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# PERCUTANEOUS ABSORPTION OF TRIACYGLYCEROLS (TAGS), TOCOLS AND CAROTENOIDS: COMPARISON STUDIES OF CRUDE AND REFINED PALM OIL

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*Studies were conducted to assess the percutaneous absorption of the triacyglycerols (TAGs), tocols and carotenoids present in crude and refined palm oil. In vitro experiments using upright Franz diffusion cells were employed to investigate the permeability of these compounds across full thickness human skin and into the receptor solution. Cetrimide, a cationic surfactant was chosen to be used as a solubilising agent in the receptor phase with an optimum concentration of 3.0 mg/mL and was able to provide sink conditions throughout the permeation. TAGs, tocols and carotenoids all permeated human skin from crude palm oil (CPO), whereas only TAGs permeated when refined palm oil (RPO) was used. Of the TAGs, oleic acid-containing TAGs was preferentially absorbed despite palmitic acid being the most prevalent fatty acid (FA) in TAGs. Tocols in the form of α-$T_3$ showed the highest permeation followed by γ-$T_3$, α-T and the lowest permeation was observed for δ-$T_3$. Carotenoids (α-carotene and β-carotene) also showed an appreciable amount of permeation from CPO.*

***Keywords:*** Franz cell, Tocol, Carotenoids, Cetrimide, Percutaneous

## INTRODUCTION

Palm oil produced from the fruit of *Elaeis guineensis* tree is the most widely used of all vegetable oils. Initially introduced to Malaysia as an ornamental plant, the first commercial planting of oil palm tree was in 1977 (Sambanthamurti *et al*. 2000). The palm oil tree is unique in producing two types of oil; crude palm oil (CPO) obtained from the fleshy mesocarp and is used mainly for its edible properties and, the palm kernel oil produced from the kernel (seed) which has wide applications in the oleochemical industry (Sundram 2000). The major constituents present in CPO are fatty acids (FAs) in the form of triacyglycerols (TAGs). Palm oil is different from other vegetable oils as it contains 50% saturated FAs, 40% monounsaturated FAs and 10% polyunsaturated FAs (Sambanthamurti *et al*. 2000). The palm fruit also contains phytonutrients that can endow the oil with nutritional and health beneficial properties. These phytonutrients are present in the form of tocols [tocotrienols ($T_3$) and tocopherols (T)], carotenoids (α-, β- and γ-carotenes) and sterols. CPO contains approximately 78%–82% tocotrienols and 18%–22% tocopherols as well as 3.3%–54% of carotenes. α- and β-carotenes are the major carotenes present in the CPO (Mortensen 2005).

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The presence of tocols and carotenoids in palm oil suggests that there could potential benefits in using this oil as an excipient in cosmetic and topical pharmaceutical formulations. It may even be possible to deliver therapeutic amounts of these nutrients via the transdermal route. Tocotrienols are structural analogues of the tocopherols and they are present in greater amounts in CPO than any other vegetable oil (Schwarts *et al.* 2007). Tocotrienols are known to possess a higher antioxidant activity than the tocopherols and they may have benefits in protecting the skin against free radical peroxidation (Zafarizal and Ismail 2009) as well as having cardioprotective, neuroprotective and anticancer properties (Aggarwal *et al.* 2010; Sen *et al*. 2007, 2005). The transdermal delivery of palm oil constituents such as tocols and carotenoids has been largely overlooked even though these substances have often been incorporated in topical formulations for their antioxidant activity. Consequently, the skin seemed to be a promising portal through which to deliver these components for a systemic effect. Moreover, these highly lipophilic compounds have a very low aqueous solubility, whilst there is also the possibility that they may be degraded in the hostile stomach environment (Anatoly and Vladimir 2004) . It is possible that these issues could be overcome if the transdermal route is considered.

The present study was therefore initiated to investigate the feasibility of delivering palm oil derived phytonutrients beyond the skin for systemic purposes. At the same time, it was also important to consider the fact that other compounds in palm oil, such as myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid and linolenic acid, could also potentially be absorbed if palm oil is used as an excipient. So from a toxicological perspective it was important to evaluate TAG absorption at the same time. As palm oil constituents are highly lipophilic the solubility of these compounds was investigated prior to the commencement of the permeation experiments in order to ensure that sink conditions in the receptor phase of the diffusion cells were maintained throughout the experiment. It was important to ensure that the permeant concentration in the receptor phase did not exceed 10% of the saturation solubility (Scheindlin 2004).

## METHODS

### Materials

CPO was obtained from Havys Oil Mill (Selangor, Malaysia) and refined palm oil (RPO) was purchased from The Store Sdn. Bhd. (Semenyih, Malaysia). Phosphate buffered saline (PBS) with pH 7.4 was prepared according to the British Standard International (BSI) method (British Pharmacopoeia 2010). Supelco standard (reference-07631-1AMP) of fatty acid methyl esters (FAME) was purchased from CNG Sdn. Bhd. (Selangor, Malaysia). Tocol and carotenoid standards were a gift from Carotech Bhd. (Perak, Malaysia). Virkon®, the disinfectant used during all experimental work involving human skin, was obtained from Labsystem Scientific (Cheras, Malaysia). Ex vivo human skin samples were obtained from a local private hospital, post-abdominoplasty (tummy-tuck). Cetrimide, used as a solubilising agent and all buffer chemicals were purchased from Fisher Scientific (Selangor, Malaysia).

