Original Research Article

Transdermal Delivery of L-Arginine in lipophilic formulations: A novel approach for muscle protein synthesis.

ABSTRACT

Aims: To develop and evaluate a lipophilic emulsion gel for transdermal delivery of L-Arginine, enabling effective, non-invasive post-exercise muscle protein synthesis while reducing renal risks associated with high dietary protein intake.

Study Design: This experimental study focused on developing and evaluating a lipophilic emulsion gel for the transdermal delivery of L-Arginine. Franz Diffusion Cell experiments were conducted to assess and compare the permeability of L-Arginine under controlled conditions.

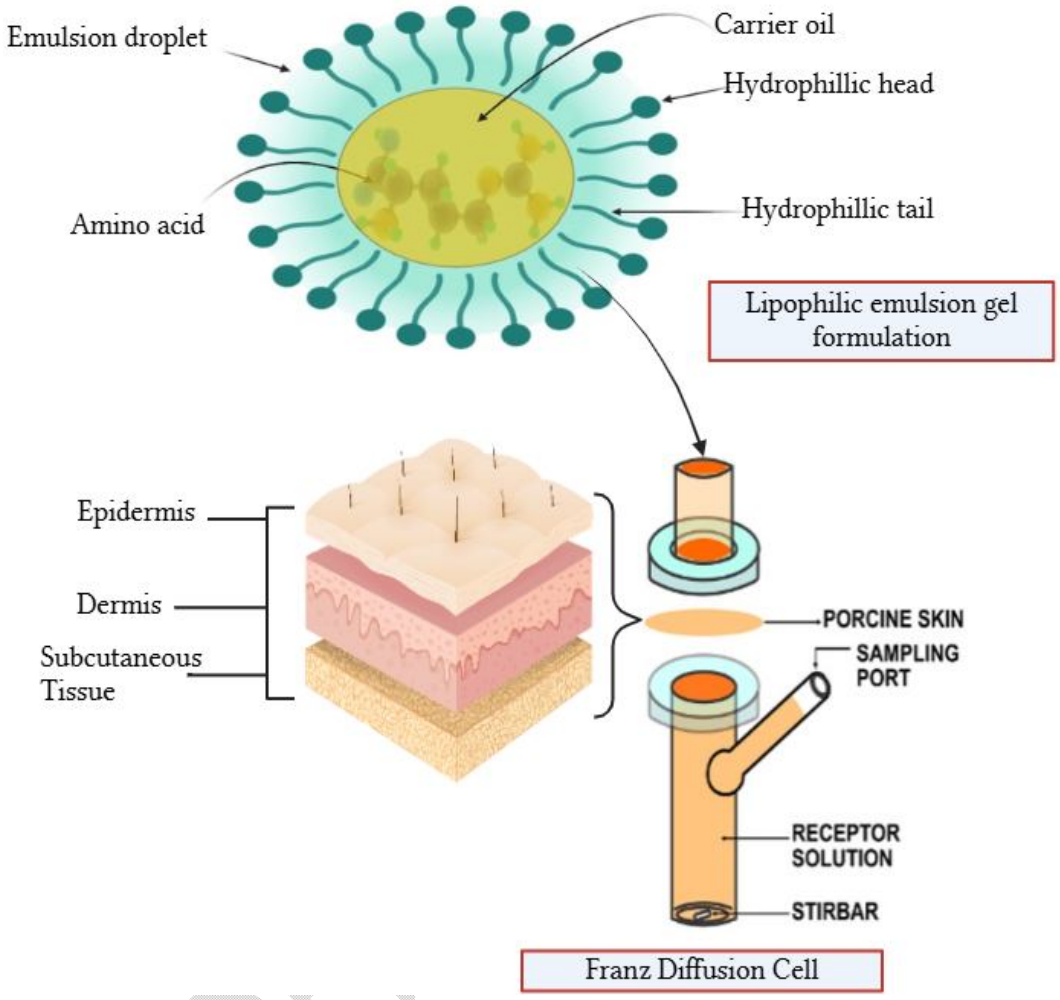
Place of Study: Department of Chemistry and Forensic Science, School of Science and Technology, Nottingham Trent University, United Kingdom, between May 2014 and December 2014.

Methodology: The study employed a two-phase emulsion system consisting of a gel phase and an emulsion phase to develop lipophilic formulations of L-Arginine. Porcine skin was used as the membrane for Franz Diffusion Cell experiments to evaluate transdermal delivery efficiency. TEM imaging was used to analyze droplet size and morphology. LC-MS quantified the permeation of L-Arginine and calculated parameters such as flux and permeability coefficient to evaluate formulation efficacy.

Results: The results demonstrated that L-Arginine successfully permeated porcine skin, with formulations containing an emulsion phase showing a higher permeation rate (77.77%) compared to formulations without it (30.66%). TEM imaging revealed spherical droplets within the nanoemulsion range, enhancing stability and absorption.

Conclusion: This study revealed that L-Arginine successfully permeated, indicating that topical amino acid delivery could be a viable method to bypass first-pass metabolism and avoid renal overload, aiding in faster muscle recovery. Further studies should discuss how this approach might influence mTOR signaling pathways and further support its value as a therapeutic option.

Key Words: Transdermal delivery; L-Arginine; Franz Diffusion Cell; LCMS Analysis; mTOR signaling; Renal failure; Non-invasive.



1. INTRODUCTION

Topical products range between conventional topical pharmaceuticals and cosmetic products termed Cosmeceuticals, which are classified into creams, ointments, gels, lotions, sprays, transdermal patches, and powders, based on their physical state acting as vehicles for the delivery of APIs into the body.[1,2]. Research on the efficacy of Topical Drug Delivery Systems (TDDS) has attracted serious attention, resulting in a bloom in the pharmaceutical industries as researchers are beginning to investigate new formulations and techniques that can effectively deliver trending topological therapeutics. Effective absorption, the rate of absorption, and permeation efficiency of APIs through the SC to other layers of the skin to exert its therapeutic effect are crucial factors to be considered for the potency of any topical formulation, resulting in little or no adverse effect.[3,4]

There has been a remarkable improvement in understanding the properties of topical formulations aimed at producing more stable products for effective permeation to deliver the required dose of APIs.[5]. However, early research has reported remarkable results on the effectiveness of topological formulation across the globe [6,7,8] and recent research still aims to improve more on the permeation of APIs for the treatment of various ailments or skin maintenance in the cosmetic industries because is key for the viability and sustainability of topical drug delivery systems.[9]

Emphasizing dermal and transdermal drug delivery, in this present research, the efficiency of emulsion gel formulations as a lipophilic drug delivery system was studied. The term lipophilic means it is oil-loving or has an affinity for oil, fats, or lipids. A drug is lipophilic when it has a good affinity for a lipid environment. Lipophilicity has presently become a very relevant drug property because it facilitates the uptake of APIs as well as its metabolism. A lot of lipid-based formulations have been developed and studies have revealed that emulsion gels are excellent lipophilic drug delivery systems, as APIs are easily absorbed into the lipids on emulsification.[10] The SC is a formidable force to consider especially for lipophilic drugs, however, literature suggests that oil/water emulsions are effective carrier systems due to their solubility in a lipid phase,[11,12] aiding the permeation through the transcellular route.[13]

The dual function of the emulsion and the cross-linked structures of the gel formed promotes controlled drug delivery of APIs as reported by Stanos and co-workers in 2007, in his study of the use of topical analgesics in the treatment of musculoskeletal pain.[14] Michel Steiger also shared the same view in his patent report on the topical pharmaceutical formulation of diclofenac sodium salt.[15] More studies also reveal that emulsion gels have an effective controlled drug release system due to the synergism of the gel phase and emulsion phase,[15,17,18,19] and are used as topical forms for the delivery of APIs in different dermatological and cosmetic products.[15]

There has been an expanded series of research on amino acids in various aspects of medicinal science exploring their antioxidant properties, collagen synthesis, skin cancer treatments, antiaging, *etc.*[20,21] and are generally safe in many medicinal applications. However, this present study proposes L-Arginine emulsion gels as topical therapeutic formulations for post-workout recovery and muscle protein synthesis.

AAs are known to activate the mToR causing muscle growth and muscle protein synthesis. [22,23,24,25] Studies by two different research groups, Fluckey, and his colleagues in 2006 and Dreyer and his team in 2006, showed that a decrease in the 4E-BP1 phosphorylation during intense exercise was unchanged during post-exercise recovery and increased after ingestion of AA supplement or diet. [26,27] This report was further validated after more investigations two years later by Dreyer and his team on the use of L-leucine-rich supplements for the activation of mTOR signaling after resistant exercise.[26] Drummond and his researchers in

2008 also shared the same views after their evaluation and comparative studies of mTOR signaling and phosphorylation in both young and old people after resistance exercise and intake of a high protein diet.[28]

MPS has emerged as a promising area in pharmaceutical formulations as it addresses weight loss, anti-aging, and anti-inflammatory, especially in chiropractic and sports health care. [29,30,31] Several research have reported an anabolic response of skeletal muscles to AAs, which has resulted in an advocacy to incorporate more protein-rich foods in diets,[32,30] and in line with the emphases, a lot of protein-rich diets and AAs post-workout supplements have emerged. Earlier research by Philip and his team in 1997 and 1999 revealed that intense exercise causes muscle breakdown as well as muscle synthesis, his reports also showed that the level of muscle breakdown after post-exercise is higher than muscle synthesis.[33,34] This effect can be equalized by ingesting AA supplements staying on a restrictive protein diet or immediately after workouts to activate MPS, which is aimed at the recovery process to avoid damage to the muscles.[35,36,37,38] The impact of high protein intake has been extensively investigated and the reports revealed changes in kidney health and normal renal function.[39] Regrettably, evidence from past and present research has revealed a correlation between renal failure and excessive loss of muscle mass due to excessive intake of protein-rich diet.[40,41,42] Other serious illnesses such as uremia and other catabolic diseases have been reported due to amino acid oxidation and degradation of proteins.[40,43,44]

Despite the giant strides by researchers in studies relating to muscle protein synthesis, there are no emphases on the need for a rethink on the development and investigation of new methods and technologies to achieve mToR signaling without causing any adverse side effects. Over the years, research focus has been on dietary and nutritional considerations to maximize post-exercise anabolism, but there is no propriety literature on topical delivery and permeation studies of amino acids for safe and effective post-workout therapy. Considering these limitations and drawbacks, topical formulations such as the emulsion gel presented in this have an advantage over post-workout supplements or protein powders because it is a target delivery system that bypasses first-pass metabolism and is easy to apply.

