Transdermal delivery of anticholinergic bronchodilators
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Chapter 3

**Standardization procedure for the in vitro permeation of anticholinergics**

### 3.0. Summary

Seven anticholinergics (atropine, atropine sulphate monohydrate, benztropine mesylate, dexetimide hydrochloride, oxyphencyclimine hydrochloride, scopolamine hydrobromide trihydrate, and tropicamide) were selected for in vitro permeation experiments using static Franz diffusion cells. Pig epidermal membranes were used because these membranes could easily be prepared by suspending full thickness skin from the pig ear in water of 60°C for 2 min. The donor solution consisted of isotonic phosphate buffered saline pH 7.4 with ethanol, propylene glycol and Azone® as additional solvent components and as penetration enhancers. Tritium labelled dexetimide was added as an internal standard. Ratios were calculated by dividing the percentage of permeated anticholinergic by the percentage of permeated $[^3]$H)dexetimide. For all anticholinergics, the use of ratios decreased the variations which shows the usefulness of $[^3]$H)dexetimide as an internal standard to correct for variations in the skin.

For all anticholinergics, the lag times were comparable, however the fluxes differed about a factor 6 between the highest and lowest value. These differences in permeation data were found not to correlate with the molecular weight and octanol/water partition coefficient or octanol/buffer partition coefficient. The differences in permeation between atropine base and atropine sulphate might be explained by differences in solubility and pH of the donor solution. The use of pig skin which had been frozen and stored for 2 months at -80°C, resulted in a higher permeability without any lag time, and therefore only fresh skin should be used.
3.1. Introduction

Transdermal drug delivery of anticholinergics may be useful in the treatment of obstructive airways diseases because sustained, constant and controlled levels of the drug in the blood may result in a prolonged duration of action [1]. It will also lead to better patient compliance by eliminating frequent dosing. Therefore, we performed in vitro experiments with anticholinergics to determine their permeation characteristics through the skin. Seven anticholinergics were selected for these experiments because of their high affinity towards the muscarinic receptor (Chapter 2).

The permeation of chemicals through the skin can be studied using in vitro techniques. It is widely believed that the in vitro permeation is a good representation of the in vivo situation [2-4]. However, in vitro experiments with human skin are difficult to conduct due to the scarcity of this material and the fact that gender, race, site, age and skin condition of the donor cannot be controlled satisfactory [2]. Therefore, various animal skin alternatives have been used to predict the percutaneous absorption through human skin.

We selected pig skin for the in vitro experiments, because previous reports indicate it has histological properties comparable to human skin with similarities in epidermal thickness and composition, pelage density, dermal structure, lipid content, and general morphology [5-10]. Furthermore, in a number of permeation studies, pig skin showed to be a good model for human skin permeability [11-18]. Because the dermis in full-thickness skin may act as an additional barrier to lipophilic drugs, we prepared epidermal membranes from the pig ear [11, 19] and used these in the in vitro experiments.

The present study describes the in vitro permeation of seven anticholinergics through pig epidermal membranes using static Franz diffusion cells. The donor solution consisted of isotonic phosphate buffered saline pH 7.4 with ethanol, propylene glycol and Azone® as additional solvent components and as penetration enhancers. Because large variations in permeability between skin samples are often observed, we decided to use an internal standard (tritium labelled dexetimide) to correct for variations in the skin. This standardization is discussed and the permeation data are examined for their correlation with a number of physicochemical parameters (Chapter 2). The suitability of pig skin after frozen storage is also studied and compared with fresh skin.

3.2. Materials and Methods

3.2.1. Materials

[\(^3\)H]Dexetimide hydrochloride (\(^3\)H)dex, 15 Ci/mmol) was obtained from Janssen Pharmaceutica N.V.(Beerse, Belgium). [\(N\)-methyl-\(^3\)H]Scopolamine methyl chloride (\(^3\)H)NMS, 81.5 Ci/mmol) was obtained from Du Pont NEN (Du Pont, Wilmington, DE, USA). The anticholinergics atropine, atropine sulphate monohydrate,
benztropine mesylate, dextimid hydrochloride, oxyphencyclimine hydrochloride, scopolamine hydrobromide trihydrate, and tropicamide were all of pharmaceutical quality and obtained from local wholesalers. Sigmacoat® was obtained from Sigma (St. Louis, MO, USA). 1-Dodecylazacycloheptan-2-one (Azone®) was kindly supplied by Nelson Research (Irvine, CA, USA). Propylene glycol was purchased from Brocacef (Maarssen, The Netherlands). All other chemicals and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany). Polyethylene tubes (12 ml) were obtained from Greiner (Alphen a/d Rijn, The Netherlands). The GF/B glassfibre filters were from Whatman (Maidstone, UK). Rialuma was used as scintillation liquid, obtained from Lumac (Olen, Belgium), in combination with mini-scintillation counting vials from Packard (Groningen, The Netherlands).

