

Original Article

In vitro and *in vivo* percutaneous absorption of seleno-*L*-methionine, an antioxidant agent, and other selenium species

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Aim: To investigate the *in vitro* and *in vivo* percutaneous absorption of seleno-*L*-methionine (Se-*L*-M), an ultraviolet (UV)-protecting agent, from aqueous solutions.

Methods: Aqueous solutions of Se-*L*-M were prepared in pH 4, 8, and 10.8 buffers. The pH 8 buffer contained 30% glycerol, propylene glycol (PG) and polyethylene glycol (PEG) 400. The *in vitro* skin permeation of Se-*L*-M via porcine skin and nude mouse skin was measured and compared using Franz diffusion cells. The *in vivo* skin tolerance study was performed, which examined transepidermal water loss (TEWL), skin pH and erythema.

Results: In the excised porcine skin, the flux was 0.1, 11.4 and 8.2 $\mu\text{gcm}^{-2}\text{h}^{-1}$ for the pH 4, 8, and 10.8 buffers, respectively. A linear correlation between the flux and skin deposition was determined. According to permeation across skin with different treatments (stripping, delipidation, and ethanol treatments), it was determined that the intracellular route comprised the predominant pathway for Se-*L*-M permeation from pH 8 buffer. Aqueous solutions of seleno-*DL*-methionine (Se-*DL*-M), selenium sulfide and selenium-containing quantum dot nanoparticles were also used as donor systems. The *DL* form showed a lower flux (7.0 vs 11.4 $\mu\text{gcm}^{-2}\text{h}^{-1}$) and skin uptake (23.4 vs 47.3 $\mu\text{g/g}$) as compared to the *L* form, indicating stereoselective permeation of this compound. There was no or only negligible permeation of selenium sulfide and quantum dots into and across the skin. With *in vivo* topical application for 4 and 8 h, the skin deposition of Se-*L*-M was about 7 $\mu\text{g/g}$, and values were comparable to each other. The topical application of Se-*L*-M for up to 5 d did not caused apparent skin irritation. However, slight inflammation of the dermis was noted according to the histopathological examination.

Conclusion: Se-*L*-M was readily absorbed by the skin in both the *in vitro* and *in vivo* experiments. The established profiles of Se-*L*-M skin absorption will be helpful in developing topical products of this compound.

Keywords: seleno-*L*-methionine; selenium; percutaneous absorption; topical delivery; skin

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Introduction

Skin is constantly exposed to prooxidant environmental stresses from an array of sources, such as air pollutants, solar ultraviolet (UV) light, chemical oxidants, microorganisms, and ozone^[1]. Reactive oxygen species (ROS) were implicated in the etiology of several skin disorders including skin cancer and photoaging. Skin cancer constitutes about 30% of all newly diagnosed cancers in the world and solar UV radia-

tion, particularly the UVB component, is an established cause of about 90% of skin cancers^[2]. In recent years, particular antioxidants have gained considerable attention as means of neutralizing ROS. This group includes vitamins (C and E), phytochemicals (carotenoids and polyphenols), and minerals (zinc and selenium)^[3]. There is much evidence to suggest that selenium has an important role in protecting skin from the harmful effects of UVB. Selenium, an essential trace element, is found in many foods, including meat, fish, eggs, dairy products, and grains^[4]. The UVB protection afforded by selenium compounds is attributed to their ability to increase activities of glutathione peroxidase and thioredoxin reductase which are

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antioxidant selenoenzymes^[5]. In humans, a low selenium status is associated with up to a 4-fold increased risk of developing skin cancer^[6]. Topical selenium compounds also increase the minimum dose of UV radiation required to cause skin reddening and protect against skin damage caused by UVB^[7, 8]. Seleno-*L*-methionine (Se-*L*-M), the major component of dietary selenium, represents an organic form of selenium which may provide enhanced protection of the skin^[9]. Se-*L*-M also decreases the oxidative stress of neurodegenerative diseases, rheumatoid arthritis, and HIV/AIDS^[10, 11].

Most ingested selenium, whether in organic or inorganic form, is converted by liver metabolism^[3]. In addition, selenium toxicity can produce gastrointestinal problems^[12]. Delivery of pharmacologically active compounds via the skin is an attractive alternative to oral dosing for numerous reasons including stable plasma concentrations, bypass of first-pass effects, and reduction of some side effects^[13, 14]. Topical delivery via the skin may provide direct selenium targeting to attain sufficient activity for skin prevention/therapy. Topical administration may also be a suitable route for selenium to achieve systemic bioavailability to maintain daily necessities and treat systemic disorders.

No evidence currently exists to support the notion that significant amounts of Se-*L*-M can be delivered into and across the skin. The number of specific metals that was investigated for skin permeation is also limited^[15]. The aim of this work was to establish skin permeation profiles of Se-*L*-M from various vehicles. The permeation via the skin of other selenium species, such as seleno-*DL*-methionine (Se-*DL*-M), selenium sulfide (SeS₂), and cadmium selenium (CdSe) quantum dots was also evaluated. Both *in vitro* and *in vivo* skin absorption experiments were performed in this study. To study the *in vitro* potential of these compounds, a permeation study was performed with Franz diffusion cells using excised porcine and nude mouse skin. The optimal pH and type of vehicles for better absorption of Se-*L*-M were investigated. Possible pathways of this antioxidant via the skin were elucidated using porcine skin treated by stripping, delipidation, oleic acid, and α -terpineol to indicate the specific routes of skin absorption. Possible skin irritation of Se-*L*-M was studied by histopathology and *in vivo* bioengineering techniques using nude mice as the animal model.

