Ultraviolet A Irradiation Increases the Permeation of Fullerenes into Human and Porcine Skin from C$_{60}$-Poly(vinylpyrrolidone) Aggregate Dispersions

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Key Words
Fullerene · C$_{60}$ · Nanomaterial · Poly(vinylpyrrolidone) · Absorption · Penetration · Permeation · Skin

Abstract
Aim: The purpose of this study was to characterise C$_{60}$-poly(vinylpyrrolidone) (PVP) dispersions, to analyse the cutaneous absorption of fullerenes as well as to evaluate whether UVA radiation (UVA-R) could modify its permeation profile. Methods: Dispersions were characterised according to their pH, particle size, zeta potential, and morphology. Skin absorption studies were performed using porcine or human skin under UVA or sham irradiation. Results: The C$_{60}$ aggregate size was 129 ± 54 nm (as determined by nanoparticle tracking analysis) and the zeta potential was $-4.93 \pm 1.72$ mV. The C$_{60}$ aggregates presented an irregular shape (as measured by transmission electron microscopy) and permeated through human and porcine skin. Conclusions: C$_{60}$-PVP aggregates were adequately characterised. Human skin was less permeable than porcine skin, and the presence of UVA-R increased the C$_{60}$ content up to the dermis.

Introduction
Recently, the European Commission published a recommended definition of ‘nanomaterial’ in order to identify materials for which specific aspects of risk management should be considered [1]. Nanomaterials are widely applied in the electronics, clothing, and cosmetics industries [2, 3]. The increasing launch of products containing nanoscale materials [4] has become a matter of concern due to the large specific surface area (SSA) of these materials, which allows a greater biological reactivity compared with larger particles. Therefore, a detailed evaluation of the effects of nanomaterials must be conducted and combined with an adequate characterisation of nanoscale systems [3, 5, 6].

Fullerene C$_{60}$ is a spherical 60-carbon-atom cage molecule with a diameter of approximately 1 nm that was first discovered by Kroto et al. [7] in 1985. Its unique chemical features allow potential uses in various fields of biomedicine, particularly due to its reactive oxygen species-quenching/producing ability [8]. This dual ability strongly depends on the modification of the molecule to increase water solubility [9]. Pristine fullerenes can form stable colloidal aggregates via extended mixing in water [10], solvent exchange [11, 12], and stabilisation using surfactants [13] or polymers [14].
Fullerene C60 in the form of aggregates stabilised by poly(vinylpyrrolidone) (PVP) has been studied by various research groups [15–17], and its radical scavenging properties have led manufacturers to use this nanomaterial in cosmetic matrices to produce ‘anti-aging’ and whitening effects on the skin [18–20]. Amounts ranging from 1 to 43 μg C60/g product were quantified in commercially available cosmetics [21, 22], although some manufacturers report fullerene contents of up to 1% [18]. However, further studies are needed to characterise the dermal absorption profile of C60 in order to ensure the safety of this nanomaterial in cutaneous applications [23].

In vitro dermal absorption studies have been widely used to predict the in vivo absorption of substances. Human skin is the ‘gold standard’ for these studies; the most suitable animal skin model is porcine skin, due to physiological and structural characteristics similar to those of human skin [24]. However, the latter is not generally used, due to its limited availability, and some studies have demonstrated that there are discrepancies between human and porcine skin absorption profile results [24, 25].

An important factor influencing cutaneous absorption is skin exposure to UV radiation as numerous cosmetics are intended for daytime use. Some studies have demonstrated that UVA radiation (UVA-R) can cause chemical alterations to structural lipids of the stratum corneum and locally reduce their packing [26]. These modifications can result in an increase in the dermal absorption of substances, making it necessary to determine the effect of UV radiation on dermal absorption in order to properly evaluate the safety of chemicals, xenobiotics, and cosmetic formulations [27], especially those containing nanomaterials such as fullerene C60-PVP aggregates.