This study reports the use of emulsion gel as a lipophilic drug device, for the dermal delivery of amino acids. Lipid excipients comprising carrier oil (Almond oil) and essential oils (ginger oil and moringa oil) were used to create an emulsion of an amino acid. The presence of the phenolic compounds that are widespread in plant oils contributes to the anti-inflammatory and antioxidant properties which makes them an excellent emollient, which can also help to improve the bioavailability and uptake of the drugs in topical formulations.[45] Non-ionic surfactant; Polysorbate 80 and solubilizer; Propylene glycol was also employed to stabilize the emulsion and enhance the permeability of the topical formulation.

Emulsion gel formulations enriched with AAs presented in this research could be adopted as an alternative to the conventional ingestion of protein-rich food for post-workout recovery and MPS, which are needed for the repair, growth, and solidification of muscle fibers after intense exercise. This topical approach, which is non-invasive and convenient to use, can improve on existing methods, thus, reducing the possible negative health effects such as renal disorders, uremia, and other catabolic diseases because of excess protein intake after intense exercise.

2. METHODOLOGY

All the chemicals, reagents, and amino acids used in this research were of analytical grade and purchased from Sigma Aldrich. They were used without any form of purification.

Topical lipophilic amino acid formulations were produced as a two-phase emulsion system; the gel phase (Phase A) and the emulsion phase (Phase B) by direct dispersion method with a high-speed homogenizer, following gelation and neutralization with Triethanolamine (TEA). [45] in a 100.00 mL scale.

The emulsion phase comprised of a carrier oil, essential oil, and other excipients; Tocopherol as a preservative and an antioxidant, Glycerin solubilized with an emulsifier Polysorbate 80 and Propylene glycol after the addition of the amino acids. The gel phase was prepared with Poly (acrylic acid) polymer, Carbomer 940, and Tripple-filtered Ultra High-Quality Water (UHQW) at 18.2MΩ. Carbomer 940 acting as the gelling agent was used to improve the consistency of topical formulations. The resulting emulsion in phase B was combined with the previously prepared gel phase A with a homogenizer with continuous stirring at 1.698 x 1000 rpm. The two phases; A and B were completely mixed after 1 minute and an off-white-coloured emulsion gel was formed. Finally, for pH adjustment and neutralization of the resulting formulation, TEA was added dropwise until the desired pH and emulsion gel consistency were achieved.

The pH of the respective emulsion gel samples produced were taken with a pH meter HANNAH Instruments H1 2211 pH/ORP Meter after calibration and then stored in the laboratory refrigerator Lec+ at 4°C before analysis and other property determinations. Transmission Electron Microscope (TEM 2100 Plus Germany) was used to observe the size and shape of the amino acid complex formed in the emulsion phase. Image J Software was used to determine the size of the emulsion droplets encapsulating the amino acid dispersed in the emulsion phase.

To study the permeability of the amino acids in the emulsion gel formulations, a Franz Diffusion Cell (FDC) with a diffusion area of 1.56cm^2 and 7.20 mL receptor capacity was employed using porcine skin as the cell membrane. During the diffusion experiment, 1.00mL

aliquots were taken at time intervals into LC vials and further analyzed with LC-MS (Acquity Xevo G2-XS QToF) to quantify the concentration of amino acids that crossed the SC.

2.1 PERMEATION STUDIES

The FDC is used for studying the permeation and in vitro drug release in different types of pharmaceutical topical formulations. The FDC is composed of two compartments: the donor and the receptor compartment separated by a membrane. The test sample which is the topical formulation is applied to the membrane through the donor compartment and from the receptor compartment, test fluids are taken at regular intervals to quantify the API that has diffused into the receptor solution.

Porcine skins were used as the membrane to evaluate the permeation of the AA present in the respective topical formulation. The porcine skin was cut into square sections measuring 4.000 x 3.000 cm and 0.500 cm thick and stored in the freezer. Before the experiment, frozen porcine skin was defrosted in 0.0005M phosphate buffer solution, pH 7.400. The skin was placed in between the donor compartment and the receptor compartment of the FDC with a diffusion area of 1.560 cm2 and 7.200 mL receptor capacity. 1.000 mL of the gel sample was placed in the donor compartment and sealed with parafilm. The receptor compartment of the FDC was filled with phosphate buffer solution, used as the receptor solution, and then sealed with parafilm.

The permeation study of L-Arginine in the respective topical formulations was done under physiological conditions at 37°C ± 1 between 4-48 hours respectively under magnetic stirring with a stir-hot plate at 800 rpm. During the diffusion experiment, 1.00 mL aliquots were taken at time intervals from the receptor compartment with a syringe needle and replaced with the receptor solution immediately to maintain sink conditions which is aimed at enabling a steady-state diffusion of the amino acid in the sample placed in the donor compartment and maintain a standard volume. The samples taken were further analyzed with LC-MS (Acquity Xevo G2-XS QToF) to quantify the concentration of the amino acid that has crossed the porcine skin per time.

2.2 SAMPLE PREPARATION

1.00 mL of the respective Emulgel formulation was added to 20.00 mL centrifuge tubes and labeled; and to the respective sample tubes, 5.00 mL methanol was added. Each sample tube containing the analyte and methanol was mixed with a whirling mixer for 3 minutes until they were completely mixed and dissolved and further sonicated with an Ultra wave water bath for 10 minutes in water at 40°C. 1.000 mL was taken from the sample tube and placed in LC Vial for LC-MS Analysis. Serial dilutions of amino acid standard solution were also

prepared from 10.000 mg/mL stock solutions of the respective amino acids and analyzed with the LCMS. The retention time, chromatograms, and elemental composition of the AAs were noted before the analysis of the samples taken during the FDC experiment.

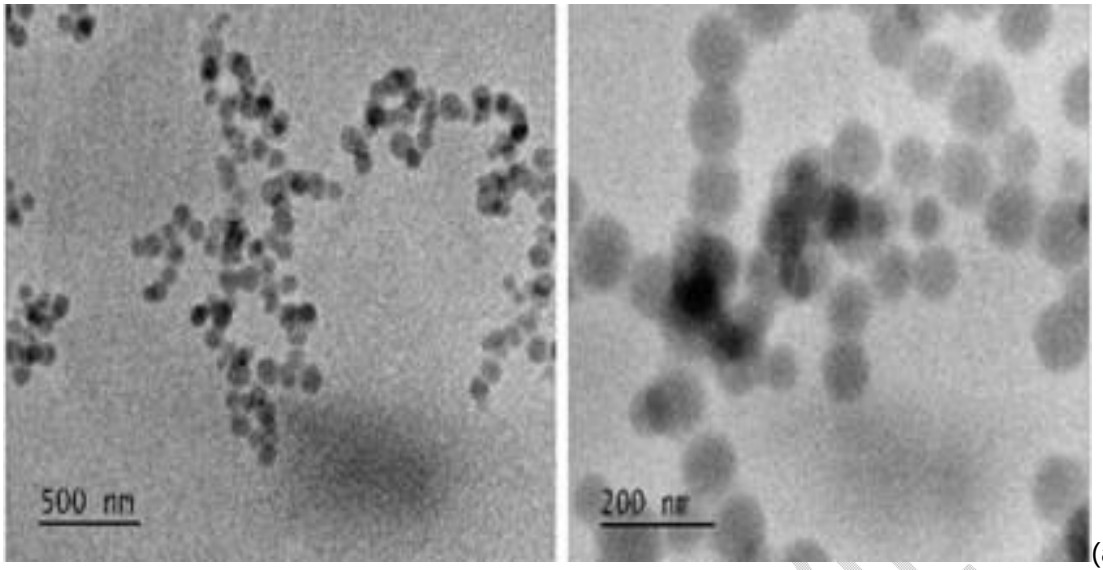
2.3 LC-MS ANALYSIS: DRUG CONTENT DETERMINATION, CALIBRATION STANDARD SOLUTIONS, AND PERMEATION TEST SAMPLES.

The LC-MS combines the ability of both the liquid chromatography (LC) and the mass spectrometer (MS). The LC carries out the physical separation based on their size, polarity, or charge, while the MS carries out the mass analysis of the analyte of interest by identifying and quantifying the molecules according to their mass-to-charge ratio. The LC-MS was used to identify the peaks of the respective amino acids in the topical formulations. The sensitivity of the instrument to the amino acids with the working method was also assessed for consistency in terms of retention time and peak area of the chromatograms, as well as the elemental composition presented by the mass spectrophotometer. Column Raptor C182.7 µL 100 × 2.1mm, was employed, and the inlet method: Mobile phase A: 10.00% MeOH in water 0.10 % Formic acid; Mobile phase B: 0.10% Formic acid with Flow rate: 0.30ml/minute; Elution: Gradient for 15 minutes.

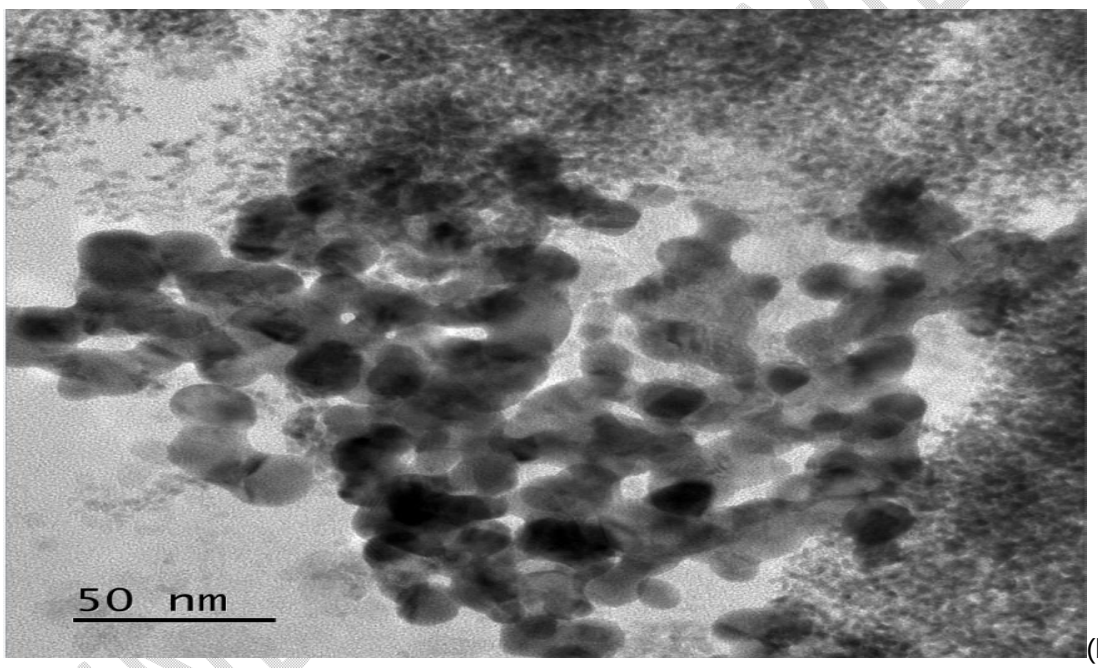
3. RESULTS AND DISCUSSION

3.1 CARBOMER 940 GEL BASE FOR TOPICAL FORMULATIONS

Cosmetic-grade Polyacrylic acid with the trade name Carbomer 940, which is a crosslinked acidic polyacrylic polymer was used as the gel base for the amino acid lipophilic topical formulations. Carbomer 940 readily absorbs water, becomes hydrated, swells, and is very effective for the slow and systemic release of active ingredients[46] It contains a large amount of carboxylic acid (Fig. 2) which makes it an un-neutralized acidic dispersion, hence to obtain the desired viscosity and pH of the final topical formulation, a neutralizer such as TEA or Sodium Hydroxide (NaOH) can be added to the carbomer dispersion to convert the acidic carbomer into a salt.

