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Permeability and Retention Studies of (-)Epicatechin Gel Formulations in Human Cadaver Skin

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ABSTRACT (-)Epicatechin (EC) is a major antioxidant component of grape seed extract which has become increasingly popular in topical skin preparations. This study assessed the following: (1) the permeability through cellulose membranes of EC in three different gel formulations (Carbopol®940, Klucel®, and Ultrez™10); (2) the effect of three different antioxidants (butylated hydroxytoluene (BHT), alpha-tocopherol (VE), and ascorbic acid (AA)) on the stability and penetration properties of EC; and (3) the permeability and retention of EC in Ultrez™10 gels, supplemented with BHT or VE, on human cadaver skin. Permeability studies through cellulose membranes showed that different gelling agents do not significantly affect the permeability of EC ($n = 7$/gel; $p > 0.05$). BHT and VE have antioxidant properties superior to AA ($p < 0.05$) and preserve 100% of the initial content of EC for 28 days. Permeation studies on cadaver human skin, following application of two anhydrous gel formulations (0.5% EC in Ultrez™10 containing BHT or VE), showed that EC was not detectable in the receiving solution. However, the EC amount in viable skin increased with time, indicating that EC penetrated and was retained in the upper part of the skin for approximately 1% and 3% of the dose for the formulations containing BHT and VE, respectively.

KEYWORDS (-)Epicatechin, Permeability, Human cadaver skin, HPLC

INTRODUCTION

In the past few years, the use of natural products rich in proanthocyanidins, (OPCs) such as grape seed extract, green tea, dark chocolate, or cranberry juice, has become increasingly popular in topical skin preparations. The rationale is that the well-known oxygen radical scavenger activity of these polyphenolic compounds is beneficial in preventing skin aging and improving skin tone and appearance. While epidemiologic studies suggest that orally administered OPCs are potentially effective in the prevention of cancer and cardiovascular disease, and as anti-inflammatory agents (Cos et al., 2004), their efficacy in topical preparation is still debatable due to the lack of rigorous studies on formulation stability and penetration profiles.

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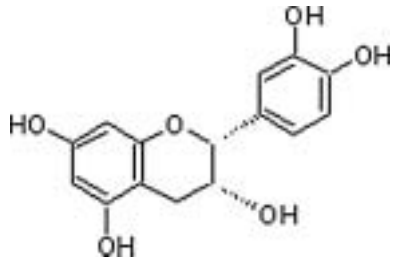

FIGURE 1 **Structural Formula of (-)Epicatechin (MW 290).**

This study evaluates the stability and permeability through human cadaver skin of (-)epicatechin formulated in different gels for topical use. Epicatechin (Fig. 1) was selected because it is one of the major antioxidant components of the proanthocyanidins (OPCs) (Joshi et al., 2001) present in grape seed extracts as well as in green tea. Typically, OPCs are a mixture of gallic acid, the monomeric flavan-3-ols (catechin, epicatechin (EC), gallocatechin, epigallocatechin (EGC), and (-) epigallocatechin gallate (EGCG)), procyanidin dimers and trimers, and more highly polymerized procyanidins. Natural products usually contain a mixture of different proportions of these components. Since the release and stability of each component may be affected by the presence of the others, we chose to systematically study the release of a single component at a time. This approach will help to determine the interaction among the different compounds in natural products.

Dvorakova et al. (1999) studied the stability and in vitro pharmacokinetics of (-)epigallocatechin gallate (EGCG), a major component of OPCs, and found that topical application of EGCG in hydrophilic ointment USP to human or mouse skin resulted in substantial intradermal uptake, up to 1–20% of the applied dose. However, transdermal penetration was detected only in mouse skin. Epicatechin has a lower molecular weight (MW = 290) compared to EGCG (MW 458), and its hydrophilic/lipophilic balance (logP = 1.5) (Schroeder et al., 2003) indicates that it is soluble in aqueous as well as in lipophilic solvents. These properties suggest that EC may penetrate more deeply and in larger amounts into the human skin compared to EGCG.

In this report, the permeability of EC from three different gel formulations (Carbopol® 940, Klucel®, and Ultrez™ 10) was determined through cellulose membranes and human cadaver skin. Gel formulations were selected because they are miscible with water and they feel less greasy on the skin than cream or lotion. The three gelling agents used in this research differ in efficiency at building viscosity, and in their physical and chemical properties.

In addition, EC is a very reactive antioxidant; as such, it is necessary to protect EC from oxidation. The efficacy of three different antioxidants (butylated hydroxytoluene (BHT), alpha-tocopherol (VE), and ascorbic acid (AA)) on the stability and penetration property of EC was evaluated. BHT was selected because it was successful (Dvorakova et al., 1999, Proniuk et al., 2002) in preventing oxidation of (-)epigallocatechin gallate (EGCG). Ascorbic acid and vitamin E were selected because, being vitamins, they are usually well accepted by consumers.