**Analysis of TAG**

Analysis of TAG was performed by converting it into FAME. FAME standard was prepared by dissolving 100 mg in 1 mL of hexane and stored at –20°C according to Tang's Malaysian Palm Oil Board (MPOB) test method (Tang 2004). This method employs a base-catalysed transesterification process. In this process, 50 mg of oil was dissolved in 0.95 mL of hexane. Vials were shaken using a vortex mixer for 5 seconds in order to dissolve the oil. Then, 0.05 mL of sodium methoxide solution was pipetted into the vials and vortex mixed again for 5 minutes. Two distinct layers were observed at this stage and the upper layer, known to contain the methyl esters was transferred to new vials prior to gas chromatography (GC) analysis.

Analysis of FAME was carried out using a Perkin Elmer Clarus 500 GC (Perkin Elmer Sdn. Bhd., Petaling Jaya, Selangor, Malaysia) equipped with flame ionisation detector (FID). 0.5 µL of each FAME sample was manually injected into the injector port using a 5 µL glass injector syringe. Separation of FAME was carried out using a 30 mm x 0.32 m, 0.2 µm film fused capillary column coated with a chemically bonded stationary phase. The oven temperature was programmed at 185°C for 8 minutes at isothermal condition, the injector and detector temperature were both set at 240°C with a flow rate of 0.6 mL/min with a split ratio of 1:100. Peak area measurements were carried out using TotalChrom Navigator (TCNav) software (version 6.01).

**Tocols Analysis**

Quantification of the tocols was carried out with a Perkin Elmer 200 series HPLC system (Perkin Elmer Sdn. Bhd., Petaling Jaya, Selangor, Malaysia) complete with a quaternary pump and Hitachi fluorescence detector. A Supelco Ascentis C18 reverse phase column (5 µm, 25 cm x 4.6 mm) was used with a mobile phase of acetonitrile and methanol (50:50 by volume). The flow rate was 1 mL/min and the injection volume was 20 µL throughout. The excitation wavelength was 295 nm and the emission wavelength was 325 nm. Peak responses were measured using TotalChrom Navigator (TCNav) software (version 2.0).

**Carotenoids Analysis**

Carotenoids were analysed using the same HPLC system equipped with a Perkin Elmer UV/Vis detector (tungsten lamp). A mobile phase consisting of 70% acetonitrile, 25% methanol and 5% dichloromethane (by volume) was used and the flow rate was set at 2 mL/min. A Supelco Ascentis C18 column (5 µm, 25 cm x 4.6 m) was used and the injection volume was 20 µL throughout. Peak responses were measured at 450 nm by TotalChrom Navigator (TCNav) software (version 6.01).

**Receptor Phase Development**

***Determination of the Critical Micelle Concentration (CMC) of Cetrimide in PBS***

Cetrimide, a cationic surfactant, was chosen as a receptor phase additive with the intention that it would be able to act as a solubilising agent for the highly lipophilic palm oil constituents. The critical micelle concentration (CMC) of cetrimide in PBS was determined using a dynamic light scattering method. The Malvern Zetasizer Nano, APS

software (version 2) [DKSH Technology, Petaling Jaya, Selangor, Malaysia] was used to determine the CMC of cetrimide in PBS. Concentrations in the range of 0.05 mg/mL to 2.5 mg/mL of cetrimide in PBS were prepared and degassed before being analysed with the Zetasizer to detect the formation of micelles and also to measure their particle size in the form of hydrodynamic diameter. At each cetrimide concentration, three particle size measurements were taken and the z-average hydrodynamic diameter was calculated. A graph of intensity of scattered light (%) and z-average hydrodynamic diameter was plotted as a function of the cetrimide concentration in PBS (mg/mL).

***Solubility Studies of Palm Oil Constituents in PBS Containing Cetrimide***

The solubility of 'palm oil' in PBS containing cetrimide was evaluated using standard methodology. Technically, it was the concentrations of the individual palm oil components (TAG, tocols and carotenoids) in aqueous samples saturated with palm oil that were being measured. Four solutions of cetrimide in PBS were prepared (0.5, 1.0, 2.5 and 5.0 mg/mL) and 4 mL of CPO was added to 8 mL of each solution. After agitation for 48 hours using an orbital shaker, 2 mL of the lower aqueous layer was removed and extracted. Triplicate analysis was performed for each cetrimide. Extraction for each compound was performed as mentioned in the analysis of TAGs method.

**Transdermal Diffusion Studies**

***Skin Preparation***

Human abdominal skin was obtained from a local hospital following abdominoplasty surgery on four female patients. Ethics approval was granted by the Joint Ethics Committee of Lam Wah Ee Hospital and Universiti Sains Malaysia (Pulau Pinang, Malaysia). Subcutaneous fat was removed from the tissue by blunt dissection with care being taken not to damage the epidermis. The full-thickness skin samples were cut into squares of approximately 2 $cm^2$, wrapped in aluminium foil, placed into self-sealing polythene bags and stored at –20°C until required.

