Analysis of the oral delivery of **vitamin D3** from BetterYou DLux spray formulations

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Abstract | **Oral delivery of vitamin D3 from BetterYou DLux spray formulations**

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Vitamins are essential nutrients that one requires to sustain normal body functions and growth. They can be obtained from variable and multiple sources. Vitamin D 3 (VD 3) is an important, fat soluble vitamin needed for growth and development of the human systems. It is mainly obtained through a photochemical reaction that occurs via the skin in the presence of UV light. The progression of a westernised culture and poor diet regimes, has led to vitamin supplementation playing a vital role in maintaining the required levels of nutrient uptake. This research has probed the efficacy of sublingual VD 3 supplementation from an oral spray, via the in-vitro permeation of VD 3 through the excised sublingual membranes; the efficacy of VD 3 across the buccal and soft palate membranes is also included. Comparative studies against the sprays were conducted using two simple oil formulations which contained VD 3 in a 9:1 ratio of olive oil: 1-methyl-2pyrrolidinone with a variant of 5% menthol. There is no scientific evidence as yet to show that this supplement works however, it is known that sublingual sprays offer a faster onset action in comparison to a tablet which would require dissolution.[1] This investigation also takes into account the differences between the different types of non-keratinised membranes in relation to the permeation of VD 3.[2]

Permeation studies were conducted using all glass Franz Diffusion Cells, where each experimental set up was run over a period of 12 h with sampling taking place after every 2 h. The receptor phase used in the cells was Cetrimide at a concentration of 0.03%. The samples obtained were analysed using reverse phase HPL with a mobile phase of methanol, ethanol and phosphoric acid at 1%. Calibration curves for vitamin D have been carried out using Cholecalciferol dissolved in ethanol. Porcine membranes have been used due to their similarities to human membranes.[3] Two membrane extraction techniques were used to excise the porcine membranes; blunt dissection to excise the ventral tongue surface membrane and lower soft palate, and heat separation for the buccal membranes. The in-vitro analysis for each membrane was carried out separately. For each experimental run cells were exposed to 200 µL of either the commercial or the simple oil preparation.

Permeation across the sublingual membranes was compared using varied concentrations of the commercial sprays and the two simple oil formulations. The commercial sprays showed an overall better delivery across all membranes. The permeation profiles for the ventral surface of the tongue showed linearity, whilst the other two membranes (lower soft palate and buccal)

showed a non-linear permeation profile of VD 3. Comparative studies of the different formulations showed that the commercial micro-emulsion spray permeated the membranes better than the simple oil formulations. The flux values of the commercial sprays of three different concentrations across the ventral surface of the tongue showed no significant difference showing that permeation was rate limiting. Three application techniques were assessed to estimate VD 3 permeation from a spray plume of 8.55 cm^2, over an available surface area of 214.7 cm^2 ± 12.9 cm^2 [4]; the buccal membrane showed the best permeation profile for all three techniques assessed.

The results confirmed the permeation of VD 3 across oral membranes; however there was a vast difference in the extent of permeation seen with each membrane. The differences in the permeation can be attributed to structural differences and/or location in the oral cavity. However with the formulations being so different it can be assumed that the difference seen is due to the number of excipients used. The buccal region is seen to have the best permeation profile with the commercial spray. The overall conclusion is that the oral commercial spray depending on the type of technique used, delivers an overall potential absorption within the mouth of ~37%, with buccal permeation delivering the highest individual level of ~20% of the dose administered.

1. Marmor, A., 1990.
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The permeability of the human oral mucosa and skin to water. *Journal of Dental Research. Vol. 68. No. 9 Pp.1345-1349.*

3. Squier, C.A., Cox, P., Wertz, P.W., 1991.
Lipid content and water permeability of skin and oral mucosa. *J. Invest. Dermatol. 96, 123-126.*

4. Collins, L.M.C., Dawes, C., 1987.
The surface area of the adult human mouth and thickness of the salivary film covering the teeth and oral mucosa. *J. Dent. Res. 66(8), pp. 1300-1302.*

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Introduction

1.1 Overview

Sublingual drug delivery utilises the permeability of the mucosal membrane located on the ventral side of the tongue. The sublingual membrane is a preventative barrier for the permeation of many compounds into systemic circulation. The membrane therefore is a difficult route to utilise for the delivery of drugs. However in comparison to other delivery routes this pathway provides several advantages, as discussed later.

Vitamins are essential nutrients that humans require to sustain life. Each vitamin has a specific and vital role in the body and can be obtained from a variety of sources (food, drinks, sunlight and supplementation). There uptake and utilisation is intricate and relies upon a delicate balance of overall nutrition.

There are two main classes of vitamins:

1. Fats soluble vitamins; these can be obtained from fatty foods, they are stored in the liver and fatty tissues and therefore do not require a daily intake. The vitamins A, D (D1 and D2), E and K are included in this category.

2. Water soluble vitamins; these vitamins, not stored in the body, require daily intake. These are mainly acquired through the consumption of fruits, vegetables and grains. Included in this category are the B range of vitamins, vitamin C and folic acid.

Some vitamins although obtained from the diet can also be obtained through non-dietary sources e.g. Vitamins such as biotin and Vitamin K are naturally synthesised in the gut, Vitamin D3 (VD3) is mainly obtained via sunlight.

The progression of a westernised culture and poor diet regimes, has led to vitamin supplementation playing a vital role in maintaining the required levels nutrient uptake.

This research probed the efficacy of the sublingual VD3 supplementation from an oral spray via the in-vitro permeation of VD3 through the excised sublingual membranes; the efficacy of VD 3 across the buccal and soft palate membranes is also included.

1.2 Vitamin D3 (VD 3)

Vitamin D is classed as a fat soluble vitamin that is naturally found in a few foods. There are two types- Vitamin D2 known as Ergocalciferol and Vitamin D3 known as Cholecalciferol. Both are produced from pre-vitamins.

VD 3 is mainly synthesized in the skin through a photochemical reaction of ultra-violet rays from the sun. VD3, as absorbed naturally, is metabolised within the liver and kidneys to the active form Calcitriol (Institute of Medicine. National Academy Press, 2010.) as shown in Figure 1. In its metabolised form it plays an important role in the homeostatic control of calcium and phosphate; this is important in the development of bones, neuromuscular (Dhesi et al. 2004) and immune functionality and modulating skeletal cell proliferation.