EXPERIMENTAL

Chemicals

Epicatechin, alpha-tocopherol, sodium phosphate dibasic anhydrous, sodium ethylene-diamine-tetraacetic acid (EDTA), ascorbic acid, and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Glycerin, phosphate buffered saline ultrapure, and propylene glycol were purchased from Spectrum Chemical (New Brunswick, NJ). Methanol and alcohol anhydrous reagents were from J.T. Baker (Phillipsburg, NJ). Trifluoroacetic acid (TFA), HPLC-grade acetonitrile, and water were obtained from EM Science (Gibbstown, NJ), while sodium phosphate monobasic monohydrate was supplied by EM Science, Merck KGaA (Darmstadt, Germany). Carbopol® 940 and Carbopol Ultrez ™10 were from Noveon (Cleveland, OH). Klucel® was from Hercules Inc. (Wilmington, DE).

Preparation of Gel Formulations

The gels were prepared in a beaker at room temperature (25 ± 1°C) by slowly dispersing the appropriate gelling agent (Carbopol® 940, Ultrez™, or Klucel®) into a mixture of propylene glycol and ethanol (2:1). The beaker was covered with Parafilm® (American National Can™, Chicago, IL) and aluminum foil during the entire procedure. The gel was continuously stirred for at least 30 min with a Teflon™-coated magnetic bar until a visually homogenous gel formed. Then, 10 mL of the antioxidant solution were added

TABLE 1 Compositions of Gel Formulations (%w/w)

ID	Carbopol 940	Ultrez 10	Klucel	Propylene glycol	Ethanol	Water	BHT	AA	VE	EC	Glycerin q.s. to make
C[1]BHT[4]	0.60			20	30		0.10			0.50	100
U[2]BHT		0.60		20	30		0.10			0.50	100
K[3]BHT			0.60	20	30		0.10			0.50	100
CAA[5]	0.60			20	20	10.00		5.00		0.50	100
UAA		0.60		20	20	10.00		5.00		0.50	100
KAA			0.60	20	20	10.00		5.00		0.50	100
CVE[6]	0.60			20	30				5.00	0.50	100
UVE		0.60		20	30				5.00	0.50	100
KVE[7]			0.60	20	30				5.00	0.50	100

[1]C = Carbopol® 940, [2]U = Ultrez ™ 10, [3]K = Klucel®, [4]BHT = Butylated hydroxytoluene, [5]AA = Ascorbic acid, [6]VE = Alpha-tocopherol; [7]KVE formulation was unstable from the beginning and was withdrawn from the study.

drop-wise to the gel. Finally, 10 mL of the 5% (-)epicatechin ethanol solution was added. The mixture was continuously stirred for 20 minutes or until a homogeneous gel formed. Table 1 shows a synopsis of the composition of the gel formulations prepared. The formulation containing Klucel® and vitamin E (KVE) was unstable from the beginning and was withdrawn from the study.

HPLC Analysis of Epicatechin

The High Performance Liquid Chromatography (HPLC) analyses were performed on a Waters 717 plus Auto sampler, Hitachi L-4250 UV-VIS Detector, Hitachi L-6200A Intelligent Pump, Perkin Elmer Nelson 900 Series Interface, and the Perkin Elmer Software TotalChrom®navigator (PE Nelson-Version 6.2.1) data-handling system. Separation was achieved on a C18 column (Alltima® HP, ESP, 3 μm particle size, 150 × 4.6 mm inner diameter), eluted with a mobile phase consisting of acetonitrile, methanol, and a pH 2.5 water solution of TFA (2.94% v/v) (16: 1.5: 82.5). Flow rate was 0.8 ml/min. A 10-μL sample was injected onto the column. Column eluate was monitored at a 215 nm wavelength. The HPLC assay used in this research was based on the work by Dvorakova et al. (1999) who developed an isocratic method for the determination of (–) epigallocatechin gallate. The detector wavelength was changed to 215 nm, EC's maximum absorbance. The mobile phase was adjusted to provide baseline separation between EC and ascorbic acid, always present in the samples as the antioxidant agent. Typical chromatogram of EC-spiked samples showed a sharp and symmetric peak (Tailing Factor: 1.14) at 3.2 minutes. The CV (%) for inter-day assays at 0.5 μg/mL and 60 μg/mL were 11 and 1.6 respectively.

Preparation of (-)Epicatechin Standard Solution

EC standard solutions were freshly prepared each day in distilled water containing 20 μL of an ascorbic acid/EDTA solution per 1 mL of final volume as antioxidant agents. The ascorbic acid/EDTA solution consisted of a mixture of ascorbic acid (20% w/v) and EDTA (0.1% w/v) dissolved in a sodium phosphate buffer (0.4 M, pH 3.6) (Dvorakova et al., 1999). Working standard solutions were then prepared by dilution at the following concentrations: 0.5, 4, 6, 8, 10, 40, 60, and 80 μg/mL and stored away from light.