**Permeation Studies**

***Assessment of CPO and RPO***

Permeation studies were performed using upright Franz-type glass diffusion cells (Permegear, USA). Prior to mounting the skin, the thickness of each excised skin was measured using a digital caliper. The average thickness of the excised skin was 0.34±0.24 mm. High vacuum silicone grease was applied to the flanges of both halves of the diffusion cells in order to prevent leakage of the donor or receptor phases. The receptor compartments (approximate volume: 2 mL) were filled with PBS, containing cetrimide (3 mg/mL). Cetrimide also possesses antimicrobial properties, therefore no additional antimicrobial agent was used. After an equilibration period of 1 hour, 2 mL of CPO, i.e. an infinite dose was added to the donor compartment. The donor compartments for the control cells were left empty and occluded with parafilm to mimic the occlusive effect of the CPO. The cells were placed on a submersible magnetic stirring block and immersed in a water bath at 37°C. Throughout the experiment the receptor phases were agitated with

magnetic stirring bars. At predetermined time intervals up to 72 hours, 400 µL samples were withdrawn from the receptor compartments and stored at –20°C prior to analysis. An equivalent volume of pre-warmed receptor phase was returned to the receptor compartment of each diffusion cell after sampling. The same permeation experiment was repeated with the exception that RPO was used as the donor phase.

***Samples Analysis***

To determine the permeation of FA (from TAG), 100 µL of the samples from the permeation experiment were extracted 3 times with 100 µL of hexane. FAME was subsequently prepared from the hexane extracts as described in the analysis of TAG method. However, the concentration of TAG in the permeation samples (as shown by the analysis of the 72 hours samples) was too low to be detected, as such, it was necessary to concentrate the samples. The 300 µL (combined extracts) of hexane were left in a fume cabinet to facilitate solvent evaporation. After 4 to 5 hours, the residues were dissolved in a smaller volume (100 µL) of hexane and FAME was prepared as described and quantified in the analysis of TAG method. For tocol analysis, the permeation samples were immediately subjected to HPLC analysis as described in tocol analysis method. For carotenoids analysis, 200 µL of the permeation samples were extracted 3 times with hexane before being subjected to HPLC analysis as in the tocol analysis method.

**Data Analysis**

Because TAG from CPO and RPO could only be detected in 56 and 72 hour samples it was not possible to construct the type of permeation profiles that would normally be used to illustrate results. The total amount of TAG permeated from CPO and RPO after 56 hours was therefore presented using line graphs. The pseudo-flux was calculated for each FA by determining the concentration gradient from the plotted line graph. The permeation of tocols and carotenoids through the excised full thickness human skin from CPO and RPO was represented by plotting a graph of the cumulative amount permeated (µg/cm$^2$) versus time (hours). The flux for each compound is determined individually by calculating the concentration gradient from the plotted graph.

Statistical analysis was performed using SPSS for Windows 16, which was used to make a comparison between the permeation of α-$T_3$ and α-T as these compounds were both present at approximately the same concentration in CPO. An independent sample T-test was used to analyse the significance of the difference in the flux of these two vitamin E compounds. A $p$ value of $<0.05$ was considered statistically significant.

**RESULTS**

**Qualitative and Quantitative Analysis of TAG, Tocols and Carotenoids**

The amount of individual FA, tocols and carotenoids present in CPO and RPO was quantified as in Table 1. The five main FAs found in CPO and RPO were myristic acid (C14), palmitic acid (C16), stearic acid (C18), oleic acid (C18:1) and linoleic acid (C18:2). Palmitoleic acid (C16:1) and linolenic acid (C18:3) were present in both CPO and RPO, but these FAs were found in much smaller quantities. Four major tocols (α-$T_3$, γ-$T_3$, δ-$T_3$ and

α-T) were detected in the CPO sample. These same tocols were also detected in RPO but in much smaller quantities (Table 1). The remaining minor carotenoids were not detected in the sample. The same isomers were also detected in RPO but at very low levels. Two carotenoids, α- and β-carotene, were detected in the CPO sample.

**Table 1:** Composition of FAs (from TAGs), tocols and carotenoids quantified from CPO and RPO.

| Components | Composition in CPO (%) | Composition in RPO (%) |
|---|---|---|
| **FAs ( from TAGs)** | | |
| Myristic acid | 1.1 | 0.8 |
| Palmitic acid | 45.0 | 43.2 |
| Palmitoleic acid | 0.8 | 0.3 |
| Stearic acid | 3.7 | 2.7 |
| Oleic acid | 38.2 | 37.6 |
| Linoleic acid | 11.6 | 10.2 |
| Linolenic acid | 0.5 | 0.1 |
| **Tocols** | | |
| α-T | 20.1 | 5.0 |
| α-$T_3$ | 20.8 | 5.3 |
| β-/γ-$T_3$ | 46.0 | 20.4 |
| δ-$T_3$ | 12.2 | 2.5 |
| **Carotenoids** | | |
| α-carotene | 32.4 | 21.5 |
| β-carotene | 54.2 | 36.8 |

**CMC of Cetrimide in PBS**

Figure 1 illustrates the changes in the size of particles present in the PBS containing cetrimide as the cetrimide concentration is increased. The average hydrodynamic diameter (d.n.m) and the intensity of scattered light by the particles present in the solution were measured as a function of the different cetrimide concentrations (mg/mL) in PBS. It was observed that at cetrimide concentrations below 1.0 mg/mL the size of particles present and the intensity of scattered light were almost constant. The size of particles present in solution started to increase as cetrimide concentrations rose above 1.0 mg/mL. Similarly, the increase in the intensity of light scattered by the particles showed a change in gradient above the same concentration. The increase in particle size detected above 1.0 mg/mL was attributed to the formation of cetrimide micelles (Fig. 2). These two empirical observations taken together strongly suggest that the CMC of cetrimide in PBS is approximately 1.0 mg/mL.