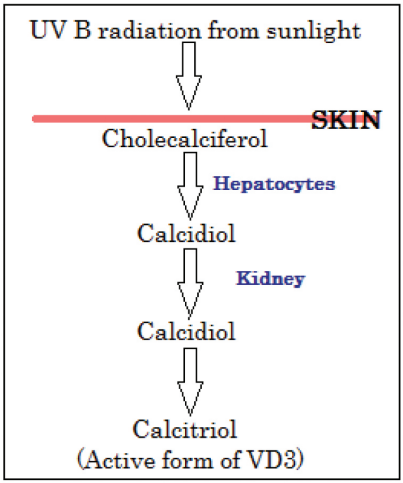


Figure 1 Conversion pathway of the formation of the active form of VD 3.

A lack of Vitamin D is known as Hypovitaminosis D. It can lead to Rickets (juvenile osteomalacia), osteomalacia (adults) and osteoporosis. It can also result in poor development of bones and immune system among many others. VD 3 supplementation is common in the elderly and pregnant. The recommended daily allowance varies between age groups, race and state of health.

1.3 Sublingual Drug Delivery

Sublingual drug delivery is defined as the permeation of a drug through the sublingual mucosal membranes, which cover the ventral side of the tongue and the soft palate; these membranes are part of the non-keratinised epithelia found in the oral cavity.

The total surface area of the oral cavity has been found to be approximately 214.7cm2 ± 12.9cm2. Of this surface area, 30% is found to be non-keratinised epithelia, involved in sublingual and buccal delivery. Non-keratinised epithelia line: the inner part of the cheeks (inside the mouth), the ventral part of the tongue and the soft palate (Collins and Dawes 1987).

An approximation of the non-keratinised membranes is:

(214 x 30) / 100 = 62.2 cm^2

60% of this surface area is represented by the sublingual membranes (the soft lower palate and the ventral side of the tongue) (Wilson 2005; Chen et al. 1999).

(62.2 x 60) / 100 = 37.32 cm^2

Of this, 13 cm2 makes up the ventral surface of the tongue (Ong and Heard 2009), with the rest making up the floor and soft palate. Therefore the rest of the surface area, 24.32 cm^2 makes up the floor and soft palate of the mouth.

Permeation is easily affected by substances such as alcohols and therefore permeability is classed as selective. This is a limiting factor in the selection of excipients for sublingual formulations.

The permeation of lipophilic compounds is greatly hindered by mucosal membranes, including the non-keratinised membranes, and therefore permeation enhancers are required. VD3 is a highly lipophilic compound, and the excipients found in oral formulations are to aid permeation, solubility, taste and appearance. Formulations commonly known to be used in sublingual delivery are sprays, the most popular one being the Glyceryl Trinitrate spray (GTN) used to provide relief in angina. (BNF62).

As part of the oral cavity, these membranes are exposed to an abundant supply of saliva which is constantly secreted and continuously flushes the cavity. Continual movement of the tongue, speech and salivary secretions coupled with the swallowing reflex lead to a limited time period of application. Aspects of delivery such as particle size and the physico-chemical nature of a formulation (eg combinations of permeation enhancers (Sudhakar et al. 2006) and mucoadhesives etc) greatly affect the flux of a compound.

1.4 Buccal Delivery

Buccal delivery involves the membranes that line the inner cheek, inside the upper and lower lips in the oral cavity. It forms approximately 40% of the non-keratinised epithelia found in the oral cavity (Wilson 2005). This can be calculated from the overall surface area:

(40 x 62.2) / 100 = 24.88 cm²

The oral mucosal membrane, comprising of the buccal and sublingual membranes, varies in thickness and permeability. The buccal membrane is thicker, approximately 580 µm (in comparison to the sublingual membrane which is approximately 190 µm) and is generally less permeable (Squier and Wertz 1996) (Squier and Hall 1985b; Lesch et al. 1989).

This route of delivery is common for muco-adhesive formulations, enabling a longer application time. Similar to sublingual delivery, buccal delivery is affected by salivary secretion and mucus turnover. However, the increased application time in this area is due to the lower susceptibility of tongue movement.

1.5 Advantages of Sublingual and Buccal Delivery

Application of drugs onto the sublingual and buccal membranes have proven to be an easy alternative to those individuals who are incapable of ingesting formulations (i.e. patients who are nil-by-mouth, experiencing episodes of nausea and vomiting) or those that do not like or have difficulty taking tablets or liquid formulations (Narang et al. 2011). This route is non-invasive and is not as intimidating as injectable or rectal and vaginal routes.

The membranes are surrounded by a good vasculature which provides easy access into the systemic circulation bypassing the gastro-intestinal (system); this avoids any lag time of drug activation which is often experienced when dosing orally. The effects of drugs administered through theses membranes are therefore a lot more rapid and are not dependent on factors that commonly affect oral routes (stability of drugs in G.I fluid). These areas are easily accessible for application and can be ideal for sustaining prolonged delivery. In case of any unwanted effects, the dosage form can be easily removed restricting delivery almost immediately.

Sublingual sprays offer a faster onset action in comparison to tablet which would require dissolution (Parker et al. 1986; Marmor 1990)

1.6 Hypothesis and Aims

This study tested the efficacy of the delivery of Vitamin D3 in vitro through the tissues of the buccal cavity from an oral spray.

"Vitamin D3 can be effectively delivered through the tissues of the buccal cavity from an oral spray"

1. Evaluation of the permeation of VD3 from commercially available preparations and compare that to laboratory made formulations through the buccal cavity – sublingual, buccal and soft palate membranes.

2. To estimate the total VD3 delivery to the system across these membranes.

2 Materials and Methods

2.1 Materials

Table 1. Materials used and their site of origin.

Material/Chemical	Origin
Vitamin D3 (Cholecalciferol – Lot#: 051M1682V	Sigma-Aldrich Company (Poole, UK)
Menthol	
Cetrimide (Myrustyltrimethylammonium bromide, 99%; Tetradecyltrimethylammoniumbromide, 99%)	Fisher Scientific UK Ltd. (Loughborough, UK)
Methanol	
Ethanol	
Phosphoric acid	
Vitamin D3 sublingual sprays: DLUX, Daily Vitamin D Oral Spray, 3000 IU, 1000 IU and 400IU	Better You Ltd. (Sheffield, UK)
High vacuum grease	Dow Corning (Michigan, USA)
Porcine tissues (tongues)	Local abattoir
porcine heads - buccal and soft palate membranes	Local butchers

2.2 Methods

In this study *in-vitro* analysis of sublingual delivery was carried out separately – ventral membrane of the tongue and the soft palate respectively. In reference to the main formulation concerned, an oral spray, it is hard to restrict delivery to only the sublingual membranes. Therefore we must account for delivery through other non-keratinised epithelia found in the cavity.

