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In vitro transdermal delivery of caffeine, theobromine, theophylline and catechin from extract of Guarana, *Paullinia Cupana*

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Abstract

Extracts of guarana (*Paullinia cupana*) feature as putatively stimulating ingredients in a number of foods, drinks and dietary/herbal supplements. The objective of this work was to investigate in vitro the transdermal delivery of the major pharmacologically active compounds contained in guarana extract. Saturated solutions of guarana were prepared in polyethylene glycol 400 (PEG400), propylene glycol (PG) and H_2O at 32 °C. Guarana extract was also formulated in Duro-tak® 2287 transdermal adhesive in a range of concentrations and the diffusional release was determined in addition to adhesive properties. Transdermal delivery across full thickness pig ear skin was investigated in vitro using Franz-type diffusion cells, with reverse-phase HPLC being used for the quantification of the permeation of theobromine (TB), theophylline (TP), (+)-catechin (C) and caffeine (CF). Based upon a combination of release and adhesive property data a patch containing 5.55 mg guarana extract cm^{-2} was deemed optimal. The general trend for the delivery of the 4 analytes was: water >5.55 mg cm^{-2} patch ≈PG > PEG400. For CF the greatest steady state flux was obtained from the water vehicle: 19 μg cm^{-2} h^{-1}, with ~420 μg cm^{-2} permeating after 24 h. This was some 6× times more than from the drug-in-adhesive patch and 10× greater than PG, a well-known penetration enhancer, and 50× that of the 'regular' excipient PEG400. A water vehicle also provided the greatest delivery of TB (0.45 μg cm^{-2} h^{-1}), TP (0.022 μg cm^{-2} h^{-1}), and C (0.10 μg cm^{-2} h^{-1}). An inverse relationship was noted between lipophilicity and k_p in each vehicle. The simultaneous transdermal delivery of the major actives of guarana was established, with permeation rates being highly concentration and vehicle dependent.
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Keywords: *Paullinia cupana*; Guarana; Transdermal; Patch; Skin; Theobromine; Theophylline; (+)-Catechin; Caffeine

1. Introduction

Paullinia cupana var. *sorbilis* (Mart) from the family Sapindaceae, is native to the Brazilian Amazon Basin (Henman, 1982). Known informally as guarana this herb was classified as an Official Medicinal Agent in the US between 1882 and 1942, although nowadays extracts of guarana (*Paullinia cupana*) feature as putatively stimulating ingredients in a number of foods, drinks and dietary/herbal supplements including transdermal patches. In the US guarana extract (from seed) is currently an approved food additive for use as a natural flavouring agent and is considered a dietary supplement under the Dietary Supplement Health and Education Act (DSHEA) of 1994.

The major chemical constituents in guarana seeds are the methylxanthine derivatives: caffeine, theophylline and theobromine, which have well-known effects on the central nervous system; there is also a high proportion of the anti-oxidant polyphenol, catechin (Fig. 1). The stimulatory effects of guarana were originally credited to the high caffeine content (Henman, 1982), although it was later indicated that the pharmacologcal effects of guarana cannot be attributed solely to the presence of the xanthine bases suggesting the importance of the antioxidants present (Mattei et al., 1998). The xanthine in highest amount in guarana is by far caffeine, approximately 2.1–6% of dry weight (Espinola et al., 1997; Mattei et al., 1998), with theobromine and theophylline at <0.2%. Theophylline produces a stronger CNS stimulant effect than caffeine, but is present in very small quantities in guarana extract. The pharmacological effects of theophylline are relaxation of smooth bronchial muscle, CNS stimulation, cardiac muscle stimulation, and diuresis (Delbeke and DeBacker, 1996). Even at low concentrations of

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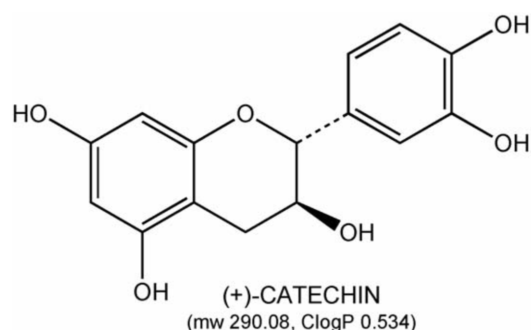