Materials and methods

Materials

Se-*L*-M, Se-*DL*-M, selenium sulfide, LumidotTM CdSe quantum dots, LumidotTM CdSe/ZnS quantum dots, oleic acid, and α -terpineol were purchased from Sigma-Aldrich (St Louis, MO, USA). Cellulose membranes with a molecular weight (Mw) cutoff of 3500 (Spectra PorTM 3) were supplied by Spectrum Laboratories (Rancho Dominguez, CA, USA). All other chemicals and solvents were of analytical grade and were used as received.

Animals

Specific pathogen-free (SPF) pigs (1 week old) were supplied

by the Animal Technology Institute Taiwan (Miaoli, Taiwan, China). Female nude mice (8 weeks old) were obtained from the National Laboratory Animal Center (Taipei, Taiwan, China). The animal experiment protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chang Gung University. Animals were housed and handled according to institutional guidelines.

Preparation of skin membranes

Full-thickness skin was excised from the dorsal region of pigs and mice. Subcutaneous fat, tissues, blood vessels, and epidermal hairs were carefully removed before use. The skin was stripped 20 times with adhesive tape in the study to obtain stratum corneum (SC)-stripped skin. To obtain delipidized skin, the SC side was pretreated with chloroform-methanol (2:1) for 2 h. Five percent oleic acid or α -terpineol in a 25% ethanol/water medium was used to pretreat the skin mounted on a Franz cell for 2 h before the *in vitro* skin permeation experiment.

In vitro skin permeation

Porcine or nude mouse skin with or without pretreatment was mounted on the receptor compartment of a Franz cell with the SC side facing upwards into the donor compartment. The receptor was filled with 5.5 mL of pH 7.4 citrate-phosphate buffer, and maintained at 37 °C under constant stirring. The donor compartment was occluded by parafilm and filled with 0.5 mL of vehicle containing Se-*L*-M or other selenium species at determined concentrations. The available diffusion area between the compartments was 0.79 cm². At appropriate intervals, 300- μ L aliquots of the receptor medium were withdrawn and immediately replaced with an equal volume of fresh medium.

At the end of the *in vitro* experiment (24 h), the skin was removed from the cell and the skin surface was cleaned with a cotton wool swab immersed in water and methanol three times each for removing any contaminant. The skin was weighed, cut with scissors, positioned in a glass homogenizer containing 1 mL of 0.1 mol/L HCl, and homogenized for 10 min at 300 revolutions per minute. The resulting solution was centrifuged for 10 min at 10000 revolutions per minute and then filtered through a polyvinylidene difluoride membrane with a pore size of 0.45 μ m. All samples were analyzed by graphite furnace atomic absorption spectrophotometry (Z-5000, Hitachi, Tokyo, Japan). The wavelength set for selenium was 196 nm. At the range 0–40 μ g/L, the concentration of selenium was linearly proportional to its absorbance. The limit of detection (LOD) of selenium was determined to be 3 μ g/L. The intra- and inter-assay precision and accuracy values were evaluated at the concentration range 0–40 μ g/L. The overall precision, defined by the relative standard deviation (RSD), ranged from 0.4% to 3.1% on average. Analytical accuracy, expressed as the percentage difference between the mean of measured value and the known concentration, varied from -3.3% to 2.1%.

In vivo skin permeation

Nude mice were used as the animal model in the *in vivo* experiment. All animals were starved overnight prior to the experiment. A glass cylinder with an available area of 0.79 cm² was placed on the dorsal skin with glue (Instant Super Glue™, Kokuyo, Tokyo, Japan). An aliquot of 0.2 mL of pH 8 citrate-phosphate buffer with Se-L-M at a concentration of 0.2% (*w/v*) was added to the cylinder. The application times of the vehicle were 4 and 8 h. The procedures for washing and extraction of the compound from the skin were the same as for the *in vitro* experiment.

In vivo skin tolerance test

A 0.6-mL aliquot of pH 8 buffer with 0.2% or 0.3% Se-L-M was uniformly spread over a sheet of non-woven polyethylene cloth (1.5 cm×1.5 cm), which was then applied to the back area of a nude mouse. The polyethylene cloth was fixed with Tegaderm™ adhesive dressing (3M, St Paul, MN, USA) and Fixomull™ stretch adhesive tape (Beiersdorf AG, Hamburg, Germany). After 24 h, the cloth was removed, and the treated skin area was swabbed clean with a cotton wool swab. The vehicle was applied daily for 5 d. After withdrawal of the vehicle, transepidermal water loss (TEWL), colorimetric parameters, and the pH of the applied skin were measured. These measurements were evaluated daily. TEWL was recorded using a Tewameter™ (TM300, Courage and Khazaka, Köln, Germany). Measurements taken at a stable level were performed 30 s after application of the TEWL probe to the skin. TEWL was automatically calculated and expressed in g·m⁻²·h⁻¹. The skin-surface pH was determined by a Skin-pH-Meter™ PH905 (Courage and Khazaka). A spectrophotometer (CD100, Yokogawa Electrical, Tokyo, Japan) was used to measure the skin erythema (a*) according to recommendations of the Commission Internationale de l'Eclairage (CIE). When recording color values, the measuring head was held perpendicular to the back skin. The reading was obtained within a few seconds on the display. The temperature and relative humidity in the laboratory were kept at 26 °C and 55%, respectively. The sample number for each experiment was six.

Histopathological examination

The dorsal skin of nude mouse was excised after topical administration of pH 8 buffer with 0.2% or 0.3% Se-L-M for 5 d. Each skin specimen was dehydrated using ethanol, embedded in paraffin wax, and stained with hematoxylin and eosin. For each skin sample, three different sites were examined and evaluated under light microscopy (Eclipse 4000, Nikon, Tokyo, Japan). Digital photomicrographs were then processed with Adobe PhotoDeluxe (Adobe Systems, San Jose, CA, USA).