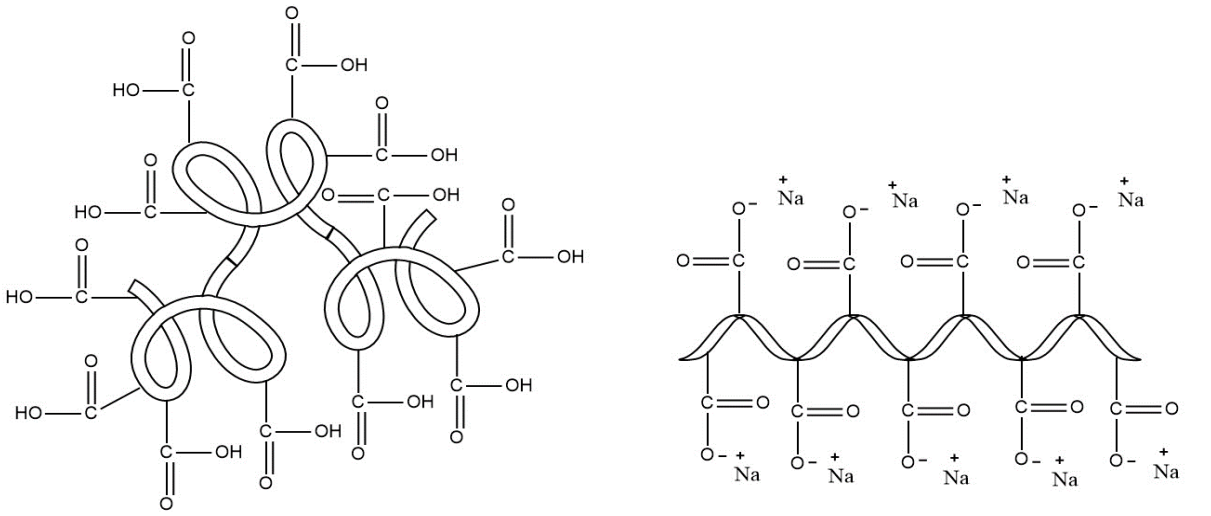


(a)



(b)

Figure 1(a-b): TEM imaging of emulsion gel formulation; Emulsion droplets of Almond and Ginger oil with 0.1 mg/mL L-Arginine with 0.200% C.940 gel base at 500 and 200nm – Phase A (a); Emulsion gel network formed after combining emulsion phase and gel phase at 50nm – Phase B (b)



A. Coilled state of Polyacrylic acid (Cabomer 940)

B. Uncoilled state of Polyacrylic acid (Cabomer 940)

Fig. 2. Unfolding of Poly (acrylic acid) polymer chains before (a) and after (b) neutralization with TEA

The surface morphology of the respective AA Emulsion gels prepared was also investigated by Scanning Electron Microscopy (SEM). Figures 3a and b show the SEM imaging of 0.10 mg/mL L-Arginine Emulsion gel formulation with varying concentrations of Carbomer 940: 0.20 and 1.00 % respectively at 10.00 µm magnification. The SEM imaging of the formulations was done after gelation and emulsification with Polysorbate 80 following neutralization with TEA. The SEM Images of the different formulations showed complete neutralization, but a different kind of gel network. The 0.20 % Carbomer 940 formulation showed a regular thin gel network compared to the 1.00 % Carbomer 940 formulation which appeared to have a bulky gel structure with high porosity. The difference in the gel network is the effect of an increase in the Carbomer 940 concentration which results in a decrease in pH resulting in a bulkier formulation as the gel network unfolds. As seen in the morphological differences, an increase in pH resulted in low viscosity of the gel which affects the structural network of the formulation.

Table 1 and 2 shows the Image J analysis of the TEM images of the Emulsion droplets presented in Figure 1. The mean and standard deviations were calculated based on the magnification of the images which gave the size of the spherical emulsions as presented as the mean length in the respective formulations.

3.2 TEM and Image J Data Analysis of Emulsion Droplets.

TEM was also used to investigate the morphology and particle size of the emulsion droplets acting as carriers of the amino acid (L-Arginine). The TEM images of the emulsion gel formulations at 50 -200 µm magnification presented in Figures 1a and b show well-defined spherical shape structures of the emulsion droplets encapsulating the amino acid. A close interface of the emulsion droplets was also observed, as they were closely linked together and well embedded in the Carbomer 940 gel network as shown in Figure 3. However, the shapes and sizes of the emulsion droplets were not completely uniform. The irregular size and shape of the emulsions were also confirmed on analysis with the Image J analytical software, to determine the size of the emulsion droplets which is presented as the Mean Length of Phase A and Phase B (Tables 1 and 2).

The data shows the size range of the emulsion droplet in Phase A (Oil Phase and L-Arginine) between 47.529 ± 18.68 nm to 28.816 ± 18.68 nm, with a mean size of 47.53 ± 18.68 nm. However, after gelation with Carbomer 940, the size of the spherical droplets reduced to 13.475 ± 5.40 nm (Table 2). This could be due to factors such as viscosity, pH, surface tension, shear force, or mixing speed and time of homogenization at the different phases of the formulation.

Moreover, the particle size analysis of the emulsion droplets was also seen to fall within the classification of Nanoemulsions which is between 20-100 nm, and Microemulsions (miscella emulsions) with diameters between 5-50 nm.[47,48,49]

Table 1. IMAGE J ANALYSIS OF THE EMULSION PHASE – PHASE A

S/N	Label	Area(nm^2)	Mean(nm^2)	Min	Max	Angle (°)	Length(nm)
1.	Mean	7.84	107.94	22.37	187.27	26.88	47.53
2.	SD	3.07	30.29	54.35	19.317	44.38	18.68
3.	Min	4.03	71.64	0.00	148.80	-44.22	24.26
4.	Max	11.20	163.01	133.32	199.11	77.47	67.90

Table 2. IMAGE J ANALYSIS OF THE EMULSION DROPLETS IN THE COMBINED PHASE – PHASE B (EMULSION GEL FORMATION)

S/N	Label	Area(nm^2)	Mean(nm^2)	Min	Max	Angle (°)	Length(nm)
1	Mean	0.89	29.68	9.99	93.12	-23.72	13.47
2	SD	0.36	15.35	10.07	55.83	34.90	5.40
3	Min	0.15	9.57	0.00	27.26	-75.96	2.17

4	Max	1.40	53.18	25.67	228.00	45.00	21.19

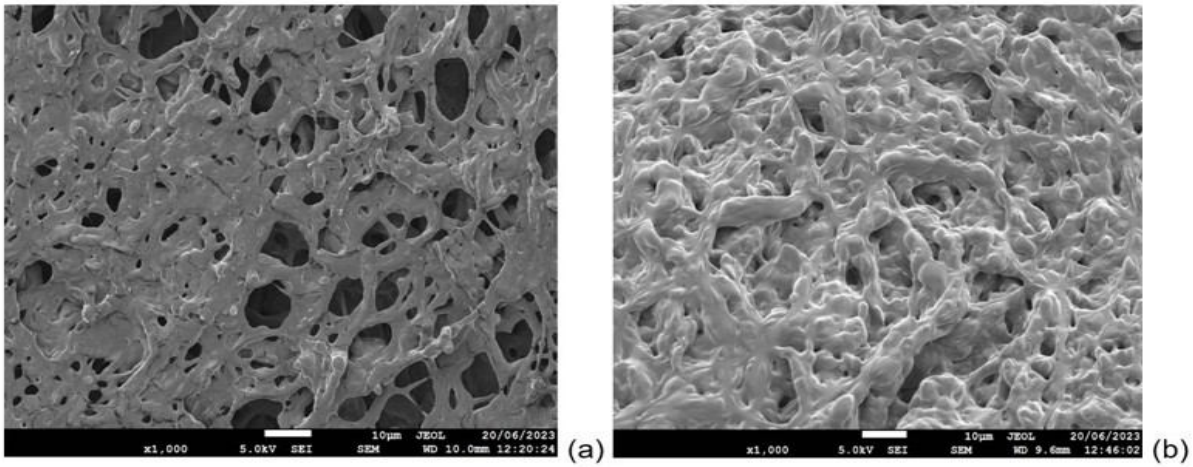
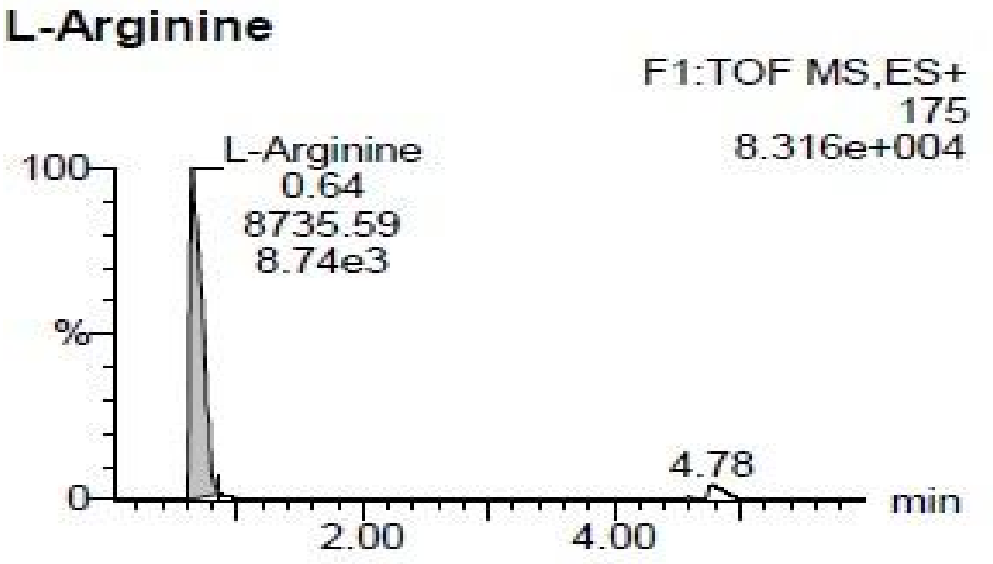


