In-Vitro Cellular Uptake, Cytotoxicity, and Permeability Assessment of Gamma Oryzanol-Loaded Self-Nanoemulsifying Drug Delivery System (SNEDDS) Using Caco-2 Cell Line

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Abstract
Gamma oryzanol exhibits significant therapeutic potential but suffers from poor aqueous solubility and P-glycoprotein (P-gp) mediated intestinal efflux, severely restricting its oral bioavailability. This study aimed to mechanistically evaluate the cellular internalization, cytotoxicity, and transepithelial permeability of an optimized Gamma Oryzanol-loaded Self-Nanoemulsifying Drug Delivery System (SNEDDS) using a fully differentiated Caco-2 human intestinal epithelial model. Biocompatibility was confirmed via MTT assay, which demonstrated that the nanocarrier maintained greater than 85% cellular viability (87.85% at 100 µg/mL), validating the formulation's safety. Confocal Laser Scanning Microscopy (CLSM) utilizing Coumarin-6 as a lipophilic probe revealed a 7.43-fold enhancement in intracellular accumulation at 4 hours, driven by the nanometric lipid matrix. Crucially, bidirectional transport studies across validated Transwell monolayers proved the formulation's ability to circumvent active biological barriers. The absorptive apparent permeability increased by 5.58-fold (from 1.15 $\times 10^{-6}$ cm/s to 6.42 $\times 10^{-6}$ cm/s). Concurrently, the Efflux Ratio plummeted from 4.21 for the unformulated drug to an optimal 1.11. In conclusion, the developed SNEDDS safely and effectively bypasses P-gp mediated extrusion, providing a robust platform for maximizing the transcellular transport of lipophilic therapeutics.

Keywords: Gamma Oryzanol, SNEDDS, Caco-2 Cell Line, P-glycoprotein Efflux, Intestinal Permeability, Cellular Internalization.

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1. INTRODUCTION

Gamma oryzanol is a natural mixture of sterol and triterpene alcohol esters of ferulic acid, primarily extracted from rice bran oil (Lerma-García et al., 2009). The compound is widely recognized for its robust antioxidant, anti-inflammatory, and cholesterol-lowering capabilities (Rong et al., 2020). Researchers have shown great interest in its potential to manage metabolic disorders and oxidative stress safely. However, translating this theoretical potential into practical clinical therapy faces a major physical roadblock. The compound is exceptionally lipophilic and practically insoluble in water (Rong et al., 2020). When administered orally, it fails to dissolve adequately in the aqueous fluids of the gastrointestinal tract (Lerma-García et al., 2009). This severe lack of dissolution leads directly to erratic absorption and very poor oral bioavailability, which sharply restricts its use in modern medicine (Rong et al., 2020). While poor solubility is a massive hurdle, it is not the only obstacle preventing effective oral delivery. Once a drug molecule finally manages to dissolve, it still has to physically cross the intestinal epithelial barrier to enter the systemic circulation (Hubatsch et al., 2007). This biological barrier is highly selective and actively defends the body against foreign substances. A critical component of this cellular defense system is P-glycoprotein, an ATP-dependent efflux pump located directly on the apical membrane of intestinal enterocytes (Hubatsch et al., 2007). Highly lipophilic compounds like gamma oryzanol are frequent substrates for this specific transporter (Hubatsch et al., 2007). As the drug attempts to diffuse into the cell, P-glycoprotein actively captures the molecules and pumps them right back out into the intestinal lumen. This continuous cycle of extrusion severely restricts the net absorption of the drug and keeps systemic bioavailability frustratingly low. To overcome both