3.2.2. Preparation of solutions

Isotonic phosphate buffered saline pH 7.4 (PBS-buffer) was prepared by dissolving 8.00 g NaCl, 0.20 g KCl, 0.20 g KH₂PO₄ and 1.44 g Na₂HPO₄·2H₂O in 1 l distilled water. PBS-buffer was used as the receptor solution. The 50 mM sodium phosphate buffer pH 7.4 (assay buffer) was prepared by dissolving 1.38 g NaH₂PO₄·H₂O and 7.12 g Na₂HPO₄·2H₂O in 1 l distilled water. Stock solutions (1x10⁻³ M) of the anticholinergic were prepared in ethanol and stored at -20°C.

Ethanol / propylene glycol / PBS-buffer / Azone® 60:20:15:5 (v/v) was used as donor solution (vehicle) [20].

The drug solution was prepared by mixing 500 µl of a solution of the anticholinergic (15 mg/ml in vehicle) with 10 µl of an ethanolic stock solution of [³H]dex (3 MBq/ml).

The tissue suspension was prepared by dissolving 5 mg of lyophilized receptors [21] in 1 ml assay buffer.

3.2.3. Preparation of pig skin

Pigs (CDL, Groningen, The Netherlands) weighing 20 kg, 8 weeks old, previously used for experimental surgery and sacrificed by termination of the resuscitation, were used. Pig ears were obtained within 30 min after termination of the resuscitation and cleaned under cold running water before whole skin membranes were removed from the underlying cartilage. Hairs were cut and the whole membranes were used immediately (fresh skin) or frozen in liquid nitrogen and stored at -80°C until further use (frozen skin). Permeation experiments were performed with epidermal membranes which were prepared by soaking the whole skin membranes in water for 120 sec at 60°C, followed by blunt dissection [11,19]. The frozen whole membranes were thawed before epidermal membranes were prepared.
3.2.4. Permeation experiments

Permeation experiments were performed using Franz diffusion cells [22-24]. These cells were made of glass with a contact area of 1.35 cm² (University Centre for Pharmacy, Groningen, The Netherlands) and pretreated with a silanizing agent (Sigracoat®). The Franz diffusion cell consists of a donor compartment and a receptor compartment. Epidermal membranes were mounted between the cell compartments with the stratum corneum towards the donor compartment and an O-ring was used to position the membrane. The two cell compartments were held together with a clamp. The receptor compartment has a volume of 4.3 ml and was filled with PBS-buffer. It was kept at 37°C by circulating water through an external water jacket. After 30 min of equilibration of the membrane with the receptor solution, 200 µl of the drug solution was applied in the donor compartment by means of a pipet. The donor compartment was covered with parafilm to prevent evaporation of the solvent. The receptor fluid was continuously stirred by means of a spinning bar magnet, at 400 rpm (Multipoint HP 15, Variomag, München, Germany). Receptor solution samples, 2.0 ml aliquots, were withdrawn through the sampling port of the receptor compartment at various time intervals and stored at -20°C until analysis. The cells were refilled with receptor solution to keep the volume constant during the experiment. The experiments were run for 25 hours.

3.2.5. Analytical procedure

To determine the amount of [³H]dexetimide present in the receptor solution, 1 ml of the receptor solution sample was added to mini-scintillation vials and mixed with 3.5 ml Rialuma. The vials were counted for 40,000 counts or 5 min in a liquid scintillation counter (Minaxi, Packard, Groningen, The Netherlands), whatever came first.

The concentrations of the unlabelled anticholinergics in the receptor solution samples were determined by means of a radioreceptor assay [21]. From the anticholinergic stock solutions, appropriate dilutions were made in assay buffer, concentrations ranging from 1x10⁻⁹ M to 1x10⁻⁵ M (calibration curve). The calibration samples, together with the receptor solution samples, were analysed as described below. Tropicamide and benztropine mesylate were analysed using RRA with pre-incubation at 0°C, and the other anticholinergics were analysed using RRA under equilibrium conditions.

