

## TESTING THE EFFECTIVENESS OF GROWTH FACTORS IN DERMAL APPLICATIONS

**Wojciech Karwowski<sup>1</sup>, Damian Dudek<sup>2</sup>, Ewa Kłodzińska<sup>3,4,✉</sup>**

<sup>1</sup> St. Lazarus Foundation, 21-300, 4, Poczтовая str., Radzyń Podlaski, Poland

<sup>2</sup> Department of Perioperative Dentistry, Ludwik Rydygier Collegium Medicum in Bydgoszcz, 85-067, 13–15, Jagiellońska str., Bydgoszcz, Poland

<sup>3</sup> Institute of Innovative Medicine, Longevity Clinic, 30-701, 25/4, Zablocie str., Krakow, Poland

<sup>4</sup> EK Bio-Med, 87-100, 26/403, Szosa Chelminska str., Torun, Poland

✉ [klodzińskaewa473@gmail.com](mailto:klodzińskaewa473@gmail.com)

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**Abstract.** This study evaluates the electrophoretic behaviour and zeta potential of fullerenes in various buffer systems, hydrogel matrices, and in combination with insulin-like growth factor-1 (IGF-1) to optimise their potential for dermal applications. The methodology involved capillary electrophoresis (CE) and zeta potential measurements to assess fullerene stability in buffer solutions with pH values of 2.5, 7, and 9.3, as well as in a hydrogel base and in combination with IGF-1. Samples were analysed at 48 and 73 hours after preparation, and electrophoretic mobility and zeta potential were measured. Pronounced aggregation and irregular migration in aqueous and buffer solutions were demonstrated under acidic and alkaline conditions. Neutral conditions provided moderate stabilization but remained insufficient to ensure long-term colloidal stability. Incorporation of fullerenes into a hydrogel matrix significantly enhanced stability. The presence of IGF-1 in fullerene dispersions further improved electrophoretic profiles, suggesting a potential synergistic stabilizing effect.

**Keywords:** fullerenes, zeta potential, capillary electrophoresis, skin delivery systems, Franz diffusion cell.

### 1. Introduction

Concerns about oxidative stress, skin ageing, and dermatological conditions, including acne, pigmentation problems, and ultraviolet (UV)-induced damage, are common and call for creative remedies. Because of their exceptional antioxidant qualities, fullerenes have become a material of promise for reducing these problems by scavenging reactive oxygen species (ROS) and avoiding skin damage. However, despite their potential, fullerenes

present a number of difficulties in real-world applications. The hydrophobic nature of pristine fullerenes restricts their solubility and bioavailability in cutaneous preparations. Furthermore, questions have not been answered regarding their long-term safety, propensity for cutaneous irritation, and capacity to reach deeper skin layers without resulting in systemic absorption. The promise of fullerene-based formulations in clinical and cosmetic applications is not fully realised due to these obstacles. To fully realise their promise in dermatological therapies, these constraints must be addressed by formulation improvements, matrix selection, and the incorporation of functionalised fullerenes, such as hydroxylated and carboxyfullerenes.

The development of effective dermal delivery systems is a critical focus in cosmetic and pharmaceutical research, as these systems must ensure the stability, bioavailability, and controlled release of active compounds.<sup>1</sup> Fullerenes, a distinctive class of carbon-based nanomaterials, have attracted considerable interest in biomedical and cosmetic research due to their exceptional physicochemical properties. Structurally, fullerenes consist entirely of *sp*<sup>2</sup>-hybridised carbon atoms arranged in hollow, cage-like molecular frameworks, with C60 and C70 being the most studied variants.<sup>2</sup> Their unique molecular architecture imparts extraordinary stability, high electron affinity, and efficient free radical scavenging ability, making them highly promising in nanomedicine and advanced dermatological formulations.<sup>3,4</sup>

Fullerenes, with their unique molecular structure and antioxidant properties, have emerged as promising candidates for use in topical formulations. However, their practical application is challenged by their tendency to aggregate and their low stability in aqueous environments, which can significantly impact their efficacy.<sup>5</sup>

Because of their exceptional antioxidant qualities, fullerenes, spherical carbon-based nanomaterials, can effectively scavenge ROS. This antioxidant action is essential for combating oxidative stress, which is a primary cause of skin ageing and a number of dermatological conditions.<sup>6</sup> Numerous studies have demonstrated fullerenes' capacity to prevent UV-induced damage and lessen ROS-induced apoptosis in skin cells. Serda *et al.*<sup>7</sup> investigated the potential use of fullerene-based nanomaterials in improving the photodynamic therapy of non-melanoma skin malignancies as an example of its protective role against UV-induced skin damage. They demonstrated that fullerene nanomaterials are powerful agents for identifying and treating cancer cells, and that they can enhance the effectiveness of photodynamic treatment by boosting ROS generation when exposed to radiation.

To cure melanoma, Kyung *et al.*<sup>8</sup> concentrated on the biochemical and computational characterisation of nanoscaled materials, such as fullerenes. The results suggest that fullerene-based treatments might gather specifically in melanoma cells, offering a new treatment option for this aggressive skin cancer. Additionally, they pointed out that fullerenes may have even greater therapeutic benefits when coupled with other therapeutic agents, such as growth factors.

The promise of fullerenes in applications like photodynamic therapy and anti-inflammatory therapies has been highlighted recently by developments in nanomedicine and tailored drug delivery systems.<sup>9</sup> Liposomal formulations and fullerene-based nanoemulsions have been found to be useful in maintaining skin moisture, which makes them important ingredients in contemporary cosmetic treatments meant to improve skin health. In their comprehensive evaluation of topical nanoparticles, Raszewska – Famielec and Flieger<sup>10</sup> examined dermatological and dermo-cosmetic products. They emphasised how fullerenes' anti-inflammatory and antioxidant qualities might lessen the appearance of skin ageing by lowering inflammation, avoiding collagen deterioration, and blocking melanin synthesis. Their study also established fullerenes as promising components for cosmetics by highlighting the multifunctional potential of formulations based on fullerenes in treating acne and other skin disorders.

Despite these developments, there is still a lack of knowledge about how fullerenes interact with biological systems, especially in practical applications. The lack of information about the long-term impacts of fullerene exposure and their specific modes of action in skin tissues reflects this gap. This study aimed to evaluate the electrophoretic behaviour and stability of fullerenes under various experimental conditions, including different pH buffer solutions, hydrogel matrices, and in combination with IGF-1. CE and zeta potential measurements were employed to assess particle dispersion, aggregation tendencies, and stabilization mechanisms.

## 2. Experimental

The research was conducted by the St. Lazarus Foundation (Fundacja Św. Łazarza). The primary objective was to investigate the stability and electrophoretic behaviour of fullerenes in different buffer systems, hydrogel matrices, and in combination with IGF-1. The study focused on understanding the colloidal stability, aggregation tendencies, and electrokinetic properties of fullerenes using CE and zeta potential measurements. To ensure the accuracy and reproducibility of the results, all experiments were performed under standardised temperature and humidity conditions (25 °C, controlled humidity 50 %). Sample preparation, instrument calibration, and data collection were carried out following Good Laboratory Practice (GLP) guidelines.

