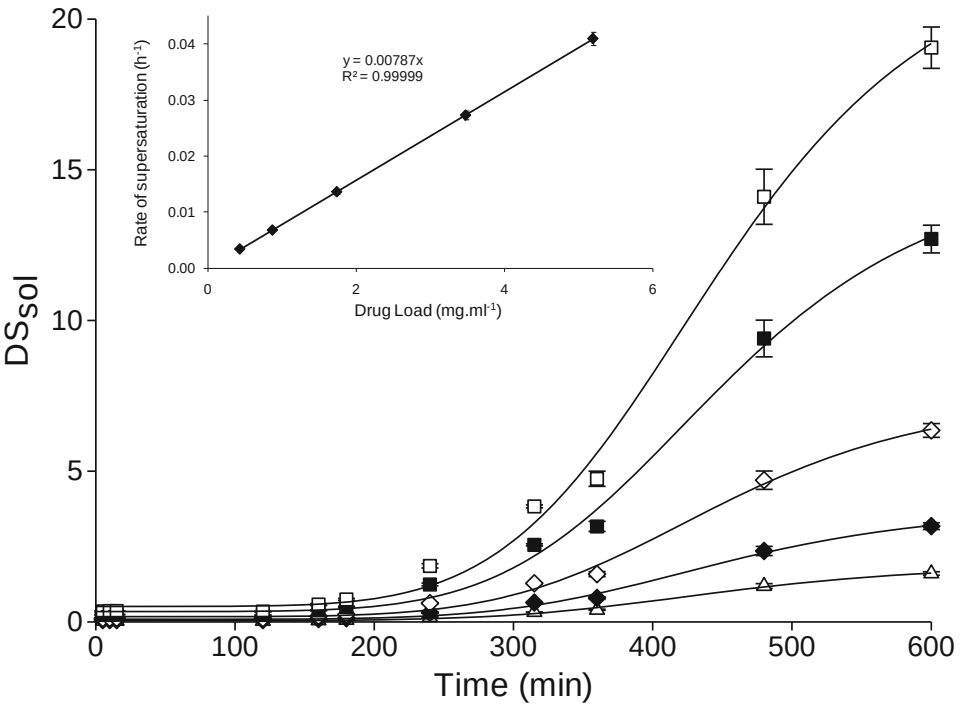

Fig. 2 Diclofenac supersaturation kinetics *in situ* when applied from the supersaturating aerosols (ISA); ISA_5 (Δ), ISA_{10} (♦), ISA_{20} (◊), ISA_{40} (■) and ISA_{60} (□). The supersaturation kinetics expressed as solution state degree of saturation (DS_{sol}) calculated gravimetrically, for the first 10 h after dose application ($n=3\pm SD$). The inset represents the rate of the supersaturation as a function of the initial drug load in each aerosol formulation.

account of the conclusions derived from the superstructure formation interpretation of the gravimetric data reported above the formulation composition at the 600 min time point most probably contained 2.95% w/w EtOH: 48.5% w/w PG: 48.8% HPMC solution (pH 3) and not a 50:50 EtOH: PG as the DS_{cal} assumed. Modelling the DS assuming that the EtOH was present changed the diclofenac solubility to 0.27 ± 0.01 mg.ml^{-1} (n=3). As this was 3-fold higher that the drug solubility used to calculate the theoretical DS at 0.087 ± 0.018 mg.ml^{-1} (n=3) it realigned the differences between the two DS values such that they were equivalent. This again supports the interpretation of the evaporation profiles and conclusion that both water and a small amount of ethanol are trapped in the suprastructured hydrophilic matrixes. Crystal growth could influence the interpretation of the data, but microscopic investigations of the final matrixes revealed no such growth and hence interpreting the data obtained from DS_{sol} and DS_{cal} using the triphasic model of mass loss was considered theoretically sound.