**RRA with pre-incubation**

Fifty µl of receptor solution samples or 50 µl aliquots of calibration samples were added to polyethylene tubes in duplicate. Then 400 µl tissue suspension were added. The tubes were vortexed and incubated during 60 min at 0°C before 50 µl of [³H]NMS (4x10⁻⁹ M) were added. The tubes were vortexed again and incubated for another hour at 0°C. After the addition of 4 ml iced cold assay buffer, the samples were immediately filtered through Whatman GF/B glass fibre filters under vacuum...
using a filtration apparatus (48S, University Centre for Pharmacy, Groningen, The Netherlands). The tubes were rinsed twice with 4 ml iced cold assay buffer, which was also filtered. The total filtration and rinsing process, taking place in approximately 15 s, was carried out on each tube in turn. The filters were transferred into mini-scintillation vials and dispersed in 3.5 ml scintillation cocktail by shaking for 120 min. The vials were counted for 40,000 counts or 5 min in a liquid scintillation counter (Minaxi, Packard, Groningen, The Netherlands), whatever came first. Fifty µl of the used [³H]NMS (4x10⁻⁹M) solution were added to 2 mini-scintillation vials and counted as well to measure the total activity added. Calibration curves were fitted with the Ligand curve fitting program [25]. The obtained binding values (Bq) of the receptor solution samples were introduced in the calibration curves and the unknown concentrations of the anticholinergics were calculated. When the final concentration of the receptor solution samples exceeded the upper limit of quantitation of the calibration curves, the receptor solution samples were diluted (10-10,000 fold) and reanalysed.

**RRA under equilibrium conditions**
Fifty µl of receptor solution samples or 50 µl aliquots of calibration samples were added to polyethylene tubes in duplicate. Then 50 µl of [³H]NMS (4x10⁻⁹M) and 400 µl tissue suspension were added to the polyethylene tubes. The tubes were vortexed and incubated for 30 min at 37°C. The filtration, washing and counting procedures were performed as described above.

### 3.2.6. Data analysis

The amount of drug permeated through the pig epidermis at a certain time interval was calculated based on the measured concentrations in the receptor compartment, which were corrected for the sampling dilution, and volume. The results of the permeation experiments were plotted in graphs showing the percentage of permeated anticholinergic versus time or the percentage of permeated [³H]dextemimide versus time.
Flux values were calculated by linear regression from the steady state portion of the permeation curve and expressed in nmol.cm⁻².h⁻¹. The lag time was determined by extrapolation of the steady state portion of the curve to the intercept of the time axis. Permeability coefficients of the anticholinergics were calculated as the flux divided by the applied concentration in the donor compartment (Chapter 1).

### 3.3. Results and Discussion

#### 3.3.1. Membrane preparation

A membrane used in an in vitro experiment should simulate as closely as possible the barrier function of the skin. Often (part of) the dermis needs to be removed,
Figure 3.1. Permeation of the anticholinergics in the presence of [³H]dexetimide (A) and permeation of the internal standard [³H]dexetimide in the presence of the anticholinergics (B) through fresh pig epidermal membranes.

- • = atropine; □ = atropine sulphate monohydrate; ▽ = benztropine mesylate; △ = dexetimide hydrochloride; ▼ = oxyphencyclidine hydrochloride; ■ = scopolamine hydrobromide trihydrate; ★ = tropicamide.

and split-thickness skin or isolated epidermal membranes should be prepared, to
resemble the distance from skin surface to blood stream in vivo [3, 26].
We tried different methods to prepare split-thickness skin because a dermatome was not available. First, full-thickness skin was cut using a microtome, however disadvantages are that the skin must be frozen and that it is difficult to prepare skin sections of reproducible thickness. Besides, the actual thickness of the prepared membranes, measured by using a micrometer, can only be checked after thawing. Recently, Olinga et al. [27] used a Krumdieck slicer to cut liver slices of reproducible thickness (200-300 µm) and high viability [28]. Therefore, we investigated the use of this slicer for the preparation of split-thickness skin membranes. With a little practising it was possible to prepare skin sections of reproducible thickness from pig ear skin, but not from pig abdominal skin or human skin. Because the microtome was operated partially submerged in an appropriate isotonic medium (PBS-buffer), the tissue became rather soft, especially the fatty tissue of the abdominal pig skin and human breast skin, and therefore difficult to cut.

