Development and evaluation of the novel topical formulation containing Bakuchiol for enhanced skin delivery

Ali Asram SAGIROĞLU 1*, Beyza Sumeyye AYDIN 2

1 Department of Pharmaceutical Technology, Faculty of Pharmacy, Istanbul University-Cerrahpasa, 34500 Istanbul, Turkey.
2 Department of Biotechnology, Institute of Health Sciences, Bezmiâlem Vakif University, Istanbul, Turkey

* Corresponding Author. E-mail: a.a.sagiroglu@gmail.com.

Received: 30 October 2023 / Revised: 30 November 2023 / Accepted: 01 December 2023

ABSTRACT: This study investigates the encapsulation of bakuchiol, a renowned compound for hyperpigmentation treatment, utilizing transfersomes as a delivery system. The transfersomes were fabricated using the thin-film hydration technique and subjected to thorough characterization across various parameters. The resulting transfersome formulation, after optimization, demonstrated notably high encapsulation efficiencies, with bakuchiol registering at 86.72±3.6%, accompanied by a narrow particle size distribution (179.9 ± 5.4 nm). In vitro release studies unveiled cumulative release percentages of 71.98±6.52% for bakuchiol over a 24-hour period. Ex vivo permeation studies further affirmed heightened retention of bakuchiol within the skin layers compared to control group. Stability assessments indicated that the optimized transfersome remained stable at 5°C for at least 90 days. These findings underscore the remarkable potential of transfersomes in facilitating the delivery of bakuchiol, offering a compelling and viable alternative for hyperpigmentation formulations.

KEYWORDS: Transfersomes; Bakuchiol; Hyperpigmentation; Skin permeation; Topical delivery.

1. INTRODUCTION

Hyperpigmentation is a common issue amongst people of all ages and backgrounds within the field of skincare. Characterized by the darkening of certain areas of the skin due to excess production of melanin, hyperpigmentation can be attributed to various factors, including sun exposure, hormonal fluctuations, and inflammation [1–3]. Over the years, a diverse array of strategies has been employed in the quest to address hyperpigmentation, each with its own set of advantages and limitations. Chemical peels and laser therapies have played pivotal roles in hyperpigmentation treatment regimens. While these methods offer targeted and controlled intervention, they are not without their disadvantages. Chemical peels may entail downtime and carry the risk of post-inflammatory hyperpigmentation, particularly in individuals with darker skin tones [4,5]. Laser therapies, though effective, may pose the risk of post-treatment erythema or even hyperpigmentation in susceptible individuals. Traditional approaches to hyperpigmentation management have predominantly relied on topical agents such as hydroquinone, retinoids, and corticosteroids. These agents have shown efficacy in regulating melanin production and distribution within the skin. However, their application is not without drawbacks, including potential side effects such as skin irritation, photosensitivity, and, in some cases, paradoxical hyperpigmentation. As the pursuit of effective and safe treatments for hyperpigmentation continues, a promising natural alternative has emerged [6–9].

Bakuchiol, derived from the Psoralea corylifolia plant, has garnered significant attention in recent years for its potential to address a wide range of dermatological concerns, including hyperpigmentation [10,11]. Often dubbed as a "natural alternative to retinol," bakuchiol exhibits a unique profile, offering both antioxidant and anti-inflammatory properties without the typical irritative effects associated with traditional retinoids. However, what sets Bakuchiol apart is its unique ability to deliver similar results with a reduced likelihood of irritation and sensitivity commonly associated with retinoids. This distinction positions Bakuchiol as an attractive option for individuals seeking a gentler yet potent solution to hyperpigmentation.
The therapeutic potential of Bakuchiol in hyperpigmentation management lies in its multifaceted approach. Bakuchiol has demonstrated remarkable potential in inhibiting tyrosinase activity, an enzyme crucial in the production of melanin. By modulating this key enzymatic process, Bakuchiol helps restore balance to melanogenesis, effectively lightening hyperpigmented areas. Its antioxidant properties also serve to shield the skin from environmental stressors that can exacerbate hyperpigmentation. One of the most compelling aspects of Bakuchiol's role in hyperpigmentation management is its suitability for a wide range of skin types, including those with sensitive or reactive skin. Its gentle yet effective nature makes it an inclusive option for individuals who may not tolerate more aggressive treatments [14,15].