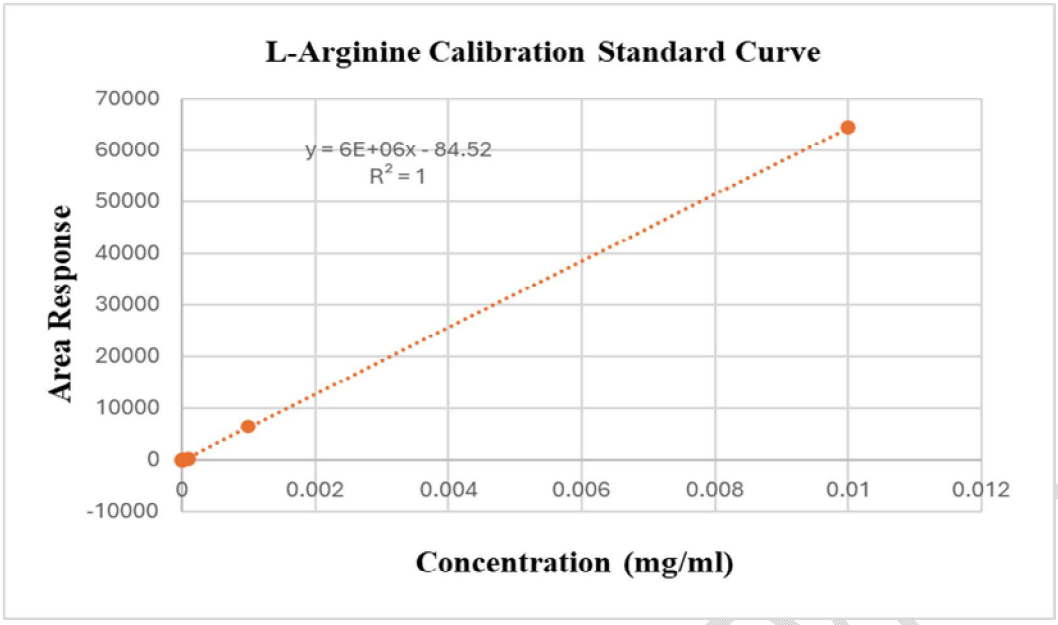
Fig. 3. SEM imaging of Emulsion gels with different Carbomer 940 concentrations. 0.20% C.940 Emulsion gel with 0.10 mg/mL L-Arginine: Test Sample A pH=7.60 (a) SEM imaging of 1.00% Carbomer 940 Emulsion gel with 0.10 mg/mL L-Arginine: Test Sample B pH=5.33 (b).

3.3 LC-MS ANALYSIS AND TRANSDERMAL EVALUATION

LC-MS Method was used for the analyses of L-Arginine in the topical formulation, the calibration standard solution, and the aliquots collected at time intervals during the FDC experiment. The ionization mode of the Mass Spectrophotometer (MS) was set to positive.



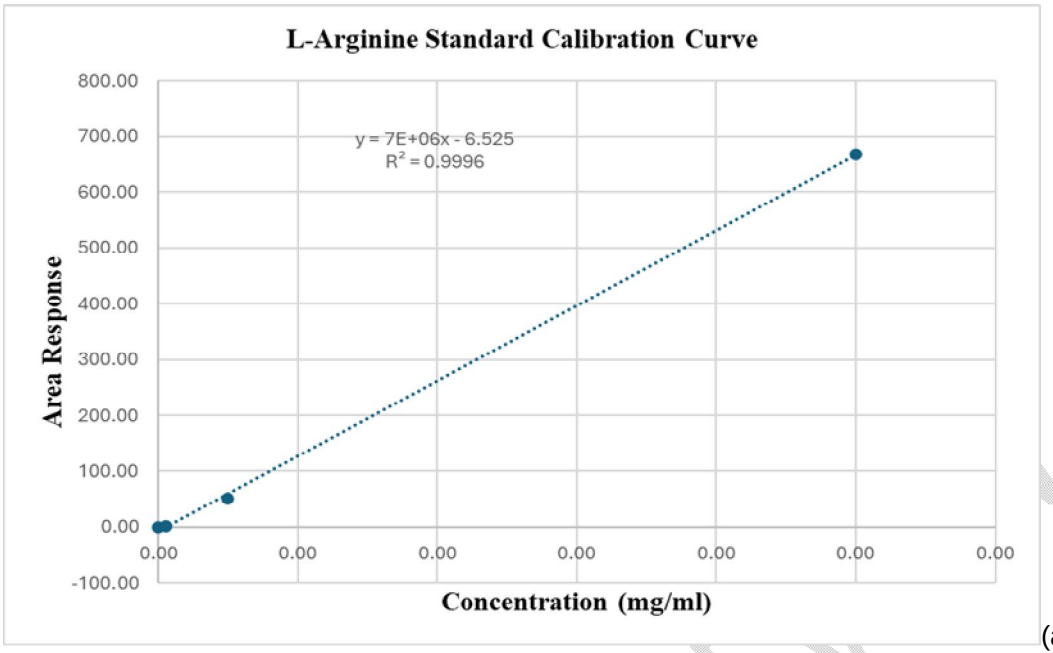
(a)



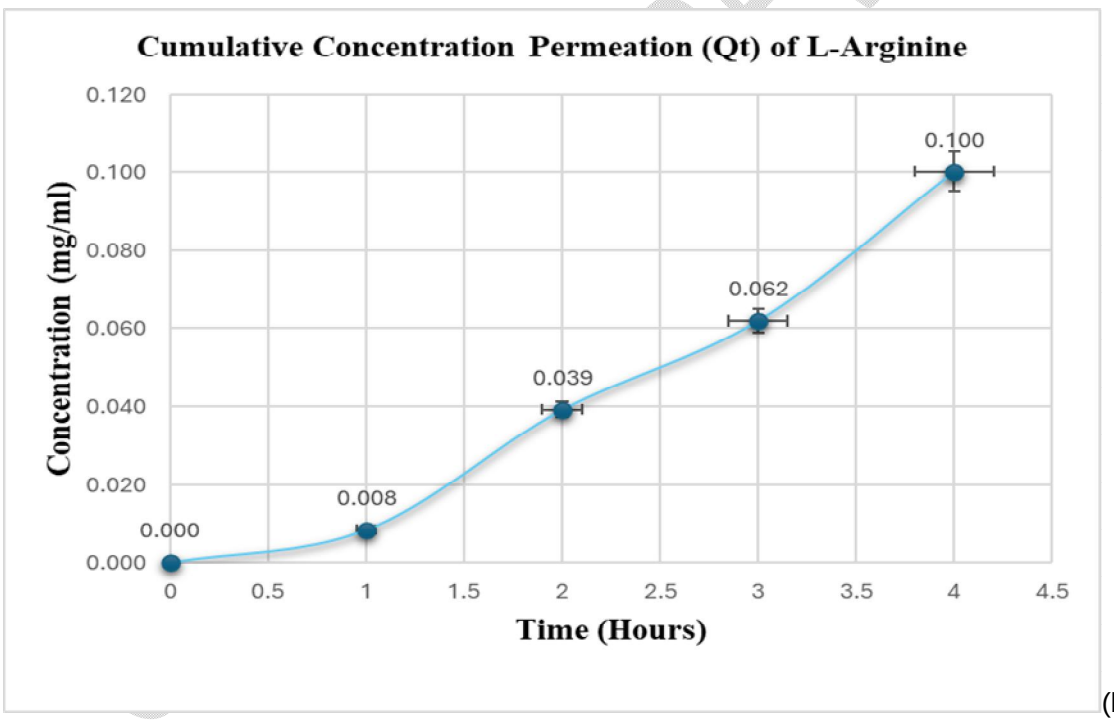
(b)

Fig. 4 and b. The Chromatogram from the LCMS Analysis of L-Arginine ($C_6H_{14}N_4O_2$) in the lipophilic topical formulation, retention Time (minutes) observed at 0.64; (b). L-Arginine Standard Calibration Curve; 1 in 10 Serial dilutions of 10mg/ml L-Arginine Stock Solution (b).

Permeation experimental trials were first conducted using filter paper as the cell membrane to evaluate the diffusion pattern of L-Arginine. Test Samples A and B were used respectively for the filter paper membrane trial. 1.000 mL of each test sample formulation containing 0.100 mg/mL L-Arginine with varying concentrations of C.940 (0.200 and 1.000% respectively) was loaded into the donor chamber and aliquots were taken from 1-5 hours from the receptor chamber and analyzed with the LCMS to quantify the permeation of L-Arginine per time. The cumulative concentration permeation (Qt), and % permeation of L-Arginine per time respectively through the filter paper membrane are shown in Figures 5 and 7.