2.2.1 Preparation of Porcine Membranes

Porcine sublingual membranes were used to perform in-vitro studies. Human and porcine oral membranes are similar in structure (Squier 1991), composition (Heaney 1978) and permeability (Squier 1996). The sublingual area is comprised of 2 parts: the floor of the mouth and the ventral surface of the tongue. Previous studies conducted have shown that permeation via the ventral surface of the tongue is greater than through the floor of the mouth (Ong and Heard 2009). However, this does not rule out delivery through the soft palate and therefore must be accounted for. Buccal membranes were also used.

2.2.1.1 Sublingual membranes – Blunt dissection technique

Porcine tongues were collected from the local abattoir as soon as they were excised and transported immediately to the laboratory for membrane extraction.

The ventral surfaces of the porcine tongues were excised using blunt dissection. Separation of the membrane required careful scalpel dissection from the ventral surface before the membrane was cut into approximately 1cm^2 pieces ready to be used on Franz-diffusion cells (FDC) for permeation studies as shown in Figure 2. Each piece was microscopically examined to ensure its full intactness. The same technique was also used to extract membranes from the lower palate.

2.2.1.2 Buccal membranes – Heatseparation technique

The buccal membranes were cut and separated using heat separation. The porcine cheeks were excised from the inner cheek region of the porcine head and were placed in DI H_2O at 80^{o}C for 60s. This allowed the membrane to be peeled away from the muscle using a forceps. This must be done carefully in order to extract large sections of the membrane for use on FDC's. Cells with a larger diffusional area were used because these membranes are thicker and tougher. The membranes extracted were cut up into approximately 2.5 cm^2 pieces, and microscopically examined before use.

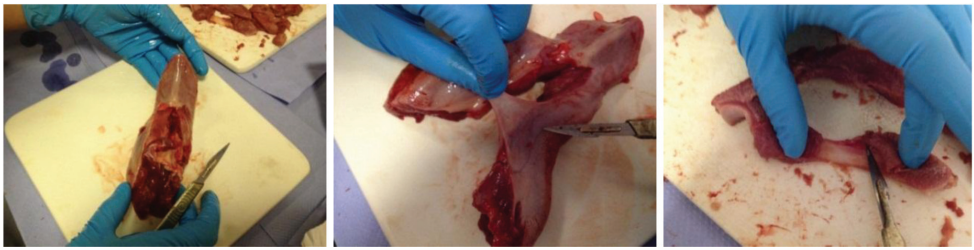
2.3 Preparation of Donor and Receptor Phase solutions

2.3.1 Donor Phase Solutions

The donor phases consisted of 200 µL of: Three commercial micro-emulsions VD 3 supplement sprays (each at a different concentration) and a simple oil formulation. Water in the donor phase was used as a control.

The simple oil VD3 supplement was prepared using Cholecalciferol, olive oil and 1-methyl-2-pyrrolidinone. Two solutions were made with a ratio of 9:1 – Olive oil: 1-methyl-2-pyrrolidinone with the variant being the addition of 5% menthol in one of them. 1-methyl-2-pyrrolidinone was

Figure 2. Ventral side of the tongue (left), sublingual membrane (middle), display of the blunt dissection technique (right).



selected as it is a suitable solvent which0 acted as a mild penetration enhancer. Toxicology studies have shown that it is relatively safe over a range of concentrations and its metabolism does not lead to the formation of toxic compounds (Paulsson et al. 1997).

2.3.1.1 In-house Preparation of 9:1 – Olive oil: 1-methyl-2-pyrrolidinone donor phase

1 µg of Cholecalciferol was weighed into a 1.5 mL mini-centrifuge tube. 0.9 mL of olive and 0.1 mL of 1-methyl-2-pyrrolidinone was measured using separate pipettes and added to the Cholecalciferol. The contents were mixed using a vortex mixer and sonication was used to ensure complete dissolution of VD3.

2.3.1.2 In-house Preparation of 9:1 – Olive oil: 1-methyl-2-pyrrolidinone + 5% menthol donor phase

1 µg of Cholecalciferol was weighed into 1.5 mL mini-centrifuge tube. 0.9 mL of olive oil and 0.1 ml of 1-methyl-2-pyrrolidinone was measured using separate pipettes and added to the Cholecalciferol. 75 µg of menthol was weighed out and added to the oil and Cholecalciferol mixture. Mixing was carried out as previously mentioned in section 2.3.1.1.200 µL of each preparation was used on the respective cells during the experiments.

2.3.2 Receptor Phase Solution

Cetrimide, at a concentration of 30 µg mL-1 was used for the receptor phase. 12 g of cetrimide was weighed out and dissolved in 400 mL of de-ionised water. The solution was stirred using a magnetic stirrer until all the cetrimide had dissolved. This was added to each FDC together with a magnetic stirrer before application of the donor phases.

Cetrimide has no detrimental effect on the tissue or effect permeation. It acts as a sink for the compounds that permeate the membrane.

2.4 In Vitro Permeation Studies

The permeability of each type of membrane to VD3 was determined using all-glass FDC's. Two sizes of cells were used: Small size cells with a receptor volume of 2.4 mL and a diffusion area of 0.1 cm^2, large size cells with a receptor volume of 3.9 mL and a diffusional area of 1.1 cm^2. The cell flanges for both the cells were greased with high performance vacuum grease prior to the mounting of the membranes.

Prepared membranes were then mounted in between the receptor and donor compartments covering the diffusional area. They were positioned with the mucosal surface facing the donor compartment, with metal clamps holding the membrane in place between the cell top (donor compartments) and cell body (receptor compartment) together.