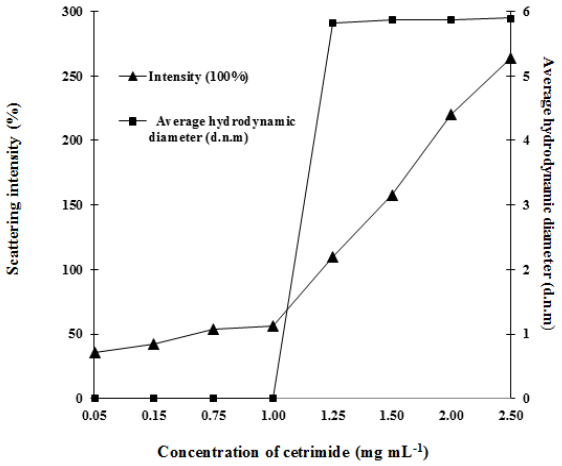


**Fig. 1:** Graph showing the light scattering intensity and average hydrodynamic diameter of particles present against concentration of cetrimide in PBS.

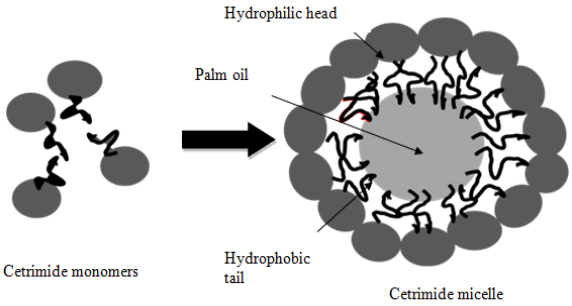


**Fig. 2:** Formation of a cetrimide micelle above the CMC.

**Solubility Studies of Palm Oil Constituents in PBS Containing Different Concentrations of Cetrimide**

Figures 3, 4 and 5 illustrate that the different palm oil constituents were solubilised in all four concentrations of cetrimide in PBS to a varying extents. It was noted that higher cetrimide concentrations were generally able to solubilise greater amounts of TAG, tocols and carotenes. For the FA present in TAG, the solubility of palmitic acid was the highest followed by oleic acid, linoleic acid and then the rest of the FAs. The sequence of FA solubility was in accordance with the composition of the FA present in the palm oil TAG.

For the tocols, the solubility of γ-$T_3$ was the greatest and this was followed by α-$T_3$, α-T and then δ-$T_3$. This corresponded with the concentrations of each tocol present in palm oil. The solubility of β-carotene increased steadily as the concentration of cetrimide increased, however, the solubility of α-carotene did not increase at the same extent. Based upon these solubility data and the CMC value of cetrimide, 3 mg/mL of cetrimide in PBS was selected as an appropriate concentration to be included in the receptor phase with the aim of ensuring satisfactory sink conditions throughout the permeation experiments.

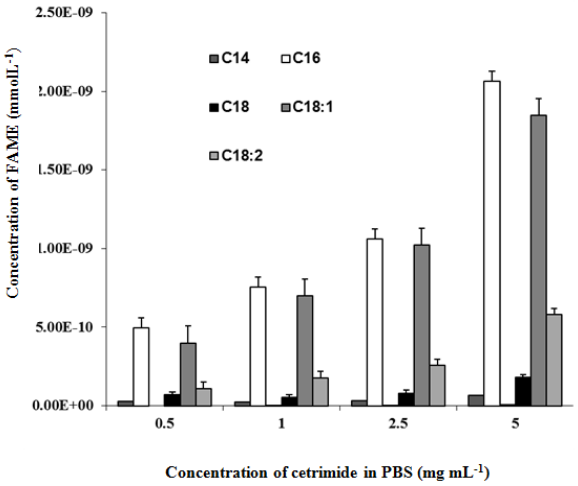


**Fig. 3:** Solubility of FA (from TAG) in different concentrations of cetrimide in PBS (the error bars show n=3, +SE).

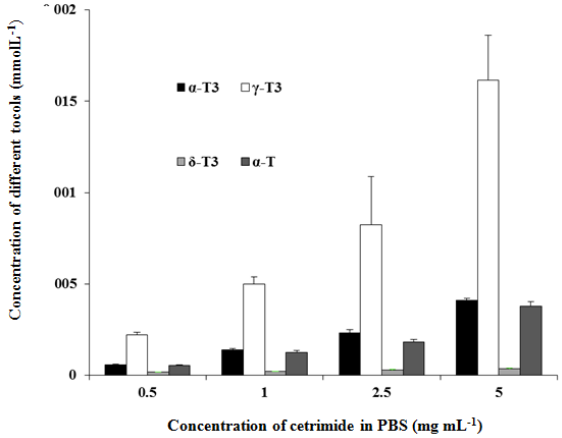


**Fig. 4:** Solubility of tocols in different concentrations of cetrimide in PBS (the error bars show n=3, +SE).

**Permeation Studies Using CPO and RPO**

***Permeation of FA in the Form of TAGs***

Table 2 shows the 'pseudo-fluxes' of the different FA (as a proxy for TAG) present in CPO and RPO across full thickness human skin. The amounts which permeated the skin from CPO were higher than that from RPO. With both CPO and RPO, only five TAG-derived FA were detected in the receptor phase samples. The FA which permeated the skin were

myristic acid, palmitic acid, stearic acid, oleic acid and linoleic acid (Figs. 6 and 7). The pseudo-flux values calculated were highest for oleic acid followed by palmitic acid, linoleic acid, stearic acid and then myristic acid (Table 2). The same rank order was observed when RPO was applied to the skin (Table 2).