L-Arginine Standard Calibration Curve
y = 7E+06x - 6.525
R² = 0.9996
Area Response
Concentration (mg/ml)
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
-100.00
0.00

(a)


Cumulative Concentration Permeation (Qt) of L-Arginine
Concentration (mg/ml)
Time (Hours)
0.120
0.100
0.080
0.060
0.040
0.020
0.000
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0.000
0.008
0.039
0.062
0.100

(b

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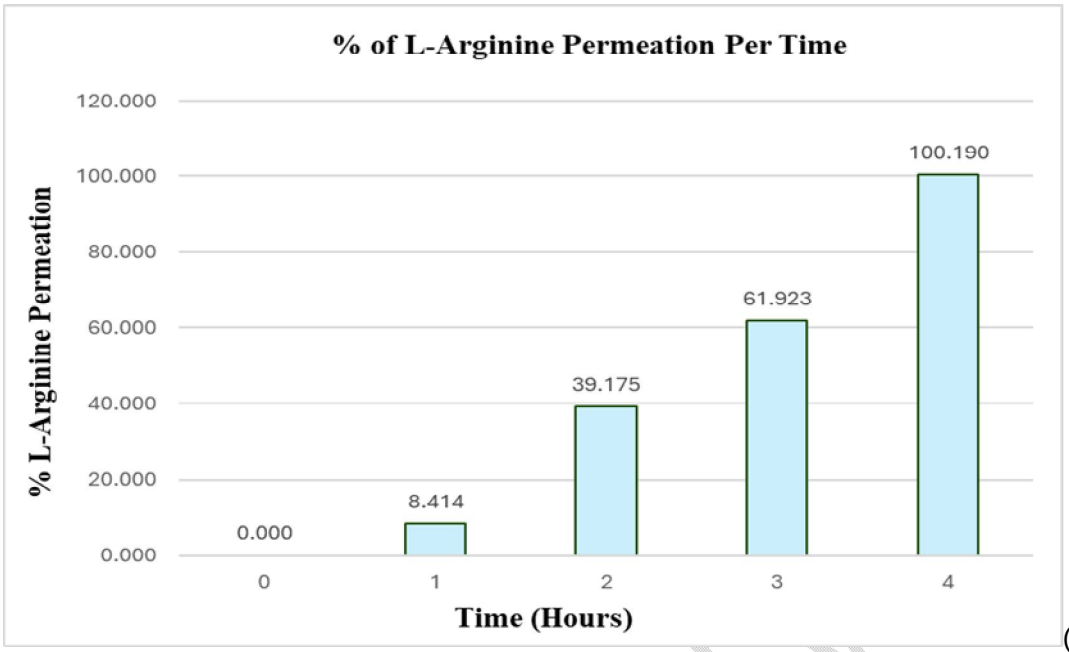


(c)

Fig. 5(a-c). Test Sample A: L-Arginine Calibration Curve (a); Cumulative Concentration Permeation (Qt) of L-Arginine Versus Time (b); % Permeation of L-Arginine through the filter paper after 4 hours(c).

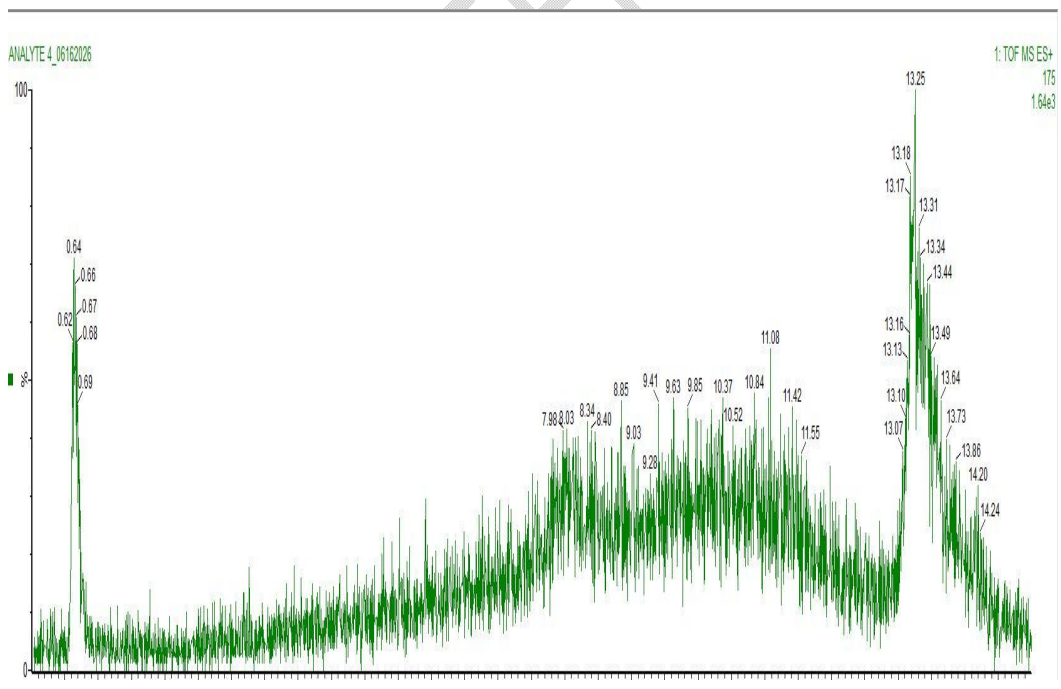
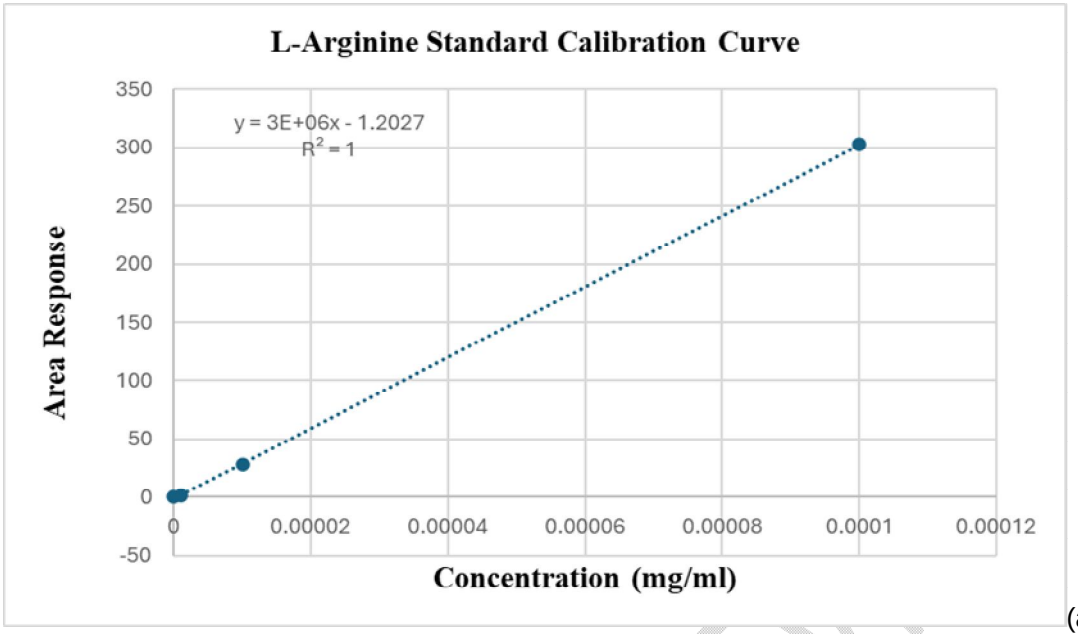
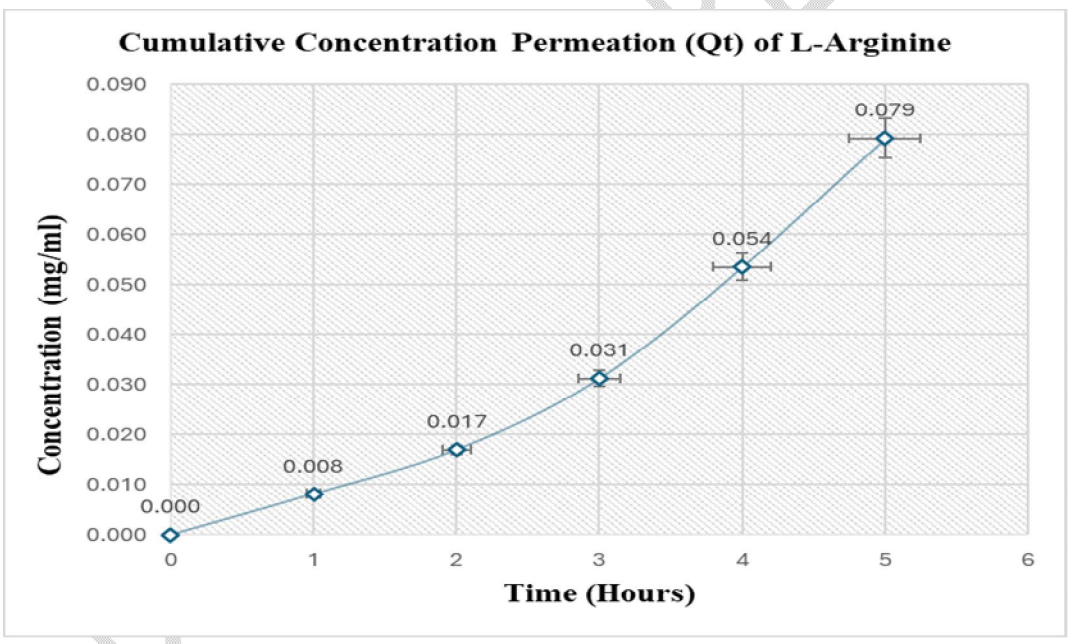


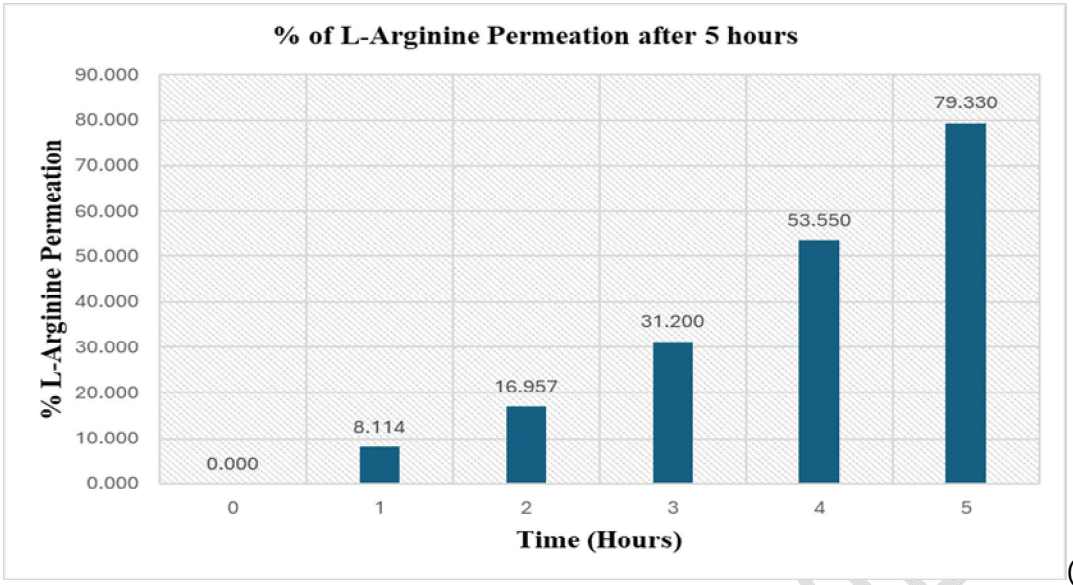
Fig. 6. Chromatogram of L-Arginine after FDE.