CE is a high-resolution analytical technique used to separate charged particles in an electric field based on their size-to-charge ratio. In this study, CE was employed to evaluate the migration behaviour, stability, and aggregation tendencies of fullerene dispersions in different buffer systems, hydrogel matrices, and in combination with IGF-1. To complement the electrophoretic behaviour analysis, zeta potential measurements were used to provide a quantitative assessment of colloidal stability. Zeta potential is a key parameter that reflects the electrostatic interactions between dispersed particles, which significantly influence their aggregation and dispersion stability.

The experiments utilised Hewlett Packard 3D Capillary Electrophoresis (HP3DCE) system (Agilent Technologies, Waldbronn, Germany) with a diode array detector (DAD). The fused silica capillary from Composite Metal Services (Worcester, UK; 365 µm outer diameter, 75 µm inner diameter; total length 33.5 cm, effective length 25 cm) was conditioned with 1M NaOH, followed by deionised water and appropriate running buffers (20 mM borate at pH 9.3 or 50 mM phosphate at pH 7.0). Fullerenes were dispersed in buffer solutions with pH values of 2.5, 7.0, and 9.3 to investigate the effect of pH on their electrophoretic mobility. The samples were incubated for 48 and 73 hours to observe time-dependent changes in aggregation and dispersion properties.

Incorporation of fullerenes into a hydrogel matrix revealed notable changes in electrophoretic patterns, with reduced agglomeration and enhanced stability as evidenced by a zeta potential value of -45.9 mV. Comparatively, fullerene suspensions in pure buffers demonstrated significant aggregation and sedimentation, with migration influenced by the EOF and random clustering of particles. The hydrogel base provided a stabilizing environment, minimising agglomeration and enabling more uniform particle distribution.

### 3. Results and Discussion

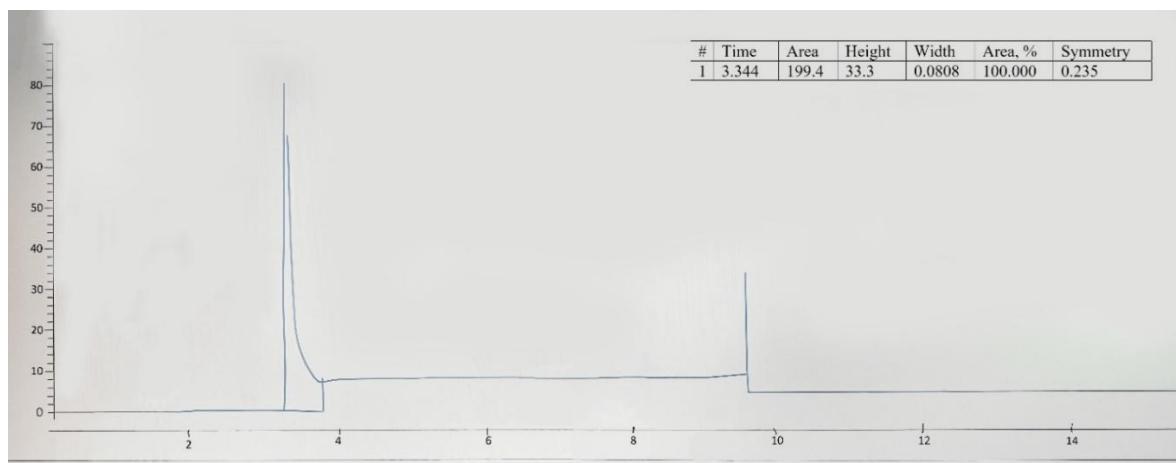
#### 3.1. Electrophoretic behaviour of fullerenes in phosphate buffer (pH 2.5 and pH 7.0)

In phosphate buffer at pH 2.5, fullerenes exhibited strong aggregation and slow migration, as evidenced by broad, irregular electrophoretic peaks (Table 1). The highly acidic environment likely contributed to charge neutralisation, leading to increased particle clustering and sedimentation.

**Table 1.** Electrophoretic behaviour of fullerenes in different buffer

Buffer type	pH	Electrophoretic behaviour
Phosphate	2.5	Strong aggregation, slow migration
Phosphate	7.0	Peaks are sharper but still irregular, indicating partial stabilization
Borate	9.3	Irregular electrophoretic patterns

The electropherogram showed multiple scattered peaks, indicating a polydisperse sample with varying particle sizes (Fig. 1).



**Fig. 1.** The electropherogram of a fullerene sample in phosphate buffer (pH 2.5)

The electropherogram of the fullerene sample in phosphate buffer (pH 2.5) exhibits a prominent initial peak, signifying substantial aggregation of the fullerenes. The width of this peak indicates that the fullerenes are inadequately dispersed in the solution, probably owing to the low pH. The absence of dispersion aligns with the propensity of fullerenes to cluster in acidic environments, where diminished surface charge restricts electrostatic repulsion among particles. The irregularity in the migration pattern following the initial peak further substantiates this, suggesting that the particles are forming clusters or aggregates, resulting in erratic migration. This behaviour underscores the instability of fullerenes in low-pH environments and emphasises the necessity for stabilization strategies in formulations utilising fullerenes, particularly in dermal or pharmaceutical applications.

At a low pH of 2.5, the capillary surface acquires a positive charge due to the ionisation of silanol groups (Si-OH) present on the inner surface. This generates an electric field towards the cathode (negative terminal), which would generally facilitate the movement of negatively charged entities such as fullerenes. The aggregation of fullerenes under acidic conditions leads to

increased particle sizes and irregular migration patterns. The diminution of electrostatic repulsion among particles results in clustering and a deceleration of migration. The presence of EOF hinders the migration of fullerenes due to the increased resistance encountered by larger aggregates during movement.

At pH 7.0, the migration patterns improved slightly, with moderate migration and reduced aggregation (Table 1). However, the electropherogram still displayed some peak broadening, suggesting that fullerenes were not fully stabilized in this medium. The results indicate that while neutral conditions mitigate extreme aggregation, additional stabilization is required to achieve uniform dispersion (Fig. 2).

Fig. 2 presents the electropherogram of a fullerene sample in phosphate buffer at pH 7.0, displaying a sequence of distinct peaks that signify the migration of fullerene particles under the influence of the applied electric field. In contrast to the outcomes at pH 2.5, the migration pattern observed here is more uniform, and the peaks are more defined, indicating that the fullerene particles are more effectively dispersed in the neutral pH milieu.