the rigid solubility limit and the biological efflux barrier simultaneously, lipid based formulations have emerged as a highly effective approach. Self-nanoemulsifying drug delivery systems represent a specialized and powerful category of these lipid carriers (Pouton, 2000). They are isotropic mixtures of oils, surfactants, and co-surfactants that spontaneously form fine oil-in-water nanoemulsions upon encountering mild agitation in gastrointestinal fluids (Date & Nagarsenker, 2007). The nanometric size of these droplets generates a massive interfacial surface area, which keeps the hydrophobic drug fully solubilized and accelerates its presentation to the absorbing membrane (Pouton, 2000). However, improving physical solubility is only half the battle. We must rigorously validate how these lipid nanodroplets interact physically with intestinal enterocytes (Date & Nagarsenker, 2007). There is evidence that the specific non-ionic surfactants used in these formulations can temporarily alter the fluidity of the cell membrane. This fluidization might be the exact mechanism needed to sneak the drug past active efflux transporters without causing permanent cellular damage. Because this interaction is highly complex, we cannot assume that a formulation with good physical stability will automatically perform well in the human body. Thorough in-vitro biological characterization is absolutely mandatory to prove its clinical worth. The primary objective of this research is to perform a strict mechanistic evaluation of a previously optimized gamma oryzanol loaded self-nanoemulsifying drug delivery system. We aim to understand exactly how this formulation interacts with human intestinal tissues using the fully differentiated Caco-2 cell line. This specific cell line serves as the gold standard for mimicking the human intestinal epithelial barrier because it naturally expresses functional tight junctions and active transport proteins like P-glycoprotein (Hubatsch et al., 2007). Our investigation is broken down into three critical phases. First, we will assess the baseline safety and biocompatibility of the nanocarrier using targeted cytotoxicity assays. It is vital to confirm that the selected excipients do not compromise cellular viability (Srinivasan et al., 2015). Second, we will monitor the cellular internalization dynamics of the nanoemulsion droplets using advanced fluorescent imaging techniques to visually track cellular uptake. Finally, we will conduct rigorous bidirectional permeability studies. By measuring drug transport in both the absorptive and secretory directions across tight monolayers validated by transepithelial electrical resistance, we will quantify the exact efflux ratio (Srinivasan et al., 2015). This specific permeability assay will definitively prove whether the optimized lipid formulation can successfully bypass the active efflux pumps and meaningfully enhance the transcellular transport of the drug (Hubatsch et al., 2007).

3. MATERIALS AND METHODS

3.1 Materials and Reagents:

The Caco-2 cell line (passage numbers 25-40) was procured from NCCS, Pune, and cultured using high glucose DMEM supplemented with 10% heat-inactivated FBS and 1% Penicillin-Streptomycin. Essential chemicals including the MTT reagent, Coumarin-6, and Transwell polycarbonate inserts were utilized for the biological assays. The optimized SNEDDS (Olive oil, Tween 20, PEG 400) used in this evaluation was previously formulated and physically characterized by our group. All procedures were performed under standard aseptic conditions to ensure optimal cell viability and barrier integrity.

3.2 Cell Culture and Maintenance

The Caco-2 cell line acts as a reliable model for the intestinal epithelium, requiring careful handling to maintain robust growth and stable morphology (Hubatsch et al., 2007). Cells are maintained in a controlled incubator environment set at 37°C supplemented with a 5% CO2 atmosphere (Hubatsch et al., 2007). Regular sub-culturing and medium replenishment are performed to preserve the functional properties and tight junction competence required for downstream transport assays (Hubatsch et al., 2007).

3.3 In Vitro Cytotoxicity (MTT Assay)

To determine cellular biocompatibility, an MTT assay is conducted over a 24-hour treatment window (Mosmann, 1983). Caco-2 cells are exposed to varying concentrations of plain Gamma Oryzanol, blank SNEDDS, and gamma oryzanol-loaded SNEDDS across a linear range spanning from 10 to 100 µg/mL (Mosmann, 1983). Following incubation, metabolic reduction of the tetrazolium salt quantifies cell survival relative to untreated control groups (Mosmann, 1983).

3.4 Cellular Uptake Study (CLSM)

Intracellular localization is visualized using Confocal Laser Scanning Microscopy by incorporating a fluorescent marker into the nanocarriers (Date et al., 2010). The self-nanoemulsifying systems are labeled with Coumarin-6 at a concentration of 1 µg/mL and exposed to Caco-2 cultures for distinct 2-hour and 4-hour intervals (Date et al., 2010). After fixation, nuclear counterstaining is performed using DAPI to reveal spatial internalization patterns within the cell layers (Date et al., 2010).