Transmembrane Transport

Infinite doses of the preformed diclofenac saturation calibration solutions (SS) achieved steady-state membrane permeation ($R^2 > 0.98$) almost instantly (Fig. 3a). However, sink conditions were only maintained for the first 4 h of the transport studies. After 4 h more than 12% of the applied drug had crossed the silicone membrane. When more than approximately 10% of the applied drug load is removed from the apical surface of the membrane dose depletion is thought to occur. A significant loss of drug from the donor solution decreases the drug thermodynamic activity in the donor phase and this can alter the rate of transmembrane penetration thus invalidating 'steady-state' conditions. As a consequence of the speed of drug permeation, the steady-state transmembrane transport rate of the SS solutions was determined from the linear portion of the transport profiles prior to the 4 h time point. The calibration solutions with a DS=1 did not show dose depletion, within the experimental time, and gave a steady-state transport rate of 1.69 ± 0.13 μg.cm^{-2}.h^{-1}. A direct comparison of the preformed drug supersaturated calibration solutions to the saturated solution (i.e. DS=1) showed a significant increase in transmembrane transport rate ($p < 0.05$), for each of the SS solutions. The magnitude of this relative increase, displayed as the ER value, was directly proportional to the DS_{sol} ($R^2 = 0.99$) until the DS_{sol} reached 10 (Fig. 3b). Therefore, the derived equation (y=0.96x – 0.14) in the range of 1–10 could be used as a calibration curve to determine the actual degree of drug saturation at the surface of the membrane. The supersaturated solution (SS_{15}), formulated at a DS_{cal} of 15, deviated from this linear trend and gave a transport rate

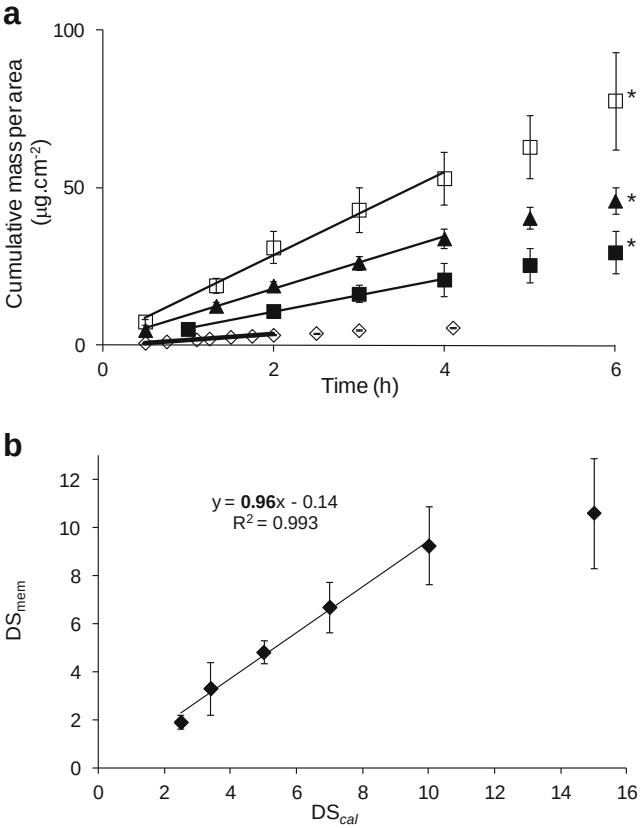

Fig. 3 (**a**) Diclofenac permeation profiles across silicone membrane at 25°C from a saturated solution of 50:50 propylene glycol (PG):phosphate buffered saline (PBS) (pH3) (control) (◊) and *in situ* supersaturation, generated from mixing drug in PG solution with PBS solution containing 3%w/v HPMC, with theoretical degrees of saturation (DS) 3.4 (■), DS 5 (▲) and DS 10 (□) ($n=5 \pm$ SD). (**b**) The correlation between the calculated DS (DS_{cal}) and the membrane interface DS (DS_{mem}) ($n=5 \pm$ SD). * represents statistical difference compared to the saturated control solution ($p < 0.05$).

that suggested that the actual DS at the membrane interface (i.e. DS_{mem}) was 10.63 ± 2.29. Such a deviation can be due to any of the conditions associated with the linear relationship described by Higuchi not being met, the most probable explanation in this case is an uneven distribution thermodynamic activity in the solutions which is more likely at such high DS values.