The receptor compartment was filled to the calibration mark with Cetrimide before adding magnetic stirrers and the sampling arm capped. The complete cells were placed in a water bath set at 37^oC for 15 minutes to allow for equilibration before the addition of .200 µL of donor phase (either the commercial sprays or simple oil VD3 supplements). The donor phase solutions varied from different concentrations of theoral spray and two simple laboratory mixed VD3 oil formulations of the same concentration, this is shown in Table 2 overleaf. Receptor phases were drawn after two hour time intervals over 12 h from the sampling ports and replaced with fresh Cetrimide 0.03%. 1 mL of the samples drawn, were then placed into HPLC vials for testing.

Table 2. Constituents of the formulations used in the donor phases.

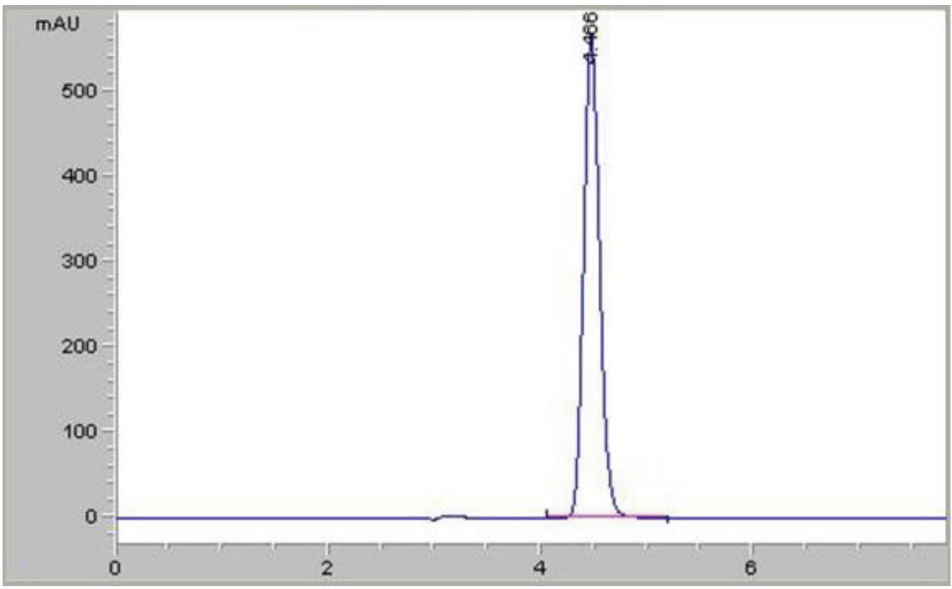
Preparation	VD3 Concentration (µg mL^{-1})	Solvents	Other
DLux400	71.43	Oil (sunflower lecithin) water emulsion	Xylitol, acacia gum, citric acid, preservative (potassium sorbate), peppermint oil.
DLux1000	178.57		
DLux3000	535.71		
9:1 (Olive oil:1-methyl-2-pyrrolidinone)	1000	Olive oil	1-Methyl-2-Pyrrolidinone
9:1 + 5% menthol	1000	Olive oil	1-Methyl-2-Pyrrolidinone, 5% Menthol

2.5 HPLC Analysis

Reverse phase HPLC was used to determine the amount of VD3 that permeated the membrane over the 12 h time period. An Agilent 1100 fitted with Gemini NX C18 column was used; the UV detector was set at 254 nm. The HPLC method used for the quantification of VD3 was developed in-house. A mobile phase of 70:30 – Methanol: Ethanol with 1% phosphoric acid was used; this aided elution of VD3 from the receptor phase.

VD3 has a retention time of 4.47 minutes (shown in Figure 3.). The LOD for VD3 was 0.25 µg mL-1.

Figure 3. HPLC of VD3 in ethanol - showing a retention time of approx. 4.5minutes



2.6 Data Processing and Statistical Analysis.

For each sample and each tissue cumulative amounts of VD3 permeated per unit area were plotted against time over 12 h. Flux values were calculated using the linear portions of these graphs.

Statistical analysis was completed using InStat 3 for Macintosh GraphPad Software, Inc. (Hercules, CA, USA). A one way ANOVA with post t-test was used to investigate differences between the data sets of the various tissues. To be considered a significant p-value of < 0.05 must be achieved. (Squier 1996).

3 Results

3.1 Sublingual permeation

Each preparation was tested on porcine sublingual membranes – this included the ventral part of the tongue and the soft palate. The volume of donor phase applied each time was the same.

3.1.1 Ventral part of the tongue

The permeation profiles of VD3 across the tongue membranes for three commercial products was carried out in order to determine whether increasing the concentration of applied VD3 would result in increased permeation. Figure 4 graphically displays the permeation profiles of the three commercial sprays.

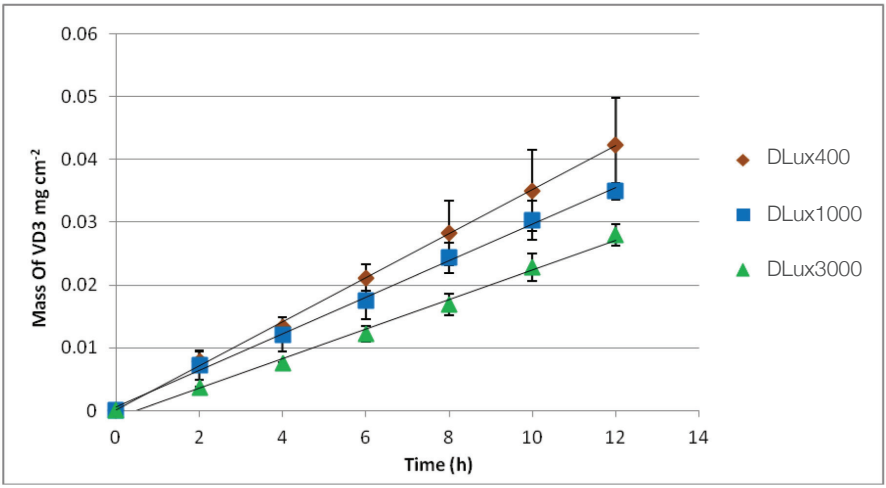
The DLux400 shows to have delivered the highest amount of VD 3 over the 12 h achieving a total mass of 0.0423 mg cm2. The DLux1000 and the DLux3000 delivered significantly less 0.0350 mg cm^2 and 0.0279 mg cm^2 respectively. All three formulations show linear permeation.

An increase in applied VD3 concentration does not increase its permeation. Therefore delivery for the commercial spray is rate limiting.