Considering the above, the primary objective of this study was to characterise C60-PVP dispersions in order to correctly evaluate the biological effects that fullerences exert on the skin. We also compared the in vitro dermal absorption processes of C60-PVP by using human and porcine skin. Furthermore, we evaluated whether UVA-R can modify the penetration/permeation profile of fullerene aggregates.

**Experimental Methods**

**Chemicals**

Fullerene C60 (≥99.5% purity) and PVP (K-30) were purchased from SES Research (Houston, Tex., USA) and Vetec (Rio de Janeiro, Brazil), respectively. Analytical-grade chloroform solvent was purchased from Labsynth (Diadema, Brazil). High-performance liquid chromatography (HPLC)-grade toluene and methanol solvents were obtained from Tedia (Rio de Janeiro, Brazil).

**C60-PVP Aggregate Dispersion Preparation**

The C60-PVP aggregate dispersions were prepared according to the method described by Yamakoshi et al. [14] as follows: 5.0 ml of a 0.8 mg/ml solution of C60 in toluene was added to 20.0 ml of chloroform containing 1.0 g of PVP under mild stirring for 15 min. The solvents were eliminated under vacuum evaporation (Büchi R-114; Büchi Laborteknik, Flawil, Switzerland), and the remaining film was sonicated with 20.0 ml of ultrapure water. The brownish dispersion formed was kept under stirring in an exhaust hood for 1.5 h to allow evaporation of the toluene residue, and the dispersion was then filtered through a PVDF membrane (0.45 μm; EMD Millipore, Billerica, Mass., USA) to select the nanometric population.

**Physicochemical Characterisation of C60-PVP Aggregate Dispersions**

The particle size distribution was determined by laser diffractometry (Mastersizer® 2000; Malvern Instruments, Malvern, UK) in order to obtain information on the nanometric and micrometric ranges of the dispersions. Measurements were carried out at 25°C, and the refractive index used was 2.20 [28]. Samples were added to the dispersion accessory (containing approx. 100 ml of distilled water) until an obscuration level between 0.02 and 0.10 was reached. The mean diameter over the volume distribution (d4,3) and the SSA were measured. The span, indicative of the polydispersity of the particle sizes, was calculated as shown in equation (1):

\[
\text{Span} = \frac{d_{90} - d_{10}}{d_{50}},
\]

where d90, d10 and d50 are the diameters at 90, 10 and 50% cumulative volumes, respectively. The results are expressed as the mean of 3 different batches.

The Z-average particle size (mean hydrodynamic diameter) and the polydispersity index were determined by dynamic light scattering (Zetasizer® Nano ZS; Malvern Instruments), which can give precise information on nanoscale diameters. Samples were diluted 200 times in filtered ultrapure water (0.45 μm; EMD Millipore) prior to analysis, which was performed at 25°C. Three measurements of 3 batches were made for each analysis.

The nanoparticle tracking analysis (NTA) method was also used to determine the particle size distribution, because it can provide a greater resolution of size distributions [29]. Moreover, NTA is one of the characterisation techniques that meet the criteria by the European Commission [1] for the definition of a nanomaterial. The analyses by NTA were carried out using a NanoSight® LM10 instrument (NanoSight, Amesbury, UK), in which samples were evaluated for 30 s with a camera shutter of 30 ms and automatic gain and blur settings. All the experiments were carried out at 1:50 dilution, leading to particle concentrations of approximately 8.09 × 10^8 ml⁻¹. The results are expressed as the mean ± SD of 3 different batches.

The mean pH values of 5 batches of C60-PVP dispersions were determined by potentiometry [Digimed® DM-22 pH meter (Digimed, São Paulo, Brazil) previously calibrated with pH 4.01 and 6.86 buffers] by immersing the probe directly in the dispersion.
Laser Doppler micro-electrophoresis was used to determine the zeta potential of the C$_{60}$-PVP dispersions (Zetasizer Nano ZS; Malvern Instruments). Measurements were performed after diluting the samples 200 times in a 10-mM NaCl solution (filtered through a 0.45-µm PVDF membrane; EMD Millipore). The average of 3 batches was used as the result.