L-Arginine Standard Calibration Curve
y = 3E+06x - 1.2027
R² = 1
Area Response
Concentration (mg/ml)
350
300
250
200
150
100
50
0
-50
0
0.00002
0.00004
0.00006
0.00008
0.0001
0.00012

(a)


Cumulative Concentration Permeation (Qt) of L-Arginine
Concentration (mg/ml)
Time (Hours)
0.090
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
0
1
2
3
4
5
6
0.000
0.008
0.017
0.031
0.054
0.079

(b)



(c)

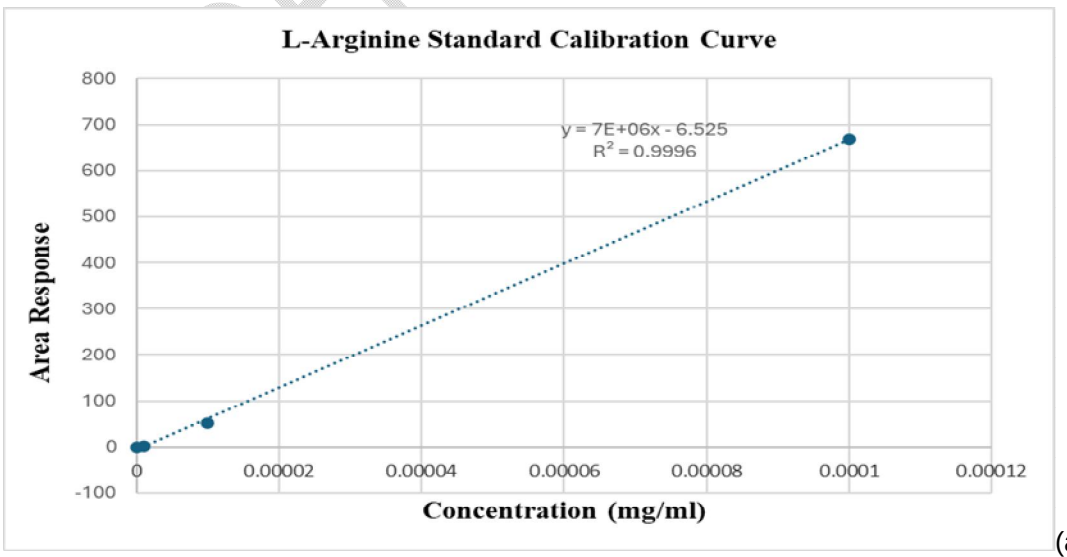
Fig. 7(a-c). Test Sample B: L-Arginine standard calibration curve (a); 1.00% carbomer 940 emulsion gel formulation(b); % Permeation of L-Arginine through the filter paper after 5 hours. (c).

The concentration of the L-Arginine that has permeation through the filter paper of the different Carbomer 940 concentrations was statiscally calculated from data obtained from standard calibration curves of L-Arginine with an R^2 of 0.9996 and 1 respectively as shown in Figure 5a and 7a respectively. Figures 5c and 7c show the bar chart data which presents the % permeation of L-Arginine in two different formulations containing the same concentration (0.10 mg/mL) but different concentrations of Carbomer 940; 0.20 (Sample A) and 1.00 % (Sample B) respectively) in a 100.00 mL scale. Sample A showed a higher % permeation of 100.19% after 4 hours than Sample B which showed 79.33 % permeation after 5 hours through the filter paper. The cumulative concentration permeated (Qt) per time showed that from the 0.10 mL in the test samples placed in the donor compartment, the same concentration was found to have fully diffused into the receptor solution after 4 hours for Sample A as compared to Sample B which showed that 0.08 mg/mL gas passed through after 5 hours. The chromatogram presented in Figure 6 shows the presence of the L-Arginine in the aliquots taken. Retention time as seen in the chromatogram, was within the retention time of L- Arginine at 0.64 ± 2.00 set for the qualitative analysis of the topical formulations. The Mass analysis of the L-Arginine, using the elemental composition quantification tool of the LCMS software which is set at positive mode (ESI +1) showed a mass of 175.119 Da was detected in all aliquots taken during the FDE. The data obtained showed the effectiveness LCMS quantification method of L- Arginine without derivatization.

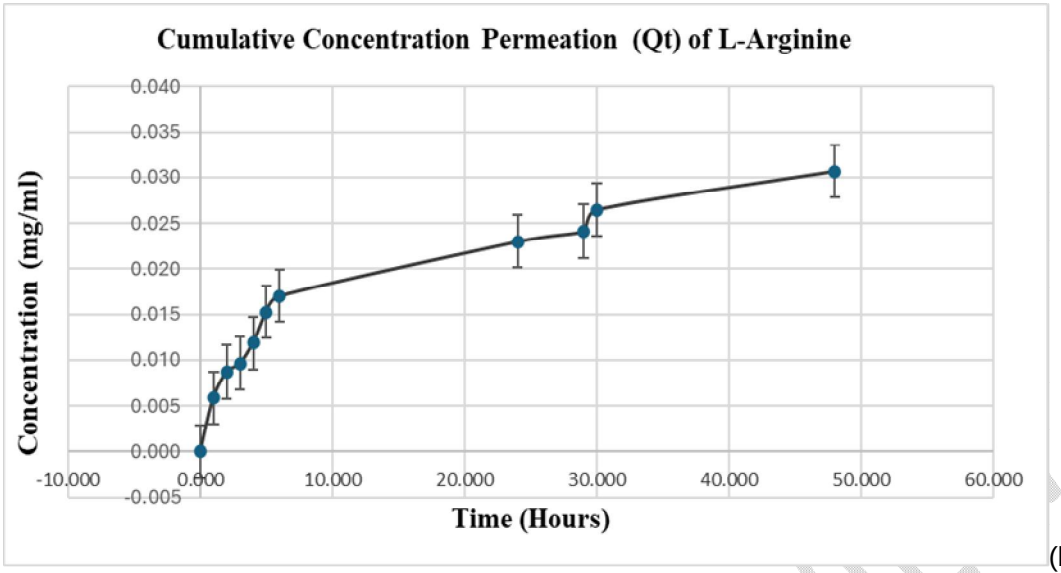
Furthermore, in the permeation studies, Porcine skin was used was used as the cell membrane to further investigate the permeation of L-Arginine. The size of the porcine skin used for all FDC experiments was uniform which was placed and clipped in between the donor chamber and the receptor chamber. The diffusion area of the FDC was 1.56 cm^2 of 7.20 mL capacity filled with 0.0005M PBS. Aliquots were also taken at time intervals between 1-48 hours.

Test Sample A which showed higher permeation during the filter paper trial experiment was used as the test sample in the final investigation, and the percentage permeation and other permeation parameters were calculated. Carbomer 940 gel base formulation with L-Arginine only neutralized with TEA to obtain a gel base formulation. This was taken as the Blank Sample (Test Sample C) with no emulsion phase and was used as the control sample. The permeation of L-Arginine in the gel base formulation through the porcine skin membrane was investigated.

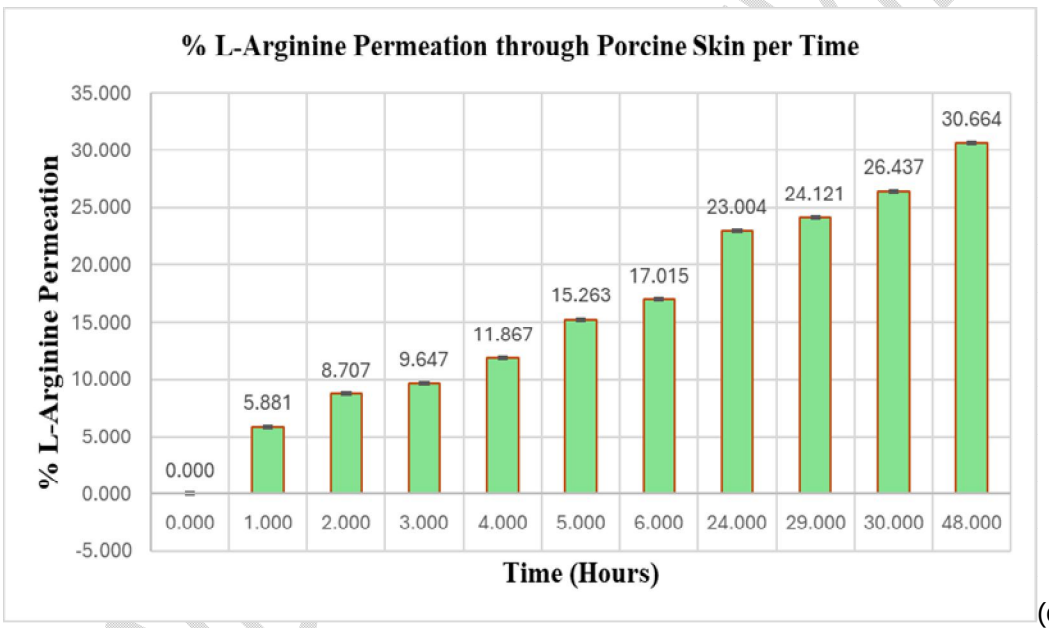
Figure 8c below presents the % permeation of the L-Arginine through the porcine skin membrane with no emulsion phase (Test Sample C). 30.66% permeation of L-Arginine through the porcine skin after 48 hours was seen after LC-MS analyses of aliquots taken during the FDC experiment. Whereas Test Sample A which had the emulsion phase showed 77.77 % permeation after 48hours (Figure 9c). Test Sample A gave a higher permeation due to the presence of the emulsion droplets created by the Oils phase (Almond and Ginger Oil) in emulsification with Polysorbate 80 which creates a lipophilic system as the emulsion droplets are formed, which acts as the carrier of the amino acid.[50]



(a)

