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# Saturation solubility of nicotine, scopolamine and paracetamol in model stratum corneum lipid matrices

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ABSTRACT

The saturation solubilities of nicotine and scopolamine bases as well as acidic paracetamol were measured in two different model stratum corneum lipid matrices. Light microscopy visualized the presence of drug above its solubility either as droplets or crystalline particles. Neither wide-angle X-ray diffraction nor DSC detected the drugs. The saturation solubilities of the nicotine and scopolamine bases are 3–5% w/w and 5–10% w/w respectively. Paracetamol strongly disrupts the lamellar phase formed by the lipids and could be dissolved to >20% w/w. Based on these results the saturation solubilities of nicotine and scopolamine in an intact stratum corneum membrane are estimated to be up to 17.5 μg and 35 μg drug per milligrams of stratum corneum membrane respectively. These concentrations are well above values obtained by tape stripping of intact stratum corneum during a permeation experiment. The drug's saturation solubility in the stratum corneum's lipid phase is therefore unlikely to be rate-limiting for permeation of these two drugs.



## 1. Introduction

If Fick's first law is applied to the problem of drug permeation through a membrane then the 'thermodynamic activity', $a^*(t)$, of the drug can be defined in both the vehicle carrying the drug and the membrane. This parameter is equal to the ratio of the drug's concentration, $c(t)$, to its saturation solubility, $c^s$, (Barry, 2001). Inserted into Fick's first law it replaces both $c(t)$ and the membrane/vehicle partition coefficient to give (Moser et al., 2001):

$$J = \frac{D \times a^*_v(t) \times c^s_m}{h}$$

where $D$ is the drug's diffusivity within the membrane of thickness $h$, and the subscripts v and m refer to the vehicle and the membrane, respectively. The only assumption made is that the drug in solution behaves ideally, i.e., $c_m(t)/c(t) = c^s_m/c^s_v$. This form of Fick's first law predicts that the flux, $J$, of the drug is determined by its thermodynamic activity and not just its concentration. Furthermore, the maximum flux of the drug achievable is dependant on the saturation solubility of the drug within the membrane, $c^s_m$. This parameter sets therefore the upper limit of membrane permeation in the absence of supersaturation of the drug in the membrane. If the latter occurs then $c^s_m$ must be replaced by the super-solubility curve of the Miers-Ostwald region (Mullin, 1993).

If this equation is applied to drug permeation through a stratum corneum membrane, then $c^s_m$ will be the saturation solubility of the drug in this heterogeneous tissue composed of regions of lipid and corneocytes. Drug concentration profiles within the stratum corneum, $c_m(x,t)$, can be measured during permeation and readily used to evaluate skin uptake (Herkenne et al., 2006). The techniques used include tape stripping (Herkenne et al., 2006) confocal laser microscopy (Alvarez-Roman et al., 2004) and FT-IR (Andanson et al., 2009). These determine drug concentration either at a particular layer-depth within the stratum corneum membrane or else localized in the lipid phase. None of these techniques has been used successfully to determine saturation solubility $c^s_m$. Indeed, it is not clear how this could be done, since sufficient drug would have to be absorbed into a stratum corneum membrane to reach saturation and cause its nucleation.

We have adopted the alternate strategy of measuring a drug's saturation solubility within an in vitro mixture of ordered stratum corneum lipids, $c^s_{lip}$. A value for $c^s_m$ can then be estimated based on the volume fraction of the lipid phase within intact stratum corneum. Stratum corneum lipid mixtures reported in the literature range from simple mixtures of partially-saponified hydrated fatty acids (Friberg and Osborne, 1985) to more complex systems containing a precise ceramide composition as found in the stratum corneum (Groen et al., 2011). For the current work we

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selected the former type of composition which can be prepared reproducibly from commercially-available lipids (Lieckfeldt et al., 1993). Two different compositions were examined: first, a hydrated mixture of partially-saponified fatty acids; secondly, a hydrated mixture of partially-saponified fatty acids plus cholesterol and ceramides. Nicotine, scopolamine and paracetamol were taken as model drugs. The first two are weak bases suitable for transdermal delivery. The latter was chosen because of its weakly acidic character which may give contrasting behavior to the bases in the partially-saponified lipid matrices. Each drug was dispersed within the stratum corneum lipid matrices after their preparation. The presence of undissolved drug was then evaluated using regular or polarising light microscopy, wide angle X-ray diffraction and differential scanning calorimetry. The results obtained illustrate first the difficulties in detecting undissolved drug within such lipid matrices. The measured values of $c^s_{lip}$ and the calculated values of $c^s_m$ are, however, plausible when compared with measured drug concentrations in intact stratum corneum during permeation experiments. They also throw some light on the question if $c^s_m$ can be rate-limiting during skin permeation.

## 2. Materials and methods

### 2.1. Materials

The fatty acids used were: oleic, linoleic, palmitoleic, palmitic, stearic and myristic, and were all purchased from Sigma Aldrich, purity > 99% (Steinheim, Germany). Cholesterol was also obtained from Sigma Aldrich in purity > 99%. Wheat flour ceramides were obtained from Chemos (Regenstauf, Germany) and comprised mainly CER3. Nicotine base (Sigma Aldrich, Steinheim, Germany), scopolamine base and paracetamol acid (Caelo, Hilden, Germany) were used as received. Water was double-distilled from an all-glass apparatus. Sodium hydroxide was obtained from Merck (Darmstadt, Germany).