The morphology of the aggregates was evaluated by transmission electron microscopy (TEM; JEM 1200 EXII; JEOL, Tokyo, Japan) operating at 80 kV [Centro de Microscopia Eletrônica, Universidade Federal do Rio Grande do Sul (UFRGS)]. The diluted dispersion was deposited on Formvar carbon films supported on copper grids (Electron Microscopy Sciences, Hatfield, PA, USA).

**Skin Absorption Studies of C$_{60}$-PVP Aggregate Dispersions**

**Porcine Skin Preparation.** Female porcine abdominal skin samples were obtained from a local slaughterhouse (Araldi, Bento Gonçalves, Brazil). The hair was trimmed off, the adipose tissue was removed, the stratum corneum was cleaned with a 1% sodium lauryl sulphate solution, and the dermis was cleaned with a 50% ethanol-50% (v/v) ether solution. Round pieces (approx. 7 cm$^2$) of skin were cut, and the skin thickness was measured with a dial thickness gauge (Mitutoyo, Kawasaki, Japan). The porcine skin samples had a mean thickness of 1.68 ± 0.21 mm. They were stored in a freezer (at –20 °C) in aluminium foil and used within 3 months.

**Human Skin Preparation.** Abdominal female human skin was obtained from elective abdominoplasty surgeries with patient authorisation (total of 4 patients) and with approval by the UFRGS Ethics Committee on Research (registration No. 12143113.4.0000.53-47). The procedures used for adipose tissue removal and cleaning were the same as those used for the porcine skin. Skin samples presenting striae were not used. The human skin samples had a mean thickness of 2.01 ± 0.17 mm. They were stored in a freezer (at –20°C) in aluminium foil and used within 1 month.

**Skin Diffusion Experiments.** The skin penetration experiments were carried out in automated Franz diffusion cell (Hanson Research, Chatsworth, Calif., USA) equipment, wherein a quintuplet of skin samples had a mean thickness of 1.68 ± 0.21 mm. The samples were stored in a freezer (at –20°C) in aluminium foil and used within 3 months. The skin penetration experiments were performed for 2, 6, and 12 h, respectively. At determined time points (0.5, 1, 2, 3, 6, and 12 h), automatic collections of the receptor medium were performed (volume: 1.5 ml).

**Skin Layer Separation Procedure.** At the end of each experiment, any remaining C$_{60}$-PVP dispersion was gently removed from the skin surface with a cotton swab. A tape stripping procedure [30] was performed to separate the stratum corneum from the viable epidermis with 18 pieces of adhesive tape (Mult Tape, Porto Alegre, Brazil), and 6 tapes each were placed in individual test tubes. The viable epidermis was separated from the dermis by placing the skin in hot water (60°C) for 45 s [30, 31]. The viable epidermis was removed with a scalpel, and the dermis was cut into small pieces that were placed in different test tubes.

**C$_{60}$ Extraction and Quantification Procedures**

**Extraction Procedure.** To quantify the C$_{60}$ in the C$_{60}$-PVP aggregate dispersion, an extraction procedure was performed to obtain C$_{60}$ in the molecular form for subsequent quantification by HPLC. The extraction of C$_{60}$ in the presence of biological samples was achieved as described by Xia et al. [32, 13], with some modifications. These authors suggested a simplified liquid-liquid extraction method using an optimal proportion of glacial acetic acid (GAA) to avoid protein and micelle interference during the quantification of C$_{60}$. Briefly, the stratum corneum of 6 tape strips each was digested in 4.0 ml of 1 M NaOH at 40°C for 1 h, followed by 1 h of sonication. The digestion solution from the tape strips (1.5 ml) was transferred to a glass extraction tube and homogenised with 4.0 ml of GAA. The extraction was performed with 1.0 ml of toluene followed by 2 min of vortexing and centrifugation at 4,120 g for 10 min. The toluene extract (300 µl) was mixed with 300 µl of methanol in an HPLC vial. The viable epidermis and dermis layers were mixed with 4.0 ml of GAA and, in the case of the viable epidermis, an additional 1.5 ml of ultrapure water, and the C$_{60}$ was extracted using the same procedure used for the stratum corneum.