In recent years, the advent of advanced drug delivery systems has ushered in a new era in hyperpigmentation management. These innovative systems offer distinct advantages over conventional methods [16,17]. By utilizing encapsulation technologies, nanoparticles, and lipid-based carriers, these systems facilitate precise and controlled delivery of drugs to the target site, minimizing potential side effects associated with systemic absorption. One of the primary advantages of these novel drug delivery systems lies in their ability to enhance the bioavailability of therapeutic agents. By encapsulating drugs within specialized carriers, these systems protect them from degradation, ensuring a higher concentration reaches the target site [18–20]. This not only amplifies the therapeutic efficacy but also reduces the likelihood of adverse effects on surrounding tissues [21,22]. Furthermore, the controlled release kinetics offered by these delivery systems allow for sustained, prolonged action. This is particularly advantageous in hyperpigmentation management, where maintaining a steady concentration of drugs is crucial for achieving optimal results. By circumventing the need for frequent applications, these systems enhance patient compliance and convenience [23,24].

Transfersomes, a specialized class of lipid-based vesicles, represent a cutting-edge innovation in drug delivery technology. These vesicles possess the unique ability to deform and squeeze themselves through intercellular gaps in the stratum corneum (SC), the outermost layer of the skin, allowing for enhanced penetration of drugs [19,25]. This remarkable capability addresses one of the primary limitations of conventional topical treatments, which often struggle to bypass the skin's formidable barrier. The utilization of transfersomes in hyperpigmentation treatment holds immense potential for several key reasons. Their phospholipid bilayer structure closely resembles the skin's lipid structure, promoting compatibility and reducing the likelihood of adverse reactions. This makes transfersomes an ideal carrier for drugs intended for dermatological applications [26–28]. Additionally, transfersomes facilitate the encapsulation of hydrophilic and lipophilic compounds alike, expanding the range of therapeutic agents that can be effectively delivered. This versatility empowers healthcare professionals to tailor treatments to individual patient needs, ensuring a more precise and targeted approach to hyperpigmentation management [28,29]. Moreover, transfersomes exhibit a unique ability to adapt their size and shape in response to environmental cues, further enhancing their penetrative capabilities. This dynamic characteristic allows transfersomes to navigate through the intricate topography of the skin, reaching even the deeper layers where melanocytes, the pigment-producing cells, reside. Through their ability to facilitate the precise and controlled delivery of drugs, transfersomes offer the potential for heightened therapeutic efficacy and reduced side effects [30].

The goal of this study was to prepare transfersomes encapsulating bakuchiol for the initial time for the purpose of counteracting hyperpigmentation. Transfersomes were manufactured using the thin-film hydration procedure, and then homogenized with sonication. Physicochemical characterization and stability experiments were conducted with the transfersome vesicles. Ex vivo permeation tests were utilized to assess the permeation capability of the formulations.

2. RESULTS AND DISCUSSION

2.1. Particle size, polydispersity index and zeta potential

Particle size, polydispersity index, and zeta potential values of the transfersome vesicles were assessed and are presented in Table 1. The particle size of transfersomes holds considerable significance in their efficacy for topical administration. Smaller particle sizes generally result in larger surface areas, facilitating better contact with the skin. This increased surface area can enhance the penetration of the active ingredients into the stratum corneum, the outermost layer of the skin, and subsequently improve their penetration into deeper skin layers. Particles below 200 nm are reported to accumulate more in the deeper layers of the skin [31,32]. The size of the transfersomes exhibited a range between 171.3 ± 6.7 nm and 221.4 ± 9.5 nm. The concentration of Span 80 in transfersome formulations can have a notable impact on the resulting vesicle size. In general, elevating the amount of Span 80 typically results in a reduction in the size.
of transfersomes. This phenomenon is primarily because of its capacity to augment the liquidity and flexibility of the lipid bilayers that form the vesicles. When the amount of Span 80 is heightened, it can reduce the surface tension and increase the fluidity of the lipid membranes, allowing for the formation of smaller, more flexible vesicles. A study by El Zaafarany et al. (2010) corroborated these findings, showing that a higher concentration of Span 80 caused smaller transfersome sizes. The authors explained that higher concentrations of Span 80 lead to a more pronounced decrease in transfersome size because of the highest hydrophobicity of edge activator [33].