Further investigations were carried out to test the efficacy of delivery of the commercial sprays (micro-emulsion) in comparison to the in-house (simple oil) formulations containing VD3. This is shown in figure 5.

Figure 7 displays the linear delivery of the commercial preparations as opposed to the delivery of the simple oil preparations which displays non-linear delivery. The DLux400 has the highest delivery of VD 3 over 12 h with a total mass of 0.0423 mg cm-2 whilst the other two commercial sprays show lower delivery. The simple oil formulations showed lower overall delivery. However, we can see that the initial delivery of the oil formulation with menthol was similar to that of the DLux400 up until the 4 h time point, achieving a total delivery of 0.0390 mg cm-2 over 12 h. The preparation without the menthol showed

Figure 4. Plot showing the cumulative delivery of VD3 across the ventral surface of the tongue for 3 commercial sprays



considerably less delivery. The DLux3000 shows a lower overall delivery in comparison to the simple oil formulations and overall worst delivery of the three sprays over 12 h time period.

The average flux values of the three preparations have been calculated and shown in Table 3.

The DLux3000 shows the highest flux of 2.80 x 10-3 mg cm-2 h-1. The difference between the flux values for the three preparations is not significantly different. This shows that a change in formulation concentration does not appreciably affect the flux values ($p > 0.05$).

Figure 5. Plot comparing the cumulative delivery of VD3 between commercial preparations and in-house made preparations across the ventral surface of the tongue.

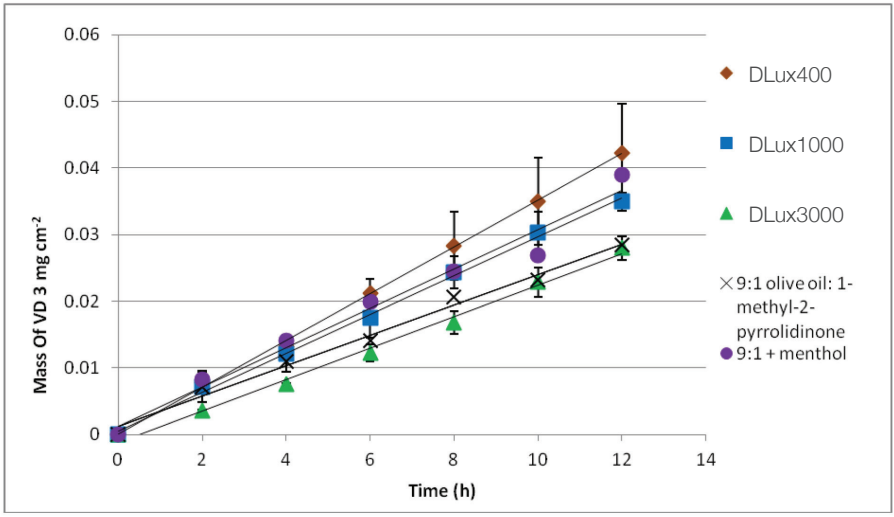
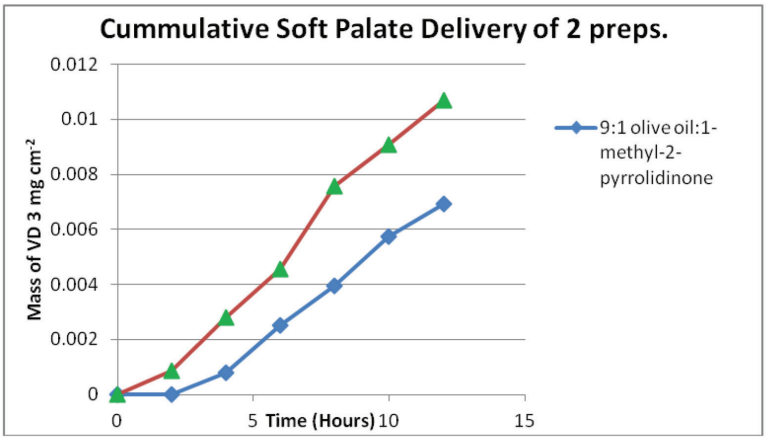


Table 3. Average steady state flux of three commercial sprays across the ventral surface of the tongue.

Preparation	Average J_{SS} ($x10^{-3}$ mg cm^{-2} h^{-1})
DLux400	2.53
DLux1000	2.67
DLux3000	2.80

Figure 6. Plot showing the cumulative mass of VD 3 that has permeated the soft palate over a 12 h time period.



3.1.2 Soft Palate

The permeation of each formulation across the soft palate has been tested over 12 h and data collected and represented graphically. This is shown in figure 6.

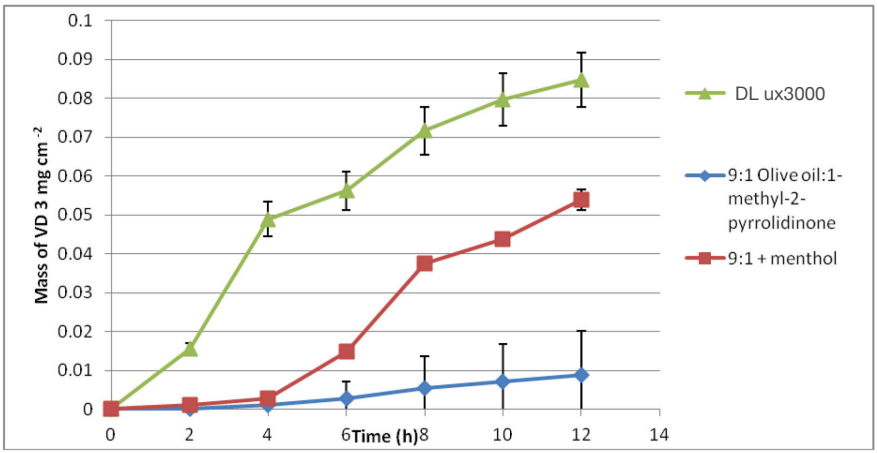
Better permeation is seen from the DLux3000, with a total mass of 0.0107 mg cm-2 permeating after 12 h. The graphs for each formulation show a lag phase followed by a linear part and then tail off. The simple oil formulation delivers approximately half the amount of VD 3 delivered by the DLux3000. Delivery from both formulations is non-linear.