Statistical analysis

Statistical analyses of differences between the various treatments were performed using an unpaired Student's *t*-test. A 0.05 level of probability ($P < 0.05$) was taken as the level of

significance. An analysis of variance (ANOVA) test was also used if necessary.

Results

Optimization of vehicles for *in vitro* skin permeation of Se-L-M

Optimization of topical drug bioavailability is an essential objective for the effective treatment of skin disorders. The pH and composition of vehicles, and the applied dose were shown to be major variables influencing the diffusivity of permeants. The effects of the pH 4–10.8 buffers on skin permeation of Se-L-M were examined. Se-L-M is an amino acid containing selenium. According to the dissociation constant (pK_a) values of Se-L-M (2.19 and 9.05)^[16], this compound is mainly in cationic, zwitterionic, and anionic forms at pH 4, 8, and 10.8, respectively. The solubility of Se-L-M in water is up to 1000 mg/mL, which can be associated with a risk of local toxicity if applied topically. The finite dose technique was applied in this study, although the maximal thermodynamic activity could not be obtained. All donor samples for testing the pH effect were prepared by dispersing 0.2% Se-L-M in buffer. Figure 1 shows the permeation profiles of Se-L-M across porcine skin and nude mouse skin as the means and standard deviations plotted against time. The flux value ($\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$) is equal to the slope of the linear permeation profile and was calculated from the experimental curves shown in Figure 1. Table 1 summarizes the Se-L-M flux from various buffer solutions. A zero-order equation was suitable for use with the curves of most

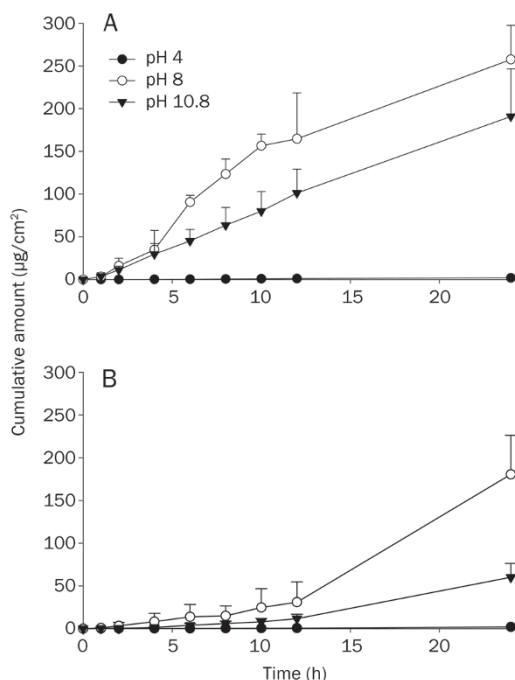


Figure 1. *In vitro* cumulative amount versus time profiles of the topical application of seleno-L-methionine permeating across porcine (A) and nude mouse skin (B) from pH 4, 8, and 10.8 buffers. All data are presented as mean±SD. $n=4$ experiments.

formulations, except the results of pH 8 buffer via nude mouse skin.

The pH of the vehicle had a significant effect on the transport of Se-*L*-M. As shown in Figure 1A, the permeability across porcine skin increased in the order of pH 8>pH 10.8>pH 4. The same trends were detected for both porcine and nude mouse skin (Figure 1A vs 1B). Based on the flux values shown in Table 1, the difference between pH 8 and 10.8 was not significant ($P>0.05$) according to a *t*-test in the case of porcine skin. For topical bioavailability purposes, the permeant retained in the strata of the skin should be included. Only considering the permeant flux reaching the receptor compartment may underestimate the true level of skin absorption. The skin deposition of Se-*L*-M by *in vitro* topical application is depicted in Table 1. The skin with no treatment (blank control) showed no selenium content above the detection limit of atomic absorption. A correlation was noted between porcine skin retention and the flux. A *t*-test revealed that there was no statistically significant difference ($P>0.05$) in the skin accumulation between pH 8 and 10.8 buffers in porcine skin. Se-*L*-M accumulation within nude mouse skin was greatest with the pH 8 buffer and least from the pH 4 buffer.

Table 1. *In vitro* flux and skin deposition of 0.2% seleno-*L*-methionine from buffers with various pH values via porcine skin and nude mouse skin. Each data represents the mean±SD. $n=4$.

Skin	pH	Flux ($\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$)	Skin deposition ($\mu\text{g}/\text{g}$)
Porcine	4	0.09±0.07	5.29±1.54
	8	11.41±1.86	47.27±8.98
	10.8	8.22±2.39	47.31±5.47
Nude mouse	4	0.08±0.01	12.36±1.53
	8	7.56±1.90	123.87±35.75
	10.8	6.56±0.66	91.30±16.96

Table 2 summarizes the permeation data of Se-*L*-M from pH 8 buffer with 30% organic solvents of glycerol, PG, and PEG400. Porcine skin was used as the permeation barrier. The permeation of Se-*L*-M from the buffer containing the organic solvents was significantly lower ($P<0.05$) compared to the neat pH 8 buffer. The flux and skin reservoir of Se-*L*-M

Table 2. *In vitro* flux and skin deposition of 0.2% seleno-*L*-methionine from pH 8 buffers with various cosolvents via porcine skin. Each data represents the mean±SD. $n=4$.

Vehicle	Flux ($\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$)	Skin deposition ($\mu\text{g}/\text{g}$)
30% Glycerol/pH 8 buffer	2.55±0.59	18.21±6.10
30% PG/pH 8 buffer ^a	4.45±1.04	12.26±2.36
30% PEG400/pH 8 buffer ^b	2.18±0.38	14.52±3.61

^aPG, propylene glycol. ^bPEG400, polyethylene glycol 400.

from pH 8 buffer were greater than those from the buffer with organic solvents by 2.5–5-fold. The general trend for the flux was PG>glycerol≥PEG400. There was no significant difference ($P>0.05$) among the skin deposition levels of the compound from the three vehicles.