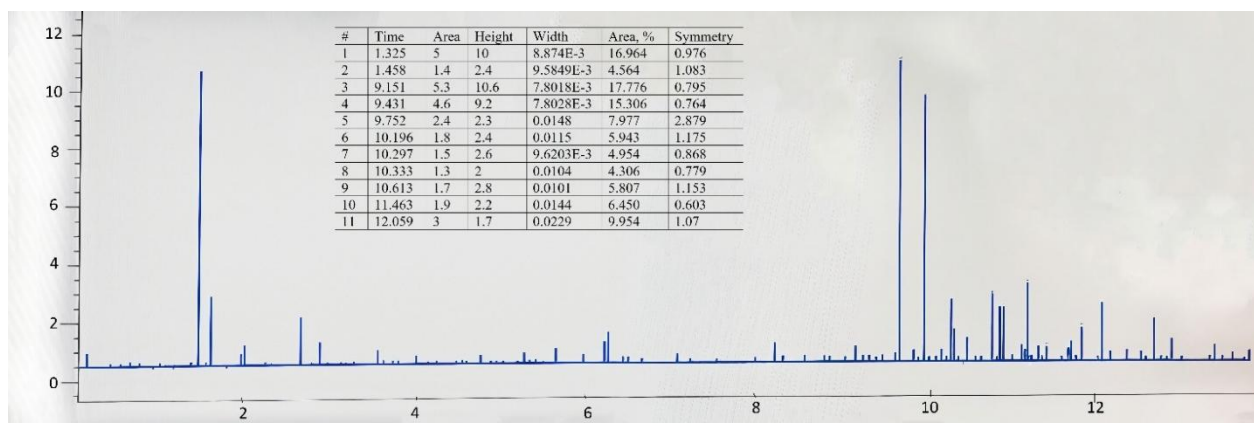


Fig. 2. The electropherogram of a fullerene sample in phosphate buffer (pH 7.0)

The initial peak is narrower than in the acidic condition, signifying a decrease in aggregation. This change is probably attributable to the neutral pH conditions, which create a more conducive environment for fullerene dispersion by inhibiting charge neutralisation that occurs at low pH levels. The supplementary peaks, while still observable, are less prominent than those at pH 2.5, indicating that a degree of aggregation remains, albeit diminished. The enhancement in migration behaviour suggests that phosphate buffer at pH 7.0 offers moderate stabilization. However, supplementary matrix or stabilizing agents may be necessary for optimal dispersion in practical formulations.

In a neutral pH environment (pH 7.0), the EOF continues to progress towards the cathode, yet the migration of fullerenes is more regulated. The electrostatic repulsion is more effectively sustained at this pH, resulting in reduced aggregation relative to acidic conditions. Nonetheless, fullerenes may still aggregate into smaller clusters, which are less problematic than those formed under acidic conditions. The EOF facilitates the migration of fullerene particles by propelling them through the capillary; however, the degree of aggregation still influences the sharpness and uniformity of the peaks. Consequently, although EOF is advantageous for migration, its impact remains ambiguous during aggregation. The findings demonstrated that phosphate buffers at pH 2.5 and 7.0 exhibited time-dependent aggregation. Fullerenes in phosphate buffer at pH 2.5 showed significant aggregation after 48 hours, which resulted in sluggish migration. Aggregation increased, and the migration rate further dropped at 73 hours, confirming fullerenes' fragility in acidic conditions. At pH 7.0, migration improved at 48 hours but worsened slightly by 73 hours due to increased aggregation. These results demonstrate that to stop aggregation in neutral buffer solutions, more stabilizing agents are required.

### 3.2. Electrophoretic behaviour of fullerenes in borate buffer (pH 9.3)

In borate buffer at pH 9.3, fullerene dispersions were highly polydisperse, with inconsistent migration patterns (Table 1). The electropherogram showed numerous irregular peaks, reflecting random clustering and fluctuating migration velocities. The instability of fullerenes in this medium suggests that high pH conditions do not provide sufficient stabilization, leading to erratic electrophoretic behaviour (Fig. 3). Fullerenes in a hydrogel matrix within borate buffer tendered agglomeration, as indicated by multiple spots in the electropherogram. However, the hydrogel provided some degree of stabilization, as confirmed by zeta potential measurements, which showed values above  $-30$  mV.

Fig. 3 presents the electropherogram of a fullerene sample in borate buffer at pH 9.3, which displays a pronounced, sharp initial peak, signifying substantial migration of fullerene particles within the buffer. The peak is comparatively narrow, indicating a degree of aggregation. The migration at pH 9.3 appears less stable than that in the neutral buffer (pH 7.0), as evidenced by the irregular secondary peaks that accompany the primary migration. These irregularities likely indicate the formation of particle clusters, resulting in inconsistent migration behaviours and a polydisperse distribution of fullerene particles.

The instability of the borate buffer underscores the propensity of fullerenes to aggregate under elevated pH conditions. The initial sharp peak, followed by scattered peaks and a broad area, indicates that the buffer fails to adequately stabilize fullerene particles. The existence of multiple peaks indicates random particle clustering, underscoring the necessity for supplementary stabilizing agents or matrices in high-pH environments to achieve more uniform dispersion. The findings indicate that,

although fullerenes can migrate in borate buffer at pH 9.3, the observed aggregation and polydispersity necessitate additional formulation modifications to enhance stability in high-pH conditions.

Fullerenes exhibited markedly irregular migration patterns at 48 and 73 hours in a borate buffer at pH 9.3. Aggregation was observed at 48 hours, leading to

irregular migration. This deteriorated at 73 hours, further evidencing that elevated pH levels do not sufficiently stabilize conditions. The instability was attributed to both the lack of electrostatic repulsion and the diminished effects of EOF in high-pH solutions. The results indicate that buffer pH influences fullerene behaviour and the efficiency of EOF in particle migration.

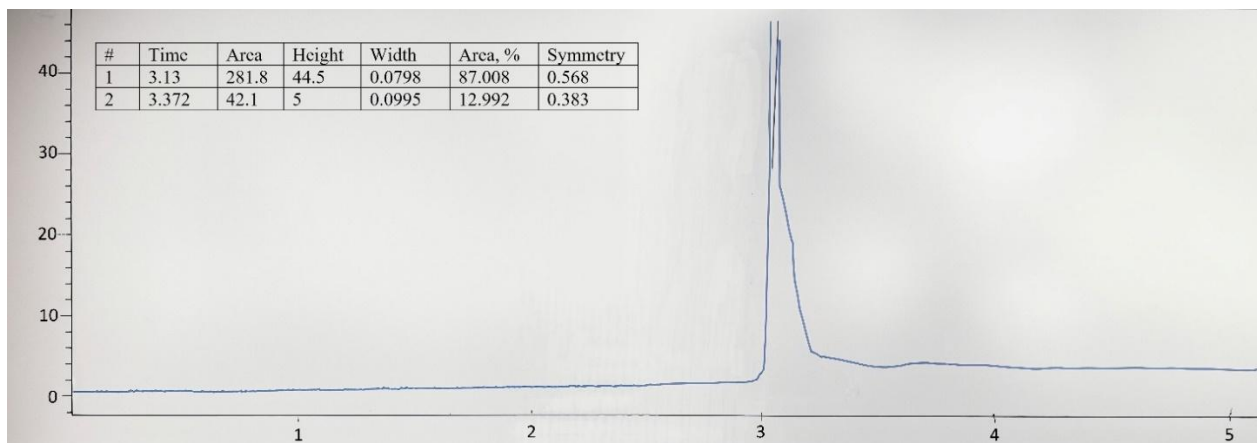


Fig. 3. The electropherogram of a fullerene sample in borate buffer (pH 9.3)

At elevated pH (pH 9.3), the capillary surface acquires a negative charge, thereby reversing the direction of EOF towards the anode (positive terminal). In this instance, EOF hinders the migration of negatively charged fullerenes by propelling the fluid in the contrary direction. At this pH, fullerenes exhibit a greater tendency to aggregate due to diminished electrostatic repulsion among particles. The elevated pH conditions fail to sufficiently stabilize the fullerenes, resulting in irregular migration patterns and erratic flow. The interplay between EOF and fullerene aggregation in this elevated pH environment leads to suboptimal and inconsistent migration, thereby diminishing the overall stability of the system.