Stability Studies on (-)Epicatechin Solutions

The stability of epicatechin solutions (4 μg/mL) was studied at various temperatures and with different amounts of the antioxidant ascorbic acid. The ratios of mole of EC to mole of vitamin C were: 0.152:0; 0.152:1, 0.152:10, 0.152:50 and 0.152:100, respectively. All samples were prepared simultaneously in a volumetric flask and then divided into 2.5 mL polyethylene eppendorfs, sealed with Parafilm® and aluminum foil to protect them from light. Samples were stored at three different temperatures: room temperature (25°C), 9°C, and –20°C. The content of EC was determined by HPLC analysis just after preparation and weekly over a four week period.

Stability Studies on Gel Formulations

Gel formulations were stored in well-sealed polyethylene (PE) containers for four weeks at room temperature. Samples of the formulations were analyzed for EC content by HPLC at 0, 7, 14, 21, and 28 days. On the day of the analysis, a known amount of the formulation was weighed in a 50-ml volumetric flask and was dissolved in water containing the ascorbic acid/EDTA solution. In addition, the physical appearance of gel samples was evaluated by visual inspection for color, odor, texture, and homogeneity.

In Vitro Permeation Using Cellulose Membrane

In vitro diffusion studies were carried out using a Modified Franz Diffusion Cell Apparatus with a diameter of 15 mm and a diffusional area of 1.76 cm^2. A Spectra/Por®7 (Laguna Hills, CA.) regenerated cellulose membrane (thickness of 60–65 μm and a molecular weight cutoff of 1000) was sandwiched between the lower cell reservoir and the glass cell-top containing the sample. The top glass sample reservoir was secured in place with a pinch clamp. The receiving compartment (volume 13 mL) was filled with Dulbecco's phosphate buffered saline (DPBS) pH 7.4 (9.6 g/L) mixed with the ascorbic acid/EDTA solution (20 μL/mL). The system was maintained at 37 ± 0.5°C by a water bath circulator and a jacket surrounding the cell, resulting in a membrane-surface temperature of 32°C (McVean et al., 1997, Suwanpidokkul et al., 2004). The receiving medium was continuously stirred (600 rpm) with a Teflon™-coated magnetic bar to avoid diffusion layer effects. A 200 μL sample of each gel formulation was accurately measured and placed in the donor compartment, which was then sealed with aluminum foil and Parafilm® (American National Can™, Chicago, IL) to prevent evaporation. 200–microliter samples were withdrawn from the receiving compartment using a micro syringe at 15, 30, and 45 min and at 1, 1.5, 2, 2.5, 3, 3.5, and 4 h. Samples were analyzed by HPLC. Sink condition of the receptor compartment was maintained by replacing the withdrawn solution with degassed, fresh solution. The experiment was carried out in seven replicates ($n = 7$) for each formulation.

In Vitro Permeation Using Cadaver Skin

The same apparatus and experimental procedure described in the previous section were used to perform cadaver human skin permeability studies. The sampling times were 2, 4, 6, 12, 24, and 48 h. The experiment was carried out in six replicates ($n = 6$) for each formulation. Full-thickness dermatomed human cadaver skin taken from the back region of Caucasian subjects was from the Ohio Valley Tissue and Skin Center (Cincinnati, Ohio). The skin was frozen in a 10% glycerol solution by the supplier and stored at −70°C until the experiments. Cadaver skin was used immediately after thawing and prepared by hydrating it in DPBS pH 7.4 for 1 h at room temperature before being cut into 3×4 cm^2 and then placed between the donor and receiving compartments. Integrity of the skin was carefully assessed by visual inspection to ensure that no holes or other imperfections were present.

Tape-Stripping Experiment

The tape-stripping method (Surber et al., 1999) was applied to estimate the amount of drug uptake in the viable skin at 2, 4, 6, 12, 24, and 48 h using the same apparatus previously described. At each sampling time, the skin tissue was rinsed with distilled water and blotted with a paper towel. The adhesive tape (Scotch®, 3M, St. Paul, MN) was then applied with uniform pressure to the skin and removed. The procedure was repeated 9 to 20 successive times for each application site. Tape-strippings were combined, extracted with water containing the antioxidant solution, and analyzed by HPLC for EC content. The remaining skin, stripped of the stratum corneum, was cut in small pieces, extracted with water containing the antioxidant solution, and analyzed by HPLC for EC content. The procedure was performed in triplicate for each data point. Thickness of the skin was measured with a micrometer caliper (Starrett, Athol, MA).