The zeta potential values, indicative of the surface charge of colloidal systems, ranged from -4.3 ± 0.5 to -8.2 ± 0.9 mV (Table 1). It was assumed that the low zeta potential value of the transfersomes was due to the presence of phosphate and carboxylic groups. The phospholipid of the zwitterion structure which was used in the research had a slightly negative charge when placed in phosphate buffer solution. This is assumed to be a result of the higher concentration of the negatively charged phosphate group in the lipid structure of transfersome, in comparison to the positively charged choline groups [34,35]. Zeta potential is a critical parameter that influences the stability and behavior of colloidal systems. When evaluated together with the stability data, it shows that the zeta potential results are suitable for the physical stability of transfersomes.

The polydispersity index, indicative of the distribution of particle sizes within the transfersome system, ranged from 0.14 to 0.28. These values suggest that the transfersome systems under investigation possess a relatively narrow size distribution and a low propensity for aggregation over time. Maintaining a low polydispersity index is crucial for the long-term stability of transfersome, as high values could lead to aggregation and compromise the efficacy of the delivery system. The observed values align with recommended standards for stable colloidal systems.

Table 1. Characterization of Transfersomes containing Bakuchiol

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Particle Size</th>
<th>Polydispersity Index</th>
<th>Zeta Potential</th>
<th>Encapsulation Efficiency(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF1</td>
<td>196.3 ± 3.5</td>
<td>0.21±0.03</td>
<td>-4.3 ± 0.5</td>
<td>61.6 ± 5.6</td>
</tr>
<tr>
<td>TF2</td>
<td>185.4 ± 8.5</td>
<td>0.28±0.05</td>
<td>-5.1 ± 0.4</td>
<td>78.2 ± 3.6</td>
</tr>
<tr>
<td>TF3</td>
<td>172.2 ± 3.6</td>
<td>0.25±0.04</td>
<td>-4.8 ± 0.7</td>
<td>70.5 ± 3.6</td>
</tr>
<tr>
<td>TF4</td>
<td>221.4 ± 9.5</td>
<td>0.17±0.06</td>
<td>-6.8 ± 0.3</td>
<td>75.8 ± 3.6</td>
</tr>
<tr>
<td>TF5</td>
<td>179.9 ± 5.4</td>
<td>0.14±0.04</td>
<td>-7.5 ± 0.7</td>
<td>86.7 ± 3.6</td>
</tr>
<tr>
<td>TF6</td>
<td>171.3 ± 6.7</td>
<td>0.25±0.11</td>
<td>-8.2 ± 0.9</td>
<td>77.5 ± 3.6</td>
</tr>
</tbody>
</table>

Overall, the characterized physicochemical properties of the transfersome vesicles in this study, including particle size, zeta potential, and polydispersity index, underscore the potential of these formulations for effective drug delivery applications in hyperpigmentation treatment. The controlled manipulation of these parameters offers a promising avenue for tailoring vesicular systems to optimize their therapeutic efficacy and stability.