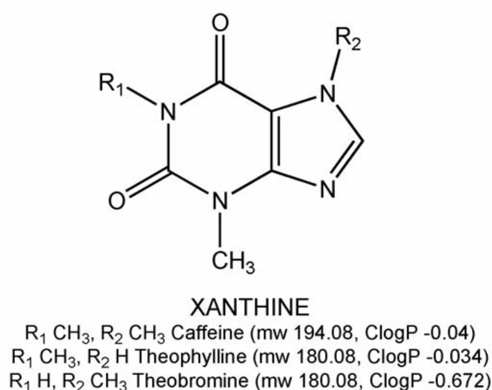

Fig. 1. Chemical structures and physicochemical properties (ChemDraw Ultra, version 9.0) of the major actives of guarana: (+)-catechin, caffeine, theobromine, theophylline.

$1.2\ \mu g\ ml^{-1}$, guarana inhibited lipid peroxidation which may be due to the known antioxidant action of the catechins or saponins, tannins and the flavonoids also present in guarana (Mattei et al., 1998; Basile et al., 2005).

Methylxanthines block adenosine receptors and inhibit phosphodiesterase that enhances the action of noradrenaline, which is released by ephedrine to induce an anorectic effect (Dulloo, 1993). Commercial preparations derived from guarana seeds are generally used as single oral formulations or as combination products, principally aimed at providing the consumer with a stimulatory or weight loss experience (Carlson and Thompson, 1998). Other reported therapeutic uses are as a tonic to overcome physical or intellectual stress, an antidiarrhoeal, or a diuretic (Mattei et al., 1998). The improved psychoactive effects of guarana were recently demonstrated (Kennedy et al., 2004). A study involving both acute and chronic dosing of rats and mice, in the range $1000–2000\ mg\ kg^{-1}$, concluded that guarana did not exert any toxic effects (Mattei et al., 1998). However, excess caffeine does have well recognised side effects.

Delivery of pharmacologically active compounds across skin is an attractive alternative to oral dosing, for numerous reasons including constant plasma concentrations and reduction of some side effects (Haller et al., 2005) through lower doses. Although a number of transdermal patches are commercially available containing guarana they tend to be mixtures containing other herbal material. Moreover, no evidence currently exists in the literature to support the notion that such formulations can deliver significant amounts of the major actives of guarana, although transdermal delivery of caffeine has been well studied both as a model permeant (Nanayakkara et al., 2005) and as a potential product (Nicoli et al., 2004). In this work we examined the in vitro transdermal delivery of caffeine in addition to theophylline, theobromine and catechin from several liquid and drug-in-glue formulations containing guarana extract.

2. Materials and methods

2.1. Materials

Guarana extract was supplied by Cambridge Commodities Ltd (Cambridge, UK). Caffeine was supplied by Aldrich (Poole, UK), theobromine by Acros organics (Loughborough, UK), and theophylline, (+)-catechin and polyethylene glycol 400 (PEG400) by Sigma (Poole, UK). Duro-tak® 387-2287 transdermal adhesive was a gift from National Starch & Chemical (Zutphen, Netherlands). HPLC grade methanol and propylene glycol (propane-1,2-diol, PG) were obtained from Fisher Scientific (Loughborough, UK). Porcine ears were obtained from a local abattoir prior to steam cleaning.

2.2. Saturated solutions

Some transdermal delivery systems are based upon 'reservoir' type patches, which typically contain active compounds in solution or gel. Saturated solutions of guarana extract in PEG, PG and H_2O were prepared overnight by standard methods in an incubator at 32 °C (skin surface temperature). Excess extract was separated by centrifugation, again at 32 °C.