Data Processing

Data obtained by HPLC analysis were corrected for sampling effects. Cumulative quantity of EC collected in the receiver (μg/cm^2) was plotted as a function of time. The flux value (J_{ss}, μg/cm^2/h) for each experiment was obtained from the slope (steady-state

portion) of the linear portion of the data fitted by regression analysis (Komatsu et al., 1979). Lag time (L) was determined from the *X*-intercept of the regression line. The apparent permeability coefficient (*P*, cm/h) was obtained by dividing J_{ss} by the donor concentration (C_d) according to Fick's First Law of diffusion. For release data analysis, cumulative permeation ($\mu g/cm^2$) was plotted as a function of the square root of time to obtain diffusion profiles in accordance with the theory of Higuchi (1962). The release rate constant was then estimated as the slope of such plots ($\mu g/cm^2/h^{0.5}$).

Statistical Data Analysis

Means, standard deviation (SD), coefficient of variation (%CV), and linear regression analyses were calculated using Microsoft Excel 2000. One-way analysis of variance (ANOVA) and Box-plots were performed with SPSS 10.1 for Windows 2000.

RESULTS AND DISCUSSION

Stability of Epicatechin in Solution and in Gel Formulations

Dvorakova et al., (1999) in a study of (-)epigallocatechin gallate (EGCG) pharmaco-kinetics used a mixture of ascorbic acid (20% w/v) and EDTA (0.1% w/v) dissolved in sodium phosphate buffer (0.4 M, pH 3.6) to prevent oxidation of EGCG standard-solutions. We used the same mixture composition for the preparation of EC standard solution and, since the shelf-life of this EC solution was not known at the time we started this project, we prepared fresh standard-solutions daily. At the same time, we performed a study of stability of EC on solutions containing different molar ratios of EC and ascorbic acid and at different storage temperatures for 4 weeks. The concentration of EC was kept constant (4 μg/mL or 0.152 M) and five levels of ascorbic acid/EDTA solution were tested: 0, 1, 10, 50, and 100 times the molar concentration of EC. The molar ratio used throughout this study and by Dvorakova et al. (1999) corresponds to the (0.152:1 or 1:1) ratio. Fig. 2 shows the concentrations of EC detected at the different storage temperatures. The results of the stability study show that temperature and amount of ascorbic acid have a significant impact on the stability of EC. At room temperature, the amount of EC in the solution without any antioxidant started to decline immediately, and less than 5% was left after 28 days. At –20°C, the solution without antioxidant started to decline after a week and only 70% was left after 28 days. The results of the stability study show that standard solutions containing a molar ratio (1:10) would be stable for 28 days if stored at 9°C.

Gels containing either BHT or vitamin E retained 100% of their initial content of EC after 28 days, while gels containing ascorbic acid showed a statistically significant loss ($p < 0.05$) of 15% EC.

In Vitro Permeation Studies Using Cellulose Membranes

In order to determine the ability of a semisolid formulation to release a drug, the release pattern and rate of release through the synthetic membrane must be known. These procedures distinguish between release from the formulation and the actual permeability through human cadaver skin. In this study, we used the permeability through cellulose membranes (MW cutoff 1000) to select the most appropriate gelling agent/antioxidant combination. Seven replicates were performed for each formulation as suggested by the FDA guidance for *in-vitro* testing of non sterile semisolid dosage forms (FDA, 1997, Shah et al., 1999). Cellulose membranes were selected because they are thin and highly permeable. Therefore, the membrane per se did not represent a barrier, and variations in the release profiles among the different formulations could be attributed solely to the different diffusion properties of the molecules in the gels. This method is usually recommended to monitor product quality because the release rates measured are formulation-specific (Shah et al., 1999). The permeability profiles obtained are shown in Fig. 3. The release-rate plots (drug released per unit membrane area versus square root of time) are shown in Fig. 4. Visual inspection of the permeability curves (Fig. 3) shows that a plateau was reached after about 2 hours. At this point, approximately 30–40% of the amount originally present in the formulation has been released, and consequently the concentration gradient across the membrane decreased. In addition, cellulose membranes are permeable to water and water will flow from a region of high water content (the receiver solution) to a region of low water content (the gel). As a result, the net release of the drug diminishes. However, in the first

