
In vitro analysis of sublingual **vitamin** **B12** permeation

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Vitamin B12 is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, the formation of blood and the reduction of tiredness and fatigue. It is one of the eight B vitamins.

Sublingual delivery: The high permeability of the mucosal membrane, coupled with the proximity of a rich vascular system offers a potentially very rapid onset of action; in some cases, close to that of intravenous administration (Zhang H, Zhang J, Streisand JB. Clin Pharmacokinet 2002).

Aim: to determine the sublingual delivery of Vitamin B12 across excised sublingual membranes from BetterYou B12 Boost spray.

1. 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 can be 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). Their uptake and utilisation is intricate and relies upon a delicate balance of overall nutrition.

There are two main classes of vitamins:

1. Fat 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 VB12 supplementation from an oral spray via the in-vitro permeation of VB12 through the excised sublingual membranes.

1.2 Vitamin B12 (VB12)

Vitamin B12 is one of the water soluble B vitamins which is bound to protein within food. It is generally obtained through dietary means from animal products such as meat, fish, milk and eggs. It is involved in the body's ability to maintain normal neurological and psychological functions, particularly those aspects of the brain and nerve functions which determine concentration, learning, memory and reasoning. VB12 plays an important role in the contribution to normal homocysteine levels, ensuring healthy red blood cell formation and has been proven to be linked to the reduction of tiredness and fatigue.

Adequate VB12 levels can be achieved through a balanced diet but certain people may have difficulty achieving these levels. VB12 is a particularly large molecule and relies upon the presence of a chemical called intrinsic factor, a glycoprotein secreted by the stomach's parietal cells, in order for it to be absorbed from food. The resulting complex undergoes absorption within the distal ileum by receptor-mediated endocytosis.

Vegans and vegetarians are typically vulnerable unless they take supplementary measures. Its role in supporting healthy cell division and folic acid metabolism also raises the importance of adequate levels within women who are pregnant or breastfeeding. Finally people who have had part of their gut surgically removed, who have bowel problems such as coeliac disease, Crohn's or ulcerative colitis will also be considered for supplementary activity.

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 $214.7\text{cm}^2 \pm 12.9\text{cm}^2$. 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:

$$(214 \times 30) / 100 = \underline{62.2 \text{ cm}^2}$$

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).

$$(62.2 \times 60) / 100 = \underline{37.32 \text{ cm}^2}$$

Of this, 13 cm^2 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.

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:

$$(40 \times 62.2) / 100 = \underline{24.88 \text{ cm}^2}$$

The oral mucosal membrane, comprising of the buccal and sublingual membranes, varies in thickness and permeability. The buccal membrane is thicker, approximately $580 \mu\text{m}$ (in comparison to the sublingual membrane which is approximately $190 \mu\text{m}$) and is generally less permeable (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 these 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)

2. Materials and Methods

2.1 Materials

Figure 1.

Material/Chemical	Origin
Vitamin B12 (labelled 1713 02783A)	Supplied by Cultech
Methanol	Fisher Scientific UK Ltd. (Loughborough, UK)
Tri-flouroacetic acid	
Vitamin B12 Boost Oral Spray	Better You Ltd. (Sheffield, UK)
Porcine tissues (tongues)	Local abattoir

2.2 Preparation of Porcine Membranes

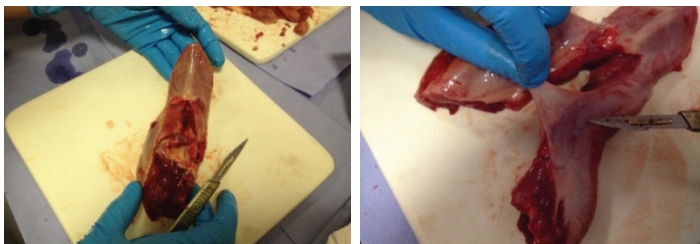
Porcine sublingual membranes were used to perform in-vitro studies. Human and porcine oral membranes are similar in structure composition and permeability, and therefore an appropriate model for human sublingual membranes.

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 1 cm² and 2 cm² 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.

(Collins and Dawes 1987).

Figure 2. Ventral side of the tongue (left), sublingual membrane excision (right)



2.3 In Vitro Permeation Studies

The permeability of the membrane by the vitamin B12 spray 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 cm², large size cells with a receptor volume of 3.9 mL and a diffusional area of 1.1 cm². 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. De-ionised water was used as the receptor phase and added to each FDC together with a magnetic stirrer before application of the donor phases. The complete cells were placed in a water bath set at 37°C for 15 minutes to allow for equilibration before the addition of 200 µL of donor phase to the small cells to represent maximal delivery or a single spray to the large cells to represent in-situ use.