The *in situ* supersaturating formulations generated drug transmembrane transport profiles that contained two distinct transport rates irrespective of the initial concentration of diclofenac applied (Fig. 4a). The first phase was from 0–10 h post dose application and the second from 10–22 h post dose application (Fig. 4b). Steady-state transport rate was measured when formulation supersaturation was established, that is, the second of these regions according to the evaporation studies. The steady-state transport rate, obtained from all the tested ISAs, was significantly greater ($p < 0.05$) than that from the preformed drug saturated control (1.69 ± 0.13 μg.cm^{-2}.h^{-1})

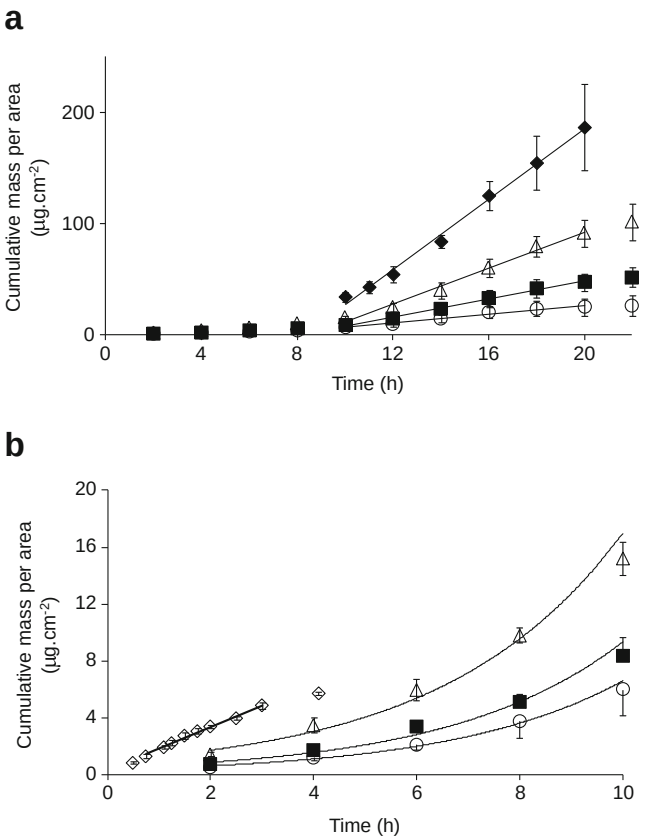

Fig. 4 (**a**) Diclofenac permeation across silicone membrane when applied to the apical surface as a saturated solution containing 50:50 propylene glycol (PG): phosphate buffered saline (PBS) (pH3) (control) (◊) and when applied using the *in situ* supersaturating aerosols (ISA) with different degrees of saturation (DS). (**b**) is an expansion of the full data set (**a**) for the 10 first hours post-dose application. (◊) represents the drug-saturated control solution, (○) ISA_5, (■) ISA_{10}, (Δ) ISA_{20} and (♦) ISA_{40} ($n=5\pm SD$). ISA_{60} was not included in the figure for clarity.