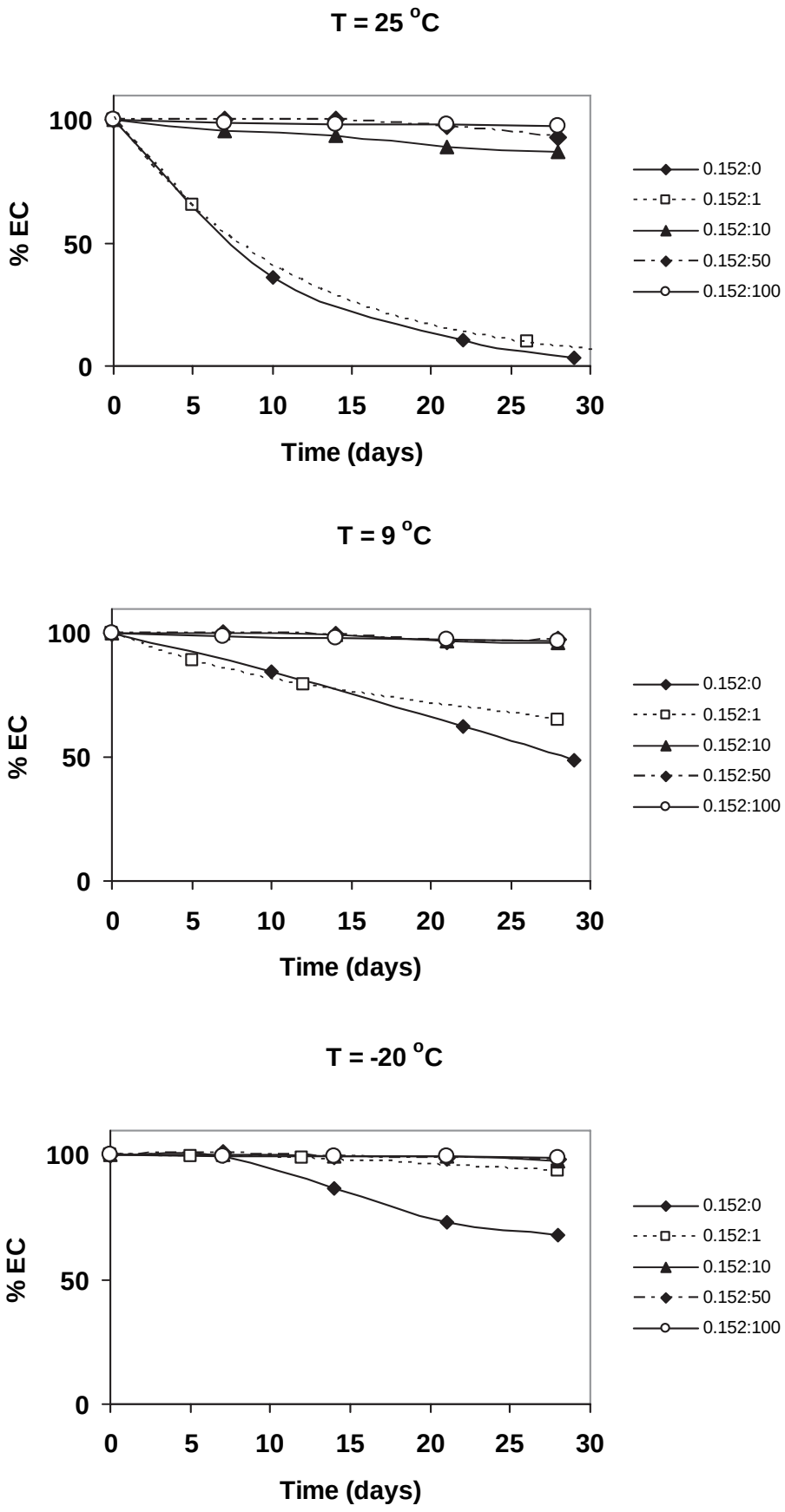

FIGURE 2 **Stability of (-)Epicatechin in Solutions Supplemented With Different Amounts of Ascorbic Acid/EDTA Solution at 25°C, 9°C, and −20°C Respectively.**

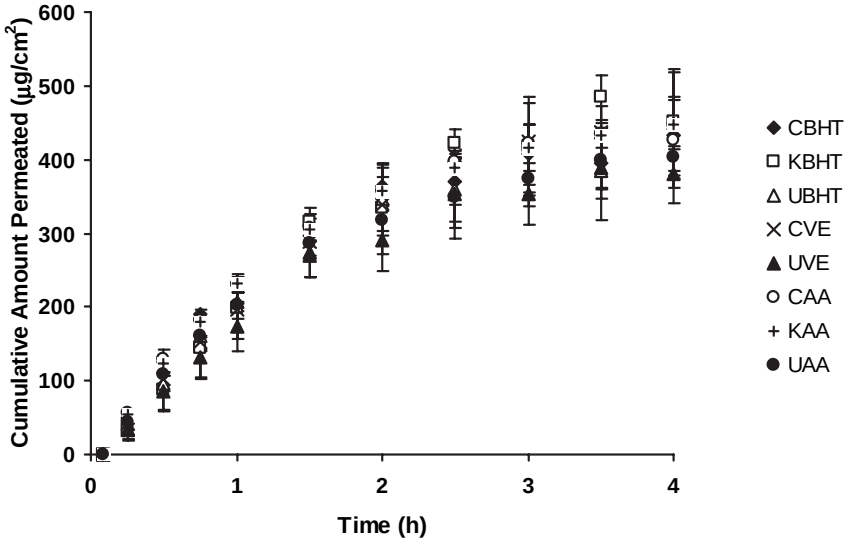

FIGURE 3 **Permeation Profile of (-)Epicatechin From Gel Formulations Through Cellulose Membranes at 32°C. Data Represent Mean ± SD (*n*=7) (For Explanation of Symbols see Table 1).**