The Se-*L*-M flux and skin deposition from pH 8 buffer with different doses of 0.05%–0.3% are respectively shown in Figure 2A and 2B. A linear correlation ($r=0.9748$) was apparent between the dose and flux across porcine skin. No significant difference ($P>0.05$) in the flux was observed between the doses of 0.2% and 0.3%. A linear relationship was not observed in nude mouse skin since a lower flux ($P<0.05$) was detected in the formulation with the 0.3% compound compared to that with 0.1% and 0.2%. As depicted in Figure 2B, the porcine skin reservoir generally increased with an increase in the Se-*L*-M dose except at 0.3%. A correlation of $r=0.7858$ was observed between the dose and porcine skin deposition. The same as the permeant flux via nude mouse skin, the skin deposition from the donor with 0.3% was relatively lower compared to that with 0.2% ($P<0.05$).

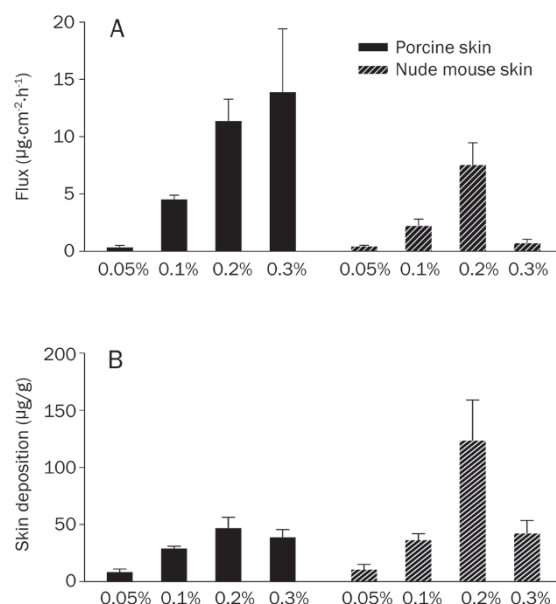


Figure 2. *In vitro* flux (A) and skin deposition (B) of seleno-*L*-methionine from pH 8 buffer with different doses of 0.05%–0.3% across porcine and nude mouse skin. All data are presented as mean±SD. $n=4$ experiments.

In vitro skin permeation of Se-*L*-M via various skin types

In order to elucidate the mechanisms involved in the skin permeation of Se-*L*-M, *in vitro* permeation experiments to examine the cumulative amount in the receptor were performed using various skin membranes. Although an examination of permeation across skin is less advantageous when targeting skin tissue, an understanding of the permeability is helpful in exploring the mechanisms or pathways of skin absorption. Figure 3 shows the cumulative amount–time profiles of Se-*L*-M from pH 4, 8, and 10.8 buffers via different skin types. Se-*L*-

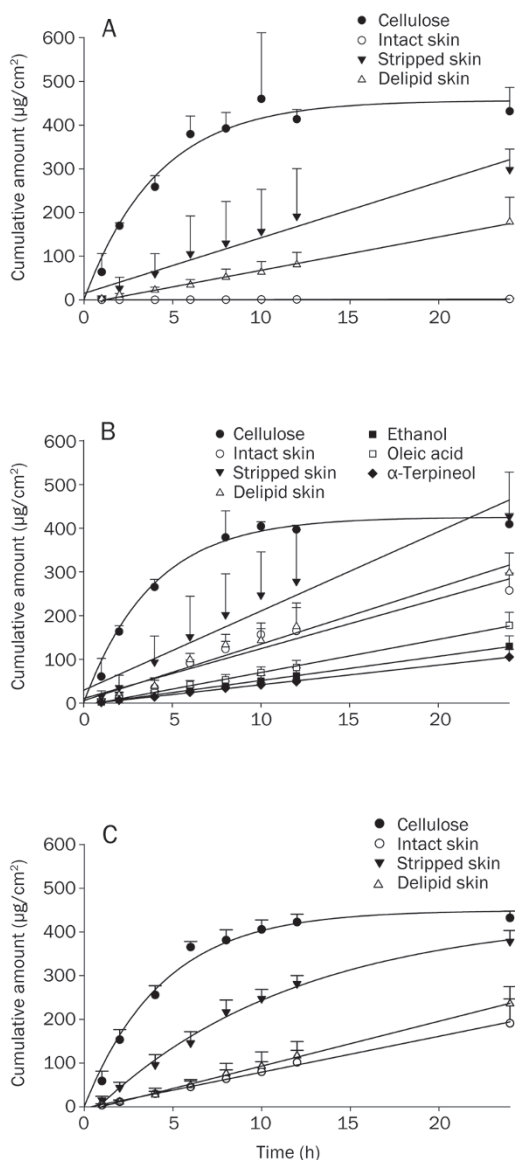


Figure 3. *In vitro* cumulative amount versus time profiles of the topical application of seleno-L-methionine permeating across various skin types from pH 4 (A), 8 (B), and 10.8 (C) buffers. All data are presented as mean±SD. *n*=4 experiments.

M diffusion across the cellulose membrane was determined to evaluate release rates from the buffers. The results indicated that the release of the permeant was faster than permeation across porcine skin. The release kinetics showed an initial burst (0–4 h), followed by a sustained burst (4–8 h), and a plateau (8–24 h). This trend and the level of release were similar for all buffers tested. Among the permeation curves, Se-*L*-M permeation across SC-stripped skin mostly approximated the data across the cellulose membrane, followed by delipidized skin and intact skin. According to the cumulative amount at 24 h (the end of the experiment), Se-*L*-M permeation across stripped skin was 1.58-, 1.7-, and 2.0-fold greater ($P<0.05$) than that across intact skin from pH 4, 8, and 10.8 buffers, respec-

tively.

