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Comparison of Caffeine (CAF) Penetration Using PLGA Nanoparticle and Aqueous Formulations: An In Vitro Study with Franz Diffusion Cell

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ABSTRACT

Caffeine (CAF) is a non-selective adenosine A1 receptor antagonist which predominates in fat cells. When CAF binds to adenosine receptors, it increases cyclic adenosine monophosphate; inhibiting adipogenesis and inducing fat lipolysis. This study aimed to evaluate using the method of Franz diffusion cells, the caffeine (CAF) releasing profiles of two forms: A nanoparticle PLGA and a pure water for injection. Drug release analysis was carried out under physiological conditions (pH: 5.6 to 7.4; ionic strength 0.15 M; at 25 °C) for 8 h. One independent vertical Franz cells were used with a nominal volume of the acceptor compartment of 12.0 mL and a diffusion area of 1.77 cm2. A transdermal simulation type (Strat-M®) type membranes is used. The CAF permeation profiles demonstrated on the membrane type and the vehicle used, the permeation is strongly affected. High permeation efficiencies were obtained for the CAF nanoparticle form, and low effect was observed for CAF water for injection formulation. The permeation studies membranes represent a reproducible method, which is easy to implement for pre-formulation stage or performance evaluation of pharmaceutical products for topical purposed administration.

KEYWORDS: Caffeine, Permeation Assay, Franz Cells, Nanoparticle Systems, Transdermal.

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INTRODUCTION

Caffeine (CAF) is a methylxanthine alkaloid, which is chemically known as 1, 3, 7-trimethylpurine-2, and 6-dione. It is mainly found in coffee, tea, and many drinks for energy drink and performance (1-2). Incorporation of CAF in various topical cosmetic products was found to prevent excessive fat accumulation in the skin manage gynoid lipodystrophy or cellulite. It was reported that CAF causes fat lipolysis and manages cellulite by inhibiting phosphodiesterase enzyme activity (3). It is well known that the skin permeation of drugs mainly relies on their physicochemical properties (e.g., lipophilicity, solubility, and molecular weight). Formulation studies are essential for drug effectiveness reliability, both in approved drugs and new pharmaceutical formulations (4). The development of innovative pharmaceutical products worldwide is valuable and the suitable methods need to be established to evaluate the fundamental parameters such as permease and required in pre-formulation studies. Simple methods must be implemented for determining the main permeating parameters including solubility, partition coefficient and dissolution profiles. In the case of topical formulations, for which the drug is released through the skin, evaluation of the permeation is critical to establish bioavailability and thereby make an approximation of the effectiveness (5). Additionally, nanoparticle dosage forms aimed for topical or transdermal application, methodologies to test and verify the performance in product pre-formulation stages are essential to avoid

The development of effective drug delivery systems is a critical aspect of pharmaceutical research, particularly for compounds intended for topical or transdermal administration. Caffeine (CAF), a well-known bioactive compound with diverse therapeutic applications (6- 7), presents challenges in achieving optimal delivery due to its physicochemical properties (8). To address these challenges, advanced formulations such as nanoparticle-based systems have been explored for enhancing drug release and permeation (9-10).

The Franz diffusion cell method is widely recognized as a reliable in vitro technique for evaluating drug release and permeation profiles. This system enables the simulation of physiological conditions to assess the performance of various pharmaceutical formulations (11). In this study, the Franz diffusion cell method was utilized to compare the permeation profiles of CAF in two formulations: a nanoparticle-based PLGA system and pure water for injection.

METHOD

Franz Diffusion Cell Method

A comparative test of caffeine penetration between a PLGA nanoparticle-based formulation and a water-for-injection-based

formulation was conducted using a Strat-M membrane in a Franz diffusion cell. The diffusion area was 1.77 cm^2, and the receptor compartment volume was 12.0 mL. Phosphate buffer solution at pH 7.4 was used in the receptor compartment, maintained at a temperature of 25 ± 0.5 °C.