Epidermal membranes can be prepared using chemical separation or heat separation. Chemical separation can be achieved by soaking full thickness skin in a 2 M solution of sodium bromide at 37°C [3, 26]. For pig skin, we found that the exposure times to the soaking solution had to be 5-24 hours, depending on the animal and anatomical site (ear, abdomen). This severely affected the viability of the resulting membranes.

Using heat separation, epidermal membranes could be prepared by suspending full thickness pig skin in water of 60°C [11, 19]. The ease of separation varied somewhat between the different pig ear membranes, yet an exposure time of only two minutes was found to be long enough. For pig abdominal skin, heat separation was useless because after splitting the hair shafts remained in the dermis and created holes in the epidermal membranes. Therefore, only epidermal membranes prepared from pig ears were used in the permeation experiments.

### 3.3.2. Permeation experiments: Use of an internal standard

The mean cumulative percentages permeation of the anticholinergics and radiolabelled [³H]dexetimide are plotted in Figure 3.1. Table 3.1 shows the corresponding standard errors of the mean (sem) at t = 25 hours. The percentages of permeation show considerable variations at all time intervals for all anticholinergics, however this is not exceptional for biological membranes [29, 30]. Figure 3.2 gives an example of the variations in permeation (sem) at all time intervals of the unlabelled drug dexetimide as well as the radiolabelled drug.

Since the variations observed may be due to differences in skin preparations, we hypothesized that the use of an internal standard may eliminate these differences. Yet, this concept would be valid only if the anticholinergic and internal standard have similar permeation characteristics, i.e. following similar transdermal routes.
Table 3.1. Percentage permeation of the anticholinergics and internal standard \[^3\text{H}\]\textit{dextetimide}, and ratios anticholinergic/\[^3\text{H}\]\textit{dextetimide} at \(t = 25\) h, using fresh pig epidermal membranes.

<table>
<thead>
<tr>
<th>Anticholinergic</th>
<th>Permeation anticholinergic (%)</th>
<th>Permeation [^3\text{H}]dextetimide (%)</th>
<th>Ratio (^*1) (\text{CV}_A^2/\text{CV}_R^2)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>40.1 (9.8)</td>
<td>4.0 (0.6)</td>
<td>9.8 (1.4)</td>
<td>2.9</td>
</tr>
<tr>
<td>Atropine sulphate.H(_2)O</td>
<td>17.1 (3.5)</td>
<td>15.4 (2.3)</td>
<td>1.1 (0.1)</td>
<td>3.3</td>
</tr>
<tr>
<td>Benztropine mesylate</td>
<td>41.4 (10.3)</td>
<td>27.5 (3.2)</td>
<td>1.5 (0.2)</td>
<td>3.4</td>
</tr>
<tr>
<td>Dextetimide HCl</td>
<td>12.8 (8.0)</td>
<td>14.7 (7.9)</td>
<td>0.8 (0.1)</td>
<td>30.2*3</td>
</tr>
<tr>
<td>Oxyphencyclimine HCl</td>
<td>11.9 (5.8)</td>
<td>12.4 (4.7)</td>
<td>0.8 (0.2)</td>
<td>6.2*4</td>
</tr>
<tr>
<td>Scopolamine HBr.3H(_2)O</td>
<td>15.3 (7.9)</td>
<td>10.1 (5.1)</td>
<td>1.5 (0.2)</td>
<td>10.4*4</td>
</tr>
<tr>
<td>Tropicamide</td>
<td>20.5 (8.1)</td>
<td>12.1 (2.7)</td>
<td>1.6 (0.3)</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Data represent mean values ± standard error of the mean.

*1 Ratio = permeation of anticholinergic (%) divided by permeation of \[^3\text{H}\]\textit{dextetimide} (%).

*2 \text{CV}_A^2 = variance in permeation of anticholinergic corrected for the mean;
\text{CV}_R^2 = variance in ratio corrected for the mean.

*3 F-test: \(p < 0.05\).

*4 F-test: \(p < 0.10\).

Table 3.2. Permeation data of the anticholinergics using fresh pig epidermal membranes.