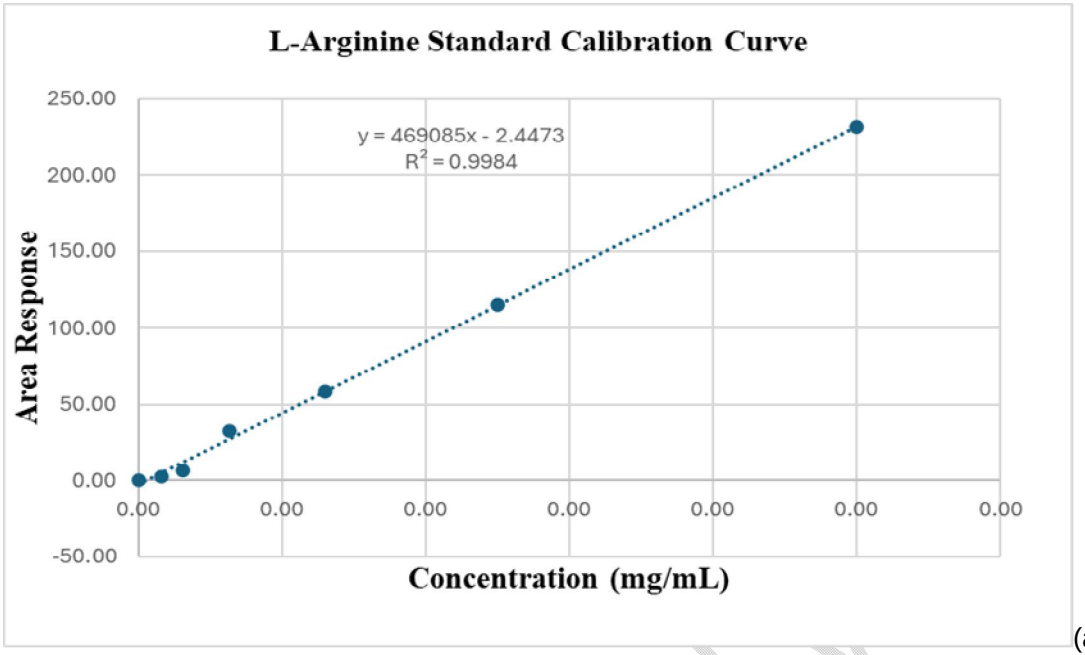


(b)

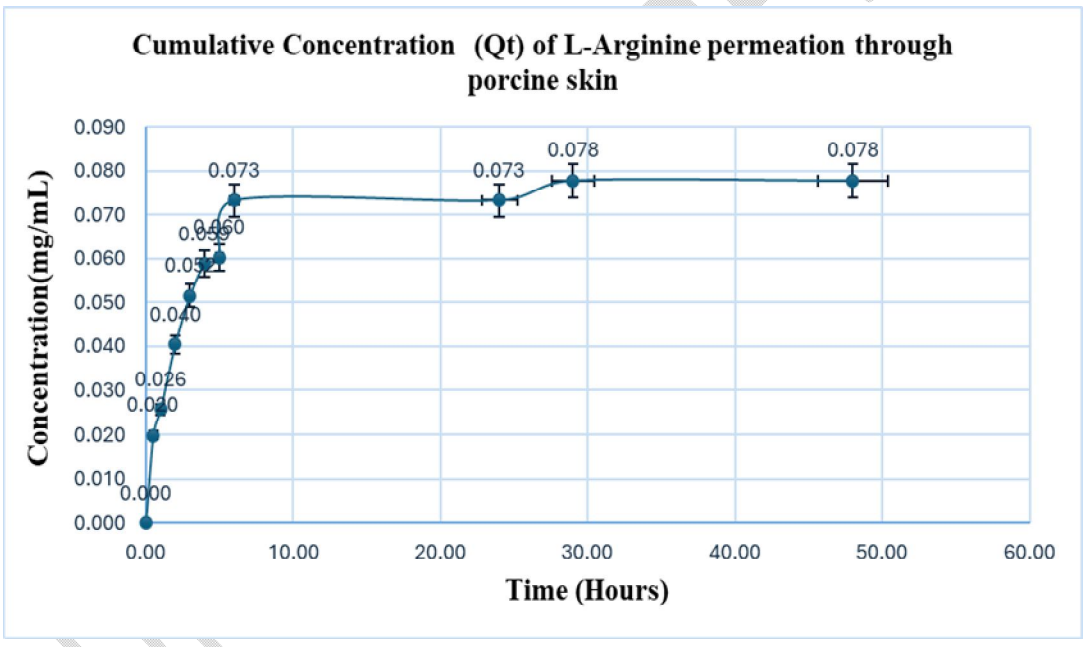


(c)

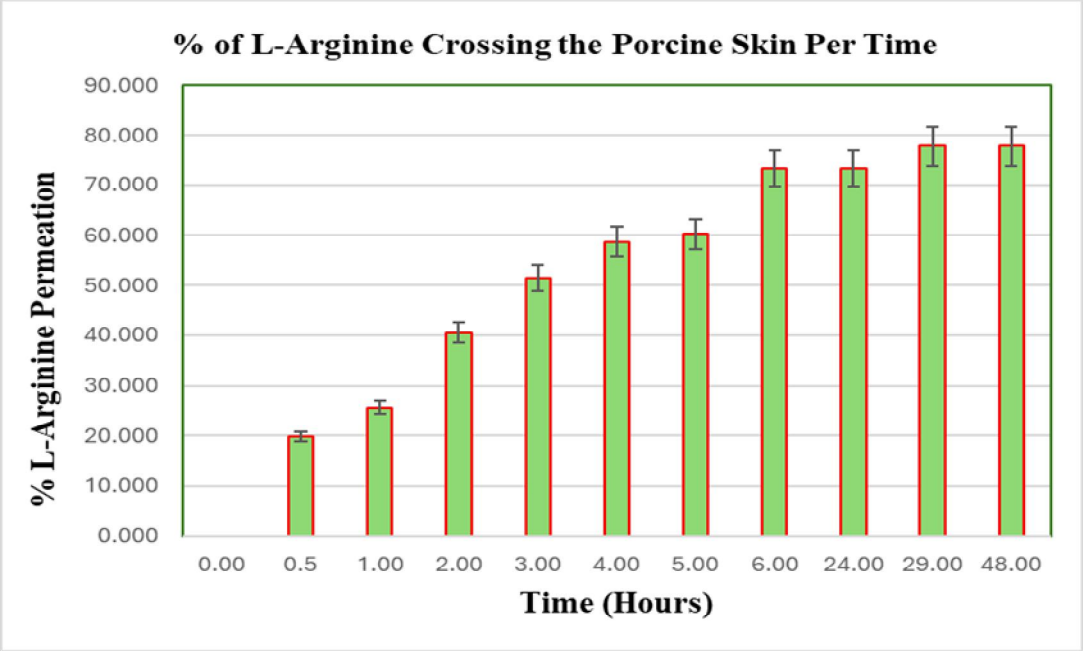
Fig. 8(a-c). Test Sample C: L-Arginine standard calibration curve(a); Cumulative Concentration Permeation (Qt) -Time Graph of L-Arginine (b); % Permeation of L-Arginine (c).


L-Arginine Standard Calibration Curve
y = 469085x - 2.4473
R2 = 0.9984
Area Response
Concentration (mg/mL)
250.00
200.00
150.00
100.00
50.00
0.00
-50.00
0.00

(a)


Cumulative Concentration (Qt) of L-Arginine permeation through porcine skin
Concentration(mg/mL)
Time (Hours)
0.090
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
0.00
10.00
20.00
30.00
40.00
50.00
60.00
0.073
0.073
0.078
0.078
0.040
0.026
0.000

(b)



(c)

Fig. 9(a-c). Test Sample A: L-Arginine standard calibration curve(a); Cumulative Concentration Permeation (Qt) -Time Graph of L-Arginine (b); % Permeation of L-Arginine (c).

The cumulative concentration of L-Arginine that has permeated through the porcine skin was calculated from the mean cumulative area response after LCMS analysis using the data obtained from the standard calibration curve using the equation below:

$$\textit{Cumulative Concentration (Qt)} = \frac{\textit{Cumulative Area Response} - \textit{Intercept}}{\textit{Slope}}$$

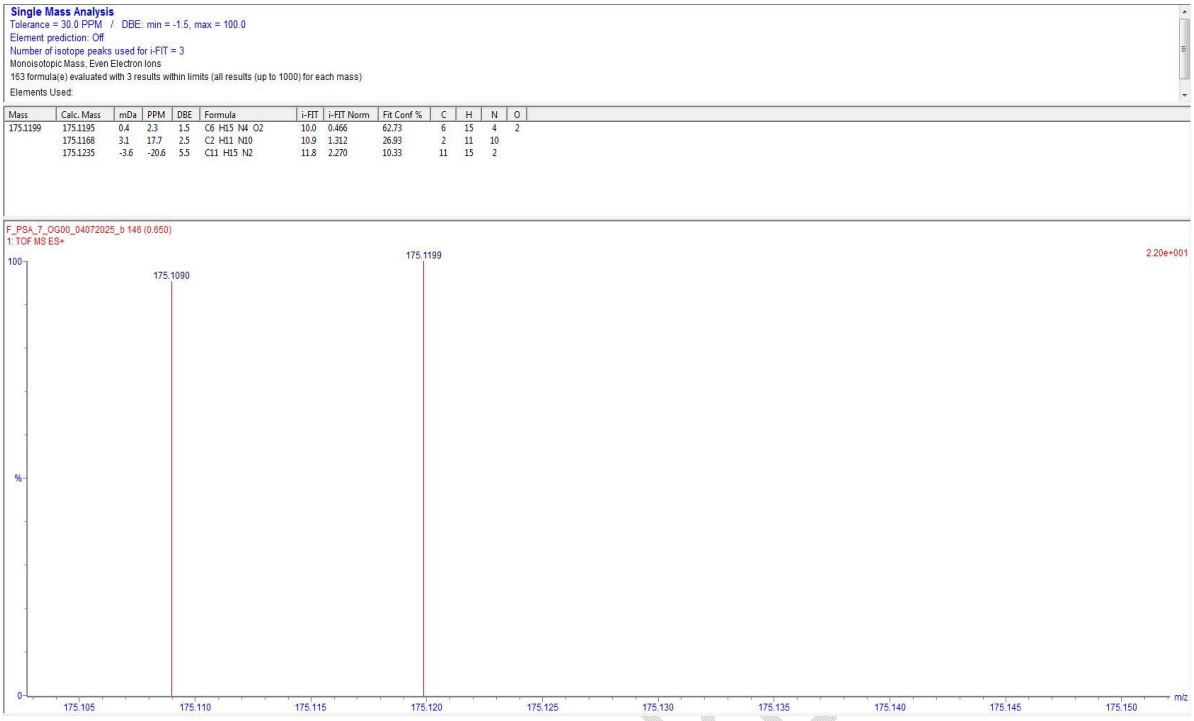


Fig. 10. Mass Spectra of L-Arginine after FDC experiments, ESI set in positive mode (174.204 +1), Retention Time (minutes) set at 0.64 ± 2.00, 174.204 +1 at 175.119 Da.