To further explore the permeation mechanisms, 3% oleic acid and α -terpineol in 25% ethanol/pH 7.4 buffer were used to pretreat porcine skin. Ethanol at 25% was used as the pretreatment medium for solubility considerations of oleic acid and α -terpineol. As shown in Figure 3B, pretreatment with 25% ethanol significantly reduced ($P<0.05$) Se-*L*-M permeation by 2-fold compared to intact skin. Treatment with oleic acid slightly but significantly increased ($P<0.05$) the cumulative amount over the 25% ethanol-treated group. On the other hand, pretreatment of the skin with α -terpineol did not further increase ($P>0.05$) permeation.

In vitro skin permeation of different selenium species

The skin delivery of other selenium species was also studied in the present work. These included Se-*DL*-M, selenium sulfide, and CdSe quantum dots. The flux and skin deposition of the selenium compounds are given in Table 3. The enantioselective transfer of selenomethionine via porcine skin was studied. Both the flux and skin deposition of Se-*L*-M increased more than those of Se-*DL*-M ($P<0.05$). From this, it is clear that the *L* form can pass more easily through the skin barrier than the *DL* form. This trend was especially significant with skin deposition since the *L* form showed 2-fold higher skin uptake than the *DL* form. Selenium sulfide could not pass across the skin into the receptor with a 24-h application. An *in vitro* uptake of 0.36 $\mu\text{g/g}$ of selenium sulfide was found within the skin. The skin absorption of two types of quantum dots, CdSe and CdSe/ZnS, was tested. No selenium was detected in either the skin reservoir or receptor as shown in Table 3.

Table 3. *In vitro* flux and skin deposition of 0.2% different selenium species from pH 8 buffer via porcine skin. Each data represents the mean±SD. *n*=4.

Species	Flux ($\mu\text{gcm}^{-2}\text{h}^{-1}$)	Skin deposition ($\mu\text{g/g}$)
Seleno-L-methionine	11.41±1.86	47.27±8.98
Seleno-DL-methionine	7.04±1.85	23.42±8.90
Selenium sulfide	0	0.36±0.13
CdSe quantum dots	0	0
CdSe/ZnS quantum dots	0	0

In vivo skin permeation of Se-*L*-M

Levels of Se-*L*-M in the skin were determined following a single application of pH 8 buffer to the dorsal surface of nude mice. *In vivo* percutaneous absorption was evaluated using nude mice because they are easy to handle. Table 4 shows the *in vivo* skin deposition of Se-*L*-M after topical delivery for 4 and 8 h. Skin uptake of the compound exhibited values of 7.8 and 7.2 $\mu\text{g/g}$, respectively. There was no significant difference ($P>0.05$) between the intradermal concentrations of Se-*L*-M at 4 and 8 h.

Table 4. *In vivo* skin deposition ($\mu\text{g/g}$) of seleno-*L*-methionine from buffers with various pH values via nude mouse skin. Each data represents the mean \pm SD. $n=6$.

Time (h)	Skin deposition ($\mu\text{g/g}$)
4	7.82 \pm 0.36
8	7.24 \pm 0.18

Bioengineering methods such as TEWL, pH, and colorimetry were conducted *in vivo* to evaluate the preliminary safety of Se-*L*-M on skin. pH 8 buffer containing 0.2% and 0.3% Se-*L*-M was applied to the dorsal skin of nude mice for 24 h. The total duration of application was 5 d. Values of TEWL, pH, and erythema (a^*) were determined every day as shown in Figure 4. Compared to the control group (pH 8 buffer without Se-*L*-M), no enhancement of TEWL and pH values was observed after application of Se-*L*-M for 5 d (Figure 4A, 4B). The same result was detected for skin erythema after topical Se-*L*-M delivery (Figure 4C). This suggests tolerable changes in the skin with topically applied Se-*L*-M. No visible disruption of the skin was observed at any point during this study of nude mice receiving Se-*L*-M.

Possible skin irritation by Se-*L*-M after 5 d of exposure was histologically investigated as shown in Figure 5. Light microscopy indicated no observable damage to intact skin in the untreated group (Figure 5A). Histopathologic analysis of skin samples isolated from the area treated with 0.2% Se-*L*-M revealed some chronic inflammatory cells such as macrophages and lymphocytes in the dermis (Figure 5B). A partial loss of the SC was exhibited with this dose. When the dose was increased from 0.2% to 0.3%, less significant inflammation was seen in the dermis and subcutis (Figure 5C). Some disarray and disarrangement of the SC were observed. Histological changes to the skin due to treatment with Se-*L*-M were generally mild.

Discussion

Cumulative and prolonged exposure to UVB is now known to induce deleterious reactions in human skin, including cutaneous aging, immunosuppression, photo-carcinogenesis, and various inflammatory skin disorders^[17]. Supplying topical