2.3. Preparation of drug-in-glue patches

The simplest form of transdermal patch is where the active compounds or extract are dispersed within a thin film of adhesive supported by an inert backing material-all currently available patches containing guarana are of this type. Model patches containing 9 different levels of extract were prepared ranging from 0.09–10.78 mg of guarana extract per patch, with a thickness of 1 mm. One set of patches were formulated from only methanol and adhesive to serve as a control. A range of concentrations was used in order to examine the incorporation of extract into a commercial transdermal adhesive. The extract was suspended in methanol and mixed with Duro-tak® 387-2287 transdermal adhesive, mixed and cast onto aluminium foil backing, then left to allow evaporation of the solvent (Batchelder et al., 2004).

2.4. Diffusional release

Diffusional release testing is useful in establishing that all major actives may be released from a formulation in the absence of other complicating factors, i.e. partitioning into and permeation across the skin (Gallagher et al., 2003). The aluminium backing of 1 cm diameter sample patches were secured to the bottoms of 10 ml screw top glass vials and 2 ml of de-gassed de-ionised water (2 ml) was added to each vial. All vials were

placed on a laboratory shaker (Gyro-Rocker, Stuart Scientific, UK). Samples of 0.5 ml were taken at 3, 5, 10.5, 24 and 48 h and the receptor phase replenished with the same amount of fresh solution. Solutions were determined for caffeine by HPLC ($n = 3$).

2.5. Adhesive properties

Adhesive properties of the model patches were determined using a Stable Micro Systems TA-XT2 Texture Analyser in the tension mode (Woolfson et al., 1998). Downward probe speed was 0.2 mm s^{-1} in the contact phase, with a pressure of 5 N. The detachment force was 2 mm s^{-1}, and the detachment distance depended upon the adhesive nature of each film. Both skin and dialysis membranes were used as substrates ($n = 3$).

2.6. In vitro skin permeation

The in vitro transdermal delivery of extract of guarana was determined using Franz-type diffusion cells (Williams, 2004) containing full thickness porcine ear skin-a generally accepted model of permeation across human skin (Meyer and Zschemisch, 2002). The skin from the dorsal side of freshly excised and cleaned pig ears was removed from the underlying cartilage and cut into 2 cm^2 samples before being placed between the pre-greased flanges of the cell and held in place by a pinch clamp. Receptor phase (de-gassed de-ionised water) was added to the receptor phase along with a micro magnetic stirrer bar and the complete cells placed in a water bath maintained at 37 °C (providing a skin surface temperature of 32 °C via heat dissipation). After 10 min, cells were dosed either by pipetting the liquid formulations or by pressing the sample patches onto the skin surface using a glass rod (Batchelder et al., 2004). Parafilm or caps occluded the donor and receptor chambers respectively. Samples of 200 μl were withdrawn from the receptor phases at 3, 6, 12, 24, 36, 48 and 72 h and replaced with fresh receptor phase. Samples were stored in the freezer at −20 °C prior to analysis. The replication for each experiment was $n = 11$.

2.7. HPLC analysis

Caffeine, theobromine, theophylline and catechin were determined using an Agilent 1100 automated chromatograph fitted with a Kingsorb 5μ C18 250 mm × 4.6 mm (Phenomenex, Cheshire, UK). The mobile phase comprised of 77:23 0.25 M potassium phosphate adjusted to pH 3.5 by phosphoric acid:methanol; and the UV detector was set at 272 nm. The injection volume was 20 μl and the flow rate was 0.8 ml min^{-1} (Carlson and Thompson, 1998). Analytes were identified through a combination of comparison of retention times with standard or, where necessary, spiking. Retention times for theobromine, theophylline, (+)-catechin and caffeine were 0.55, 0.65, 0.72 and 1 min, respectively. A run time was 60 min was employed to ensure complete elution of all species between samples. All analytes were baseline resolved under these conditions.

3. Results and discussion

3.1. Diffusional release of caffeine from drug-in-adhesive patches

Up to 8.8 mg cm^{-2}, linear relationships between the amount of caffeine liberated from the model patches per unit time were observed (data not shown), indicating 0-order release kinetics and no dump effect, despite the loadings being visibly in excess of the solubility. However, above this level dump and non-linear release effects were apparent. Fig. 2 shows a plot of patch loading versus diffusional release rate of caffeine, demonstrating a reasonably linear relationship, with a r^2 value of 0.978. Linearity up to a loading level of 8.8 mg cm^{-2} indicates that the major limiting factor in the optimum amount of extract to be incorporated within the matrix is determined by the ability of the patch to adhere efficiently to skin.