<table>
<thead>
<tr>
<th>Anticholinergic</th>
<th>Lag-time (h)</th>
<th>Flux (nmol.cm(^{-2}).h(^{-1}))</th>
<th>Permeability coefficient (\times10^6) cm.s(^{-1})</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>16.1 (0.9)</td>
<td>321 (82)</td>
<td>1.76 (0.45)</td>
<td>4</td>
</tr>
<tr>
<td>Atropine sulphate.H(_2)O</td>
<td>14.1 (0.4)</td>
<td>46 (11)</td>
<td>0.61 (0.14)</td>
<td>4</td>
</tr>
<tr>
<td>Benztropine mesylate</td>
<td>16.1 (1.9)</td>
<td>215 (24)</td>
<td>1.64 (0.18)</td>
<td>2</td>
</tr>
<tr>
<td>Dextetimide HCl</td>
<td>13.7 (1.7)</td>
<td>59 (35)</td>
<td>0.44 (0.26)</td>
<td>3</td>
</tr>
<tr>
<td>Oxyphencyclimine HCl</td>
<td>14.1 (0.7)</td>
<td>55 (31)</td>
<td>0.40 (0.22)</td>
<td>4</td>
</tr>
<tr>
<td>Scopolamine HBr.3H(_2)O</td>
<td>13.2 (0.5)</td>
<td>61 (34)</td>
<td>0.51 (0.28)</td>
<td>3</td>
</tr>
<tr>
<td>Tropicamide</td>
<td>10.1 (5.1)</td>
<td>130 (84)</td>
<td>0.70 (0.45)</td>
<td>2</td>
</tr>
</tbody>
</table>

Data represent mean values ± standard error of the mean.
**Figure 3.2.** Permeation of dexetimide (■) and [3H]dexetimide (●) through fresh pig epidermal membranes; each point represents mean and standard error of the mean (n=3).

**Figure 3.3.** Ratios of anticholinergic and [3H]dexetimide versus time.
- ● = atropine;
- □ = atropine sulphate monohydrate;
- ▼ = benztrapine mesylate;
- △ = dexetimide hydrochloride;
- ▼ = oxyphencyclidine hydrochloride;
- ■ = scopolamine hydrobromide trihydrate;
- ▲ = tropicamide.
This can be tested by plotting ratios over time, as shown in Figure 3.3. The ratios were calculated by dividing the percentage of permeation of unlabelled anticholinergic by the percentage of permeation of the radiolabelled drug. For all anticholinergics the use of ratios decreased the variations in comparison with the variations in percentage permeation of the anticholinergic alone (Table 3.1, Figure 3.2 and 3.3). However, in the beginning of the curves (t = 1-5 hours), this decrease in variation was negligible. After 15 hours, the ratios became constant which implies that the permeation routes of unlabelled and labelled drug through the skin were comparable. However, for atropine base the ratio still increased after 15 hours which may be explained by differences in transdermal routes of atropine and [3H]dextetimide. Another explanation may be the competition of the two drugs for a certain permeation route because in the presence of atropine the permeation of [3H]dextetimide decreased compared to the [3H]dextetimide permeation in the presence of other anticholinergics. This phenomenon will be investigated and discussed in more detail in Chapter 4.

3.3.3. Permeation experiments: Correlation with physicochemical parameters.

The permeation data of the anticholinergics are shown in Figure 3.1 and Table 3.2. The lag times of the curves were comparable for all anticholinergics which means that the diffusivity would be more or less the same. However, the fluxes differed about a factor 6 between the highest and lowest value which may be explained by differences in physicochemical characteristics of the anticholinergics as determined in Chapter 2.

Various models, based on Fick’s first law of diffusion, have been described to predict the relationship between percutaneous absorption and physicochemical properties of drugs [31-34]. Potts et al. [34] analysed permeability data using a model which depends only upon the size of the drug and its octanol/water partition coefficient. The following equation was used to predict skin permeability:

$$\log K_p = \log \frac{D^0}{d} + f \cdot \log K_{o/w} - \beta' \cdot MW$$

(3.1)

where:

- $K_p$ = the permeability coefficient;
- $D^0$ = the diffusivity of a hypothetical molecule having zero molecular volume;
- $d$ = the diffusion path length;
- $f$ = constant which accounts for the difference between the partitioning domain presented by octanol and that presented by the stratum corneum lipids;
Standardization procedure for in vitro permeation

\[ K_{o/w} = \text{the octanol/water partition coefficient;} \]
\[ \beta = \text{a constant which includes a conversion factor for the substitution of molecular weight for molecular volume;} \]
\[ \text{MW} = \text{the molecular weight.} \]