3.5 In Vitro Intestinal Permeability Study

Epithelial transport functionality is established through a 21-day culturing protocol where Caco-2 cells grow on Transwell polycarbonate inserts to construct a differentiated monolayer (Srinivasan et al., 2015). Monolayer quality and tight junction tightness are verified prior to experiments via

Transepithelial Electrical Resistance measurements, applying a strict acceptance threshold above 250 Ω·cm² (Srinivasan et al., 2015). Bidirectional transport assessments are then executed in both apical-to-basolateral and basolateral-to-apical directions, allowing the mathematical derivation of the apparent permeability coefficient and the corresponding efflux ratio (Hubatsch et al., 2007).

4. RESULTS AND DISCUSSION

4.1. In Vitro Cytotoxicity and Biocompatibility

The in vitro cytotoxicity and biocompatibility of the developed formulations were investigated using the MTT assay on Caco-2 cell monolayers. Cellular morphology following treatment was examined by phase-contrast microscopy, while quantitative cell viability was determined after exposure to increasing concentrations of the test formulations. Representative microscopic images are presented in Figure 1, and the comparative percentage cell viability is illustrated in Figure 2. Microscopic examination revealed that untreated Caco-2 cells exhibited their typical epithelial morphology with intact cell boundaries and uniform distribution. Cells treated with plain Gamma Oryzanol, Blank SNEDDS, and GO-SNEDDS retained normal morphology without noticeable membrane damage, cellular shrinkage, or detachment, indicating that the formulations did not induce significant cytotoxic effects within the investigated concentration range. The quantitative MTT assay further confirmed these observations. Plain Gamma Oryzanol exhibited excellent cellular compatibility, maintaining cell viability above 94% even at the highest tested concentration of 100 µg/mL. Blank SNEDDS demonstrated a slight concentration-dependent reduction in viability, decreasing from 96.85 ± 1.42% at 10 µg/mL to 86.40 ± 2.45% at 100 µg/mL. Similarly, GO-SNEDDS maintained high cell viability throughout the study, with values ranging from 97.12 ± 1.55% at 10 µg/mL to 87.85 ± 2.15% at 100 µg/mL. The percentage cell viability data for all treatment groups are presented in Table 1.

Table 1. Percentage Cell Viability (Mean ± SD, n = 3) of Caco-2 Cells Treated with Plain Gamma Oryzanol, Blank SNEDDS, and GO-SNEDDS

Concentration (µg/mL)	Plain Gamma Oryzanol Viability (%)	Blank SNEDDS Viability (%)	GO-SNEDDS Viability (%)
Control (Untreated)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
10	98.42 ± 1.15	96.85 ± 1.42	97.12 ± 1.55
25	97.65 ± 1.32	94.22 ± 1.68	95.45 ± 1.20
50	96.10 ± 1.85	91.35 ± 2.10	92.18 ± 1.84
100	94.55 ± 1.60	86.40 ± 2.45	87.85 ± 2.15

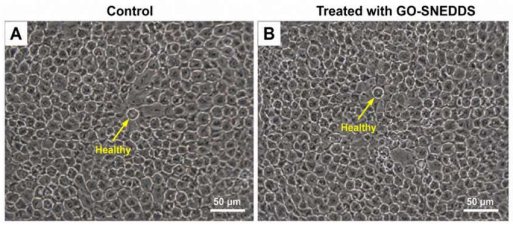

Figure 1. Representative phase-contrast microscopic images showing the morphology of untreated Caco-2 cells and cells treated with Plain Gamma Oryzanol, Blank SNEDDS, and GO-SNEDDS.

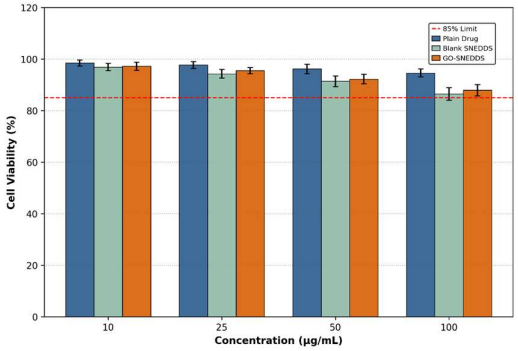

Figure 2. Comparative percentage cell viability of Caco-2 cells following treatment with Plain Gamma Oryzanol, Blank SNEDDS, and GO-SNEDDS at different concentrations.