2.2. Determination of Encapsulation Efficiency

The encapsulation efficiency of bakuchiol within transfersome formulations was assessed in this study. As presented in Table 1, the encapsulation efficiency values for the encapsulation of bakuchiol ranged from 61.6% to 86.7%. The concentration of phospholipid in transfersome formulations can significantly influence the encapsulation efficiency of drug. Generally, higher concentrations of phospholipids tend to lead to increased encapsulation efficiency. This is because the higher amount of phospholipids provides more structural material for the formation of vesicles, resulting in a greater capacity to entrap the bakuchiol. The data for encapsulation efficiency revealed that when the amount of edge activator was upped from 25 to 50 mg, there was an obvious enhancement in encapsulation efficiency. This phenomenon can be attributed to the fact that edge activator create a larger hydrophobic area within the transfersome structure. Consequently, as the concentration of edge activator increases, a greater quantity of bakuchiol can be accommodated within the available space, resulting in enhanced encapsulation efficiency. Nevertheless, when the concentration of edge activator was augmented from 50 to 100 mg, the encapsulation efficiency of the bakuchiol declined. By further increasing the Span 80 ratio in the formulation, the entrapment efficiency was adversely affected. Scientists postulated that reduced encapsulation efficiency observed in the
transfersomes at higher edge activator concentrations may be attributed to the coexistence of mixed micelles with vesicles, or the potential formation of pores within the vesicles. This finding is consistent with earlier studies in the field [33,36].

These results demonstrate the essential importance of formulation composition in influencing the encapsulation efficiency of transfersomes. The judicious selection of edge activator and cholesterol levels allows for the optimization of encapsulation, ensuring that a higher quantity of drug is effectively incorporated into the transfersome structure.

Among the formulations examined, TF5 exhibited the highest encapsulation efficiency values for bakuchiol. Additionally, it demonstrated an optimal particle size. Based on these results, TF5 was identified as the most promising formulation and was selected for further investigations.

2.3. In vitro release study

The dialysis membrane diffusion method was utilized to assess the drug-release characteristics of transfersome vesicles. The release behaviors of Bakuchiol from the transfersome formulation are depicted in Figure 1. The findings indicated an initial rapid release of Bakuchiol, with an approximate release of 44.96±4.51% occurring within the initial two-hour period. Subsequently, a more gradual release phase ensued. It was established that the lipophilic compounds were localized either within the bilayer membrane or on the external and superficial layers of the transfersomes. The initial release event is postulated to be attributed to the detachment of bakuchiol from the surface, while the subsequent release is attributed to the sustained release of the hydrophobic bakuchiol contained within the bilayer membrane and internal layers. After a span of 24 hours, a cumulative release of 71.98±6.52% for Bakuchiol was observed.

Figure 1. In vitro release profile of TF5 formulation

The assessment of drug release kinetics from pharmaceutical formulations through mathematical modeling is crucial for gaining insights into the underlying physical processes governing drug release. Given that the in vitro release profile plateaued at 8 hours, the kinetic mechanism was analyzed based on data collected during the initial 8-hour period. The determination of drug release mechanisms was made by evaluating the high correlation coefficients ($r^2$) derived from applying release kinetic models to the obtained data. As presented in Table 2, the $r^2$ values indicate that the release of Bakuchiol from the transfersome formulation aligns with Higuchi's square root kinetics. The release process is believed to be regulated by the diffusion of the bakuchiol via the lipid, according to this model. It is worth noting that while Higuchi’s model provides valuable insights into the release mechanism, it may not fully capture all the complexities of drug release from transfersomes. Factors such as vesicle size, lipid composition, and the physicochemical features of the encapsulated bakuchiol can affect the release kinetics, potentially leading to deviations from
the idealized Higuchi behavior. The findings of this study corroborate the report that the pentoxifylline was released from transfersomes in agreement with the Higuchi model [37].

**Table 2.** Regression coefficients for the kinetic release models of the optimized transfersome

<table>
<thead>
<tr>
<th>Kinetic Models</th>
<th>Bakuchiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero-order ($r^2$)</td>
<td>0.8369</td>
</tr>
<tr>
<td>First-order ($r^2$)</td>
<td>0.8996</td>
</tr>
<tr>
<td>Hixson–Crowell ($r^2$)</td>
<td>0.8798</td>
</tr>
<tr>
<td>Higuchi ($r^2$)</td>
<td>0.9662</td>
</tr>
</tbody>
</table>