We used this model to examine the permeability data of the anticholinergics although the model was only used to describe skin permeabilities of non-ionic drugs dissolved in water. Multiple regression analysis of \( \log K_p \) upon \( \log K_{o/w} \) and MW was required to provide values for \( f, \beta \) and \( \log(D^0/d) \). Octanol/water partition coefficients (\( \log K_{o/w} \)) and molecular weights of the bases (MW) were found not to correlate with permeability coefficients (\( \log K_p \)). Using the octanol/buffer partition coefficients (\( \log K_{o/b} \)) instead of \( \log K_{o/w} \), we still did not find any correlation. This lack of correlation may be due to the penetration enhancers added into the donor solution. The actions of these enhancers may explain why the model does not fit our data, and provide values for \( f, \beta \) and \( D^0/d \) which have no physicochemical significance. Ethanol has been proposed to increase skin permeability of polar solutes, propylene glycol probably solvates the intracellular proteins and occupies hydrogen bonding sites, and Azone® reduces the order of the intercellular lipids [35-38]. Also, it is known that propylene glycol promotes Azone® penetration and vice versa [38]. Apparently, the combination of these enhancers resulted in a high permeation for all anticholinergics because the passage of both hydrophilic and lipophilic drugs can be enhanced using this combination. However, for any drug/enhancer/vehicle combination, it will be difficult to predict which mechanism will predominate, and how this will interfere with the model. Another explanation for the lack of correlation may be the ionic character of the anticholinergics because only non-ionic drugs were predicted with this model. We may conclude that the model cannot be used to describe our data although the number of experiments and compounds was limited.

3.3.4. Comparison of atropine and atropine sulphate

Figure 3.1 and Table 3.2 present the permeation characteristics of atropine and atropine sulphate. The lag times of the two curves, 16 hours for atropine and 14 hours for atropine sulphate, indicate that the diffusion coefficients of the two drugs are comparable. However, the flux of atropine is approximately six times higher compared to the flux of atropine sulphate and the permeability coefficient is approximately three times higher. These differences in permeation may be explained by differences in solubility and pH of the donor solution. The apparent pH of atropine base in the donor solution was 9.8 compared to 6.5 for atropine sulphate (Chapter 2) which means that the concentration of non-ionized species in the donor solution of atropine base will be
increased compared to atropine sulphate (pK\textsubscript{a} = 9.9). This may explain the higher flux and permeability of atropine base, because in general the flux of the non-ionic species will be greater. However, it should be realized that the measured flux is the flux of both ionic and non-ionic species and therefore the differences between atropine base and salt can be smaller than expected on the basis of pH [39].

### 3.3.5. Effects of freezing on permeation

Where some authors suggest that freezing affects skin permeation, other authors show no effect of freezing on permeability data [40-45]. To examine the effects of frozen storage, the experiments with anticholinergics and internal standard were also performed using pig skin which was frozen and thawed. An example is given in Figure 3.4, which shows the permeation of dextemide and \(^{3}\text{H}\)dextemide using pig skin which has been stored for 2 months at -80°C. When we compare these profiles with the results on fresh skin as depicted in Figure 3.2, the storage of pig skin resulted in a higher permeability without any lag time. This indicates the loss of viability of the skin and changes in physical and chemical properties [42-45]. It has been suggested that the greater the storage times, the greater the permeation is [12]. These results show that at least for anticholinergics only fresh pig skin should be
used, because frozen storage clearly affects the permeation.

3.4. Conclusions

Pig epidermal membranes were prepared using heat separation because of the ease of separation. Only pig ears should be used because with pig abdominal skin holes can be created. The use of an internal standard and expressing the permeation as the ratio of drug permeated over internal standard permeated decreased the variations in comparison with the variations in percentage of the anticholinergics alone. This shows the usefulness of \(^{3}H\)dextemimde as an internal standard to correct for variations in the skin. The permeability coefficients of the anticholinergics were found not to correlate with the molecular weight and octanol/water partition coefficient or octanol/buffer partition coefficient. The differences between the permeation of atropine base and atropine sulphate might be explained by differences in solubility and pH of the donor solution. The use of pig skin which had been frozen and stored for 2 months at -80°C, resulted in a higher permeability without any lag time and therefore only fresh skin should be used.

3.5. Acknowledgement

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3.6. References

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