3.2 Buccal permeation

Buccal membranes include the membrane that lines the inner part of the lips and the inner cheeks.

VD 3 is able to permeate the buccal membranes.

Figure 7. Plot showing the cumulative delivery of VD 3 across the buccal membranes over 12 h.



Preparation	Average J_{SS} ($x10^{-3}$ mg cm^{-2} h^{-1})
DLux3000	0.00870
9:1 olive oil:1-methyl-2-pyrrolidinone	0.00130
9:1 + menthol	0.00125

Table 4. Average steady state flux of three preparations (1 commercial spray and 2 simple oil formulations with a menthol variant) across the porcine buccal membrane.

This is shown in Figure 7.

Delivery of VD 3 from all three preparations is non-linear. The DLux3000 has the best permeation profile, achieving maximum delivery of 0.0848 mg cm-2 after 12 h. The simple oil preparations show much lower delivery; however the preparation containing the menthol displays a similar delivery profile to that of the commercial spray, delivering a total mass of 0.0538 mg cm-2 of VD 3. The 9:1 olive oil:1-methyl-2-pyrrolidinone delivers the least VD 3 across the membrane with a delivery profile which looks a lot more linear in comparison to the other two preparations. It delivers almost ten times less the amount of VD3 (0.0087 mg cm-2) after 12 h in comparison to the DLux3000

The average flux values of the three preparations have been calculated and shown in Table 4.

The DLux3000 shows the highest flux across the buccal membrane with a value of 0.0087 x10-3 mg cm-2 h-1 which is approximately eight times greater than the flux of the simple oil preparations.

3.3 Estimation of the VD 3 delivered from oral spray

The spray plume of a single dose covers an area of 8.55 cm^2 , with the spray distance being approximately one inch from the application site. Permeation will differ depending on the position of the device when spraying which determines the membrane area that is exposed. It is also apparent that a proportion of the dose will target other membranes and/or be swallowed.

Table 5. Area that is covered during sublingual administration of spray and the percentage permeation of that dose.

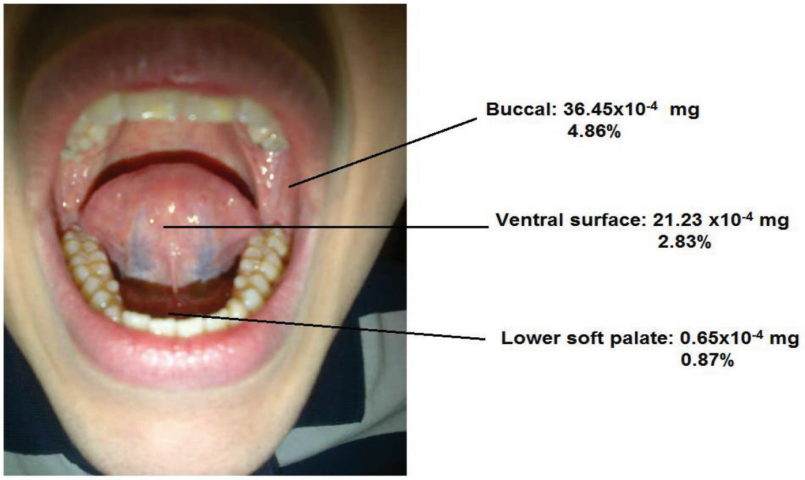
Membrane	Membrane area over which dose is distributed (cm^2)	VD 3 permeated after 0.25 h ($x10^{-4}$ mg)	VD 3 permeated after 0.25 h (%)
Ventral surface	3.85	21.23	2.83
Lower soft palate	2.90	0.65	0.87
Buccal (membranes on inner cheeks)	1.80	36.45	4.86

3.3.1 Spray aimed at the ventral tongue surface

Here, the dose is sprayed directly at the ventral surface of the tongue and an estimation of the dose distribution is shown in table 5.

With this type of application method a total of 8.56% (58.33x10-4 mg) of the overall dose permeates the membrane with the rest of the dose being swallowed. The data in table 5 is displayed in figure 8.

Figure 8. Permeation values of VD 3 when spray is directed to the ventral tongue surface.



3.3.2 Spray applied directly to one of the inner cheek lining (buccal)

Having determined that the buccal membrane is significantly more permeable than sublingual, it is worthwhile considering the outcome should the spray be directed solely at the inner cheek. This would involve facing the device at the buccal membrane; either, the right or left cheek, directly exposing only one buccal membrane. In this case the amount that would reach the other areas would be lower, especially as there would be greater retention between the cheek and gums, and where saliva is less likely to wash the dose away. An estimated dose distribution for this is shown in table 6.

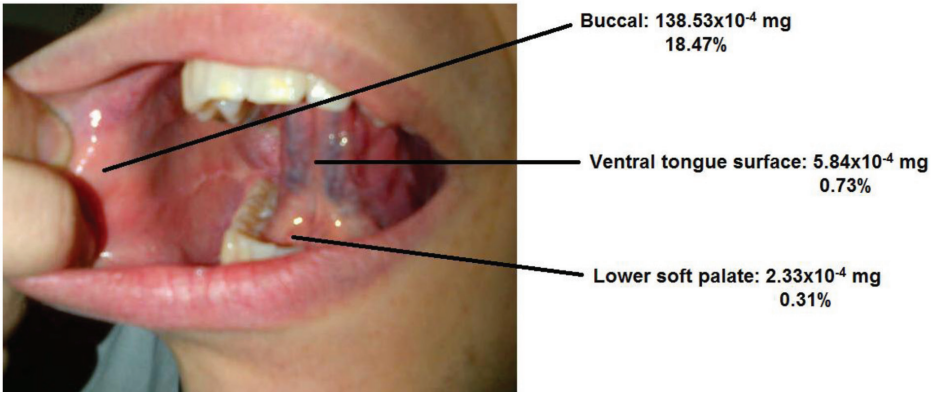
Table 6. Area that is covered by buccal administration and the percentage permeation of that dose

Membrane	Membrane area over which dose is distributed (cm^2)	VD 3 permeated after 0.25 h ($x10^{-4}$ mg)	VD 3 permeated after 0.25 h (%)
Ventral surface	~1	5.48	0.73
Lower soft palate	~1	2.33	0.31
Buccal (membrane on one inner cheek)	6.84	138.53	18.47

This method shows a much higher permeation percentage in comparison to the sublingual technique. We already know that the commercial spray shows a higher degree of permeation through the buccal membrane. However, even with this dosing method the overall percentage permeation achieved is only 19.51% (146.33x10-4 mg), less than one fifth of the dose administered.