3.2. Adhesive properties

The adhesive properties of the model patches were determined on two substrates, porcine skin and an artificial membrane (dialysis tubing). Although the latter lacks the complex architecture of the stratum corneum, it was the porcine skin that provided greater reproducibility. However, the differences were not great and the trends were very similar, confirming previous suggestions that the artificial membrane is an adequate substrate for modelling skin, in a purely comparative sense. Fig. 3 shows that increasing drug loading clearly affects the adhesion of the patches. However, a significant modulation in adhesive properties was observed between 5.55 and 8.8 mg cm^{-2}, at which point a plateau was reached. This reflects the general pattern observed in the diffusional release experiment, ostensibly due the increased disruption of the polymer matrix and its ability to interact with the substrate surface. This has the potential

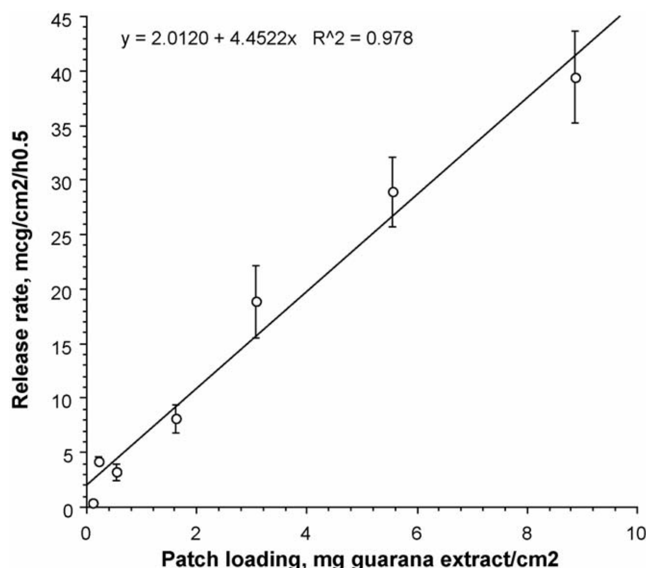

Fig. 2. Correlation between guarana loading in drug-in-adhesive patches and release rate of caffeine ($n = 4$, ±S.D.).

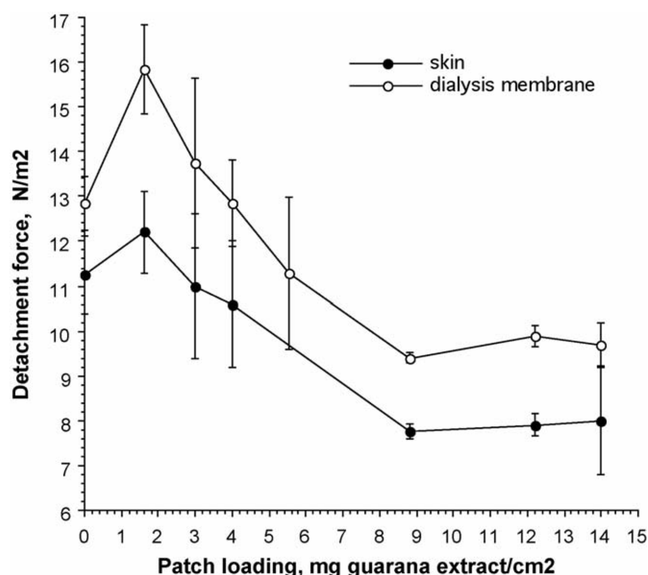

Fig. 3. Plot of detachment force vs. loading for guarana patches applied to porcine ear skin and dialysis membrane ($n = 3$, ±S.D.).