**Quantification Procedure.** The quantitative analysis was performed by HPLC with UV detection (PerkinElmer® Series 200 HPLC; PerkinElmer, Waltham, Mass., USA), as described elsewhere [13], with some modifications. The analysis was carried out with a C$_{18}$ 5-µm 4.6 × 150 mm column (Phenomenex, Torrance, Calif., USA) with a mobile phase of 50% toluene and 50% methanol (v/v), detection at 333 nm, a flow rate of 1 ml/min, and a running time of 8 min. The sensitivity of the HPLC analysis was improved by injecting a volume of 150 µl. The method was validated by the following parameters: (1) linearity in the range of 0.03–15 µg/ml for C$_{60}$ solutions in toluene (C$_{60}$-Tol); (2) repeatability and intermediate precision of C$_{60}$-Tol; (3) quantification of C$_{60}$-PVP in the absence of biological samples and its specificity; (4) specificity with each medium used in the extraction procedure, including PBS, porcine and human total skin, and 1 M NaOH, and (5) total recovery and precision of extraction from each medium described in (4).

**Statistical Analysis**

The results of the skin penetration studies are reported as means ± SD. The data were statistically analysed using the non-parametric Kruskal-Wallis test, after Levene’s test for equality of error variances, via the software SPSS version 18 (UFRGS).

**Results and Discussion**

**Physicochemical Characterisation of C$_{60}$-PVP Aggregate Dispersions**

The proper physicochemical characterisation of a nanomaterial has become a matter of discussion for agencies concerned with consumer products containing nanoscale materials [33, 34]. Thus, this study has addressed the attributes of C$_{60}$-PVP aggregate dispersions that can affect the distribution and interaction of fullerenes with the skin. In this context, the size of a particle is considered a key parameter in determining its interactions with living...
The laser diffraction pattern of the C_{60}-PVP aggregate dispersion demonstrated that no further aggregation occurred after filtration ($d_{4.3}$: 142 ± 4 nm; span: 1.13 ± 0.030). In addition, the laser diffraction information provided an SSA of 50.9 ± 1.3 m$^2$/g. Dynamic light scattering (DLS) provides an accurate intensity-weighted size distribution when the scattering pattern comes from spherical nanoparticles with a narrow distribution [36], which is not the case for the C_{60}-PVP aggregates (Z-average: 173 ± 24 nm; polydispersity index: 0.288 ± 0.039). Nevertheless, the number mean determined by DLS (116 ± 18 nm) is in agreement with the mean diameter determined by NTA, as described below.

NTA is a more recently developed system for assessing the size distribution of heterogeneous samples illuminated by a laser beam. Each particle is individually visualised through a microscope, and its Brownian motion is analysed by the software on a particle-by-particle basis. The size distribution of the C_{60}-PVP aggregate dispersion determined by NTA is shown in figure 1. For the first time, C_{60}-PVP aggregates were analysed by NTA, revealing a non-homogeneous size distribution (mean: 129 ± 54 nm), most likely due to the non-controllable aggregation dynamics as a result of the preparation method.