The MTT assay demonstrated that both the optimized GO-SNEDDS formulation and its corresponding blank formulation possessed excellent cytocompatibility toward Caco-2 cells. Cell viability remained above 85% across all investigated concentrations, indicating that the formulations did not exert any significant toxic effect on intestinal epithelial cells. According to the widely accepted criteria for in vitro biocompatibility, cell survival exceeding 80–85% is generally considered indicative of a non-cytotoxic formulation suitable for oral administration. Plain Gamma Oryzanol exhibited the highest cell viability throughout the study, reflecting the inherent safety of the bioactive compound. Although Blank SNEDDS and GO-SNEDDS produced a modest concentration-dependent decrease in viability, the reduction remained limited and did not compromise overall cellular integrity. This slight decline is commonly associated with the presence of non-ionic surfactants and co-surfactants used in self-nanoemulsifying systems, which can temporarily increase membrane fluidity and enhance epithelial permeability without causing irreversible cellular damage. Importantly, the viability profile of GO-SNEDDS closely resembled that of Blank

SNEDDS, indicating that incorporation of Gamma Oryzanol within the nanoemulsion system did not introduce additional cytotoxicity. The absence of marked differences between the two formulations suggests that the encapsulated drug remains compatible with the lipid carrier and does not induce cellular stress beyond that associated with the formulation excipients. The microscopic observations further supported the quantitative findings, as treated cells maintained normal morphology, intact cell boundaries, and uniform monolayer organization without evidence of membrane disruption, cell lysis, or excessive detachment. These findings collectively demonstrate that the optimized GO-SNEDDS possesses excellent biocompatibility and is suitable for oral delivery applications, providing a safe carrier system capable of enhancing drug delivery while preserving the structural and functional integrity of intestinal epithelial cells.

4.2. Cellular Internalization and Uptake Dynamics

The intracellular uptake of the optimized self-nanoemulsifying drug delivery system (GO-SNEDDS) was investigated using confocal laser scanning microscopy (CLSM) in Caco-2 cell monolayers. Since Gamma Oryzanol does not possess intrinsic fluorescence, Coumarin-6 (C-6), a lipophilic fluorescent marker, was incorporated into the formulation to monitor cellular internalization and intracellular distribution. Representative CLSM images illustrating the uptake of Plain C-6 suspension and C-6-loaded SNEDDS are presented in Figure 3.

Confocal microscopic observations revealed a marked difference in the cellular uptake patterns between the two formulations. Cells treated with the Plain C-6 suspension exhibited weak fluorescence that was mainly confined to the cell periphery, indicating limited penetration across the plasma membrane. Even after 4 hours of incubation, only a slight increase in intracellular fluorescence was observed, suggesting poor cellular internalization. In contrast, cells exposed to the C-6-loaded SNEDDS displayed intense and uniformly distributed green fluorescence throughout the cytoplasm. The fluorescence intensity increased noticeably with incubation time, demonstrating rapid and efficient uptake of the nanoemulsion system by Caco-2 cells. The homogeneous intracellular fluorescence distribution confirmed successful transport of the fluorescent probe into the cells by the optimized lipid-based carrier. Quantitative analysis of the confocal images was performed by measuring the mean fluorescence intensity (MFI). As shown in Table 2, the MFI of the C-6-loaded SNEDDS reached 58.75 ± 4.35 after 2 hours, corresponding to a 4.71-fold enhancement compared with the Plain C-6 suspension. Following 4 hours of incubation, intracellular fluorescence further increased to 112.40 ± 6.80, representing a 7.43-fold improvement in cellular uptake. These findings clearly demonstrate the superior internalization efficiency of the optimized GO-SNEDDS formulation.