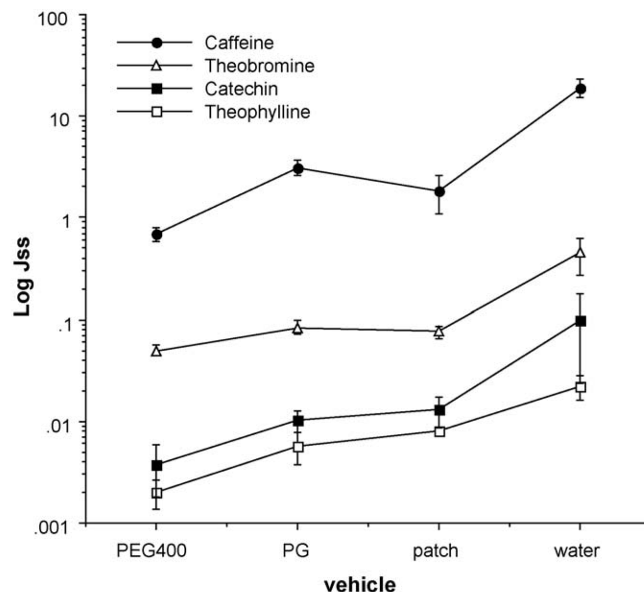

Fig. 4. Plot of log steady state flux across pig ear skin vs. vehicle for (+)-catechin, caffeine, theobromine, theophylline ($n = 11$, ±S.E.M.).

to cause a device to prematurely detach from the location to which it was affixed. Consequently, 5.55 mg cm^{-2} was selected as optimal loading level, as a balance between loading and adhesion.

3.3. In vitro transdermal delivery

Table 1 contains a summary of the permeation data. Each of the four permeants were delivered from each of the vehicles examined, although the amounts varied greatly. Fig. 4 is a plot of log J_{ss} versus vehicle for the four permeants. The permeation rates generally reflect the proportion of each active in guarana extract and the proportions dosed. The general trend for each permeant is: water >5.55 mg cm^{-2} patch ≈PG > PEG400. For caffeine the greatest steady state flux was obtained from the water vehicle: 19 μg cm^{-2} h^{-1}, with ~420 μg cm^{-2} permeating after 24 h. This was some 6× times more than from the drug-in-adhesive patch and 10× greater than PG, a well-known penetration enhancer, and 50× that of the regular excipient PEG400. A water vehicle also provided the greatest delivery of theobromine, with a flux of 0.45 μg cm^{-2} h^{-1} which was some 5× that of PG

Table 1
Summary of data for the permeation of the major actives of guarana across full-thickness pig ear skin ($n = 11$, ±S.E.M.)