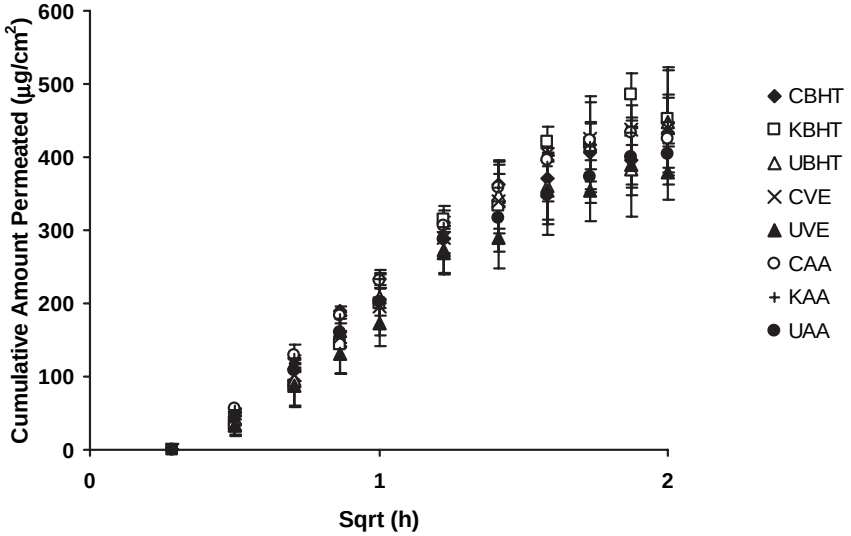

FIGURE 4 **In Vitro Release of (-)Epicatechin From Gel Formulations Through Cellulose Membranes at 32°C. Data Represent Mean ± SD (*n* = 7) (For Explanation of Symbols see Table 1).**

part of the curve, the penetrated amount of EC per unit area increased linearly with time, therefore flux (J_{ss}) could be estimated from the slope of the linear part of the plot. The values estimated for flux, permeability coefficient, and release rate are reported in Table 2.

Analysis of variance showed that the types of gels used did not significantly affect the flux, permeability, and release rates (Fig. 5). This behavior was expected, since the three types of gelling agents are very similar. However, there was a statistically significant effect of antioxidant on flux ($p > 0.05$). Flux of formulations containing BHT was significantly larger than the flux from the formulations containing vitamin E by an average of 41 µg/cm^2/h, while the ascorbic acid formulations were somewhere in between. The effect of antioxidant was independent of the gel used indicating that there was no interaction between the different gelling agents and the antioxidants. The reasons for the variation in results between the three antioxidants can be attributed to their different concentrations and molecular sizes. Indeed, BHT is present in much lesser amounts (0.1% vs. 5% for vitamin E) and it has a smaller molecular size (MW = 220.34 vs. 430.71 for vitamin E). Ascorbic acid is present at 5% but has the smallest MW (176.12).

TABLE 2 Flux (J_{ss}), Permeability Coefficient (K_p) and Release Rates of (-)Epicatechin From Gel Formulations Across Cellulose Membranes

	Mean ± SD							
	CBHT	*KBHT*	*UBHT*	*CVE*	*UVE*	*CAA*	*KAA*	*UAA*
J_{ss} (μg/cm^2/h)	251 ± 7	257 ± 15	232 ± 23	207 ± 25	190 ± 16	230 ± 9	235 ± 6	210 ± 10
K_p (cm/h)	0.050 ± 0.001	0.052 ± 0.003	0.046 ± 0.005	0.041 ± 0.005	0.038 ± 0.003	0.046 ± 0.002	0.047 ± 0.001	0.042 ± 0.002
Release Rate Constant (μg/cm^2/hr$^{0.5}$)	659 ± 31	613 ± 83	671 ± 31	634 ± 83	567 ± 78	620 ± 78	649 ± 62	591 ± 63