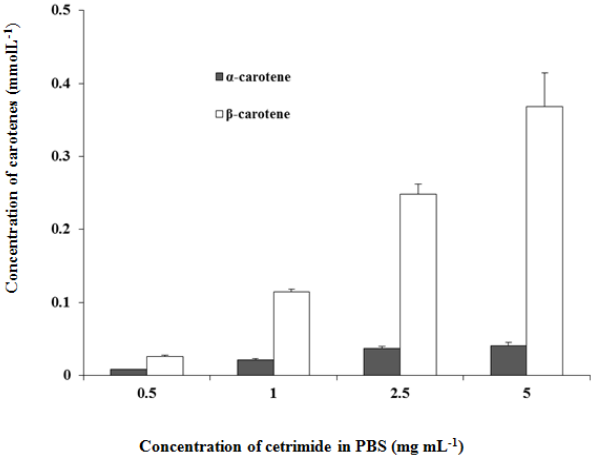


**Fig. 5:** Solubility of α-carotene and β-carotene in different concentrations of cetrimide in PBS (the error bars show n=3, +SE).

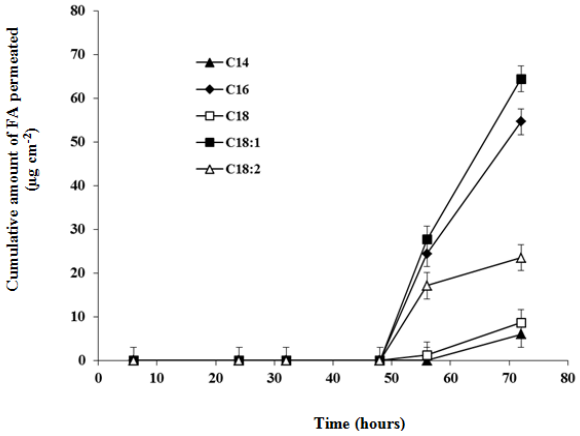


**Fig. 6:** Permeation of FA from TAG across full thickness human skin following CPO application (n=8, ±SEM).

**Table 2:** FA from TAG: Chain length, 'pseudo-fluxes' across full thickness human skin and proportion of total FA that permeated full thickness human skin (n=8; ±SEM).

| FA | Chain length | Pseudo-flux (ng/cm²/h) of CPO across full thickness membrane | Pseudo-flux (ng/cm²/h) of RPO across full thickness membrane | Proportion of total FA that permeated the skin (%) from CPO after 72 h | Proportion of total FA that permeated the skin (%) from RPO after 72 h |
|---|---|---|---|---|---|
| Myristic acid | C14 | 0.34±0.10 | 0.14±0.09 | 4.1±0.02 | 2.9±0.22 |
| Palmitic acid | C16 | 2.82±0.36 | 0.58±0.27 | 34.5±0.19 | 21.3±0.63 |
| Palmitoleic acid | C16:1 | – | – | – | – |
| Stearic acid | C18 | 0.41±0.08 | 0.10±0.14 | 5.6±0.56 | 3.1±0.15 |
| Oleic acid | C18:1 | 3.22±0.50 | 0.77±0.37 | 41.1±0.03 | 32.6±0.55 |
| Linoleic acid | C18:2 | 0.55±0.17 | 0.14±0.004 | 14.7±0.15 | 4.7±0.14 |
| Linolenic acid | C18:3 | – | – | – | – |

**Permeation of Tocols and Carotenoids from CPO and RPO**

The permeation of tocols and carotenoids across full thickness human skin was only noticed from CPO sample. The permeation of these compounds was not detected in the receptor phase when the RPO was used as the donor phase. Figure 8 illustrates the cumulative permeation profiles of the three $T_3$ and α-T from CPO. The greatest flux was observed for α-$T_3$ followed by γ-$T_3$ (Table 3). The flux of δ-$T_3$ was the lowest of all the tocotrienol forms. The flux of α-T was slightly higher than that of δ-$T_3$. It was noted that the flux of α-$T_3$ was 9-fold higher than that of α-T. The flux of α-$T_3$ was also observed to be 3-fold higher than that of γ-$T_3$.

The permeation of α- and β-carotene across full thickness human skin was also investigated since these compounds are present in appreciable quantities in CPO (Fig. 9). The flux of β-carotene was almost twice that of α-carotene (Table 3), which was as expected given that the concentration of β-carotene in palm oil is much greater than that of α-carotene.