### 3.3. Electrophoretic behaviour of fullerenes in water

Fullerenes suspended in deionised water showed random clustering and significant peak broadening in the electropherograms, suggesting poor electrostatic stabilization. The lack of ionic content in water likely contributed to low electrostatic repulsion, leading to particle aggregation (Fig. 4). The migration of fullerenes was erratic, reinforcing the fact that hydrophobic fullerene molecules tend to form clusters in aqueous environments.

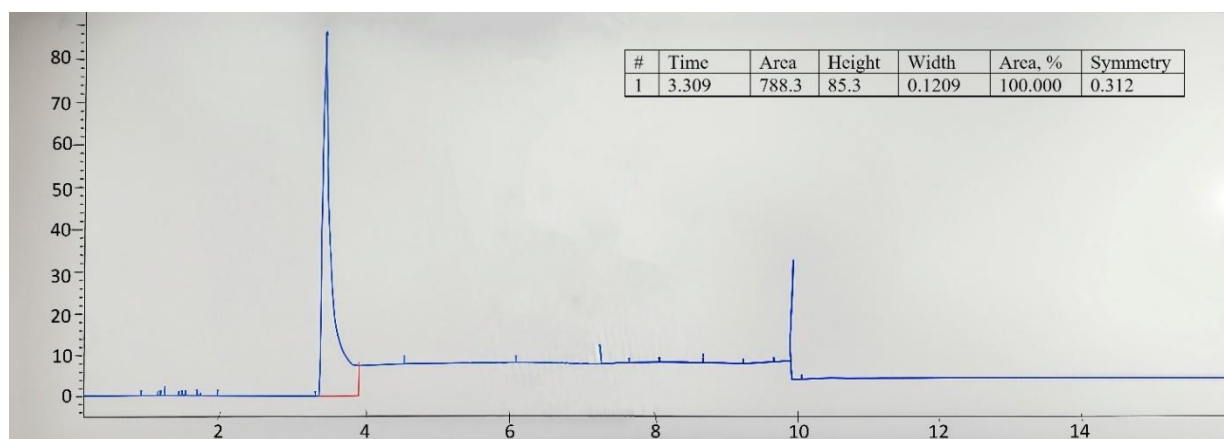


Fig. 4. The electropherogram of a fullerene sample in water (phosphate buffer, pH 7)

Fig. 4 illustrates the electropherogram of a fullerene sample in water (phosphate buffer at pH 7), exhibiting a broad and irregular peak at the beginning of migration. This indicates that the fullerene particles are undergoing considerable aggregation, as evidenced by the dispersed nature of the electrophoretic peak. The peak's width signifies the presence of diverse particle sizes in the sample, indicative of an unstable colloidal dispersion.

The irregularities in the migration pattern following the primary peak further substantiate the hypothesis that the fullerenes are aggregating, resulting in unpredictable migration behaviour. The absence of uniform migration results from the hydrophobic characteristics of fullerenes, leading to their aggregation in aqueous environments. The lack of adequate electrostatic repulsion in water leads to inadequate dispersion, highlighting the necessity for stabilizing agents or matrices to enhance the dispersion and stability of fullerenes in aqueous settings.

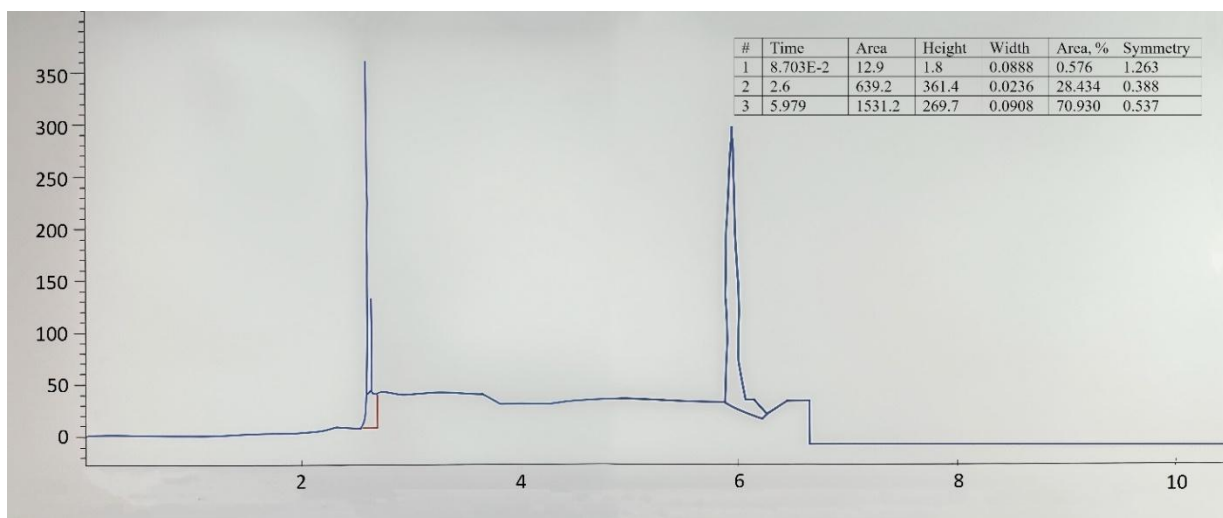
The electropherogram in water demonstrates that fullerenes possess low stability and show an increased tendency for aggregation, resulting in erratic migration. This discovery highlights the significance of selecting

suitable stabilization techniques or matrices for fullerene dispersions in aqueous settings.

Fullerenes dispersed in deionised water exhibited considerable aggregation and erratic migration patterns. At both 48 and 73 hours, the migration exhibited erratic behaviour, signifying inadequate electrostatic stabilization. The deficiency of ionic content in water led to diminished electrostatic repulsion, facilitating particle aggregation. This observation corroborates the findings from the pH 2.5 phosphate buffer, underscoring the significance of pH and matrix conditions in stabilizing fullerene dispersions.

### 3.4. Electrophoretic behaviour of fullerenes with IGF-1

The addition of IGF-1 to fullerene dispersions in borate buffer (pH 9.3) resulted in improved electrophoretic migration patterns compared to fullerenes in buffer solutions alone. The electropherograms showed sharper, more defined peaks, indicating reduced aggregation. This suggests that IGF-1 may have contributed to the steric or electrostatic stabilization of fullerene particles (Fig. 5).



**Fig. 5.** The electropherogram of a fullerene sample in Genfactor with IGF-1 (borate buffer, pH 9.3)

Fig. 5 illustrates the electropherogram of a fullerene sample in Genfactor with IGF-1 in borate buffer at pH 9.3, demonstrating markedly enhanced migration characteristics relative to the prior sample without IGF-1. The initial peak is prominent and clearly delineated, indicating diminished aggregation and enhanced particle mobility. IGF-1 seems to exert a stabilizing influence, probably via electrostatic or steric interactions, as indicated by the reduced peak widths and increased symmetry values. The evidence suggests that IGF-1 facilitates the distribution of fullerenes in high-pH

conditions, thereby enhancing their stability and mobility, which is essential for their prospective use in dermal and pharmaceutical formulations.