Table 2. Quantitative Mean Fluorescence Intensity (MFI) of Caco-2 Cells Treated with Plain Coumarin-6 Suspension and Coumarin-6-Loaded SNEDDS (Mean ± SD, n = 3)

Formulation	Incubation Time (h)	Mean Fluorescence Intensity (MFI)	Uptake Enhancement Ratio
Plain C-6 Suspension	2	12.45 ± 1.82	1.00
Plain C-6 Suspension	4	15.12 ± 2.10	1.00
C-6 Loaded SNEDDS	2	58.75 ± 4.35	4.71
C-6 Loaded SNEDDS	4	112.40 ± 6.80	7.43

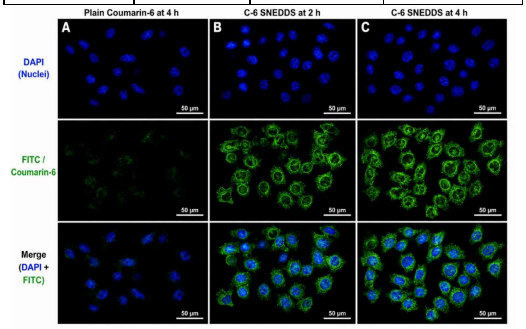

Figure 3. Confocal laser scanning microscopy images of Caco-2 cells showing the intracellular uptake of Plain Coumarin-6 suspension and Coumarin-6-loaded SNEDDS after 2 and 4 hours of incubation.

The CLSM study demonstrated that incorporation of Coumarin-6 into the optimized SNEDDS markedly enhanced intracellular delivery compared with the conventional suspension. The weak fluorescence observed with the Plain C-6 suspension indicates limited cellular permeation, which is expected because poorly water-soluble compounds exhibit low dissolution and reduced interaction with the intestinal epithelial surface. In contrast, the strong and time-dependent increase in intracellular fluorescence produced by the C-6-loaded SNEDDS confirms efficient cellular internalization of the nanoemulsion droplets. The nanometric droplet size generated after self-emulsification provides a large interfacial surface area, allowing intimate contact

with the apical surface of Caco-2 cells and facilitating rapid cellular uptake. In addition, the lipidic components and non-ionic surfactants present in the formulation improve membrane wettability and promote interaction with the phospholipid bilayer, thereby enhancing endocytic transport and intracellular accumulation. The progressive increase in fluorescence intensity from 2 to 4 hours indicates that uptake of the nanoemulsion is both efficient and time dependent. Importantly, the substantially higher fluorescence intensity achieved by the C-6-loaded SNEDDS compared with the Plain C-6 suspension confirms the ability of the optimized formulation to deliver lipophilic molecules into intestinal epithelial cells more effectively than conventional formulations.

4.3. Intestinal Permeability and Efflux Pump Bypass

The intestinal transport behavior of the optimized Gamma Oryzanol-loaded self-nanoemulsifying drug delivery system (GO-SNEDDS) was evaluated using differentiated Caco-2 cell monolayers cultured on Transwell inserts. Before initiating the transport study, the integrity of the epithelial barrier was confirmed by measuring transepithelial electrical resistance (TEER). The recorded TEER values remained consistently above 250 Ω·cm² throughout the experiment, confirming the formation of an intact and functional epithelial monolayer. The TEER profile and cumulative transport data are presented in Figure 4. Bidirectional permeability studies were performed to determine the apparent permeability coefficient (Papp) in both the absorptive (apical-to-basolateral, A→B) and secretory (basolateral-to-apical, B→A) directions. Plain Gamma Oryzanol exhibited relatively poor absorptive permeability, with a Papp (A→B) value of $1.15 \pm 0.22 \times 10^{-6}$ cm/s. In contrast, the secretory permeability was considerably higher, with a Papp (B→A) value of $4.85 \pm 0.41 \times 10^{-6}$ cm/s, resulting in an efflux ratio of 4.21. These findings indicate that Gamma Oryzanol is subjected to significant active efflux across the intestinal epithelium. Encapsulation of Gamma Oryzanol within the optimized SNEDDS markedly improved its absorptive transport characteristics. The Papp (A→B) increased to $6.42 \pm 0.35 \times 10^{-6}$ cm/s, corresponding to a 5.58-fold enhancement compared with the plain drug. Although the secretory permeability also increased to $7.15 \pm 0.48 \times 10^{-6}$ cm/s, the calculated efflux ratio decreased substantially to 1.11, indicating a pronounced reduction in transporter-mediated drug efflux. The bidirectional permeability parameters are summarized in Table 3.