**Table 3:** Fluxes of tocols and carotenes that permeated full thickness human skin from CPO and RPO (n=8; ±SEM).

| Compound | MW | *C Log P | Flux (ng/cm²/h) CPO | Flux (ng/cm²/h) RPO |
|---|---|---|---|---|
| α-$T_3$ | 424.66 | 11.92 | 28.05±5.00 | – |
| γ-$T_3$ | 410.60 | 11.38 | 9.56±4.58 | – |
| δ-$T_3$ | 396.60 | 10.83 | 2.73±1.27 | – |
| α-T | 430.71 | 12.18 | 3.17±0.97 | – |
| α-carotene | 536.87 | 12.78 | 208.88±44.97 | – |
| β-carotene | 536.87 | 12.78 | 317.5±75.31 | – |

*Note:* *partition coefficient

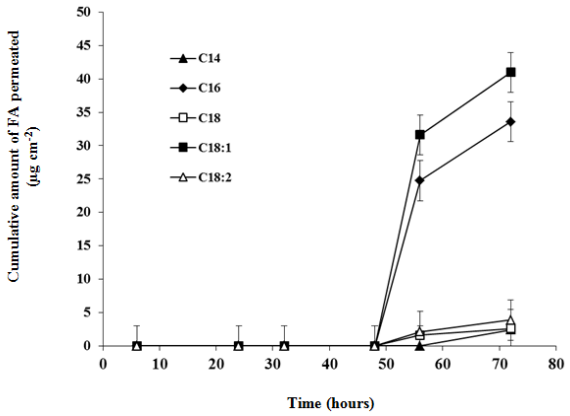


**Fig. 7:** Permeation of FA from TAG across full thickness human skin following RPO application (n=8, ±SEM).

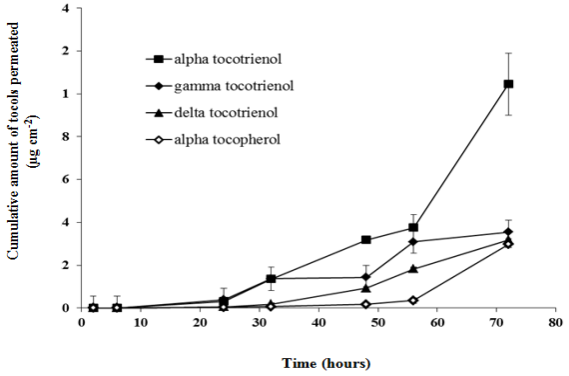


**Fig. 8:** Mean cumulative amount of α-$T_3$, γ-$T_3$, δ-$T_3$ and α-T that permeated full thickness human skin from CPO (n=8, ±SEM).

## DISCUSSION

These studies have demonstrated that palm oil constituents such as FA (from TAG), tocols (tocotrienols and tocopherols) and carotenoids (α- and β-carotenes) can all permeate full thickness human skin. The highly lipophilic nature of these palm oil constituents meant that a suitable receptor phase was required that could mimic physiological conditions and also provide the necessary sink conditions. PBS is often employed as a receptor phase in diffusion studies and in this case it was modified by adding cetrimide, a cationic surfactant, which was shown to be capable of solubilising palm oil. Researchers have often considered the use of organic solvents, in particular ethanol, as a means of enhancing sink conditions during permeation experiments. In the receptor phase, ethanol acts as a co-solvent and can facilitate the dissolution of many hydrophobic drugs (Sartorelli *et al*. 2000).

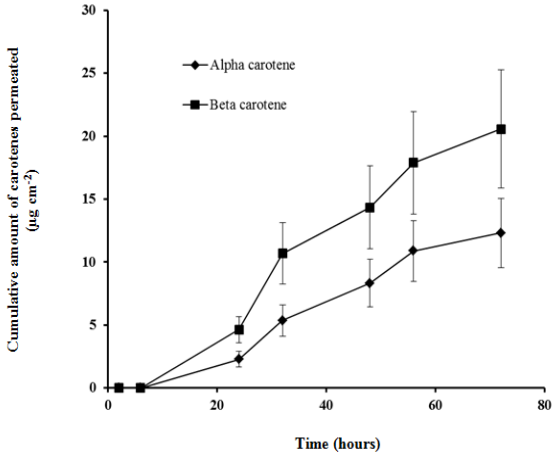


**Fig. 9:** Mean cumulative amount of α-carotene and β-carotene that permeated full thickness human skin from CPO (n=8, ±SEM).

However, adding high concentrations of ethanol into the receptor phase may also have detrimental effects on the skin. Exposing the skin to ethanol for long periods can perturb the barrier properties of the stratum corneum (SC) as ethanol tends to disrupt the lipid bilayers therein and reduce the integrity (Megrab *et al*. 1999). This could, in turn, lead to an overestimation of the permeant flux in vitro.Thus, to avoid the complications of using ethanol, surfactants were considered as means of increasing the solubility of the permeants in the receptor phase. However, adding significant higher amount of cetrimide in the receptor phase could contribute to the same implication as using ethanol; therefore the amount of cetrimide to be added into the receptor phase should be minimised and at the same time be able to maintain sink conditions throughout the permeation studies. In order to obtain the minimum amount of cetrimide to be added into the receptor phase without violating the sink conditions, the formation of cetrimide micelles and the solubility of palm oil in different cetrimide concentrations were performed and optimised.