The data in table 6 is displayed in figure 9.

Figure 9. Permeation values of VD 3 when spray is directed to the buccal region (one cheek).



3.3.3 Spraying into the oral cavity (as alluded to by Instructions)

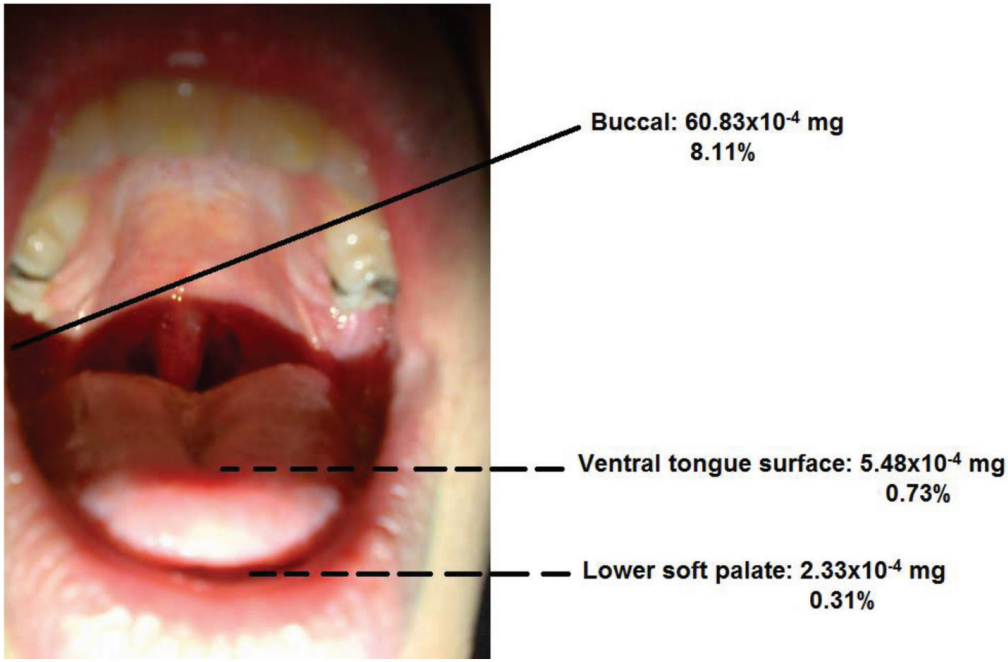
Spraying directly into the cavity would result in most of the dose being sprayed on the surface of the tongue or hard palate - in both these areas absorption is very poor due to their keratinised nature. We can assume that a small proportion of the sprayed dose will be deposited on the buccal membranes. Table 7 shows an estimate of the dose distribution areas.

Table 7. Area that is covered by spraying directly into the oral cavity and the approximate percentage permeation of the dose administered.

Membrane	Membrane area over which dose is distributed (cm^2)	VD 3 permeated after 0.25 h ($x10^{-4}$ mg)	VD 3 permeated after 0.25 h (%)
Ventral surface	<1	5.48	0.73
Lower soft palate	<1	2.33	0.31
Buccal (membranes on inner cheeks)	3	60.83	8.11

Even by spraying the dose directly into the cavity we do achieve a small percentage of permeation, approximately 9.15% (68.63x10-4 mg). Most of the dose, 90.85% (681.38x10-4 mg) will follow the oral route. The tabulated data is shown in figure 10.

Figure 10. Permeation values of VD 3 when the formulation is sprayed directly into the oral cavity.



4 Discussion

4.1 Permeability of porcine oral membranes

Delivery through the membranes of the oral cavity occurs through passive diffusion (Kurosaki et al. 1998). The design of the formulation is a major important factor in achieving permeation; molecular weight, size, degree of lipophilicity and charge. Absorption into the systemic circulation occurs via the jugular vein; this is shown in figure 11.

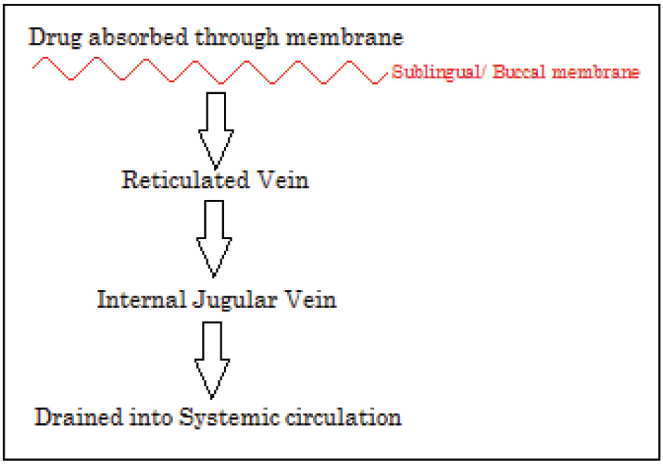


Figure 11. Absorption pathway from the membrane into the systemic circulation.

Drug characteristics are an important factor for permeation; VD 3 has a molecular weight of 384.6 and a logP of 9.1 making it a good compound for sublingual and buccal permeation. Both membranes are lipophilic in nature, favouring the permeation of lipophilic compounds, which penetrate the membranes faster than hydrophilic compounds (Hiroshi et al. 1993). However the drug must be able to permeate the surface membrane and then the mucosal membrane; therefore a highly lipophilic drug such as VD 3 needs to be dissolved in suitable solvent to achieve permeation (Loftsson et al. 2002).

4.1.1 Comparison of the oral membranes involved in the delivery of VD 3.

Sublingual and buccal delivery are both forms of topical delivery, although permeation across each of the membranes varies. Studies on the different membranous regions of the oral cavity have shown this. Sublingual membranes have the highest permeability, of which the ventral surface of the tongue is more permeable than the lower soft palate, followed by the buccal membrane which is the least permeable (Lesch et al. 1989). The buccal membrane does not provide the same rapid absorption and bioavailability which is seen with the other two membranes (Singh et al. 2011). This however can be argued based on the variation seen in this study, where the buccal permeation is greater than the sublingual, as shown in Section 3.