Table 3. Bidirectional Apparent Permeability (Papp), Efflux Ratio (ER), and Absorptive Enhancement of Plain Gamma Oryzanol and GO-SNEDDS (Mean ± SD, n = 3)

Formulation	Papp (A→B) (×10⁻⁶ cm/s)	Papp (B→A) (×10⁻⁶ cm/s)	Efflux Ratio (ER)	Absorptive Enhancement Ratio
Plain Gamma Oryzanol	1.15 ± 0.22	4.85 ± 0.41	4.21	1.00
GO-SNEDDS	6.42 ± 0.35	7.15 ± 0.48	1.11	5.58

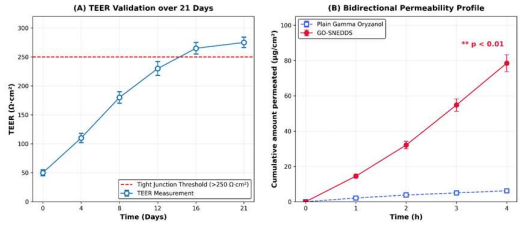

Figure 4. Transepithelial electrical resistance (TEER) measurements and bidirectional permeability profile of Plain Gamma Oryzanol and GO-SNEDDS across differentiated Caco-2 cell monolayers.

The Caco-2 permeability study demonstrated that plain Gamma Oryzanol possesses limited intestinal permeability, as reflected by its low absorptive Papp value and high efflux ratio. An efflux ratio greater than 2 is generally considered indicative of active transporter-mediated extrusion, suggesting that Gamma Oryzanol is a substrate for intestinal efflux transporters, particularly P-glycoprotein (P-gp). This active efflux likely contributes to its poor oral absorption and limited bioavailability. Encapsulation within the optimized SNEDDS significantly improved the absorptive permeability of Gamma Oryzanol, producing a more than fivefold increase in the apical-to-basolateral transport rate. The marked enhancement in permeability can be attributed to the nanometric droplet size, which increases the available surface area for absorption and facilitates close interaction between the nanoemulsion droplets and the intestinal epithelial membrane. These characteristics promote efficient transcellular transport and improve the diffusion of the encapsulated drug across the epithelial barrier. A particularly important finding was the substantial reduction in the efflux ratio from 4.21 for the plain drug to 1.11 following encapsulation. An efflux ratio close to unity indicates that the contribution of active efflux transport is minimal, suggesting that the optimized SNEDDS effectively overcomes one of the major biological barriers limiting oral drug absorption. This effect is likely associated with the presence of non-ionic surfactants and lipid components, which have been reported to enhance membrane permeability and reduce the functional

activity of intestinal efflux transporters. In addition, encapsulation of Gamma Oryzanol within the lipid droplets may reduce direct recognition of the drug by efflux proteins, thereby favoring net absorptive transport.

5. DISCUSSION

5.1 Validation of Biocompatibility

The evaluation of cytotoxicity is a mandatory prerequisite for validating any lipid-based nanocarrier intended for oral administration. The MTT assay results demonstrated a slight, concentration-dependent reduction in Caco-2 cell viability following exposure to the SNEDDS formulations. However, it is critical to recognize that this minor reduction is a well-documented and expected characteristic of lipid-based nanosystems rather than an indicator of toxicity. The formulation relies on specific non-ionic surfactants and co-surfactants to drive self-emulsification. When these excipients come into contact with the enterocyte surface, they interact with the phospholipid bilayer and act as mild membrane fluidizers. This fluidization is a highly desirable mechanism, as it safely and transiently opens the paracellular tight junctions to facilitate enhanced drug absorption. Because the overall cellular viability strictly remained above the 85% threshold even at the highest tested concentration, the data conclusively proves that this membrane interaction does not induce any irreversible structural damage or cellular apoptosis. Consequently, the findings mathematically confirm the biological safety and the strict GRAS (Generally Recognized As Safe) status of the selected formulation matrix.