The incorporation of IGF-1 into fullerene dispersions within a borate buffer (pH 9.3) markedly enhanced the migration characteristics of fullerenes. Following incubation periods of 48 and 73 hours, more pronounced and distinct peaks were noted, signifying diminished aggregation. This indicates that IGF-1 may facilitate the stabilization of fullerene particles via electrostatic or steric interactions. These findings



correspond with prior studies regarding the function of growth factors in improving the stability of nanoparticle formulations and indicate a possible direction for further enhancement.

### 3.5. Electrophoretic behaviour of fullerenes in a hydrogel matrix

When fullerenes were suspended in a hydrogel matrix, the electropherograms showed stable and uniform migration patterns, indicating minimal aggregation. The peaks were well-defined, suggesting that the hydrogel environment provided steric hindrance and electrostatic stabilization, preventing the formation of large aggregates. This result strongly supports the use of hydrogel-based formulations for dermal applications (Fig. 6).

Fig. 6 illustrates the electropherogram of a fullerene sample with a hydrogel matrix in phosphate

buffer at pH 7.0, demonstrating remarkably stable and consistent migration characteristics. The initial peak is pronounced, and the following peaks are distinctly defined with minimal broadening, suggesting that the fullerene particles are uniformly dispersed within the hydrogel matrix. The sample's stability is indicated by the lack of substantial aggregation, as demonstrated by the narrow and symmetrical peaks. This indicates that the hydrogel matrix offers effective stabilization, inhibiting particle aggregation and ensuring a homogeneous distribution of fullerenes. The findings indicate that hydrogels serve as an ideal medium for preserving fullerene stability, especially in dermal applications where uniform dispersion is essential for effectiveness. Fig. 7 displays the electropherogram of a fullerene sample with a hydrogel base in borate buffer at pH 9.3, demonstrating a notable enhancement in the stability and dispersion of fullerene particles relative to the pure buffer.

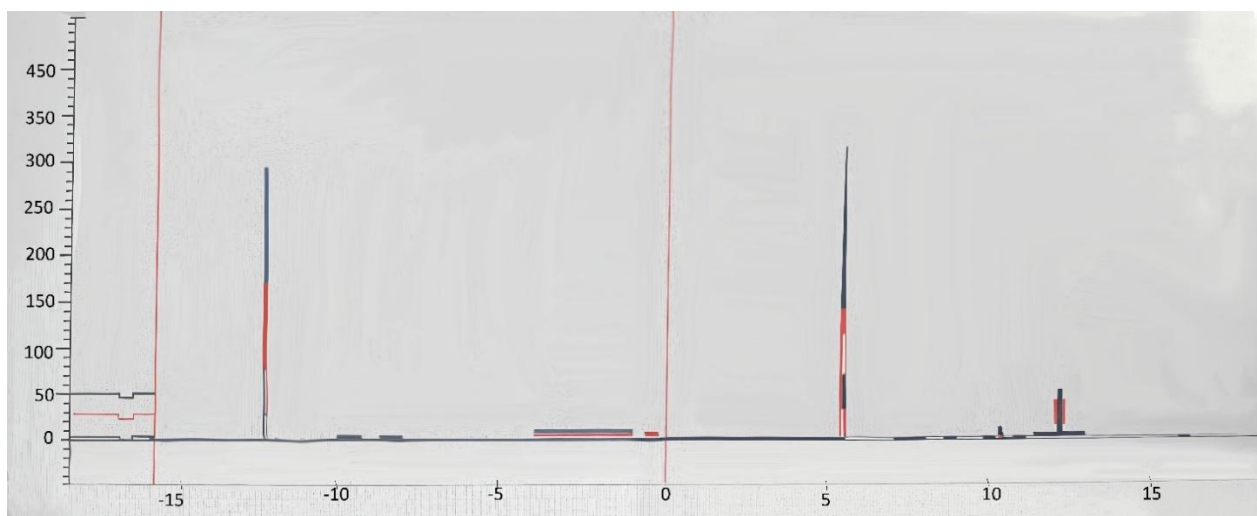


Fig. 6. The electropherogram of a fullerene sample with the hydrogel base (phosphate buffer, pH 7)

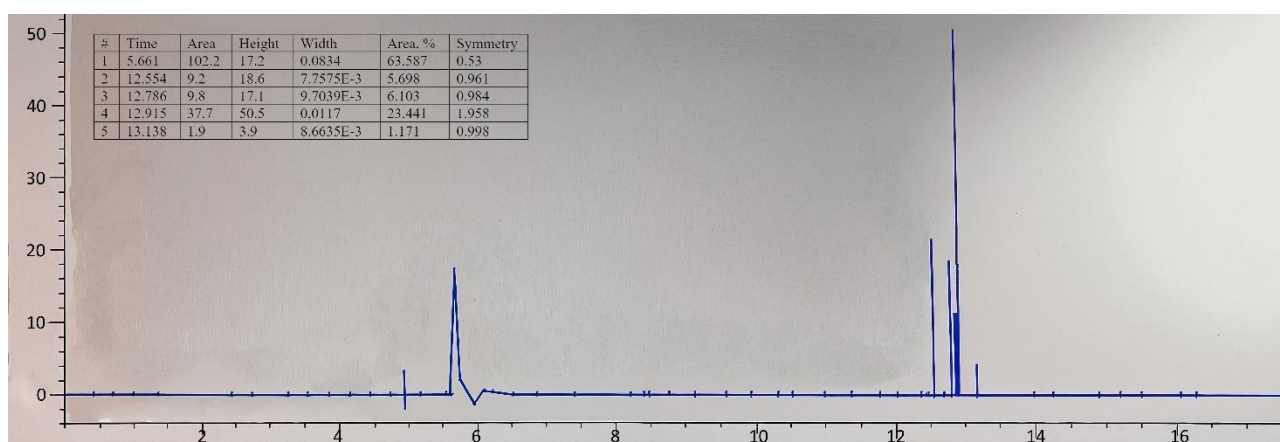


Fig. 7. The electropherogram of a fullerene sample with the hydrogel base (borate buffer, pH 9.3)

In Fig. 7, the initial peak is acute and distinctly delineated, meaning that the hydrogel proficiently stabilizes the fullerenes, inhibiting aggregation. The ensuing peaks exhibit clarity and symmetry, with negligible broadening, indicating uniform migration and effective dispersion throughout the analysis. This stability differs from earlier samples in borate buffer lacking the hydrogel base, which exhibited irregular peaks and aggregation. The findings indicate that the hydrogel matrix effectively stabilizes fullerenes in high-pH conditions, ensuring uniform particle distribution and thus rendering it an optimal medium for dermal or pharmaceutical applications.