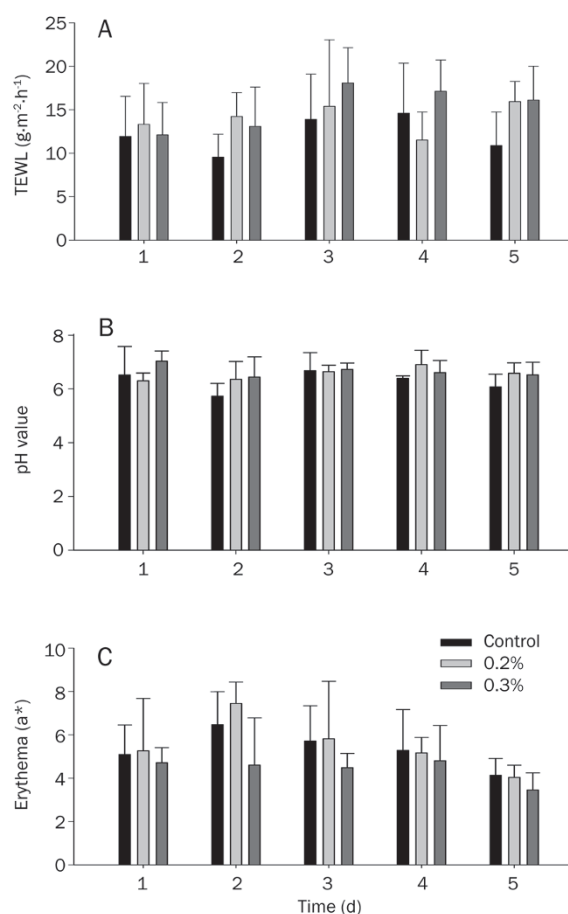


Figure 4. *In vivo* skin tolerance examination determined by transepidermal water loss (TEWL) (A), skin surface pH (B), and erythema (a^*) (C) after an application of topically applied seleno-*L*-methionine from pH 8 buffer at a dose of 0.2% or 0.3% for 5 d. All data are presented as the mean \pm SD. $n=6$ experiments.

exogenous antioxidants to the skin can prevent or minimize UVB-induced damage^[18]. Despite various reports linking many of the beneficial properties of selenium to its use, no comprehensive study has been conducted to investigate the skin absorptive ability of related compounds. Moreover, penetration of the skin still needs to be separately determined for each metal species because of the large diversity permeation characteristics of various metal via the skin^[15]. The present

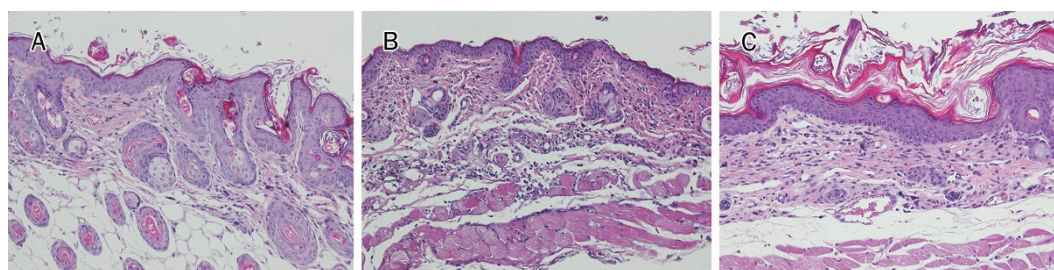


Figure 5. Histological examination of nude mouse skin stained with hematoxylin and eosin with no treatment (control group) (A), treatment with seleno-*L*-methionine from pH 8 buffer at a dose of 0.2% for 5 d (B), and treatment with seleno-*L*-methionine from pH 8 buffer at a dose of 0.3% for 5 d (C).

results showed that Se-*L-M* exhibited considerable absorption into the skin, especially with pH 8 buffer. The preliminary safety of Se-*L-M* to the skin was also evaluated. The results showed negligible irritation of the skin after *in vivo* topical administration, suggesting its feasibility for dermal use.

Porcine skin was initially used as the permeation barrier in this study because of its similarity to human skin. The skin of rodents is most commonly used for *in vitro* and *in vivo* skin permeation studies. There are a number of hairless species (eg, nude mice and hairless rats) in which the absence of a hairy coat mimics human skin better than hairy skin^[19]. Hence nude mice were also utilized as an animal model in this study. The experimental results showed similar flux values between nude mouse skin and porcine skin in our case. This suggests that nude mice can be a successful model for evaluating the skin permeation of selenium compounds. Se-*L-M* in the buffers with higher pH values was found to penetrate more easily into and through the skin. The accumulation of a compound within the skin reflects the skin deposition after topical application. On the other hand, the concentration of a compound received in the Franz cell predicts the amount distributed to plasma or other organs in an *in vivo* status^[20]. Both parameters were meaningful for Se-*L-M*. This selenium compound can prevent or treat UVB-related disorders in skin. The essential uptake for systemic levels of selenium in daily life was also achieved by a transdermal route.

Skin absorption of a permeant is determined by its physicochemical characteristics, in particular, its Mw and lipophilicity, which play major roles in the skin permeation process. Finnin and Morgan^[21] indicated that molecules with a Mw of <500 Da can penetrate across the skin because of their small molecular volumes. Se-*L-M* fits this criterion (Mw 196.1 Da). Poor absorption is more likely when the octanol/water partition coefficient (logP) is >5 or <-1^[17]. Se-*L-M* showed a logP of -3.01^[22], which does not fulfill the criterion. This can explain the extremely low permeation of Se-*L-M* from pH 4 buffer. However, Se-*L-M* still demonstrated a considerable penetration in pH 8 and 10.8 buffers. Se-*L-M* is predominantly in a zwitterion form in pH 8 buffer, which is beneficial due to its lipophilicity. The neutral form of a compound always shows higher skin partitioning compared to the ionic form because of the lipophilic characteristics of the SC^[23, 24]. The present findings suggest that the corresponding anions at pH 10.8 also showed high skin permeation. Increased pH can ionize a greater part of the intercellular fatty acids, changing the phase behavior and packing of the barrier lipid mixture^[25]. The SC may be much more permeable to molecules in an alkaline vehicle^[15]. This effect was not observed below pH 9 since the evidence indicates buffers at pH <9 did not compromise the barrier function^[26].