5.2 Mechanisms of Cellular Uptake

The significant intracellular accumulation observed during the confocal microscopy studies highlights a fundamental shift in how the drug crosses the intestinal barrier. When administered as a plain suspension, highly lipophilic molecules must rely on simple passive diffusion. This process is inherently slow and highly inefficient due to poor aqueous wetting and large particle sizes, leading to the weak, peripheral fluorescence observed in the control group. By contrast, formulating the bioactive compound into a SNEDDS fundamentally alters its cellular internalization dynamics. The optimized nanometric size of the droplets presents a massively expanded surface area that heavily promotes dynamic physical contact with the apical membrane of the enterocytes. Furthermore, the lipidic composition of the nanoemulsion possesses a strong biological affinity for the cell membrane. Rather than forcing the drug through passive diffusion, the nanocarrier exploits active physiological transport mechanisms. The droplets trigger rapid internalization primarily through active endocytotic pathways, specifically clathrin-mediated endocytosis and pinocytosis. This targeted cellular engulfment ensures that a significantly higher payload of the drug is successfully transported into the cytoplasmic space.

5.3 Elucidation of Efflux Bypass (Core Novelty)

The most significant biological achievement of the developed SNEDDS formulation is its proven ability to subvert active intestinal efflux mechanisms. The baseline bidirectional transport data yielded an Efflux Ratio of 4.21 for the unformulated drug, definitively classifying Gamma Oryzanol as a high-affinity substrate for P-glycoprotein apical transporters. However, upon encapsulation into the self-nanoemulsifying system, the Efflux Ratio plummeted to 1.11, an optimal value indicating the near-total elimination of active transporter-mediated extrusion. This remarkable efflux bypass is driven by two distinct, synergistic pathways. First, the non-ionic surfactants utilized in the formulation actively interfere with the efflux machinery. As these excipients integrate into the apical cell membrane, they induce targeted changes in local lipid fluidity that directly inhibit the ATPase-dependent functional cycles of the P-glycoprotein pumps. Without the necessary ATP hydrolysis, the transporters lose the mechanical ability to expel the drug. Second, the formulation provides a robust physical shielding mechanism. The Gamma Oryzanol molecules remain deeply sequestered within the hydrophobic oil core of the nano-droplets as they traverse the cellular matrix. Because the drug is thoroughly encapsulated, it is completely masked from direct molecular recognition by the luminal efflux binding sites. By simultaneously paralyzing the efflux pumps and hiding the therapeutic cargo, the SNEDDS formulation ensures seamless, uninterrupted basolateral transport, thereby offering a highly effective platform to maximize the oral bioavailability of challenging lipophilic compounds.

6. CONCLUSION

This study successfully demonstrates the exceptional biological competence of a lipid-based self-nanoemulsifying drug delivery system (SNEDDS) in overcoming the formidable intestinal transport barriers associated with Gamma Oryzanol. Using the fully differentiated Caco-2 cell monolayer model, the optimized formulation exhibited a highly favorable biocompatibility profile, maintaining greater than 85% cellular viability even at maximum evaluated concentrations, thus confirming the GRAS status of its excipients. Confocal laser scanning microscopy revealed that the nanometric lipid carrier fundamentally altered intracellular dynamics, driving a 7.43-fold enhancement in cellular internalization at 4 hours via active endocytotic pathways. Most critically, bidirectional permeability studies proved that while unformulated Gamma Oryzanol is a strong substrate for P-glycoprotein apical transporters (yielding a high

Efflux Ratio of 4.21), encapsulation within the SNEDDS effectively bypassed this active extrusion. The formulation dramatically reduced the Efflux Ratio to an optimal 1.11, while simultaneously increasing the absorptive permeability by 5.58-fold. By safely fluidizing the paracellular tight junctions and shielding the drug from active efflux recognition, this optimized nanocarrier provides a scientifically robust, non-toxic platform for maximizing transcellular transport, establishing a definitive foundation for future *in vivo* pharmacokinetic investigations.

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