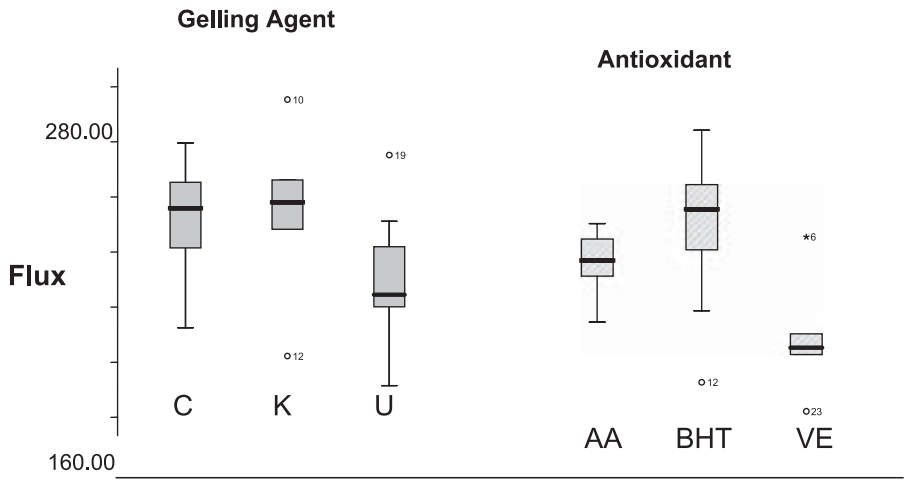

FIGURE 5 Box-plot of Flux vs. Gelling Agent (Left: *C* = Carbopol® 940; *K* = Klucel®; and *U* = Ultrez™ 10) and of Flux vs. Antioxidant (Right: *AA* = Vitamin C; *BHT* = Butylated Hydroxytoluene; and *VE* = Vitamin E).

Cadaver Skin Permeability

Insignificant differences in permeability parameters among the different gel formulations were observed in the cellulose membrane experiments (Fig. 5). As a result, we decided to evaluate the permeability properties in cadaver human skin for only one gelling agent. The selection of the gelling agent Ultrex™ 10 was based on its optimal physical characteristics, such as: smooth texture, no color change, and the observed stability at room temperature over the period of the study. The two antioxidants BHT and alpha-tocopherol were tested because they both demonstrated antioxidant efficacy superior to vitamin C in the stability study. However, the in vitro permeability showed that fluxes from gels containing either vitamin E or ascorbic acid (Fig. 5) were not statistically significant. No EC was detected in the reservoir compartment of the Franz cells during the permeability studies over a period of 48 h. EC water solubility is 50mg/mL (Herb-tech, 2005), thus poor solubility in water cannot account for the results observed. The average skin thickness was 0.82 ± 0.19 mm ($n = 18$) (including only epidermis and dermis) because the skin was from the human back, where the dermis thickness is on average 3–4 mm (Odland, 1991). Therefore, the possibility that EC could be trapped in the subcutaneous fat must be excluded. The amount of EC detected in the stratum corneum was essentially constant (slope not statistically significant) for both formulations (Fig. 6a) with an average of 340 ± 37 μg and 260 ± 28 μg for the BHT and VE formulation respectively. However, the EC amount increased with time inside the cadaver viable skin (Figure 6b). The recovery efficiency of the method used to extract EC from the dermis was not determined. Therefore, the EC amounts reported in Fig. 6b are actually the lower limit for the amount of EC possibly present in dermis.

Interestingly, it can be observed from the plots of EC penetrated into the skin vs. time that the diffusion rate of EC in the skin is higher in the first 4–6 h. The flux and permeability coefficients calculated for this initial period are reported in Table 3. This boost in penetration may be due to the presence of ethanol and propylene glycol in the gel formulations. Both ethanol and propylene glycol are well-known penetration enhancers (Ota et al., 2003). Therefore, it is possible that they diffused through the stratum corneum rapidly, helping the EC to go through. Once the gel formulation is depleted of ethanol and propylene glycol, this effect disappears and the diffusion of EC decreases. Furthermore, from the permeation curves shown in Fig. 6b, it is evident that the formulations containing vitamin E penetrate faster and in larger amounts into the skin than those containing BHT. It is, therefore, evident that vitamin E not only acts as an antioxidant but also as a penetration enhancer, while BHT is only an antioxidant.

The results of this study show that EC can penetrate into the human cadaver skin. However, once there, EC does not move forward into the receiving solution of the Franz' cells in detectable amounts during the 48 h of the experiment. Dvorakova et al. (1999) found a similar behavior for (-)epigallocatechin