The majority of publications on the size distributions of fullerene aggregates in water have used DLS and TEM for the measurements, producing variable results. For instance, the mean particle size of Radical Sponge® was approximately 688 nm for DLS, but TEM images showed particle sizes between 150 and 250 nm [15]. Because this aqueous solution was filtered through 0.1-μm pores, the large difference between the two methods may indicate that this formulation is able to re-aggregate after preparation. In another study, TEM images indicated particle size-

### Table 1. Particle size distribution parameters of each method used for their determination

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser diffractometry</td>
<td>$d_{4.3}$, nm$^a$</td>
<td>142 ± 4</td>
</tr>
<tr>
<td></td>
<td>Span$^b$</td>
<td>1.13 ± 0.030</td>
</tr>
<tr>
<td>DLS</td>
<td>Z-average, nm$^c$</td>
<td>173 ± 24</td>
</tr>
<tr>
<td></td>
<td>Number mean, nm</td>
<td>116 ± 18</td>
</tr>
<tr>
<td></td>
<td>Polydispersity index$^d$</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>NTA</td>
<td>Mean, nm</td>
<td>129 ± 54</td>
</tr>
<tr>
<td></td>
<td>$D_{50}$, nm$^e$</td>
<td>123 ± 8</td>
</tr>
</tbody>
</table>

$^a$ Volume-weighted mean.

$^b$ Measurement of the width of the distribution ($d_{0.9} - d_{0.1})/d_{0.5}$.

$^c$ Mean hydrodynamic diameter (intensity mean).

$^d$ Width parameter.

$^e$ Fifty percent of all particles analyzed are below the size.
es of approximately 10–25 nm [37]. Finally, the size of the C₆₀-PVP agglomerates was evaluated by TEM, revealing particles 50–100 nm in diameter [21]. Taken together, these results demonstrate that small modifications in the preparation method can dramatically alter the characteristics of a formulation, even for the same components. One of the most important parameters is the filtration process as the pore size should select the population of particles to be analysed for a biological purpose.

The fullerene dispersion presented a clear yellowish-brown colour and had a pH value of 4.52 ± 0.54. This value is appropriate for topical application due to the slight acidity of the skin surface [38]. The mean zeta potential value of the C₆₀-PVP aggregate dispersion, as obtained by an electrophoretic mobility assay, was −4.93 ± 1.72 mV. The few existing reports on the surface charge of C₆₀ aggregates suggest they are negatively charged [13, 39], but values as high as −40 and −50 mV were found in these studies. A recent study showed more than one zeta potential band for fullerenes stabilised with PVP, one of which was −2.21 mV [40]. This value is more consistent with the results of the present work and was attributed to a stable surface layer of C₆₀ encapsulated by PVP. Because different methods of preparation were used in each study, it is not unreasonable to expect different zeta potential profiles.

The C₆₀-PVP dispersion was further characterised in terms of the morphologies of the sample aggregates via TEM. Figure 2a shows 100- to 200-nm aggregates, while figure 2b shows an aggregate of approximately 400 nm under the same magnification, demonstrating the polydispersity of the sample, which corroborates the size distribution observed by NTA. The presence of PVP is evident in both images due to the differences in electron density between PVP and C₆₀, with the latter presenting a higher degree of darkness. On the TEM images, the irregular shapes of the C₆₀-PVP aggregates are clearly demonstrated, which can be attributed to the non-controllable assembly dynamics.

C₆₀ Extraction and Quantification Procedures

To validate the quantification of C₆₀ from each skin layer sample, several parameters were evaluated. The quantification of C₆₀-Tol by HPLC showed linearity with \( r = 0.9998 \), and repeatability and intermediate precisions of 2.24 and 2.64%, respectively. In the case of the C₆₀-PVP dispersion, the quantification in the absence of biological samples was carried out by the extraction of 20, 50, and 100 μl of dispersion in a total volume of 1.5 ml of water, which resulted in a concentration of C₆₀ of 160 μg/ml (approx. 222 μM) of dispersion and up to a 5% relative SD for all volumes extracted.