### 2.2. Methods

#### 2.2.1. Production of model stratum corneum lipid matrices

This was performed using the capillary centrifugation technique originally described by Friberg and Osborne, 1985. The compositions of the fatty acid matrix (FFA) and the fatty acid/cholesterol/ceramide matrix (FCC) are given in Table 1. In each case sufficient NaOH solution was added to saponify 41 mol% of the fatty acids at a water content of 32% w/w. A drug-free matrix contained in the capillary was first heated in a water-bath to 80 °C and centrifuged 15 times under $N_2$ back and forward through its constriction. After this the drug as either a liquid (nicotine) or powder (scopolamine and paracetamol) was added followed by a further 15 heating and centrifugation cycles at a temperature below the melting point of the drug (not nicotine) being used. Each matrix was examined for the presence of undissolved drug <24 h after its preparation.

**Table 1**
Compositions of the stratum corneum lipid matrices.

| Substance | | (mol%) | FFA-matrix (mol%) | FCC matrix (mol%) |
|---|---|---|---|---|
| FFA mixture | Stearic acid | 9.35 | 100.0 | 45.4 |
| | Palmitic acid | 38.72 | | |
| | Oleic acid | 31.63 | | |
| | Linoleic acid | 12.04 | | |
| | Palmitoleic acid | 3.77 | | |
| | Myristic acid | 4.49 | | |
| Cholesterol | | | 0.0 | 26.2 |
| Ceramides | | | 0.0 | 27.4 |

#### 2.2.2. Detection of undissolved drug

Regular light microscopy (LM) and polarized light microscopy (PLM) were performed by spreading a small sample of a matrix onto a microscope slide which was then covered with a cover slip and examined on an Olympus IMT-2 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) with or without crossed polarizers at room temperature. This temperature was used to avoid the experimental difficulties involved in reproducibly examining the matrices by LM or PLM at stratum corneum temperature, i.e., 32 °C (Gonneke et al., 1999). Photographs were taken with a Pixelink PL-A662 camera. Wide angle X-ray diffraction (WAXS) was performed on a 2 mm thick layer of matrix sample contained in an Al holder. This was examined on an X'Pert X-ray diffractometer (Philips, Kassel, Germany) at an acceleration voltage of 40 kV and an anode current of 40 mA. Scans were performed from $2\theta$ = 0.5–40°. For differential scanning calorimetry (DSC) the experiment was performed on a Mettler Toledo DSC. Approximately 8 mg of matrix was sealed in an Al pan and temperature-cycled from room temperature down to −20 °C and subsequently up to 80 °C at a heating rate of 4 °C/min.

#### 2.2.3. Characterization of lipid matrices

Small angle X-ray diffraction (SAXS) was used to identify the lamellar ordered structures formed by the lipid mixtures (Lieckfeldt et al., 1994). It was performed on the same X'Pert diffractometer described above but with the scan covering $2\theta$ = 0.5–10°.

## 3. Results and discussion

The three drugs examined show differing interactions with the stratum corneum lipid matrices. Their saturation solubilities in the two lipid matrices also differ.

### 3.1. Nicotine

The SAXS diffractograms of the drug-free hydrated fatty acids matrix (FFA) and the drug-free hydrated fatty acids/cholesterol/ceramides matrix (FCC) shown as the lower profiles in Fig. 1a and b both show a lamellar periodicity of 1: 1/3: 1/4: 1/5. The repeat distances are 4.18 nm and 4.26 nm, respectively, in agreement with previous measurements (Lieckfeldt et al., 1994). The additional reflections at 3.33 nm and 1.69 nm in the FCC diffractogram (Fig. 1b) are typical for crystalline cholesterol which is evidently not fully incorporated into the lamellar structure of the partially-saponified fatty acids. Fig. 1a and b also shows that the addition of up to 2% w/w nicotine weakens the lamellar reflections of both matrices, especially the primary reflection and to a lesser extent the third reflection. The lamellar structures are evidently at least partially disrupted by the nicotine molecules. This disruption could be a result of saponification of the nicotine with the fatty acids. It is known that nicotine carboxylates are formed with aliphatic monocarboxylic acids (Perfetti, 1983) including stearic, palmitic, oleic and myristic acids (Casanova et al., 2005). NMR studies have shown that a molar ratio of nicotine/fatty acid of 1:3 exists in these nicotine carboxylates – one fatty acid molecule binds to the *N*-methypyrrolidone nitrogen of the nicotine whilst the other two dimerize and bind to the pyridine nitrogen (Perfetti, 1983). The p$K_a$'s of nicotine are 8.02 and 3.12, respectively, which means that the nicotine's *N*-methypyrrolidone nitrogen will be almost 100% monopronotated at the pH of 5.0–5.5 (Morie, 1972) existing in the lipid matrices and can interact with the fatty acids. The nicotine carboxylates up to and including oleic ($C_{18:1}$) are highly water