### 2.4. Ex vivo Permeation Studies

The strategy of achieving dermal permeation, a crucial step for localized therapeutic effect, encompasses the release of the drug from the vesicular system, its permeation through the SC and its targeting of the viable epidermis/dermis layers. The permeation efficacy chiefly depends on the physicochemical properties of the drug and the vehicles. The aim of this research was to raise the level of bakuchiol in the epidermal layers and block it from entering the bloodstream. Notably, bakuchiol was not detected in the receptor medium upon the conclusion of the 6-hour permeation experiment. The accumulation of bakuchiol in the skin after 6 hours of application is presented in Figure 2, detailing its distribution within the SC and viable epidermis/dermis layers. The outcomes of ex vivo skin permeation assessments revealed a substantial increase in the permeation of bakuchiol from the prepared transfersomes into both the SC and viable epidermis/dermis layers, in contrast to the performance of the control group ($p<0.05$).

![Figure 2](http://dx.doi.org/10.29228/jrp.711)

**Figure 2.** Bakuchiol amount permeated in SC and viable epidermis/dermis layers (*$p<0.05$*).

The study showed that the transferomes had a retention rate that was 2.79 and 4.62 times more than that of the control group for Bakuchiol in the SC and viable epidermal/dermal layers, respectively. Transfersomes possess a highly deformable lipid bilayer structure. This characteristic allows them to alter their shape in response to pressure gradients, permitting them to pass through the tight intercellular gaps of the SC, which is the outermost layer of the skin. This deformability is a critical factor in their enhanced permeation capabilities. Additionally, the phospholipid components of transfersomes contribute to their increased fluidity compared to conventional dosage forms. This enhanced fluidity allows transfersomes to adapt more readily to the dynamic and ever-changing environment of the skin, facilitating their passage through the lipid-rich SC. When applied to the skin, these polar lipids interact favorably with water, creating an osmotic gradient. As a result, transfersomes are propelled into the skin, particularly into the SC and deeper layers, in an attempt to counteract the partial dehydration caused by water loss through evaporation [38–40].
2.5. Stability Studies

To make sure the stability of the formulated transfersomes, a comprehensive stability study was conducted over a period of 90 days, subjecting the transfersomes to two different storage conditions: 25±2 °C, 60±5% relative humidity and 5±3 °C. The physicochemical properties including particle size, zeta potential, polydispersity index, and physical controls were monitored throughout the study. At 25±2 °C and 60±5% relative humidity, the transfersomes exhibited signs of instability, with noticeable sedimentation leading to an increase in particle size. This observation indicated that this storage condition was not conducive to maintaining the stability of the transfersomes. In contrast, when stored in a refrigerator at 5±3 °C, the transfersomes demonstrated remarkable stability. The particle size, zeta potential, and polydispersity index were monitored over the course of the study. Statistical analysis revealed no significant variations in these parameters (p > 0.05), indicating that the transfersomes remained structurally intact and uniform in size distribution throughout the 90-day period (Figure 3). The stability study demonstrated that the optimized transfersomes maintained their structural integrity and size distribution when stored in a refrigerator at 5±3 °C for a period of 90 days. These results highlight the importance of controlled storage conditions in preserving the stability of transfersomes, ultimately ensuring their effectiveness as a drug delivery system for hyperpigmentation treatment.

![Figure 3. The result of stability studies of optimum transfersome formulation](image)

3. CONCLUSION

This research was the first to create and optimize transfersome systems containing bakuchiol, resulting in high encapsulation efficiency and small particle size. Thin-film hydration was used to prepare the transfersomes, which were subsequently sonicated to homogenized. The particle size, polydispersity index, and zeta potential values of the created transfersome were acceptable for topical administration of the drug. The stability assessment revealed that these transfersomes maintain their integrity over an impressive 90-day period when stored at 4°C, further affirming their potential for practical application. Additionally, the results of the in vitro release and ex vivo permeation studies indicated an extended release of the bakuchiol and improved permeation. Therefore, it can be suggested that the transfersome vesicular system is a beneficial method for achieving extended efficacy and simultaneous administration of bakuchiol. While these findings are highly encouraging, it is imperative to acknowledge that further investigations are warranted to fully unlock the capabilities of this formulation. The results obtained thus far, however, strongly indicate that transfersome-based delivery of bakuchiol holds great promise as a valuable addition to hyperpigmentation skincare regimens.
4. MATERIALS AND METHODS

4.1. Materials

Bakuchiol was procured from Zhongxin Pharmaceutical Co. (purity 98%; Tianjin, China). Phosholipon 90 G, containing 95% phosphatidylcholine derived from soybean, was sourced from Lipoid AG. Span 80 was ensured from Sigma–Aldrich, Inc. All remaining chemicals and reagents employed in this study met the criteria for analytical grade.