3.4 RATE OF PERMEATION OR FLUX (J*SS*), AND PERMEABILITY COEFFICIENT (KP).

Permeation parameters: Rate of Permeation or Flux (J*ss*), and Permeability Coefficient (Kp) were also statistically evaluated from the cumulative mean intensities. These parameters are often used to show the rate of flow and the capability of the drug to penetrate through the porous layers of the skin membrane.[51] The data summary is presented in Figures 10 a and b, showing the steady-state diffusion of L-Arginine through porcine skin.

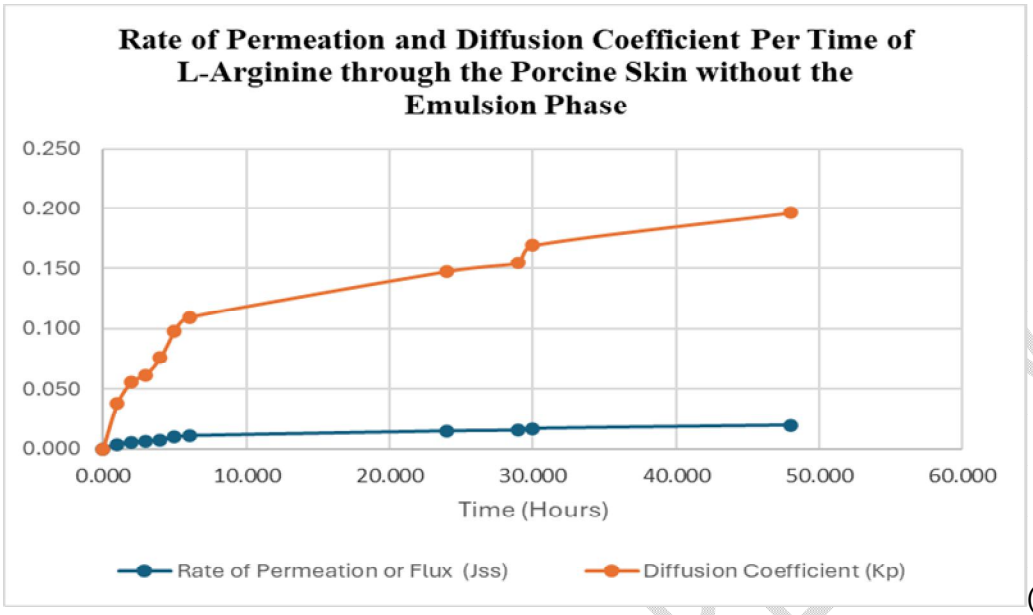
The Rate of Permeation also known as Flux (*Jss*) which represents the rate at which a volume of liquid flows across or through a membrane per unit area and time was also calculated with the equation below:

$$Jss = (Qt / Diffusion\ Area) \times Time$$

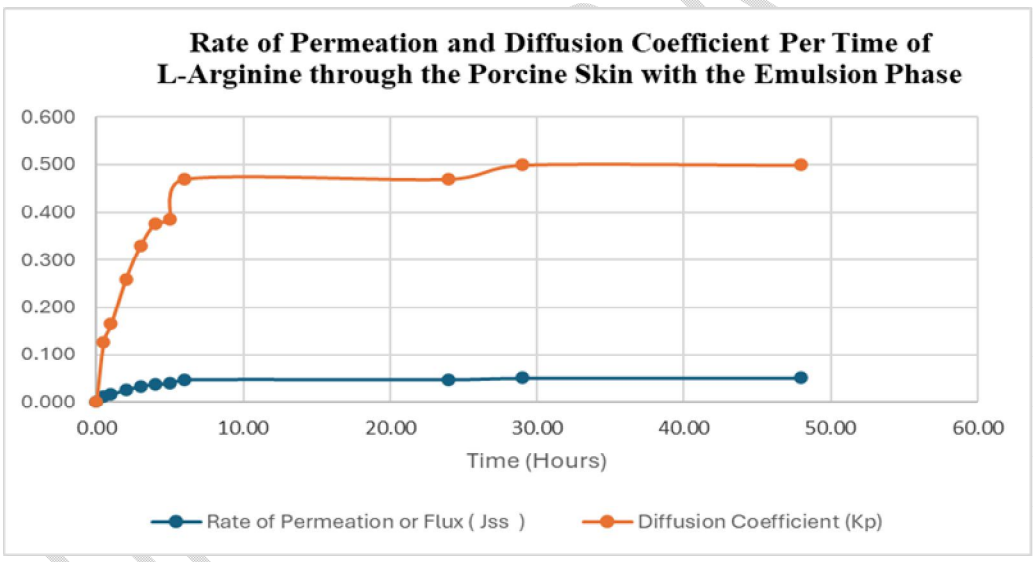
Furthermore, from the Flux, the Permeability coefficient (*Kp*) describing the flow through a porous layer of the porcine skin, based on Fick's first law of diffusion was calculated based on the equation below:

$$Kp = Jss / Co$$

Co is the initial drug concentration in the test sample loaded in the donor compartment of the FDC. The calculated values are seen in the graphical presentation in the Figure 14 below:



(a)



(b)

Fig. 11(a-b). Rate of Permeation and Diffusion Coefficient per time of L-Arginine through the Porcine Skin without and with the emulsion phase in the Carbomer 940 gel formulation.

The Test Samples A and C showed a steady state diffusion, as indicated by an increase in Kp as the Jss remained constant. Similar to a report by Korinth *et al*., in 2005 in their research on the percutaneous absorption of 2-butoxyethanol on excised human skin, the Kp Values of his test samples showed a higher value than the Flux which remained constant, as the concentration was critically considered in its evaluation.[52] As shown in Figure 11a, Test

Sample C showed a low diffusion of Kp of 0.19 ± 0.05 which correlates to the slow permeation of the L-Arginine through the Porcine skin compared to Test Sample A, which showed a higher Kp of 0.49 ± 0.13, within the same 48 hours window. The difference in Kp values, while Jss remained steady, revealed that the emulsion phase in Test Sample A enhanced the permeation from 30.64% to 77.77%.

When juxtaposed with the 0.10 mg/mL L-Arginine in the test formulation placed in the donor compartment of the FDC, an obvious difference in the cumulative concentration of L-Arginine that permeated at that time was also seen. Sample A showed 0.031 mg/mL while Sample B showed 0.078 mg/mL (Figure 7b and 8b). Moreover, the interest of the Jss and Kp data was to show the steady-state flow of the active ingredient from the donor compartment through the Porcine skin into the receptor solution relative to time.

4. CONCLUSION

The outcome of this study showed that L-Arginine was successfully encapsulated in the lipid layer of the emulsion phase as seen in the TEM imaging; hence a topical lipophilic drug delivery system was formulated. The emulsion droplets of the respective formulations were found to fall within the classification of Nanoemulsions between 20-100 nm and Microemulsions (miscella emulsions) with diameters between 5-50 nm. This size range indicates the stability of the emulsion gel. Iyer and his co-researchers also share similar reports in their studies on evaluating the effect of formulation parameters on the particle size of emulsions.[49] In their studies, emulsions having different size ranges were formulated, and emulsions particle sizes lower than 80 nm showed good performance and no phase separation was recorded.

The use of lipid-based formulations as emulsion gels to deliver lipophilic drugs has been previously reported.[53,54,55] In this study, Almond oil used as a carrier oil in combination with the essential oils proved to be an effective penetrating enhancer of the amino acids, showing 77.77% permeation of the L-Arginine.

Similar to a study by Alghurabi and his team in 2019, which also showed that Almond oil was able to enhance the permeation of Nimesulide; an anti-inflammatory drug in the gel formulation up to 99.00% and was also found to be safe without causing any skin irritation.[55] Findings in a review by Herman *et al.,* in 2015 also revealed that essential oils are capable of penetrating deeper layers of the skin, promoting the absorption of lipophilic drugs.[56] The Kp data presented in this study showed a good steady-state diffusion of the amino acids with a constant Jss as earlier discussed, revealing the potency of the oils in the emulsion phase of the formulations when juxtaposed with the formulation without the oils. The surfactant:

Polysorbate 80 and Propylene glycol used in the emulsion formation also played key roles in creating a stable emulsion of the final formulation by reducing the surface tension and creating an interface, thus enhancing the solubility of the oil dispersion.[57] This research has shown possible permeation of L-Arginine through porcine skin membrane using the FDC. However, for further studies, the permeation study of optimized dosage forms of other L-Arginine, as well as other amino acids such as leucine and Proline, could also be evaluated with varying concentrations of Carbomer 940. The application of permeation enhancers such as liposomes or peptides in the lipophilic topical formulation could also be an innovative step. Liposomes and peptides act as cargoes of API and are known to deliver the actives faster into the different layers of the skin.[58,59] This aspect of the research will be welcoming, as it could show an enhanced permeation of the amino acids at a shorter time of less than 30-48 hours reported in this study. However, more investigation on mTOR signaling after the transdermal absorption of the respective amino acids in the topical formulations will be laudable. Studies on the increase in phosphorylation in muscle cells after mTOR signaling[28] can ascertain if MPS could occur on the application of the topical product within a time frame. This aspect of research could bring to light a very realistic, and precise therapeutic topical product for human health care, that could attract serious attention in the sports healthcare sector, cosmetics industries, and other pharmaceutical stakeholders.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

AA - Amino Acid

API - Active Pharmaceutical Ingredient

C.940 - Carbomer 940

FDC - Franz Diffusion Cell

Jss - Rate of Permeation or Flux

Kp - Permeability Coefficient

LC-MS - Liquid Chromatography-Mass Spectrometry

MPS - Muscle Protein Synthesis

mTOR - Mammalian Target of Rapamycin

PBS - Phosphate Buffer Solution

R2 - Coefficient of Determination (statistical measure)

SC - Stratum Corneum

SEM - Scanning Electron Microscopy

TEM - Transmission Electron Microscopy

TEA - Triethanolamine

TDDS - Topical Drug Delivery Systems

UHQW - Ultra High-Quality Water