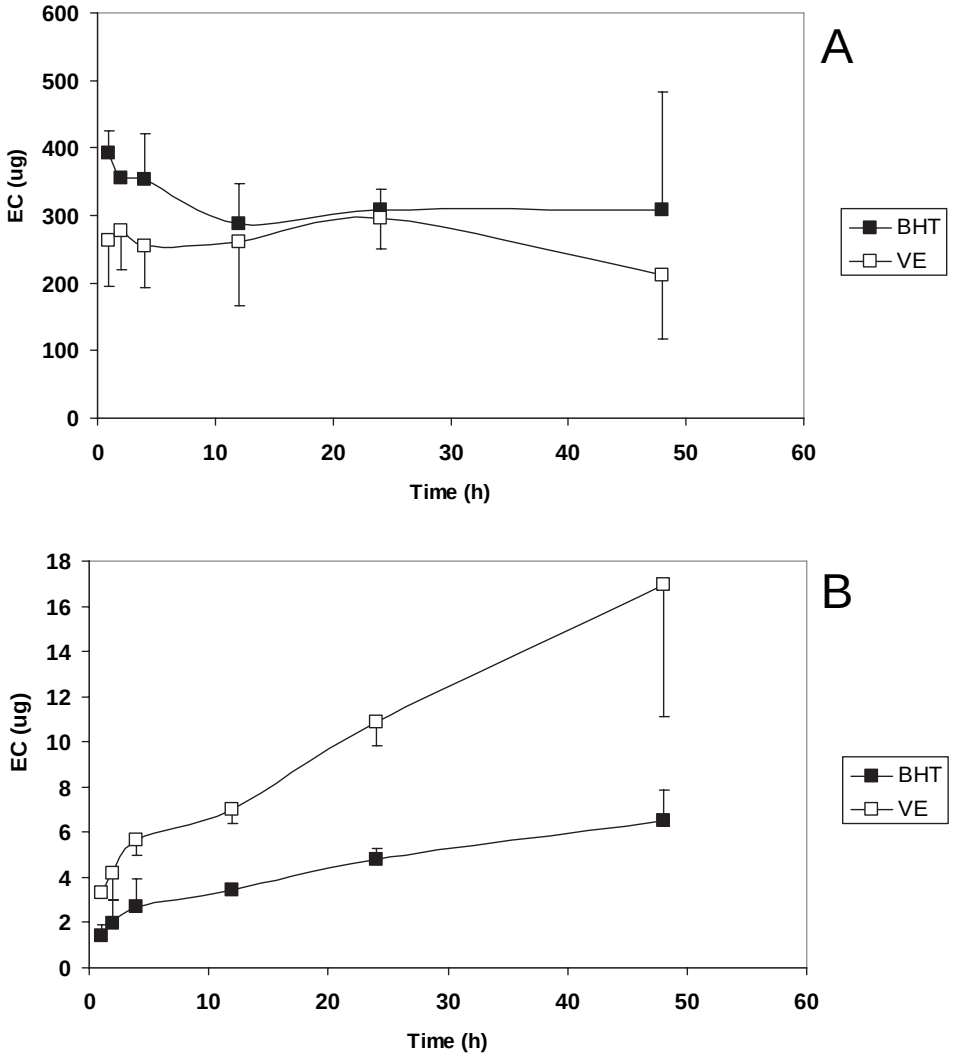

FIGURE 6 Retention of (-)Epicatechin in Cadaver Human Skin From Ultrez ™ 10 Gel Formulations at 32°C. (a) Retention in Stratum Corneum (Tape Stripping); (b) Retention in Viable Skin. Data Represent Mean ± SD (n = 3).

TABLE 3 Flux (J_{ss}) and Permeability Coefficient (K_p) of (-)Epicatechin From Ultrex™10 Gels Supplemented With Vitamin E or BHT as Preservative Agent. J_{ss} and K_p Were Estimated From the First Four Hours of Application

	Mean ± SD	
	UBHT	*UVE*
J_{ss} (μg/cm^2/h)	0.422 ± 0.150	0.785 ± 0.095
K_p (cm/ h)	8.44E-5 ± 3.1E-5	1.57E-4 ± 1.7E-5

gallate (EGCG) in hydrophilic ointment USP: it accumulates in human cadaver skin but is not detected in the receiving fluids following a 24 h exposure. However, the dose percentage of EC uptake in the skin at 24 h for the UVE gel is twice that of EGCG (EC_{24}: 1.9 % ± 0.2 vs. $EGCG_{24}$: 0.9 %), whereas the EC uptake from the BHT gels and the USP ointment are similar (EC_{24}: 0.85% ± 0.08 vs. $EGCG_{24}$: 0.9 %). Conversely, the major OPCs (EC, EGCG, and epigallocatechin) were found to pass across pig-ear skin in a similar in vitro apparatus when green tea extract permeability was assessed from different liquid formulations and a patch (Batchelder et al., 2004).

This study confirms that transdermal delivery of catechins in humans is probably difficult to achieve through conventional, topical delivery systems. However, EC formulated in Ultrex™ gel supplemented with VE can reach the skin immediately below the SC at a concentration of 38 ± 16 mg per gram of skin. The presence of vitamin E as an antioxidant shows that the skillful selection of the composition of the formulation may lead to a three-fold increase of flux compared with the formulation containing BHT. The amount of EC that reaches the skin may be sufficient to stop the

peroxyl radical propagation produced by exposure to UV photons, which could result in significant protection of the skin from photo aging and the damaging effects of UV-induced DNA mutations that may cause cancer (Gilchrest, 1991).

CONCLUSIONS

(-)Epicatechin permeability parameters are similar for the different gel formulations tested on cellulose membranes. EC is subject to oxidation in the gel formulations but when supplemented with VE or BHT, gels are stable for at least four weeks at room temperature. A single topical application of formulations of EC in Ultez™10 promotes penetration and retention in the upper layers of human cadaver viable skin of up to 3% of the dose in 48 h. Conversely, EC does not appear in the receiving compartment of the Franz cells, suggesting that the availability of EC to the dermis or blood vessels might be negligible. The results of this study show that, when properly formulated, EC is a viable candidate for an anti-aging and UV-protectant.

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