Fullerenes dispersed in a hydrogel matrix demonstrated consistent and uniform migration behaviour, with minimal aggregation at both 48 and 73 hours. The electropherograms exhibited distinct peaks, signifying successful stabilization. The hydrogel matrix presumably offered steric and electrostatic stabilization, inhibiting particle aggregation and facilitating migration. These results confirm that hydrogels effectively preserve fullerene stability in dermal formulations, even during prolonged incubation periods.

When fullerenes are incorporated into a hydrogel matrix, the effect of EOF is less pronounced. The hydrogel serves as a stabilizing agent, preventing particle aggregation through both steric and electrostatic stabilization. The matrix helps maintain consistent particle migration by minimising the impact of EOF, allowing fullerenes to move more uniformly through the capillary. This results in sharper and more defined peaks in the electropherogram, as seen in samples with the hydrogel matrix. The EOF still influences the movement of the particles, but the hydrogel mitigates the aggregation that

would typically hinder migration in aqueous or buffer solutions.

### 3.6. Zeta potential measurements of fullerenes

Zeta potential measurements were conducted to quantify the stability of fullerene dispersions. A high absolute zeta potential value ( $>30$  mV in magnitude) typically indicates strong electrostatic repulsion and colloidal stability, whereas low values suggest aggregation and instability.

Zeta potential assessments were performed at 48 and 73 hours, indicating the stability of fullerene dispersions across various media. In phosphate buffers at pH 2.5 and 7.0, the zeta potential was consistently low, signifying inadequate electrostatic repulsion and diminished colloidal stability, particularly at pH 2.5. At pH 7.0, a marginal enhancement in zeta potential was noted at 48 hours. However, the values continued to indicate moderate instability. Conversely, fullerenes within a hydrogel matrix demonstrated a substantial negative zeta potential of  $-45.9$  mV, signifying exceptional stability and negligible aggregation at both time intervals. These measurements validate the visual observations of enhanced migration and diminished aggregation within the hydrogel matrix.

Fullerenes dispersed in deionised water exhibited low zeta potential values, ranging from  $-12.2$  mV to  $-16.2$  mV (Table 2). These values confirm that fullerenes in water experience weak electrostatic repulsion, leading to aggregation and poor stability. The measured instability correlates with the broad, scattered electrophoretic peaks observed in CE, reinforcing the need for stabilization strategies.

**Table 2.** Zeta potential and stability behaviour of fullerenes

Sample	Buffer	pH	Zeta potential, mV	Stability behaviour
Fullerene + water	–	–	$-12.2$	Significant particle clustering, low colloidal stability
			$-12.4$	Increased agglomeration
			$-16.2$	Moderate instability; partial agglomeration observed
Fullerene	Phosphate	2.5	$-2.97$	Highly unstable
		2.5	$-6.91$	Unstable
		7	$-24$	Moderate stability, but some degree of aggregation remains
		7	$-25$	Moderate stabilization, minimal agglomeration
	Borate	9.3	–	Polydisperse
Hydrogel	–	–	$-16.9$	Improved stability
Fullerene + hydrogel			$-45.9$	Excellent stabilization, minimal aggregation

At pH 7.0, the zeta potential improved to  $-24$  mV to  $-25$  mV, indicating moderate stability (Table 2). While this level of electrostatic repulsion partially prevented aggregation, it was not sufficient to maintain full colloidal

stability over time. The electropherograms also reflected reduced, but still present, clustering, reinforcing that neutral buffer conditions alone do not ensure stable fullerene dispersion.



The zeta potential of fullerenes in water was measured, revealing low values between  $-12.2$  mV and  $-16.2$  mV at both 48 and 73 hours, indicating weak electrostatic repulsion and a propensity for aggregation in deionised water. The instability evident in the electropherograms, characterised by random clustering and broad peaks, corresponds with the low zeta potential values, underscoring the necessity for stabilization in aqueous environments.

In phosphate buffer at pH 2.5, fullerenes exhibited high instability, with zeta potential values of  $-2.97$  mV and  $-6.91$  mV (Table 2). These low values indicate rapid agglomeration, consistent with the slow migration and strong clustering observed in electrophoretic analysis.

At pH 2.5 in phosphate buffer, the zeta potential values after 48 hours were  $-2.97$  mV and  $-6.91$  mV, respectively, signifying inadequate stability and considerable agglomeration. These values diminished further at 73 hours, corroborating the heightened instability over time. At pH 7.0, the zeta potential was marginally elevated, attaining  $-24$  mV to  $-25$  mV at both intervals, signifying moderate stability yet remaining susceptible to aggregation. These findings underscore the significance of optimising pH conditions for fullerene dispersions.

The zeta potential of fullerenes in borate buffer at pH 9.3 could not be consistently measured due to the highly polydisperse nature of the sample (Table 2). This aligns with the irregular electrophoretic peaks observed in CE, confirming that fullerenes in high-pH environments exhibit significant aggregation and instability. In a borate buffer at pH 9.3, the zeta potential of fullerenes could not be reliably quantified owing to the significant polydispersity of the sample at both 48 and 73 hours. This corroborates the electrophoretic findings, wherein anomalous migration patterns were noted, and further substantiates the instability of fullerenes in high-pH conditions.

The most stable dispersion was observed in hydrogel-based formulations. The zeta potential of fullerenes in hydrogel was measured at  $-45.9$  mV, indicating excellent stability and minimal aggregation (Table 2). This high negative charge suggests that the hydrogel provided strong electrostatic and steric stabilization, effectively preventing particle clustering and sedimentation. The uniform migration observed in electrophoretic measurements supports this finding, demonstrating that hydrogels are highly effective stabilizing agents for fullerene dispersions.

The zeta potential of fullerenes within a hydrogel matrix was measured at  $-45.9$  mV at both 48 and 73 hours, indicating superior colloidal stability. The sustained elevated zeta potential values indicate that the hydrogel matrix offers efficient electrostatic and steric stabilization, inhibiting aggregation and guaranteeing uniform particle dispersion for optimal dermal application.

### 3.7. Solubility and dispersion challenges of fullerenes in various solvents

The study successfully demonstrated that fullerenes exhibit strong aggregation in aqueous and buffer solutions, particularly at extreme pH levels. However, incorporating fullerenes into a hydrogel matrix significantly improved their stability, as evidenced by sharper electrophoretic peaks and a high zeta potential of  $-45.9$  mV, indicating strong electrostatic and steric stabilization. These results confirm that hydrogel matrices provide an optimal medium for maintaining the dispersion of fullerenes, making them highly suitable for cosmetic and pharmaceutical applications.

Additionally, the analysis of fullerenes in combination with IGF-1 revealed improved migration behaviour, suggesting that IGF-1 may contribute to fullerene stabilization. However, due to the lack of zeta potential data for IGF-1-stabilized fullerenes, further research is required to fully determine their long-term stabilizing effects and potential synergistic role in bioactive dermal formulations.

Through a systematic evaluation of electrophoretic migration and electrostatic stability, this study successfully achieved its goal of identifying suitable dispersion conditions for fullerene formulations, demonstrating that hydrogels provide superior stability, while unmodified aqueous and buffer suspensions result in rapid aggregation. The findings serve as a foundation for future research on optimising fullerene-based formulations, particularly in terms of transdermal penetration, functionalisation strategies, and long-term biocompatibility assessments.