The receptor phases were drawn at 0.5, 1, 2, 3, 6, 12 and 24 hours for the maximal delivery test. The in-situ analysis receptor phases were drawn at 10, 15, 20, 25, 30 and 60 minutes.

2.4 HPLC Analysis

Reverse phase HPLC was used to determine the amount of vitamin B12 that permeated the membrane over the time periods. An Agilent 1200 fitted with Gemini NX C18 column was used; the UV detector was set at 278 nm. The HPLC method used for the quantification of vitamin B12 was developed in-house.

The mobile phase used a gradient elution timetable represented in figure 3. Vitamin B12 has a retention time of 6.87 minutes. The LOD for vitamin B12 was 1.75 ng mL⁻¹.

2.5 Data Processing and Statistical Analysis.

For each sample and each tissue cumulative amounts of vitamin B12 permeated per unit area were plotted against time. Flux values were calculated using the linear portions of these graphs.

Figure 3

Time (mins)	% MeOH	%H ₂ O (0.1%TFA)
0	20	80
20	20	80
25	80	20

3. Results

Figure 4 shows the cumulative permeation of vitamin B12 across sublingual membranes from an INFINITE dose of Boost oral spray as **nanomoles/cm²**.

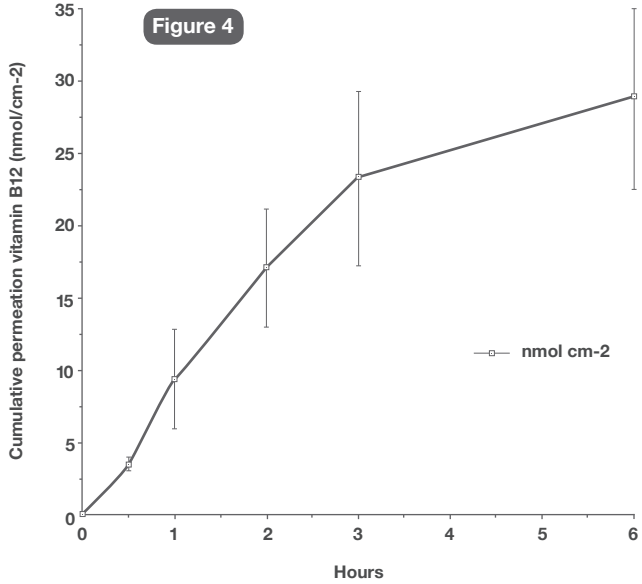


Figure 5 shows the cumulative permeation of vitamin B12 across sublingual membranes from an INFINITE dose of Boost oral spray as **micrograms/cm²**.

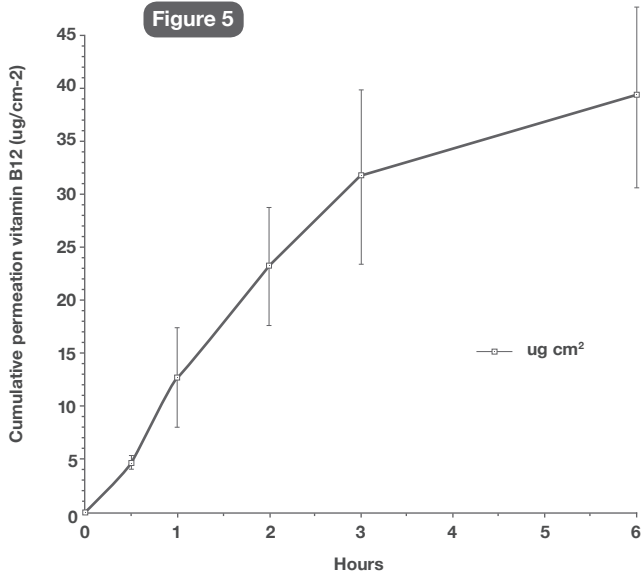


Figure 6 shows the cumulative permeation of vitamin B12 across sublingual membranes from a FINITE (SINGLE SPRAY) dose of Boost oral spray as **nanomoles/cm²**.