The extraction procedure was conducted with all extraction media. The placebo chromatograms obtained with water, PBS, porcine and human total skin, and 1 M NaOH, using a PVP solution instead of the C₆₀-PVP dispersion, showed no peaks that could interfere with C₆₀ quantification, which demonstrates the specificity of the proposed method (fig. 3a shows human total skin spiked with a PVP solution, and fig. 3b shows human total skin spiked with C₆₀-PVP dispersion). The total recovery of C₆₀ from each extraction medium, using the same volumes of dispersion described above and considering the concentration of 160 μg/ml to be 100%, was nearly constant at approximately 89%, with a precision of up to 10% for all the concentrations tested in all the media. This set of results indicates that the quantification and extraction methods were adequate for the application proposed.
Skin Absorption Studies of the C₆₀-PVP Aggregate Dispersion

Figure 4 shows the skin absorption profiles of the C₆₀-PVP aggregates through porcine (fig. 4a, c, e) and human skin (fig. 4b, d, f), showing the C₆₀ content in each layer of skin (rows). The results obtained in the presence of UVA-R or sham irradiation, represented by light and dark bars, respectively, are shown.

Porcine stratum corneum does not seem to be an effective barrier to the penetration of C₆₀ aggregates in comparison with human stratum corneum, as shown in figure 4a and b. This was evident because the C₆₀ content (up to 0.706 ± 0.081 μg/cm²) remained constant (no significant differences between groups) with time from the initial hours of exposure for both the sham and UVA-R conditions. Figure 4c shows that after flux maintenance in the stratum corneum, the C₆₀ reached the porcine viable epidermis in smaller quantities (up to 0.128 ± 0.034 μg/cm²), being constant at 2 h of exposure in the presence of UVA-R and becoming significantly different (letter ‘a’) at 12 h (UVA-R condition) in comparison with the value observed at 2 h (sham), suggesting that the UVA-R alters the porcine skin structure. The C₆₀ permeation through the dermis (fig. 4e) was approximately 2-fold higher than the quantity measured in the viable epidermis (up to 0.244 ± 0.070 μg/cm²). A significant difference (letter ‘b’) was obtained in the presence of UVA-R after 12 h of exposure in comparison with 2 and 6 h of exposure under the same conditions. No presence of C₆₀ in the receptor medium was detected.

Irradiation by UVA-R in the experiments using human skin did not modify the C₆₀ content in the human stratum corneum as observed for porcine skin, maintaining a constant quantity up to 0.297 μg/cm² (fig. 4b). However, the C₆₀ penetration in the sham-irradiated group was slower, being significantly different (letter ‘c’) after 12 h of exposure in comparison with 2 and 6 h of exposure under the same conditions. In the case of the human viable epidermis, despite having values different from 0 after 12 h (fig. 4d), the groups were not significantly different from each other, which demonstrates that the human viable epidermis could be functioning as a passageway for...
C$_{60}$ to permeate to the dermis. This is demonstrated in figure 4f, which shows $C_{60}$ penetration quantities of up to 0.149 ± 0.080 μg/cm$^2$ after 12 h in the presence of UVA-R, which was significantly different (letter ‘d’) from those observed after 2 and 6 h under the same conditions and from those observed (letter ‘e’) after 2 and 6 h of sham irradiation. As observed for porcine skin, $C_{60}$ was not quantified in the receptor medium. The absence of $C_{60}$ in the receptor medium does not mean that systemic absorption will not occur as the dermis has lymphatic and blood vessels. The receptor medium is normally modified with surfactants and/or solvents to enhance the partitioning of lipophilic substances; however, these modifications are also responsible for skin viability disruption [41]. In this study, because the $C_{60}$-PVP aggregates were stabilised in water, an aqueous physiological pH medium was chosen for the skin absorption experiments. As a result, this medium did not impair $C_{60}$ diffusion through the skin.