The results obtained in this study provide crucial insights into the electrophoretic behaviour and stability of fullerenes in different buffer solutions, hydrogel matrices, and in combination with IGF-1. The data highlight how pH, dispersing medium, and stabilization agents influence the colloidal stability, migration behaviour, and aggregation tendencies of fullerene particles.

The solubility and dispersion of fullerenes in diverse solvents, including aqueous and buffer solutions, presented difficulties owing to their hydrophobic characteristics and propensity to aggregate. This study corroborated that, akin to prior research, fullerenes demonstrated inadequate dispersion in aqueous and buffer solutions, especially at extreme pH levels. Nonetheless, integration into a hydrogel matrix markedly enhanced their stability, indicating that matrix selection is vital for optimising fullerene dispersion in dermal applications.

The primary goal of this study was to assess the electrophoretic behaviour and stability of fullerenes under different buffer conditions, hydrogel matrices, and in

combination with IGF-1 to optimise their potential use in dermal and transdermal applications. This goal was successfully achieved through a comprehensive analysis of migration patterns in CE and zeta potential measurements, which provided key insights into fullerene stability and aggregation behaviour.

The findings confirmed that fullerenes exhibit strong aggregation tendencies in aqueous and buffer solutions, particularly at extreme pH levels, which compromise their stability. However, the incorporation of hydrogel matrices significantly improved dispersion and prevented aggregation, as evidenced by sharper electrophoretic peaks and a high zeta potential of  $-45.9$  mV. The observed enhanced stabilization within hydrogels suggests that they provide an effective medium for fullerene-based formulations, ensuring long-term stability and uniform dispersion in cosmetic and pharmaceutical applications.

Additionally, the electrophoretic analysis of fullerenes with IGF-1 revealed improved migration patterns, indicating that IGF-1 may contribute to particle stabilization. However, further investigation is required to fully understand its role in electrostatic stabilization and long-term dispersion behaviour.

The results confirm that hydrogel-based delivery systems are optimal for fullerene formulations, successfully addressing the key challenge of aggregation and instability that has historically limited their practical applications. These findings lay a strong foundation for further research on functionalised fullerenes, transdermal penetration studies, and advanced matrix stabilization techniques, ultimately contributing to the development of next-generation cosmetic and therapeutic products.

The study's principal findings underscore the necessity for stabilizing matrices in fullerene formulations. Fullerenes in buffer solutions at extreme pH levels (both acidic and alkaline) exhibited considerable aggregation and suboptimal migration behaviour, whereas hydrogel matrices ensured optimal stabilization. The findings indicate that IGF-1 may provide further stabilization, enhancing the migration and dispersion of fullerenes. These findings have significant implications for the formulation of fullerene-based products in the cosmetic and pharmaceutical sectors, especially for applications aimed at dermal delivery systems.<sup>11</sup> The research facilitates subsequent investigations into functionalised fullerenes and sophisticated matrix stabilization methods to improve their bioavailability and effectiveness.

Thus, the research successfully met its objective by demonstrating that matrix selection plays a critical role in stabilizing fullerenes, improving their dispersion, and enhancing their suitability for dermal applications. Future work will focus on Franz diffusion cell studies to assess skin penetration and bioavailability, further optimising the delivery of fullerenes in biomedical formulations.

### 3.8. Discussion

Due to their exceptional physicochemical characteristics, fullerenes, in particular C60 and C70, have received a lot of interest in both biological and cosmetic applications. Fullerenes' potential for cutaneous applications, particularly their anti-inflammatory and antioxidant properties, has been investigated in recent research. In order to maximise fullerenes' performance for cutaneous applications, this study assessed their electrophoretic behaviour and zeta potential in a variety of buffer systems, hydrogel matrices, and in conjunction with IGF-1.

The work of Kausar,<sup>12</sup> who investigated the uses of fullerene nanocomposites in a variety of industries, such as cosmetics and medical, is directly tied to the current study. With an emphasis on their stability and distinctive chemical interactions, Kausar's study investigated the potential of fullerene-based nanocomposites to improve the qualities of materials in biomedical domains. However, this work expands on the knowledge of fullerene behaviour in dermal formulations, specifically about their stability and aggregation tendencies in buffer solutions and hydrogels, whereas Kausar's study focused on fullerene composites in material sciences.

The results of this study are also supported by investigation of Romero Robles *et al.*<sup>13</sup> on the antioxidant potential of fullerenes in cosmetic formulations. Romero Robles *et al.* concentrated on the advantages of fullerenes in skincare products, showing how they may shield skin from oxidative damage. This is consistent with recent studies that demonstrated fullerenes' low stability in aqueous solutions while also confirming their antioxidant properties. By providing a useful method for enhancing fullerene dispersion in dermal applications, the current study expands on Romero Robles' work by using hydrogel matrices to improve fullerene stability and functioning.

Fullerenes in the current research showed a considerable propensity to aggregate when suspended in different buffer solutions, particularly in alkaline (pH 9.3) and acidic (pH 2.5) environments. This outcome confirms previous research by Francis Luther King and Sherin Nithya,<sup>14</sup> who talked about the difficulties in obtaining stable fullerene dispersions in various solvent systems. Like their results, the current study shows that buffer pH has a substantial impact on fullerene stability. At high pH levels, decreased zeta potential values are seen, which suggests poor dispersion and an aggregation propensity.

Fullerenes, on the other hand, were more stable when added to a hydrogel matrix; their zeta potential was  $-45.9$  mV, indicating good dispersion and little aggregation. Biswas *et al.*<sup>15</sup> investigated the utilisation of fullerene derivatives in biological applications, including

their capacity to stabilize inside polymer matrices, and this supports their results. The new work builds on previous research by demonstrating that hydrogels are perfect for dermal applications because they promote more uniform particle distribution in addition to improving fullerene stability.

Furthermore, enhanced migratory patterns were seen when IGF-1 was added to fullerene dispersions, indicating a possible stabilizing impact. The study of Mulqueen *et al.*,<sup>16</sup> who emphasised the possibility of mixing nanoparticles with growth factors to improve their performance in medicinal and cosmetic applications, is consistent with this conclusion. IGF-1 stabilizing impact, as shown in this work, may improve the performance of fullerene-based dermal systems by providing further advantages in skin regeneration and antioxidant defence.

The difficulties of using fullerenes in dermal formulations, especially with regard to their propensity to congregate in aqueous conditions, are supported by the results of this work. The findings suggest that fullerenes need stabilization techniques such as matrix incorporation to get optimum dispersion, which is in line with the study of Mchedlov – Petrossyan *et al.*<sup>17</sup> The findings of Kyzyma,<sup>18</sup> who showed that the use of hydrogels for nanoparticle dispersion might increase the stability and bioavailability of fullerenes in dermal systems, are supported by the current study's conclusion that hydrogel matrices offer greater stabilization.

Furthermore, although previous research, such as that conducted by Shinde *et al.*,<sup>19</sup> highlighted the potential of fullerenes in anti-aging and skin rejuvenation applications, this study goes beyond those conclusions by offering a thorough examination of the circumstances in which fullerenes show the best stability. The new work adds to the body of literature by showing that hydrogels and IGF-1 can improve the stability and dispersion of fullerenes, providing useful information for the creation of pharmacological and cosmetic products based on fullerenes.