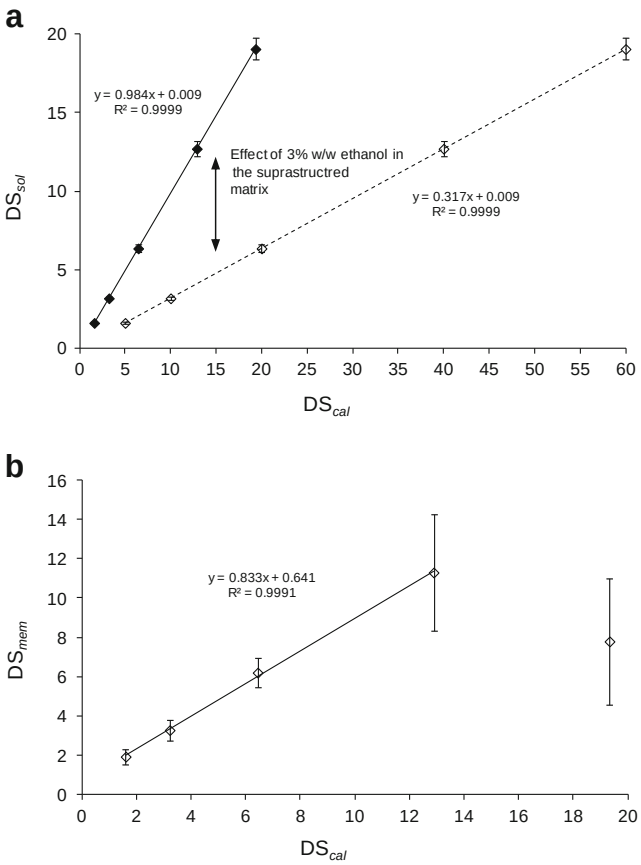

Fig. 5 (**a**) The correlation between the solution state degree of saturation (DS_{sol}) and the theoretically calculated DS (DS_{cal}) before accounting for the ethanol remaining in the deposited suprastructured matrix (◊) and after correcting for the ethanol remaining in the matrix (♦). (**b**) The correlation between the membrane interface degree of diclofenac saturation (DS_{mem}) and the corrected DS derived from the theoretical calculation (DS_{cal}) of drug supersaturation generated from the *in situ* supersaturating aerosols, at 25°C ($n=3\pm SD$ for DS_{sol} and $n=5\pm SD$ for DS_{mem}).

(Fig. 4a). Increasing the drug loading of the spray, hence the DS_{cal} of the residual phase produced a consequent increase in the steady-state transport rate of diclofenac. This relationship was linear (R^2=0.999), up to theoretical DS_{cal} 40 (data not shown graphically). When the DS_{cal} was corrected for the presence of ethanol and plotted against DS_{sol} it was directly proportional (R^2=0.98, gradient =0.99), likewise the DS_{mem} showed a linear correlation to DS_{cal} but it was not directly proportional (R^2=0.99, gradient=0.83) (Fig. 5). The discrepancies in DS were due to losses in translation of the thermodynamic activity in the transport process and this could have been as a consequence of drug partitioning effects or a slowing of the drug diffusion when passing through the silicone membrane. The highest DS_{mem} attained for diclofenac was 11 in PG/water. Previous studies have reported maximum DS values for testosterone of 2.5 (35), 4.8 for hydrocortisone 4 (36) and 13 for estradiol (2) using supersaturation and in this context the diclofenac sprays appear to be relatively efficient at delivering the agent across the membrane using the *in situ* approach. The unique aspect that self assembling matrixes provided was the ability to prolong the supersaturation process and tune the DS speed and extent using the suprastructure matrix and this is a promising advance in this field of drug delivery.

CONCLUSION

In this study, it was hypothesised a HFA spray that formed a supramolecular structured hydrophilic matrix would be an efficient strategy to support drug supersaturation for extended periods of times. The PG/water mixture was deemed to be an appropriate matrix since the extensive inter-molecular bonding between the PG and water molecules has been demonstrated in previous work (21,33,37). The results support the hypothesis in that they showed formulating a supersaturating metered dose aerosol system using a highly superstructured solvent mixture provides a favourable supporting matrix for *in situ* supersaturation; wherein the

readily formed hydrogen bonds between the formulation constituents not only sustains high levels of supersaturation by preventing nucleation, but also permits rapid drug transmembrane transport. The *in situ* drug supersaturation and matrix self-assembly was driven by the volatile solvent evaporation kinetics however the supersaturation rate was dictated by the drug load upon dose application.

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