Each sample (1 g) was weighed and applied to the surface of the Strat-M membrane in the donor compartment. From the receptor compartment, 1 mL of sample was periodically withdrawn at specific intervals over 8 hours (0.5, 1, 2, 4, 6, and 8 hours) using a 1 cc syringe. The withdrawn volume was replaced with an equal amount of phosphate buffer solution (pH 7.4) modified from Salamanca et al. (12).

The collected samples were homogenized and transferred to a 10 mL volumetric flask, where 3 mL of methanol was added. The solution was diluted to the mark with methanol, forming an extract solution. Subsequently, 2 mL of this extract was pipetted and diluted to 50 mL with methanol in another volumetric flask. A 20 µL volume of the resulting solution was injected for analysis. The analysis was performed using HPLC equipped with a photodiode array (PDA) detector, a reversed-phase C18 column, and an isocratic pump system. The mobile phase consisted of methanol and distilled water (35:65) at a flow rate of 0.75 mL/min. The injection volume was 20 µL, the column temperature was maintained at 35°C, and the detection wavelength was set to 275 nm. The caffeine concentration in the PLGA nanoparticle-based formulation was compared to that in the water-for-injection formulation, and the flux and cumulative penetration were calculated.

Releasing efficiency was defined in terms of the mass flux (*J*), which describes the change of drug permeation with respect to time in aqueous systems. In our study, the mass flux ($mol \cdot cm^{-2} \cdot h^{-1}$) was determined using the AUC of the permeation profile recorded at a specific time interval and is related to the rectangular area (*R*) described by 100% of the permeation process at the same time interval modified from (24 h) to (8 h) Salamanca, 2018. Mass flux can be calculated from Flux (J)=$\int t0y$ dty100t×100%

RESULTS

The drug release profiles of caffeine (CAF) from two different formulations, PLGA nanoparticle-based and water for injection (WFI), were assessed using the Franz diffusion cell method. This method provides a controlled environment for evaluating transdermal permeation over a set period. Table 1. Showed parameters evaluated during the standardisation of the methodology to quantify levels of caffeine (CAF) through UV-Visible spectrometry.

Table 1. Dosage form and Linier Equations of PLGA-CAF and CAF Water for Injection

Dosage Form	Linearity	
	Linier Equations	R^2
PLGA-CAF	Y= 1.0964x – 0.0086	R^2 = 0.6626
CAF Water for Injection	Y= 0.2284x -1.2176	R^2 = 0.7584
Caffeine (CAF Standard)	Y=0.7951x – 2.7894	R^2= 0.9998

Table 2 presents the results for the PLGA nanoparticle formulation treated, showing the lag-time, flux value, and cumulative release after 8 hours.

Table 2: Caffeine (CAF) Release Profile from PLGA Nanoparticle Formulation

Permeation Parameters	Formula		Result
Lag-Time (TI) (h)	AUC/(J)	2.7894/4.4108	5.27 Hours
Mass Flux (J)			4.4108 mcg/cm^2/h
Cumulative Amount at 8 Hours			12.02 mcg/cm^2

Table 3 provides the same parameters for the CAF water for injection formulation, showing the lag-time, flux value, and cumulative release after 8 hours.

Table 3: Caffeine (CAF) Release Profile from Water for Injection Formulation

Permeation Parameters	Formula		Result
Lag-Time (TI) (h)	AUC/ (J)	2.7894/0.7951	3.50 Hours

Permeation Parameters	Formula	Result
Mass Flux (J)		0.7951 mcg/cm²/h
Cumulative Amount at 8 Hours		3.57 mcg/cm²

In the Figure 1 and Figure 2 showed graph curve the results of the in vitro permeation profiles of PLGA nanoparticle formulation and provides the results of the in vitro permeation profiles of CAF water for injection .