Analyte	C_{sat} (mg cm^{-3})	J_{ss} (μg cm^{-2} h^{-1})	k_p (cm h^{-1})	Q_{24} (μg cm^{-2})
PEG400				
TB	3.73	$5.1 \times 10^{-3} \pm 6.0 \times 10^{-4}$	$1.34 \times 10^{-5} \pm 1.61 \times 10^{-6}$	6.74 ± 2.70
TP	2.01	$2.03 \times 10^{-3} \pm 1.03 \times 10^{-3}$	$1.01 \times 10^{-6} \pm 5.12 \times 10^{-7}$	$1.97 \times 10^{-2} \pm 1.89 \times 10^{-2}$
(+)-C	7.69	$3.78 \times 10^{-3} \pm 2.24 \times 10^{-3}$	$4.91 \times 10^{-7} \pm 2.91 \times 10^{-7}$	$5.69 \times 10^{-2} \pm 4.45 \times 10^{-2}$
CF	324.46	0.69 ± 0.11	$2.13 \times 10^{-6} \pm 3.39 \times 10^{-7}$	8.34 ± 1.33
PG				
TB	3.8	$8.38 \times 10^{-2} \pm 1.3 \times 10^{-2}$	$2.20 \times 10^{-5} \pm 3.42 \times 10^{-7}$	4.09 ± 1.51
TP	0.49	$5.70 \times 10^{-3} \pm 2.12 \times 10^{-3}$	$9.90 \times 10^{-6} \pm 4.32 \times 10^{-6}$	$0.10 \pm 5.63 \times 10^{-2}$
(+)-C	44.56	$3.03 \times 10^{-2} \pm 2.54 \times 10^{-3}$	$6.70 \times 10^{-7} \pm 5.69 \times 10^{-8}$	$0.21 \pm 8.16 \times 10^{-2}$
CF	320.37	3.13 ± 0.52	$9.80 \times 10^{-6} \pm 1.64 \times 10^{-6}$	40.64 ± 13.17
Water				
TB	9.90	0.45 ± 0.18	$4.52 \times 10^{-5} \pm 1.22 \times 10^{-5}$	9.81 ± 4.73
TP	1.20	$2.2 \times 10^{-2} \pm 6.07 \times 10^{-3}$	$1.83 \times 10^{-5} \pm 3.42 \times 10^{-6}$	0.23 ± 0.13
(+)-C	83.61	$0.100 \pm 7.6 \times 10^{-2}$	$1.19 \times 10^{-6} \pm 9.1 \times 10^{-7}$	2.27 ± 0.64
CF	726.92	19.13 ± 3.75	$2.63 \times 10^{-5} \pm 5.16 \times 10^{-6}$	419.19 ± 103.05
5.55 mg cm^{-2} Patch				
TB	–	$7.6 \times 10^{-2} \pm 1.1 \times 10^{-2}$	–	1.36 ± 0.19
TP	–	$8.18 \times 10^{-3} \pm 2.16 \times 10^{-3}$	–	$0.58 \pm 7.79 \times 10^{-2}$
(+)-C	–	$1.3 \times 10^{-2} \pm 4.66 \times 10^{-3}$	–	1.71 ± 0.27
CF	–	1.83 ± 0.73	–	73.44 ± 15.55

C_{sat} = concentration in saturated solution; J_{ss} = steady state flux, k_p = permeability coefficient, Q_{24} = cumulative permeation after 24 h, TB = theobromine, TP = theophylline, (+)-C = catechin, CF = caffeine.

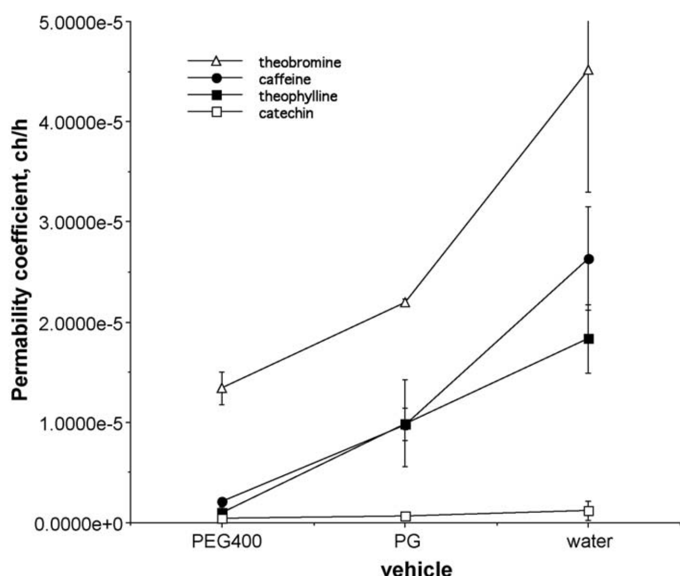

Fig. 5. Plot of permeability coefficient, k_p across pig ear skin vs. vehicle for (+)-catechin, caffeine, theobromine, theophylline ($n = 11$, ±S.E.M.).