The difference in permeability can be seen with reference to the flux values of the DLux3000 between the ventral membrane of the tongue and the buccal membrane. The flux across the ventral membrane of the tongue is approximately 321 times greater than that of the buccal

membrane. This difference can be attributed to glucosylceramide, which is an important mucosal membrane constituent. Studies have shown that the greater the glucosyleceramide content the poorer the membrane permeability (Squier et al. 1991). The amount of glucosylceramide present in the buccal mucosa is almost 3 times more than in the sublingual membranes. This allows us to understand the difference that is seen with the permeability results obtained (Wertz et al. 1986).

However when the entire membranous region is considered, the overall delivery of the commercial spray through the buccal membranes is significantly higher than through the sublingual. This can be seen in Figure 8 showing an increase in buccal delivery which is almost twice that of the sublingual. Looking at the 9:1 olive oil:1-methyl-2-pyrrolidinone preparation the results are reversed. The percentage permeation is higher in sublingual than in buccal, but not significantly. This may be attributed to the type of formulation and the variation in the selectivity of the membrane.

Comparisons of the sublingual membranes have shown that permeation across the ventral surface of the tongue is almost double to that of the lower soft palate. The DLux3000 and 9:1 olive oil:1-methyl-2-pyrrolidinone respectively have both shown increased permeation through the ventral surface of the tongue, with permeation being approximately 2.6 times and 2.9 times higher respectively. This can be attributed to the difference in the lipid composition of the epitheliums (Squier et al. 1986).

Several permeation studies, when specified as sublingual, do not consider drug permeation occurring in other parts of the oral cavity. Most drugs delivered through membranes in the oral cavity will be exposed to an abundant supply of saliva, which will ultimately result in parts of the drug being moved to other membranous regions, hence the basis of this study. This study has shown the difference in permeability in the prominent regions of the oral cavity, proving that delivery can occur all over but to different extents. Whilst vascularity is important, it is not the limiting factor in this type of delivery.

4.2 Preparation analysis: spray (DLux400, DLux1000, DLux3000) and simple oil ((9:1) olive oil: 1-methyl-2-pyrrolidinone and 9:1 + menthol)

The commercial preparation is classed as an oral spray, with no specific guidance on use. It is difficult to control the area of application as the spray plume would vary depending on individual use. The excipients used in all three preparations remain constant ruling out variability in the delivery of VD 3. Oils have been used as solubilising agents due to the lipophilic nature of VD 3. The purpose of the other excipients is outlined below:

Xylitol – A sugar alcohol that is commonly used as a sweetener. It is safe and known to reduce the incidence of tooth decay (Lynch 2003) and has some permeation enhancing effects and as a solubilising agent (Nep et al. 2011).

Acacia gum – Used as a demulcent and suspending agent in several pharmaceutical preparations with some mild permeation enhancing effect, and is known to inhibit growth of periodontic bacteria.

Peppermint oil – It is famously known that peppermint and menthol oils are commonly used for taste and as permeation enhancers (Abdullah et al. 1996).

The lipophilic nature of VD 3 makes choosing a solvent a lot harder. VD 3 is freely soluble in ethanol and methanol, but, these are not suitable solvents for permeation studies as they can alter membrane viability. 1-methyl-2-pyrrolidinone is used as a solubilising agent for poor soluble drugs (Uch et al. 1999). With a low toxicity profile it is safe to use and has slight permeation enhancing effects. VD 3 is freely soluble in olive oil and therefore the 1-methyl-2-pyrrolidinone is to aid permeation through the surface layer of the membranes, before permeating the lipid part. Olive oil is used in several traditional commercial

VD 3 supplements, “oral drops”.

Menthol is a permeation enhancer, making up 30-55% of peppermint oil (Gelal 2008). The minty smell helps mask the formulation smell. For the in-house simple oil preparation, menthol was the permeation enhancer of choice. By keeping the oil formulations simple we were able to test the effect of adding a penetration enhancer. Based on the results seen with the commercial preparations, the results obtained verified what we expected. The preparation containing the menthol shows a better permeation profile than the preparation without, as seen in figures 7. Tables 4 and 5 show that the percentage permeation of the simple oil formulation is better suited to sublingual membranes, showing a larger permeation percentage over the whole sublingual membranes.

The results shown in Figures 5 to 7 confirms once more that the commercial micro-emulsion mixture provided much higher permeation of VD 3 in comparison to the simple oil formulations. Surprisingly, a change in concentration did not appreciably increase flux values as seen in Table 3; showing that delivery is rate limiting. The percentage permeation values shown in Tables 5,6,7 and Figures 8,9,10 show that the commercial preparations are more suited to buccal delivery.

4.3 Comparisons of the two types of preparations

The commercial spray is a micro-emulsion preparation whilst the other is a simple oil preparation, with the main difference being the types of excipients used. Both contain oils and permeation enhancers. We either expected similar permeation patterns or expected the commercial preparation to have a poorer permeation profile. This was because the commercial spray has a lot more excipients. On investigation, the commercial preparations showed better permeation profiles through all the membranes. These can be seen in figures 8, 9 and 10. This can be attributed to the micro-emulsion formulation, possibly in addition to the presence of excipients.

5 Conclusion

The current study has confirmed that VD 3 permeates the major membranes in the oral cavity from an applied spray dose. However, there was considerable variation in the permeability of VD 3 across individual membrane types.

Predicted percentage permeation values have shown that the inner cheek/buccal membrane provided significantly greater VD3 permeation from the commercial spray compared to the other membranes. Comparing the different types of preparations this work has shown that the micro-emulsion commercial preparations have a higher degree of permeation in comparison to the simple oil preparations.

Lack of specificity regarding the Instructions for use of the oral spray has the potential to lead to differing VD 3 permeation obtained by users administering in different manners. In particular, when the spray is targeted towards the inner cheek ~20% of the dose is absorbed, whereas when directed towards the sublingual region ~9% will be absorbed – approx. the same as spraying into the mouth without the tongue raised. The balance of the sprayed doses will presumably be swallowed.

VD3 can sufficiently be delivered as an oral spray, with an overall absorption potential within the mouth of ~37% and buccal permeation delivering the highest individual absorption of ~20%, easily reaching the RDA, this does not include the levels still delivered after swallowing. It can be reasonably argued that a rapid and more constant delivery is achieved through this method of application.

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