Studies comparing human skin permeability with animal skin models have been performed by some research groups [reviewed in 42, 43]. In the present study, the results for the viable epidermis and dermis penetration profiles were comparable between human and porcine skin types. Nevertheless, important observations can be made. Without radiation, the human stratum corneum acted as a superior barrier to $C_{60}$ penetration compared with the porcine stratum corneum, although, interestingly, the $C_{60}$ penetration profile in the presence of UVA-R was not different between the two skin types. Thus, some structural modifications may have occurred in the stratum corneum of human skin in the presence of UVA-R that allowed $C_{60}$ penetration from the first hours of exposure. This result corroborates findings from the literature, which have demonstrated lipid structure alterations after UVA-R exposure [26] most likely due to the formation of radical oxygen species [44, 45].

The lowest $C_{60}$ content found was in the viable epidermis skin layer for both human and porcine skin, indicating that nanomaterial diffusion occurs with a lower affinity for the viable epidermis than for the stratum corneum. The

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**Fig. 4.** $C_{60}$ content after 2, 6, and 12 h of skin absorption. Data are expressed as means ± SD. Statistical significances are represented by different letters and described along the text. **a**, **c**, **e** Porcine skin. **b**, **d**, **f** Human skin. **a**, **b** Stratum corneum. **c**, **d** Viable epidermis. **e**, **f** Dermis.
porcine and human dermis presented a greater C$_{60}$ content than that observed in the viable epidermis but with a permeation profile similar to that of the upper layer. The dermis thickness relative to the viable epidermis could explain this result, suggesting that after C$_{60}$ penetration into the stratum corneum, diffusion through the layers is impaired but still occurs. After 12 h of exposure, both the porcine and human dermis presented an accumulation of C$_{60}$ in the presence of UVA-R. Moreover, it was demonstrated that irradiation at 350 nm penetrates 60 μm in depth through the skin [46], which could explain this result.

It is assumed that nanosized materials larger than 100 nm would be safe for skin applications because most skin pores are in the range of 0.4–36 nm [reviewed in 47, 48]. The total C$_{60}$ content in the porcine and human skin was approximately 5% after 12 h of exposure, and a total of 2.7% of the fullerene aggregates were smaller than 40 nm, as measured by NTA, demonstrating that the skin pore size and C$_{60}$ aggregate size are nearly correlated. Moreover, the SSA of nanomaterials could be a potentially important parameter to consider in a skin absorption study. In this case, the SSA of 50 μg of C$_{60}$ aggregates was approximately 14-fold larger than the skin area to which the dispersions were applied (1.77 cm$^2$).

The literature contains few reports of fullerene skin absorption studies. Xia et al. [13] focused on occupational exposure to pristine C$_{60}$ in industrial solvents. C$_{60}$ was not detected in receptor solutions but was quantified in skin biopsies of the viable epidermis and dermis, as also demonstrated by our research group. Rouse et al. [49] studied the influence of mechanical flexion on the dermal penetration of a fullerene-based peptide and found that the longer the flexion time, the higher the particle penetration. Other factors affecting nanoparticle/nanomaterial skin penetration include diseased or damaged skin, the presence of penetration enhancers in cosmetic formulations, and environmental factors such as pollution and solar radiation.

Thus, studies addressing whether and to what extent external factors affect nanomaterial skin permeation are of high importance in considering their safety of use.

Conclusions

The results presented in this study show, for the first time, that C$_{60}$ aggregates stabilised with the hydrophilic polymer PVP can be more adequately characterised for size distribution by the NTA method and present a negatively charged surface and irregular shape. C$_{60}$ had the capacity for permeating in vitro through porcine and human skin; however, the stratum corneum penetration profiles were dissimilar between the two types of skin and similar between the viable epidermis and dermis. Moreover, the UVA irradiation source modified the permeation profile of the C$_{60}$ aggregate, as demonstrated by the increasing C$_{60}$ content in the lower skin layers for both porcine and human skin. C$_{60}$ was not quantified in the receptor medium. These results raise some concerns about the utilisation of daytime cosmetic formulations containing C$_{60}$-PVP. Some questions must be addressed regarding the effects of short- and long-term exposure to fullerenes on the skin.

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Disclosure Statement

The authors declare that there are no conflicts of interest.

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