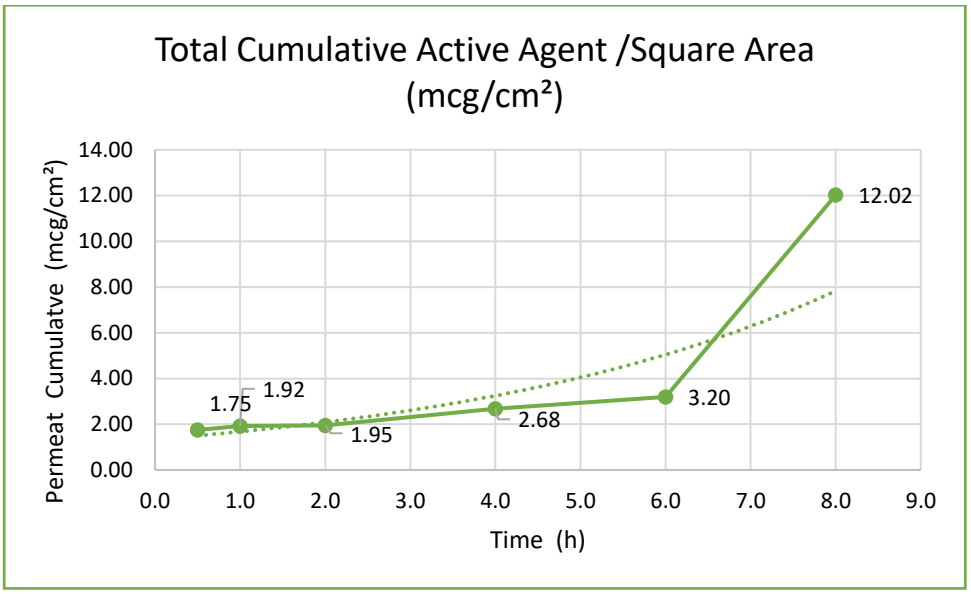

Figure 1. Curve Permeat Cumulative of PLGA nanoparticle formulation at 1,2,4,6 and 8 hours examination

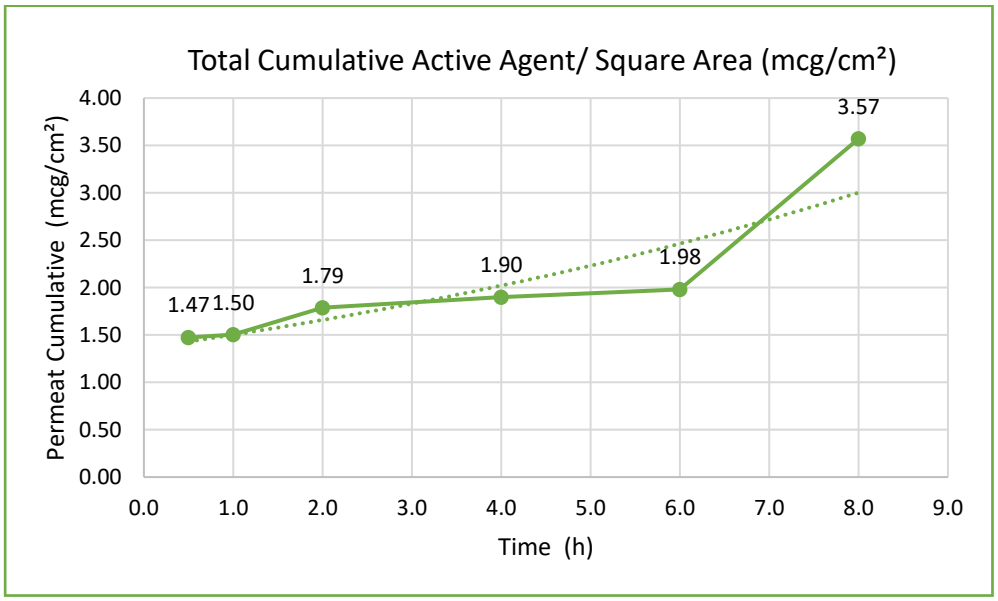

Figure 2.Curve Permeat Cumulative of the CAF water for injection formulation at 1, 2, 4, 6, 8 of hours examination .

DISCUSSION

This study aimed to compare the release profiles of caffeine (CAF) from two different formulations—PLGA nanoparticle-based and pure water for injection—using the Franz diffusion cell method. The results highlighted significant differences in the permeation efficiencies of the two formulations, with the PLGA nanoparticle formulation exhibiting superior release rates. The permeation profiles show a marked dependence on the type of dosage matrix used formulation (Figure 1 and 2) at 1, 2, 4, 6, and 8 hours examination differ from Salamanca et al., (12) that used until 24 hours examination triplicates.