A direct correlation between the skin deposition and flux was achieved for all Se-*L-M* formulations tested. The higher skin reservoir of the compound may result in high release into the receptor compartment because of the rapid diffusion due to the concentration gradient^[24]. However, differences in skin deposition among formulations were smaller compared

to those of flux values. For example, pH 8 buffer exhibited a 9-fold increase in skin deposition over pH 4 buffer, whereas a 127-fold increase was detected for the flux. The electrophilic nature of many metals determines their protein reactivity, which can result in depot formation in the SC^[15, 27]. Se-*L-M* was found to replace methionine when incorporated into proteins^[16], resulting in an abundance in the skin reservoir. A previous study^[28] suggested that protection from UVB-radiation-induced human skin cell death can be obtained with concentrations of as low as 10 nmol/L with Se-*L-M*. This value can be calculated as 0.002 ng/g, a level that simulates skin deposition. Se-*L-M* showed skin deposition which was much greater than the minimum concentration for inducing protection efficiency.

The incorporation of an organic solvent such as glycerol, PG, or PEG400 in pH 8 buffer significantly reduced the permeation of Se-*L-M*. The addition of an organic solvent may have reduced the polarity of the aqueous vehicle as the solvent is relatively lipophilic. The decreased polarity with organic solvent incorporation in the vehicle hinders the partitioning of the permeant into the SC^[29, 30]. Another explanation may be the higher viscosity of these solvents relative to the aqueous solution, making it difficult for the permeant to diffuse within the oil system. The data clearly showed an increased flux with PG compared to glycerol and PEG400. The permeation process is generally referred to as the drag-effect and may explain the effect of PG^[31, 32]. Such data may indicate a polar route of delivery for Se-*L-M*.

It can be seen that the permeation of Se-*L-M* was not commensurate with the applied concentration. A 0.3% dose did not further increase the flux or skin deposition via porcine skin compared to a lower dose (0.2%). This can be interpreted as a saturation of the skin reservoir by the selenium compound. A dose of 0.3% Se-*L-M* even showed reduced permeation via nude mouse skin. This phenomenon is commonly observed with the skin delivery of metals. This is due to the buildup of a secondary diffusion barrier as a consequence of metals forming stable bonds with proteins of the skin^[15]. In this way, a depot accumulates in the SC retarding further penetration in inverse proportion to the metal concentration. This effect may have been more pronounced for nude mouse skin than porcine skin.

With respect to drug permeation via the skin from the vehicle, a permeant should first diffuse out of the vehicle onto the skin surface. The release rate of Se-*L-M* across the cellulose membrane was significantly higher than that across porcine skin. This indicates that the skin exerted a significant barrier function against the transport of Se-*L-M*. The release profiles from various buffer systems approximated each other. Since a large discrepancy of skin penetration existed among the various buffers, the partitioning or permeation process via skin but not the release process was the rate-limiting step for Se-*L-M*. Tape stripping is a simple standard technique for evaluating the function of the SC in percutaneous absorption studies^[33]. Se-*L-M* in pH 4 buffer showed the highest enhancement of skin permeation across SC-stripped skin compared

to the other buffer systems, suggesting that diffusion through the SC layer was important for permeation in a cationic form. Se-*L-M* permeation across stripped skin did not achieve the level of release across the cellulose membrane by the end of the experiment (24 h). This suggests that permeation was hindered by the SC, and the viable epidermis/dermis beneath the SC may have contributed to the aqueous resistance to its diffusion. The epidermal-dermal tight junction is an example of an important barrier for some materials^[34]. This phenomenon was not observed for Se-*L-M* at pH 8 and 10.8 since the cumulative Se-*L-M* amount via stripped skin approximated the release amount at the end of the experiment.

Permeation through corneocytes (a transcellular pathway) and permeation through the lipid bilayers surrounding the corneocytes (an intercellular pathway) contribute to the routes of drug permeation across the SC. Delipidation greatly increased the skin permeation of Se-*L-M* at pH 4 compared to intact skin. This suggests that the intercellular lipid bilayers were the main barrier blocking the transit of Se-*L-M* in an acidic environment. The penetration rates of this compound via delipidized skin and intact skin were comparable in neutral and alkaline environments (pH 8 and 10.8). This indicates that the presence of lipid bilayers did not greatly influence the penetration of this selenium compound at these pH values. Intracellular or transappendageal routes may be pathways for Se-*L-M* delivery at pH 8 and 10.8. This result is in accordance with permeation from PG-containing vehicles. The penetration pathways of Se-*L-M* in pH 8 buffer were further investigated using ethanol, oleic acid, and α -terpineol as pretreatment media for the skin. Pretreatment with 25% ethanol resulted in a retardation of Se-*L-M* permeation. Changes in the skin's structure induced by ethanol reduced the delivery of some drugs^[24, 35]. Protein denaturation in the SC may have been involved in this reduction. This result verified that the hydrophilic pathways through corneocytes can play an important role in the skin delivery of Se-*L-M*.

Oleic acid and α -terpineol were used as permeation enhancers for dermal/transdermal drug delivery. Oleic acid acts on the lipidic tail portion of intercellular lipid bilayers, while α -terpineol is known to act on the lipid polar heads of ceramides^[36]. Oleic acid produced enhancement of the skin permeation of Se-*L-M* compared to the 25% ethanol-treated group. This suggests that the alkyl chain of the lipids could act as a barrier hindering permeation. It also confirms that the intracellular route may be essential for Se-*L-M*, since oleic acid can disrupt corneocytes^[37]. Pretreatment with α -terpineol did not enhance the activity of Se-*L-M* permeation. This may indicate that hydrogen bonds between ceramides are not important for Se-*L-M* penetration across the SC.