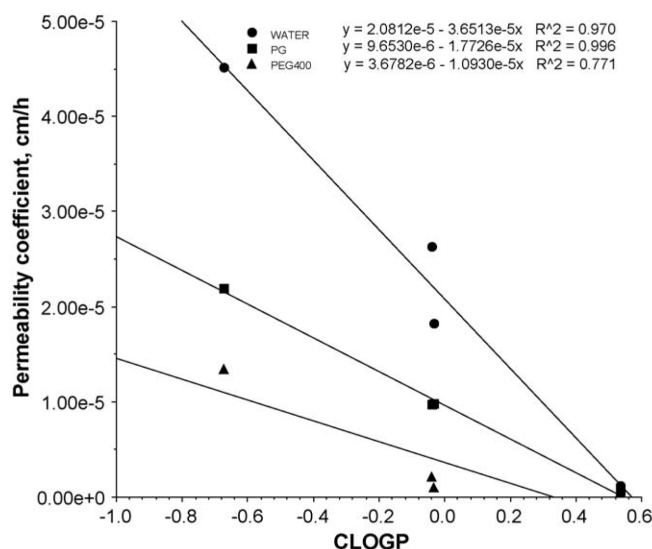

Fig. 6. Plot of mean permeability coefficient vs. c Log P for (+)-catechin, caffeine, theobromine, theophylline in PEG400, PG and water.

and the patch and 88× PEG400. Again from water, the maximum flux of theophylline was 0.022 μg cm^{-2} h^{-1} which was 2–3× PG and the patch and 10× PEG400. Similarly, water provided the greatest flux of catechin: 0.10 μg cm^{-2} h^{-1} which was ∼3× PG, 8× the patch and 26× PEG400.

From a thermodynamic activity perspective one might anticipate fluxes all to be equal in the absence of permeant/or solvent skin interactions; however these data clearly show the enhancing properties of water and PG relative to PEG400. Also from a Fickian standpoint, k_p data would also be expected to be similar. However, Fig. 5 again shows big differences and similar trends. Moreover, it is not straightforward to rationalise the transdermal delivery of multiple species from complex mixtures such as a plant extract as used here, as the behaviour of each species can potentially influence that of another. In addition to the four major actives of guarana determined in the current work, other chemical species are present in the extract.

The complexation/permeation process generally referable to as the drag-effect (Kadir et al., 1987; Bowen and Heard, 2006) may explain the enhancing effect of PG and the surprisingly large effect of water. Such data may indicate a polar route of delivery, possibly involving sweat pores, although prolonged hydration can lead to skin degradation (Bond and Barry, 1988) where such a catastrophic breakdown would also be expected to be associated with loss of steady state kinetics. It is also apparent that despite the large excess and high fluxes of caffeine, it was theobromine that demonstrated the greatest skin permeability by virtue of its consistently higher k_p. Fig. 6 is a plot of mean permeability coefficient of (+)-catechin, caffeine, theobromine, theophylline in PEG400, PG and water versus c log P. Again the ranking of relative enhancement is observed: water > PG > PEG400. However, the plot also clearly demonstrates an inverse relationship between lipophilicity and k_p in each vehicle. Such observations are at odds with the general conception that k_p should increase with lipophilicity up to a log P value of ∼2.

It was surprising to note that the drug-in-adhesive patch performed in a similar manner to a PG vehicle, although clearly this is not a case of like being compared with like. However, from a purely practical point of view a simple drug-in-adhesive system is far more attractive proposition than a reservoir patch containing PG. Whereas solution and diffusion chemistry can be used to at least partially rationalise transport from liquid formulations, the comparable performance of drug-in-adhesive systems remains more of an enigma. The compounds contained with such matrices as solid solutions and dispersions of particles ought not be readily absorbed into the skin as they are not in fluid phase. In an in vivo application the occlusion of skin, e.g. by adhesive film + backing layer leads to increased skin hydration and barrier modulation, whereby interfacial water may aid solubilise drug within the patch prior to re-absorption. However, this would assume that the permeants had appreciable aqueous solubility, which was so in this case. However, in the absence of sweat and homeostatic processes in the in vitro experimental set-up, this is clearly not the case, although it is possible that solubilisation occurred following the diffusion of receptor phase throughout the skin membrane.

4. Conclusions

The simultaneous transdermal delivery of the major actives of guarana has been established, which to a certain extent supports current products based upon this herb. The main benefit of administering simultaneously all major actives of guarana rather than caffeine alone is that the stimulatory effects of caffeine are modulated (Mattei et al., 1998; Espinola et al., 1997). However, commercial patches containing much lower amounts of guarana than that used in this work may be unable to deliver across skin adequate amounts of the lesser components.

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