The flux values obtained for the PLGA nanoparticle formulation (4.41 mcg/cm²/h) were notably higher than those for the pure

water for injection formulation (0.79 mcg/cm²/h). This is in line with several studies that have demonstrated the enhanced permeation capabilities of nanoparticle systems over conventional drug delivery forms (13). For instance, there was reported that nanoparticle formulations, due to their small size and increased surface area, enhance drug penetration through the skin, providing better therapeutic outcomes in transdermal drug delivery (14-15). Similarly, Zhang et al. (9) found that the use of PLGA nanoparticles significantly improved the bioavailability of poorly permeable drugs, highlighting their potential in controlled-release applications (16)

The cumulative release detail (Figure 1) after 8 hours was also significantly higher for the PLGA nanoparticle formulation (12.02 mcg/cm²) compared to the water for injection formulation (3.57 mcg/cm²) detail in Figure 2. This difference can be attributed to the formulation characteristics, with nanoparticles being able to overcome barriers in the skin or other biological membranes more efficiently than conventional drug forms. This observation supports findings from Sharma et al. (17), who concluded that the use of polymeric nanoparticles could facilitate higher drug permeation and provide a more controlled release profile for various drug molecules.

The lag time (TI) values indicated that the time required for the nanoparticle formulation to reach maximum flux was faster (5.27 hours) than for the pure water for injection formulation (3.50 hours). This suggests that PLGA nanoparticles provide more rapid release, which could be beneficial for certain therapeutic applications where fast action is required. A similar trend was observed by Niu et al. (18), who noted that nanoparticles, owing to their structure, facilitate quicker drug diffusion across membranes. Interestingly, although the PLGA nanoparticle formulation exhibited a longer lag time, it also showed a markedly higher flux value. This apparent paradox may be attributed to the initial hydration and swelling of the polymeric matrix, which delays the onset of drug permeation but subsequently enhances diffusion once equilibrium is achieved. The gradual polymer hydration allows sustained drug release and the formation of a diffusion pathway, resulting in higher overall flux despite the longer lag phase.

There were highlighting the differences in permeation efficiency between the two formulations. These results offer insights into the comparative performance of each formulation in terms of flux and cumulative release. The differences observed between the two formulations suggest that PLGA nanoparticles could be an effective strategy for enhancing the transdermal delivery of caffeine, as demonstrated by their higher flux and cumulative release values (19-20). These results are consistent with the findings of other studies that have emphasized the potential of nanoparticles to improve drug delivery efficiency and the overall effectiveness of therapeutic treatments (21-23).

The findings of this study are promising for the use of PLGA nanoparticle formulations in transdermal drug delivery. However, further research is required to explore the long-term stability, potential toxicity, and clinical efficacy of these systems in more complex models. Additionally, exploring various formulations of PLGA nanoparticles and their effects on skin penetration and drug absorption across different membrane types could provide further insights into optimizing their performance for drug delivery applications.

Research Limitations

This study has several limitations that should be acknowledged. Firstly, the experimental procedure was conducted using a single measurement without replication, which may limit the reliability and reproducibility of the results. Multiple trials are typically required to account for experimental variability and to strengthen the statistical validity of the findings. Another limitation of this study is the absence of particle characterization data, such as particle size distribution, polydispersity index (PDI), and zeta potential we did not show in this article. Future studies should include comprehensive nanoparticle characterization to better elucidate the relationship between formulation parameters and drug release kinetics.

CONCLUSION

This study demonstrated that the PLGA nanoparticle-based caffeine formulation exhibited significantly higher transdermal permeation compared to the pure water for injection formulation, as evidenced by the higher flux and cumulative release observed over the 8-hour period. The enhanced drug release profile of the PLGA nanoparticle formulation can be attributed to the unique characteristics of nanoparticles, such as their small size, large surface area, and ability to facilitate drug penetration through biological membranes. These results support the potential of PLGA nanoparticles as an effective and efficient.

Conflict of Interest: Authors declare there is not any conflict of interest.

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