Even though this study shows how fullerene-based formulations may be used in cutaneous applications, there are still a few areas that need more research. To assess the transdermal penetration and bioavailability of formulations based on fullerene, *in vivo* investigations are the next step. According to Dubey *et al.*,<sup>20</sup> functionalised fullerenes provide encouraging chances to get over the solubility issues that virgin fullerenes confront. Future studies might look at the long-term impacts of fullerene-based products on skin health as well as the functionalisation of fullerenes to improve their solubility, biocompatibility, and targeting ability.

To assess the stabilizing impact of IGF-1 and other bioactive compounds in fullerene formulations, more

research is also required. Even though this study found that IGF-1 improved migratory behaviour, further thorough investigation is required to completely comprehend its function in stabilizing fullerene and its possible synergistic effects in dermal systems.

The significance of matrix selection in maximising fullerene-based formulations for dermal applications is emphasised by this study's result.<sup>21</sup> By showcasing the impacts of hydrogels and IGF-1 on stability, the study offers a useful starting point for the creation of advanced fullerene-based cosmetic and medicinal goods. The optimisation of these formulations, including transdermal penetration evaluation, functionalisation techniques, and long-term biocompatibility evaluations, should be the main emphasis of future research.

The results of this study provide a strong foundation for the continued optimisation of fullerene-based formulations, particularly in the fields of dermal and transdermal applications. While this study successfully demonstrated improved stability and dispersion of fullerenes in hydrogel matrices, several critical areas require further exploration to ensure their efficacy, safety, and potential for large-scale biomedical and cosmetic applications. One of the primary challenges with fullerene applications remains their limited solubility in most solvents. Although colloidal suspensions and nanoemulsions have shown potential in enhancing fullerene dispersibility, the precise interactions between fullerenes and various solvents, surfactants, or stabilizing agents remain incompletely understood.<sup>22</sup>

The potential stabilizing effect of IGF-1 observed in this study presents a novel avenue for improving fullerene formulations. IGF-1 is widely recognised for its role in cellular regeneration and skin repair, but its interactions with fullerene nanoparticles have not been fully explored. Expanding functionalised fullerene research also holds promise in overcoming solubility limitations. Functionalisation is one of the most promising strategies for improving fullerene solubility, biocompatibility, and targeting ability. While pristine fullerenes exhibit poor solubility in aqueous environments, various chemical modifications can enhance their stability, dispersibility, and potential biological activity.<sup>23</sup>

Additionally, understanding the skin permeability and bioavailability of fullerene-based formulations is crucial for their clinical and commercial viability. Although previous studies have shown that fullerenes can penetrate the epidermis, additional research is needed to assess their long-term retention, metabolism, and potential systemic absorption. Investigating the cytotoxicity and long-term effects of fullerene-based formulations is also critical. Although fullerenes have been widely studied for their antioxidant and anti-inflammatory properties, there

are still concerns regarding their potential toxicity, long-term accumulation, and effects on skin health. Some studies have suggested that functionalised fullerenes exhibit low cytotoxicity, while others have raised concerns about ROS generation and oxidative stress in certain biological environments.<sup>24</sup> Finally, there is significant potential for the development of advanced fullerene-loaded formulations for cosmetic and pharmaceutical use. With the increasing interest in nanotechnology-driven skincare and drug delivery systems, there is an opportunity to develop new, optimised formulations that enhance the stability, efficacy, and bioavailability of fullerenes.

## 4. Conclusions

This study provided valuable insights into the behaviour and stability of fullerene particles in various experimental conditions, including buffer solutions of differing pH levels and a hydrogel matrix, and in combination with IGF-1. Through CE and zeta potential measurements, the study established critical parameters that influence the stability, dispersion, and aggregation tendencies of fullerenes, highlighting their potential for dermal and transdermal applications.

The research demonstrated that fullerene particles exhibit significant aggregation and sedimentation in aqueous and buffer solutions, particularly under highly acidic (pH 2.5) and alkaline (pH 9.3) conditions. Zeta potential measurements confirmed that at these pH extremes, the particles lacked sufficient surface charge to maintain stability. Moderate stability was observed at neutral pH (zeta potential:  $-25$  mV), but the suspensions remained prone to polydispersity. These findings underscore the importance of matrix stabilization for practical applications.

Incorporating fullerenes into a hydrogel matrix markedly improved their stability, with a zeta potential of  $-45.9$  mV indicating excellent dispersion and minimal aggregation. The hydrogel provided steric and electrostatic stabilization, ensuring uniform particle distribution and long-term stability. Electropherograms further supported this conclusion, showing reduced aggregation and consistent migration patterns. These results demonstrate that hydrogels are an optimal medium for fullerene stabilization in cosmetic and therapeutic formulations. Additionally, the combination of IGF-1 with fullerene dispersions showed improved migration patterns in CE, suggesting a potential stabilizing effect.

While the study highlights the potential of fullerene-based hydrogels, limitations include the absence of *in vivo* studies or transdermal diffusion experiments, which are necessary to validate their efficacy and safety in

practical applications. The impact of functionalisation on stability and biocompatibility warrants further investigation.

Future research should focus on optimising hydrogel formulations for targeted fullerene delivery, conducting Franz diffusion cell studies to assess skin penetration, and exploring the long-term effects of fullerene-based products on human skin. Investigating functionalised fullerenes or alternative matrices could further enhance their stability and broaden their applicability in dermal and biomedical fields.

Overall, this study confirms the feasibility of stabilizing fullerene particles in hydrogels and in combination with IGF-1, establishing a foundation for developing advanced cosmetic and therapeutic products.

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## ВИПРОБУВАННЯ ЕФЕКТИВНОСТІ ФАКТОРІВ РОСТУ В ДЕРМАЛЬНИХ ЗАСТОСУВАННЯХ

**Анотація.** У дослідженні оцінено електрофоретичну поведінку та дзета-потенціал фулеренів у різних буферних системах, гідрогелевих матрицях та у поєднанні із інсуліноподібним фактором росту-1 (IGF-1) із метою оптимізації їхнього потенціалу для застосування у дерматології. Методологія передбачала капілярний електрофорез (CE) та вимірювання дзета-потенціалу для оцінювання стабільності фулеренів у буферних розчинах зі значеннями pH 2,5, 7 та 9,3, а також у гідрогелевій основі та у поєднанні з IGF-1. Зразки аналізували через 48 та 73 години після приготування, вимірювали електрофоретичну рухливість та дзета-потенціал. У кислотних і лужних умовах продемонстровано виражену агрегацію та нерегулярну міграцію у водних і буферних розчинах. Нейтральні умови забезпечили помірну стабілізацію, але були недостатніми для забезпечення довгострокової колоїдної стабільності. Включення фулеренів до гідрогелевої матриці істотно підвищило стабільність. Наявність IGF-1 у дисперсіях фулеренів ще більше поліпили електрофоретичні профілі, що свідчить про потенційний синергетичний стабілізуювальний ефект.

**Ключові слова:** фулерени, дзета-потенціал, капілярний електрофорез, системи доставки через шкіру, дифузійна комірка Франца.