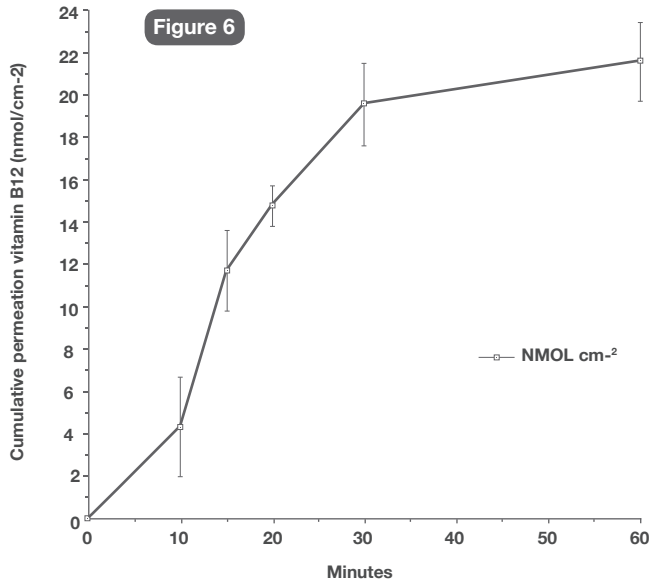


Figure 7 shows the cumulative permeation of vitamin B12 across sublingual membranes from a FINITE (SINGLE SPRAY) dose of Boost oral spray as **micrograms/cm²**.

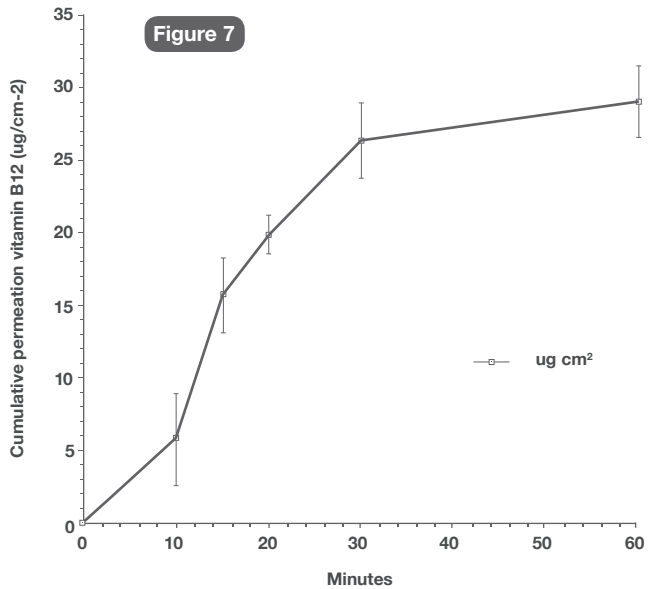
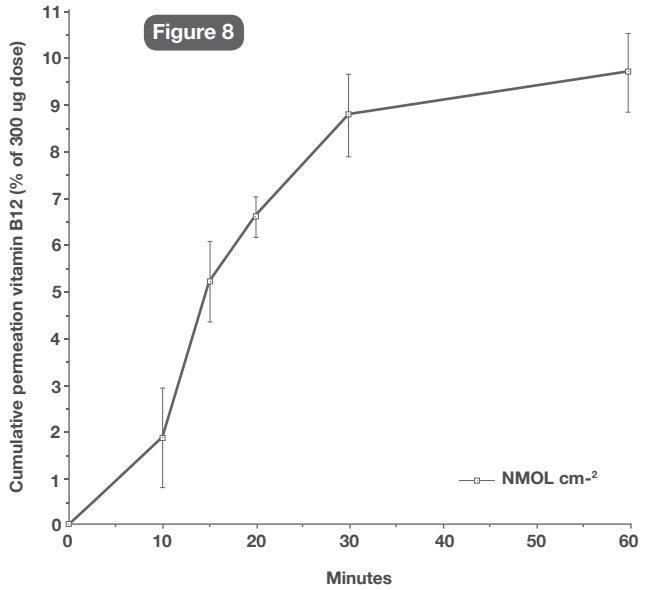


Figure 8 shows the cumulative percentage permeation of vitamin B12 across sublingual membranes from a FINITE (SINGLE SPRAY CONTAINING 300 MICROGRAMS of vitamin B12) dose of Boost oral spray as **micrograms/cm²**.



4. Conclusions

1. 'Infinite' refers to the maximal amount that can permeate the membrane; 'finite' refers to the application of a single spray as per in-use conditions.
2. Vitamin B12 readily permeated excised sublingual membranes. This result is unexpected, given it is a large water-soluble molecule, with a molecular weight of 1355.37. The micro emulsion formula and spray delivery mechanism may explain, in part, this exceptional result.
3. The dietary reference intake for an adult ranges from 2 to 3 µg per day – this figure is readily attained from a SINGLE spray application.
4. Based on the in vitro data contained herein, the B12 Boost spray product appears to be an effective supplement for the rapid attainment of recommended levels of vitamin B12.
5. In practical terms the findings indicate that at least 30mcg of vitamin B12 will enter the bloodstream one hour after a single spray application.