The amount of cetrimide required in the receptor phase was determined using a light scattering method. The mode of action of cetrimide as a solubilising agent in aqueous solutions is based upon the formation of micelles which can carry lipophilic molecules in their core above a particular surfactant concentration (Mahato 2007). The concentration at which the free cetrimide monomers start to aggregate together to form micelles is known as the CMC. Above the CMC, the insoluble palm oil constituents become incorporated in the core of the micelle and, therefore, sink conditions can be maintained throughout the duration of the permeation experiment. The solubility studies with palm oil showed that the component solubilities increased steadily as the cetrimide concentration was increased (Figs. 3, 4 and 5). Above the CMC, which was shown to be around 1.0 mg/mL, the number of micelles present would have increased with increasing cetrimide concentration and this explains the steady increase in solubility of the palm oil components. Based on these solubility studies and knowledge of the CMC value of cetrimide in PBS, a cetrimide concentration of 3.0 mg/mL was selected as appropriate to be included in the PBS receptor phase in order to prolong sink conditions. A study by Karia *et al*. (2004) showed that cetrimide at a concentration of 30 mg/mL could increase the solubility of the highly

lipophilic compounds tamoxifen and γ-linolenic acid in an aqueous receptor solution without damaging the integrity of the skin. This was also supported by Morris *et al*. (2009) who also noted that cetrimide enhanced the solubility of another lipophilic drug, haloperidol, without damaging the integrity of the skin barrier.

TAG containing all of the five major FAs present in palm oil was able to permeate the skin and be detected in the receptor phase. The amount of FA (from TAG) which permeated the skin was greater from CPO than from RPO. Interestingly, TAG containing palmitic acid, which is the major FA present in TAG, permeated the skin to a lesser extent than TAG containing oleic acid, which is present in palm oil TAG at lower level. The reason for this observation could possibly be explained by the kinked shape of the unsaturated chain of oleic acid. This unsaturated chain, due to its shape, may have disrupted the ordered intercellular lipid in the SC. This disruption would have, in turn, promoted the diffusion of TAG across the SC. This was also the conclusion of Dae and Yie (1995), who noted that oleic acid enhanced the delivery of zalcitabine. Others have shown that unsaturated FA is more effective penetration enhancers than saturated FA. Wang *et al*. (2003) showed that oleic acid and linolenic acid (both unsaturated FA) enhanced the permeation of midodrine to a greater extent than the saturated FA, lauric acid and decanoic acid. However, in the case of CPO and RPO, oleic acid was the only unsaturated component of TAG that permeated the skin to a greater extent than would have been expected from the starting concentration. Despite from having different chemical structures, the permeation of other FA (from TAG) was observed to be in the same rank order as their occurrence in palm oil TAG.

The sudden surge fluxes observed from the permeation of FA after 48 hours are attributed to the slow diffusion of the TAGs across the skin. The ability of TAGs to diffuse through the skin membranes is a function of its molecular weight. Since TAG, which is a combination of three different FAs, has greater molecular weight than the individual FA itself, this resulted in slower diffusion across the skin, therefore, prolonging the lag time of TAGs permeation across the skin. The loss of skin integrity during the prolong exposure of receptor phase could also contribute to the permeation of TAG observed in the experiment which may account to the long lag time. Moreover, TAG do not confer any significant health benefits, therefore, the percutaneous permeation of this compound is not further pursued. Investigation of TAG permeation was more for toxicology purpose because of the fact that this compound might be used as a vehicle to deliver vitamin E and carotenoids through the human skin.

The percutaneous permeation of the tocols and carotenoids was only observed from CPO. The flux of α-$T_3$ was the highest followed by that of γ-$T_3$, α-T and then δ-$T_3$, which had the lowest flux. The latter compound is only present in small quantities in palm oil so its low flux was not surprising. However, even though the tocotrienols and tocopherols do not differ greatly in structure, the permeation of α-$T_3$ was significantly greater than that of α-T and this was unexpected. α-$T_3$ and α-T possess a similar molecular weight (MW) [424.7 and 430.7 respectively] and they have the same methyl substitutions on the chromanol ring. The difference between these two vitamin E forms is the side chain and it is possible that the superior percutaneous absorption of α-$T_3$ can be explained by its unsaturated nature. The unsaturated kinked side chain of the tocotrienols may have exhibited a similar effect to oleic acid, a known penetration enhancer (Theriault *et al.* 1999). It is known that tocotrienols are more mobile within bilayers and that they are more likely to move across phospholipid membranes than tocopherols (Atkinson *et al*. 2008; Suzuki *et al.* 1993). It is quite possible that a similar effect was being seen here and that α-$T_3$ is

more likely to permeate the multiple lipid lamellae present in the SC at a faster rate than α-T for this reason.

Differences in the rates of permeation were also observed within the tocotrienol group. γ-$T_3$, which is present at the highest concentration in CPO, exhibited a lower flux than that of α-$T_3$. Since both of these permeants possess the same 'kinked' unsaturated isoprenoid side chain, the most likely explanation for this difference in flux is that the substituent at the chromanol head group could play a role in altering permeation across the skin. The percutaneous permeation of carotenoids in the form of α- and β-carotene was also observed from CPO. The permeation of β-carotene was greater than that of α-carotene, however, this can be explained by its difference chemical structure or higher starting concentration as the MW and partition coefficient was the same for the both compound.