Both inorganic and organic forms of selenium are commonly used for therapeutic and diagnostic purposes. The experimental results indicated that molecular stereochemical complexity can predominate the skin delivery of compounds. The *in vitro* skin permeation experiments demonstrated that the diffusion of the *L* and *DL* forms via porcine skin was stereoselective, with the *L* form showing higher permeation. This indicates

that the SC contributed to the barrier function against percutaneous absorption of the *D* form. Since intracellular pathways were the predominant route for selenomethionine delivery, corneocytes may produce qualitative evidence of stereoselective interaction. This chiral interaction can cause differences in diffusion rates via the skin. Se-*L-M* may favor this interaction compared to the *D* form. According to previous studies involving the skin delivery of enantiomers^[38-40], the *L* form (or *S* form) of ketorolac, ketoprofen, and selegiline also exhibited greater skin absorption than the *D* form (or *R* form).

Topical preparations containing selenium sulfide are frequently used to manage tinea versicolor, seborrheic dermatitis, and dandruff^[41, 42]. It is commonly incorporated into shampoo for treating dandruff. Our results showed that the skin was virtually impermeable to selenium sulfide. Only a very low amount of selenium sulfide was retained in the skin reservoir. This is accordance with a previous study which found that selenium sulfide in shampoo is not absorbed by the skin^[43].

Quantum dot nanoparticles have received attention due to their fluorescent characteristics and potential use in medical applications^[34, 44]. Because the skin is the main target tissue for nanoparticle exposure, assessment of the skin penetration of quantum dots has attracted a great deal of attention. A recent development is utilization of selenium as a material for quantum dot preparations. The core of quantum dots consists of cadmium and selenium (CdSe), sometimes with a shell of ZnS to make them biologically compatible. Whether the quantum dots can permeate the skin is controversial. Recent investigations reported that skin penetration by quantum dots did not occur in intact mouse skin^[45], was minimal in a murine model^[46], and was detected in the lower SC layer of a human skin equivalent^[47]. In our case, neither CdSe nor CdSe/ZnS quantum dots were absorbed by porcine skin. No or only negligible amounts in the receptor and skin reservoir were detected by analyzing selenium. This suggests that the shell of quantum dots was not damaged, and the CdSe core did not leach out. Penetration of quantum dots into the epidermal layers can result in a localized skin response, such as inflammation or cytotoxicity^[26]. Cadmium and selenium are two components of the core that are known to be toxic to cells^[34]. Our results indicated that quantum dots with a diameter of 6 nm may be safe for topical administration to skin tissue.

The electrophilic nature of many metals determines their protein interactions, which can result in depot formation in the skin. The *in vivo* skin uptake of Se-*L-M* was hence determined. The level of the *in vivo* skin reservoir was less than that in an *in vitro* condition. This was due to the significant diffusion and distribution of permeants from the skin to the systemic circulation or other tissues after *in vivo* topical administration, thus reducing the skin accumulation in an *in vivo* status. No significant difference in Se-*L-M* skin levels was observed between 4 and 8 h of administration. This can be explained by saturation of the skin reservoir. Another reason for the lower uptake of *in vivo* permeation was the shorter exposure time during the *in vivo* experiment compared to the *in vitro* experiment (24 h).

In addition to the efficiency of diffusion into the skin, the

skin tolerance is another concern for topical delivery systems. Selenium should be used with caution as a topically applied reagent since it may increase the risk of nonmelanoma skin cancer in excessive doses^[48]. TEWL was utilized to assess the degree of SC disruption, and a good correlation between the chemical damage to the skin barrier and TEWL increment was demonstrated^[49]. By evaluating established endpoints of skin irritation, the present work found that the topical application of Se-*L-M* for up to 5 d did not cause TEWL enhancement. Although Se-*L-M* caused some abrasion or disarray of the SC according to a histopathological examination, the barrier function of the SC did not fail. It was shown that the complete removal of lipids from the SC led to a 100-fold increase in water permeability^[50]. Healthy skin has a slightly acidic pH, and the acidity of the skin maintains antimicrobial activity^[18]. Disturbing this naturally acidic mantle may cause skin diseases. On the other hand, the *a**-coordinate of colorimetry correlates well with inflammatory interactions of the skin, especially viable skin^[24]. Se-*L-M* delivered by a topical route may be safe for both skin acidity and viable skin. Although inflammation occurred by monitoring skin slices, this irritation was not significant. An interesting result was that the inflammation induced by the 0.3% dose was less than that with 0.2%. This may have been due to a secondary barrier of the skin formed by the metals as cited above.

Conclusions

As demonstrated in this study, Se-*L-M* was readily absorbed by the skin in both the *in vitro* and *in vivo* experiments. Se-*L-M* in a zwitterion form revealed higher permeation compared to the other forms. A linear correlation was observed between the flux and skin deposition. The *DL* form of selenomethionine showed less skin absorption than did the *L* form, indicating a stereoselective character of this compound. Other selenium compounds including selenium sulfide and CdSe quantum dot nanoparticles exhibited no or only negligible skin delivery. Intracellular but not intercellular pathways were an important route for Se-*L-M* penetration via the SC. Summarizing the results of the present work, it was concluded that it may be suitable to develop dermal preparations of Se-*L-M* to protect against the harmful effects of UV. A preliminary safety examination of the skin indicated acceptable skin tolerance to Se-*L-M*. The established profiles of Se-*L-M* skin absorption will be helpful in developing topical products of this compound. More *in vivo* and clinical information on the efficacy and safety of the percutaneous absorption of Se-*L-M* is required to assess future practicability.

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Author contribution

Jia-you FANG and Saleh A AL-SUWAYEH designed research; Chih-hung LIN, Chia-lang FANG, and Shih-yun YANG per-

formed research; Chih-hung LIN analyzed data; and Jia-you FANG and Saleh A AL-SUWAYEH wrote the paper.

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