4.2. Methods

4.2.1. Preparation of Transfersomes

A vesicular system known as transfersomes, composed of phospholipids and an edge activator, was fabricated utilizing a thin film hydration methodology [41,42]. Phosholipon 90G was used as a phospholipid in this study. Additionally, Span 80 was utilized as an edge activator in the experimental formulation. Phosholipon 90G and Span 80 were accurately weighed and solubilized in a mixture of chloroform:methanol (3:1, v/v) to achieve the desired concentration. The solution was stirred thoroughly until a homogeneous mixture was obtained. The organic solvents were evaporated under reduced pressure at 40°C using a rotary evaporator. This process led to generation of a thin film. The thin film obtained in the previous step was hydrated at 60°C using an aqueous solution. Specifically, PBS served as the aqueous medium for this hydration process. Following hydration, the ready-made dispersion was exposed to sonication with the aid of a probe sonicator at 45% amplitude for 10 minutes. This step was crucial for decreasing the size of the transfersome incorporating bakuchiol. The components used in the transfersome formulations, including Phosholipon 90G, Span 80, and Bakuchiol, were documented and are shown in Table 2. Taking into account the particle size and encapsulation efficiency values, the most suitable transfersome formulation was chosen. The formulation that exhibited the most favorable characteristics was chosen for further evaluation.

<table>
<thead>
<tr>
<th>Code</th>
<th>Bakuchiol (%)</th>
<th>Span 80 (mg)</th>
<th>Phospholipon 90G (mg)</th>
<th>PBS pH 7.4 (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF1</td>
<td>1</td>
<td>25</td>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td>TF2</td>
<td>1</td>
<td>50</td>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td>TF3</td>
<td>1</td>
<td>100</td>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td>TF4</td>
<td>1</td>
<td>25</td>
<td>150</td>
<td>10</td>
</tr>
<tr>
<td>TF5</td>
<td>1</td>
<td>50</td>
<td>150</td>
<td>10</td>
</tr>
<tr>
<td>TF6</td>
<td>1</td>
<td>100</td>
<td>150</td>
<td>10</td>
</tr>
</tbody>
</table>

Abbreviation: PBS: phosphate-buffered saline

4.2.2. Encapsulation efficiency of transfersome vesicles

The encapsulation efficiencies of transfersomes were assessed utilizing the centrifugation technique. Each transfersome formulation was subjected to centrifugation at 20000 g for a duration of 1 hour. This step was crucial for separating the bakuchiol contained within the encapsulation system from that which remained unencapsulated [42]. After centrifugation, the supernatant containing the non-encapsulated bakuchiol within the vesicles and any unbound edge activator was carefully removed. The collected supernatant was then appropriately diluted with acetonitrile. Quantification of the encapsulated drug was conducted through validated reversed-phase high-pressure liquid chromatography (RP-HPLC) technique. This analytical method has been previously established and optimized for accurate and precise determination of the drug content within the transfersome vesicles. The subsequent mathematical expression was employed to compute the values representing encapsulation efficiency:

\[
Encapsulation\ Efficiency(\%) = \frac{\text{the amount of total drug} - \text{the amount of nonencapsulated drug}}{\text{the amount of total drug}} \times 100 \quad (1)
\]
4.2.3. Particle size, polydispersity index and zeta potential analyses