## CONCLUSION

The results have provided important data about the ability of palm oil constituents such as TAG, tocols and carotenoids to permeate full thickness human skin. A suitable receptor phase for the highly lipophilic palm oil constituents was developed using cetrimide as a solubilising agent. It was encouraging to observe that TAG, the tocols and the carotenes all permeated the skin from CPO. From RPO, only TAG permeated the skin and not the tocols or the carotenes, which were present in the oil at much lower starting concentrations. The TAG which permeated the skin, oleic acid, followed by palmitic acid was the most prevalent constituent FA from both CPO and RPO. Of the tocol group, the permeation of α- and γ-tocotrienol was observed to be superior to that of α-tocopherol. Additionally, the percutaneous permeation of α- and β-carotene was also observed to be greater from CPO than RPO.

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## REFERENCES

AGGARWAL, B. B., SUNDRAM, C., PRASAD, S. & KANNAPPAN, R. (2010) Tocotrienols, the vitamin E of the 21st century: It's potential against cancer and other chronic disease, *Biochemical Pharmacology*, 12: 1–19.

ANATOLY, N. L. & VLADIMIR, P. T. (2004) Micelles from lipid derivatives of water-soluble polymers as delivery systems for poorly soluble drugs, *Advanced Drug Delivery Reviews*, 56: 1273–1289.

ATKINSON, J., EPAND, R. F. & EPAND, R. M. (2008) Tocopherols and tocotrienols in membranes: A critical review, *Free Radiology Biology Medicine*, 44: 739–764.

BRITISH PHARMACOPOEIA (BP). (2010) British Pharmacopoeia (UK: British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare Products Regulatory Agency).

DAE, D. K. & YIE, W. C. (1995) Transdermal delivery of Zalcitabine: In vitro permeation study, *AIDS*, 9: 1331–1336.

KARIA, C., HARWOOD, J. L., MORRIS, A. P. & HEARD, C. M. (2004) Simultaneous permeation of tamoxifen and γ-linolenic acid across excised human skin, further evidence of the permeation of solvated complexes, *International Journal of Pharmaceutics*, 271: 305–309.

MAHATO, R. I. (2007) *Pharmaceutical dosage forms and drug delivery*, 3rd edition, pp. 267 (USA: CRC Press Pharmaceutical Education).

MEGRAB, N. A., WILLIAMS, A. C. & BARRY, B. W. (1999) Oestradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water co-solvent systems, *International Journal of Pharmaceutics*, 22: 101–112.

MORRIS, A. P., BRAIN, K. R. & HEARD, C. M. (2009) Skin permeation and ex vivo skin metabolism of *O-acyl* haloperidol ester prodrugs, *International Journal of Pharmaceutics*, 367: 44–50.

MORTENSEN, A. (2005) Analysis of a complex mixture of carotenes from oil palm (*Elaies guineensis*) fruit extract, *Food Research International*, 38: 847–853.

SAMBANTHAMURTHI, R., SUNDRAM, K. & YEW, A. T. (2000) Chemistry and biochemistry of palm oil, *Progress in Lipid Research*, 6: 507–558.

SARTORELLI, P., ANDERSON, H. R., ANGERER, J., CORISH, J., DREXLER, H., GOEN, T. *et al.* (2000) Percutaneous penetration studies for risk assessment, *Environmental Toxicology and Pharmacology,* 8: 133–152.

SCHEINDLIN, S. (2004) Transdermal drug delivery: Past, present and future, *Molecular Interventions*, 4: 308–312.

SCHWARTS, H., OLLILAINEN, V., PIRONEN, V. & LAMPI, A. M. (2007) Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats, *Journal of Food Composition and Analysis*, 21: 152–161.

SEN, C. K., SAVITHA, K. & ROY, S. (2007) Tocotrienols in health and disease: The other half of the natural vitamin E family, *Molecular Aspects of Medicine*, 28: 692–728.

SEN, C. K., SAVITHA, K. S. & ASHWATI, R. (2005) Tocotrienols: Vitamin E beyond tocopherols, *Life Sciences*, 78: 2088–2098.

SUNDRAM, K. (2000) Palm oil: Chemistry and nutrition updates (MPOB). PhD diss., University Malaya.

SUZUKI, Y. J., TSUCHIYA, M., WASSALL, S. R., CHOO, Y. M., KAGAN, V. E. & PACKER, L. (1993) Structural and dynamic membrane properties of alpha-tocopherol and alpha-tocotrienol: implication to the molecular mechanism of their antioxidant potency, *Biochemistry*, 32: 10692–10699.

TANG. (2004) Methods of test for palm oil products: Determination of fatty acids composition as their methyl esters by capillary column gas chromatography, 313–317.

THERIAULT, A., WANG, J. T., GAPOR, Q., ADELT, A. & KHOSROW, F. (1999) Tocotrienol: A review of its therapeutic potential, *Clinical Biochemistry*, 32: 309–319.

WANG, Y., FAN, Q., SONG, Y. & MICHNIAK, B. (2003) Effects of fatty acids and iontophoresis on the delivery of midodrine hydrochloride and the structure of human skin, *Pharmaceutical Research*, 10: 1612–1618.

ZAFARIZAL, A. A. H. & ISMAIL, R. (2009) Palm tocotrienols rich fraction in topical application, *Palm International Nutra-Cosmeceutical Conference (PINC),* 24–26 May 2009, Crowne Plaza Mutiara Hotel, Kuala Lumpur.