Particle size and polydispersity index of the bakuchiol-loaded transfersomes were determined utilizing a dynamic light scattering method employing the Zetasizer Nano ZSP (Malvern Instruments Ltd, Malvern, UK). For zeta potential determination, the laser Doppler micro-electrophoresis technique, also facilitated by the Zetasizer Nano ZSP (Malvern Instruments Ltd), was employed to measure the charge on the surface of the transfersome vesicles. To mitigate any potential impacts stemming from multiple scattering, the transfersome vesicles underwent a suitable dilution process (at a ratio of 1:50) using high-quality deionized water prior to the analytical assessment. All analyses were performed under controlled conditions, ensuring a consistent and stable ambient temperature. Each of the described analyses, encompassing particle size, polydispersity index, and zeta potential, were conducted in three times to ensure robust and reliable results. This rigorous approach to data collection guarantees the accuracy and reproducibility of the obtained measurements, essential for a comprehensive evaluation of the transfersome formulation.

4.2.4. Analytical method for Bakuchiol determination

The analysis of bakuchiol was conducted utilizing a Shimadzu LC20-AT RP-HPLC instrument which was fitted with a UV-Vis detector (Shimadzu, Kyoto, Japan). Mobile phase compositions were prepared by mixing acetonitrile and ultrapure water (containing 0.1% formic acid) in a ratio of 80:20 (v/v) for bakuchiol. The mobile phase was pre-filtered and degassed prior to use. The GL Sciences InertSustain C18 column, measuring 150 × 4.6 mm with a particle size of 5 µm, was employed for the chromatographic separation. The temperature of the column was kept at 25°C, and the flow rate of the mobile phase was maintained at 1 mL/min. Bakuchiol was identified by the UV detector set at 260 nm.

The validation of the HPLC method for bakuchiol was performed following the guidelines established by the International Conference of Harmonization (ICH) Q2 (R1). Standard curves were generated using a stock solution of bakuchiol spanning concentrations from 0.01 to 10 µg/mL, which were subsequently subjected to linearity assessment. The obtained results demonstrated a robust linear association between the peak area and the concentrations of bakuchiol (r² = 0.9993) within the specified range. Additionally, it was observed that the presence of free transfersomes in the samples did not interfere with the detection of bakuchiol, underscoring the method’s specificity. Limit of Detection (LOD) and Limit of Quantification (LOQ) values were found to be 0.005±0.001 µg/mL and 0.016±0.004 µg/mL, respectively. Further validation confirmed the accuracy, precision, and stability of the RP-HPLC method.

4.2.5. In vitro release study

The in vitro release study was conducted utilizing the dialysis technique, following established protocols [43]. Prior to experimentation, cellulose-based dialysis, which possessed a defined molecular weight cutoff within the range of 12,000 to 14,000 Da, underwent a thorough cleaning process and were subsequently soaked in distilled water to remove any residual preservatives. Transfersomes (1 mL) were carefully introduced into dialysis bags, which were subsequently sealed using standard closures. These prepared dialysis bags were then immersed in a solution consisting of 100 mL of a PBS:Ethanol mixture (1:1 ratio; pH 7.4). The experiment was carried out within a shaking incubator set to a controlled temperature of 32°C±0.5°C, with agitation at 400 rpm. Samples of 0.5 mL each were taken from the receptor phase at specified time points. To maintain sink conditions, an equivalent volume of fresh medium was immediately introduced following each sample withdrawal. The quantification of released bakuchiol was performed using a validated RP-HPLC method.

Subsequently, various mathematical models were employed to assess the kinetic mechanisms governing the release of bakuchiol from the transfersome formulations, as represented in the following equations [44–47]:

Zero-order: \( C = k_0 t + C_0 \)  
First-order: \( \ln C = \ln C_0 + k_1 t \)  
Higuchi model: \( C = k_2 t^{1/2} \)
Hixson–Crowell: \( W_0^{1/3} - W_i^{1/3} = k_1 t \)  

In these mathematical expressions, the symbol ‘C’ denotes the concentration of released bakuchiol at a precise time, \( t \). \( C_0 \) represents the initial concentration of the bakuchiol within the transfersome. The parameters \( k_0 \), \( k_1 \) and \( k_2 \) correspond to the release rate constants for zero-order, first-order, and Higuchi kinetics, respectively. \( W_0 \) signifies the initial quantity of bakuchiol in the transfersome, while \( W_i \) denotes the remaining amount of bakuchiol within the formulation at a given time, \( t \); \( k_1 \) serves as a constant that integrates the surface-to-volume relationship in this context. Finally, the determination of the ideal kinetics governing the release of bakuchiol from the transfersomes was achieved through the analysis of correlation coefficients.

4.2.6. *Ex vivo* skin permeation study

The *ex vivo* skin permeation study was conducted using the tape-stripping technique to assess the impact of topical formulations on the permeation of bakuchiol through the skin [40]. Six-month-old pig ears, sourced from a local slaughterhouse, were employed for this study. The dorsal skin was carefully dissected from the underlying cartilage using a scalpel, and uniform sections were prepared. Prior to experimentation, hair was gently excised from the skin utilizing surgical scissors, ensuring the integrity of the SC. Subcutaneous fat and tissues were meticulously separated from the skin using a scalpel. Pig skin specimens were kept at -20°C until required, after which they were allowed to equilibrate to room temperature. To ensure proper hydration, the skin samples were soaked in phosphate-buffered saline (PBS) solution for 30 minutes prior to the commencement of the experiment. In this research, the experiment involved the utilization of a double-jacketed modified Franz diffusion cell known as the Hanson Vertical Diffusion Cell, which featured a receptor phase volume of 7 ml and a diffusion surface area of 1.77 cm². The receptor phase consisted of an Ethanol 1:1 PBS (pH:7.4) solution, providing a suitable medium for the dissolution and sink conditions of the bakuchiol. The pig skin samples were then carefully positioned at the interface between the donor and receptor compartments, ensuring that the epidermal surface was oriented towards the donor compartment. Optimum transfersome formulation and bakuchiol solution containing equal amounts of drug with transfersome for comparison were applied on the skin. The receptor phase was continuously stirred at 400 rpm using a magnetic stirrer. Throughout the study, the diffusion cells were upheld at a stable temperature of 37 ± 0.5 °C over the course of the investigation. At predetermined intervals, 0.5 ml samples were taken from the receptor phase, subsequently passed through a 0.45 µm syringe filter, and substituted with an equivalent volume of the solvent mixture to uphold consistent volume and temperature conditions. After a 6-hour period, the experiment was concluded.

Following the prescribed sampling duration, skin specimens were carefully extracted from the diffusion cells, rinsed with PBS solution, and dried using cotton wool. Adhesive tape (Corneofix) strips were applied to the skin surface with mild stress, subsequently rolled to remove the SC. This process was repeated 20 times. The tape strips, along with the residual skin specimens, were placed in acetonitrile for a duration of 24 hours, agitated, subjected to agitation, and subsequently underwent sonication and centrifugation to facilitate extraction. The resulting extract aliquots were subjected to analysis using the validated HPLC method to quantify the bakuchiol content.

4.2.7. Stability studies of transfersomes

The stability of the optimized transfersome formulations was assessed under controlled environmental conditions. The formulations were stored in stability cabins set at two different temperature conditions: 25±2°C, 60±5% relative humidity and 5±3°C conditions. This storage environment was chosen to mimic a range of conditions that the transfersomes might encounter during storage and transport. The stability study was carried out over a duration of 90 days. During this period, samples were periodically withdrawn for analysis. The purpose of these analyses was to assess the stability of the transfersomes and any changes in their physical and chemical characteristics over time. To ensure the reliability and accuracy of the data obtained, the measurements were conducted at multiple time points.

4.2.8. Statistical Analysis

The trials were conducted three times, and the ensuing data were presented as mean values accompanied by the standard deviation. Statistical assessments were performed employing a one-way
analysis of variance (ANOVA) in conjunction with Tukey's post hoc test. A significance threshold of p<0.05 was employed to ascertain statistical significance. This rigorous approach ensured robust and reliable statistical evaluation of the obtained results.


**Conflict of interest statement:** The author declares no conflict of interest.

**REFERENCES**


[21] Roberts MS, Cheruvu HS, Mangion SE, Alinaghi A, Benson HAE, Mohammed Y, Holmes A, van der Hoej J, ...


